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Duckweed Uptake of Phosphorus and Five Pharmaceuticals: Microcosm and Wastewater Lagoon Studies

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DUCKWEED UPTAKE OF PHOSPHORUS AND FIVE PHARMACEUTICALS: MICROCOSM AND WASTEWATER LAGOON STUDIES

by

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ABSTRACT

Duckweed Uptake of Phosphorus and Five Pharmaceuticals:

Microcosm and Wastewater Lagoon Studies

by

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Utah State University, 2012

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Department: Civil and Environmental Engineering

Duckweed species *L. turionifera* and *W. borealis* grow on Wellsville Municipal Sewage Lagoons in northern Utah and, when harvested, contribute to the removal of phosphorus and pharmaceuticals from wastewater. Microcosm studies showed that duckweed contains an average of 1% phosphorus (dry weight) and removes 113 mg-P/m^2day under 200 μmol/m^2sec light. Duckweed in laboratory experiments reduced influent phosphorus ranging from 3.88 to 5.2 mg-TP/L to effluent concentrations of 0.88 mg-P/L in 3 days to 0.16 mg-P/L (0.32 mg-TP/L) with continual harvesting and a liquid retention time of 46 days.

Duckweed removal of pharmaceuticals was comparable to removal by membrane bio-reactors. Duckweed removed 99% acetaminophen mainly by plant uptake; 98% progesterone primarily by absorption to plant tissue; 90% fluoxetine by adsorption with some biological removal attributed to plants; and sulfamethoxazole removal varied between 25 to 90% depending on polarity. Carbamazepine did not react with duckweed.

Typical influent wastewater concentrations of the five pharmaceuticals in this study were not toxic to duckweed with an EC50 value of 614 μg/L per compound. HPLC/MS detection of pharmaceuticals in liquid samples using solid phase extraction at a neutral pH and silanized glassware produced 92-102% recoveries. Analysis of extracted solids produced lower recoveries. Solid extraction efficiencies ranged from 56-70% for samples stored for 24 hours and decreased with increasing storage time.

Field growth studies showed higher than expected duckweed growth rates in the spring compared to three models due to turion germination after the ice melts. The growth rates decreased in the fall due to turion formation in preparation for winter. Harvesting duckweed from the Wellsville Municipal Sewage Lagoons should begin after full surface coverage around June 17th and end when temperatures fall below 15 °C around September 15th. Bi-weekly harvests with a starting plant density of 75 g-dry duckweed/m^2 for the lagoons operating at 0.547 MGD and 5 mg-TP/L are required to physically remove enough phosphorus in order to meet the city's 432 kg-P/yr discharge permit. A duckweed phosphorus harvesting system in Wellsville was estimated to produce enough biomass to meet the P-discharge limit until the flow increases above 0.656 MGD around the Year 2017.

(193 pages)

PUBLIC ABSTRACT

Duckweed Uptake of Phosphorus and Pharmaceuticals: Microcosm and Wastewater Lagoon Studies

Utah State University students under the direction of Dr. R. Ryan Dupont, Environmental Engineering, have been enthusiastically involved in researching ways to improve wastewater treatment at the nearby Wellsville Municipal Sewage Lagoons. Wellsville City, along with several other Cache Valley communities, has experienced some problems with their current wastewater facility, particularly with phosphorus removal, which will become more difficult to address as the population increases in the future. Excessive phosphorus causes unappealing algae blooms in ponds and reservoirs while also posing a threat to local fish. Brittany Wilkes, Brett Housley, and Naho Orita first looked into reducing phosphorus discharge by improving management practices and harvesting duckweed. First, they recommended installing a basic bar rack and grit chamber at the headworks since it did not have any. Next, they recommended discharging wastewater in the morning before phosphorus levels peaked in the evening—preferably from lagoon 3 for highest quality effluent. Their initial duckweed studies provided a foundation for two more in-depth studies.

Jonathan Farrell's research found that *Lemna turionifera* and *Wolffia borealis* duckweed species completely cover these 23 hectare (56-acre) lagoons for 5 to 6 months of the year in this temperate climate zone. Results showed that a single harvest of these lagoons can produce 0.5 kg-dry duckweed/m^2 per 90-day harvesting season while bi-weekly harvesting can produce 1.5 kg-dry duckweed/m^2 which accounts for 30-90% removal of the annual phosphorus loading, respectively. In addition, duckweed achieved pharmaceutical removals comparable to literature reported removals by membrane bio-reactors and powder activated carbon.

Maureen Kesaano's research evaluated options for the management of the harvested biomass; after all, successful systems depend not only on growing and harvesting the duckweed, but also on the safe disposal of the harvested biomass. She discovered that anaerobic digestion of the duckweed biomass yielded 370 liters methane per kg volatile solids destroyed with a 65% methane composition. The duckweed contained less than 10% starch but could be raised to an average of 19% starch after accumulation by nutrient starvation. Fermentation for ethanol production yielded 20 to 80 mg ethanol/g-duckweed for dry and fresh material, respectively. As an animal feed option, the duckweed contained 21-38% crude protein and received relative feed values (RFVs) exceeding that of alfalfa and corn silage.

Jonathan Bay Farrell

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Opus unicum meum est conpositionem operum collegarum meorum.

I acknowledge: Dr. Dupont for his time and experience. For directing this research and yet allowing me the flexibility to try new experiments. For supporting travel to the 1st International Duckweed conference in China. Dr. Bugbee for his passion for plants and environmental instrumentation. For the times he thought about this research project while jogging along the canal or on vacation in Minnesota. Dr. Doucette for his commitment to excellence. For explaining complex aquatic and organic chemistry interactions in ways that made sense. Tom Maughan and Don Hartle from Wellsville City for duckweed and data. Mentors like Joe Stewart and Joan McLean and for funding from the Utah Water Research Laboratory. Maureen Kesaano and her enthusiasm for duckweed plants and life. Dr. Landesman at Viginia State University for collaborating with us. Brittany, Brett and Naho for their Sr. Design project with duckweed that provided a solid foundation for this and Maureen's theses. My brother Elvon for believing me when I told him it was okay to step on duckweed. Sorry about the wet pant legs, but thanks for making our first exposure to duckweed a memorable one. My uncle Dr. James Bay for always answering my questions and for helping my family feel welcome in Logan. My beautiful wife, Heather, who will talk about aquatic plants and wastewater at the dinner table—who helped me realize that the humanities exist in engineering too. And for my kids who are perhaps the youngest experts on duckweed this side of the Mississippi. My parents who have believed in me and supported me since day one. My Heavenly Father who has provided me with opportunities to explore His creations here on Earth—for the awesome beauty of those creations.

Jonathan Bay Farrell

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CHAPTER 1

INTRODUCTION

This study was designed to evaluate the performance of a duckweed system to remove phosphorus and pharmaceuticals from small wastewater treatment lagoons such as the Wellsville (UT) Municipal Sewage Lagoons (U.S. EPA Storet #490560). These lagoons were commissioned in 1974 (JUB Engineering 2008) and cover 23 ha (56-acres). Currently, this is a 0.547 MGD system that is expected to increase flow during the next 10 years to the point that the Utah Department of Environmental Quality (UDEQ) is concerned that it will not be able to meet its allowable 432 kg-P/yr discharge permit (UPDES #UT0020371). Total Maximum Daily Load (TMDL) reports from Cache Valley waterways, including the Little Bear River, showed that phosphorus was the leading chemical causing eutrophication (Utah Division of Water Quality D.o.E.Q. 2000, 2006). The Little Bear River is receiving water for agricultural runoff and wastewater discharge. Phosphorus was the principal contaminant in the TMDL and therefore the principal chemical examined in this study.

In addition to phosphorus, certain pharmaceutical chemicals have been detected in Wellsville City wastewater and are of growing interest to those concerned with water quality and in the environment in general. Five of the compounds selected for evaluation come from a larger list of 13 compounds currently being studied by the Utah Water Research Laboratory and measured in Wellsville City wastewater effluents (see Appendix A.1 Table 22). The pharmaceutical compounds selected for this study represent a range of environmentally relevant pharmaceuticals from differing classes, and having various chemical properties under typical environmental conditions. The pharmaceutical

compounds selected were Carbamazepine, Sulfamethoxazole, Acetaminophen, Fluoxetine, and Progesterone. Incidentally, the concentration of these compounds detected in Wellsville City effluent was typically lower than concentrations from nearby mechanical treatment plants pointing to a possible correlation between pharmaceutical removal and duckweed plants.

A duckweed system for phosphorus and pharmaceutical removal seems promising for lagoon based municipal wastewater treatment systems, and in particular for the system at Wellsville for three principal reasons: 1) native duckweed plants (a mixed culture of *Lemna turionifera* or *L. minor* and *Wolffia borealis*) already cover the entire surface of the lagoons for at least 6 months (May-November) of the year; 2) Wellsville has weak wastewater approximately 4 mg-P/L that results in a loading of approximately 13.3 g-P/m^2·yr. that is in the recommended < 20 g-P/m^2·yr. Range (Kadlec and Wallace 2009) for the application of duckweed based systems; and 3) duckweed floats on the surface of water. Although studies on duckweed growth and phosphorus uptake have been done for decades in areas with 9 month growing seasons, this one contributes to the research because it takes place in a temperate climate with a shorter growing season. Research on pharmaceutical uptake by duckweed has become more popular in the past decade, but few studies exist correlating duckweed growth and pharmaceutical uptake.

The performance of a duckweed system to remove phosphorus and pharmaceuticals was evaluated based on laboratory microcosm studies measuring chemical uptake by the plants, and on growth rate studies conducted both at the laboratory and field scale. In the microcosm studies, the uptake, sorption, and transformation of phosphorus and a selected set of pharmaceuticals: Acetaminophen

(ACT), Sulfamethoxazole (SLF), Fluoxetine (FLX), Carbamazepine (CRB), and Progesterone (PRG) were measured from a nutrient solution made to simulate Wellsville City wastewater. The microcosms contained either whole-, macerated-, or no-plants. Results generated from these studies were used to determine the fraction of removal by photolysis, plant sorption, plant uptake, attached microorganisms, and sorption to glassware. In addition, plant water partition coefficients and sorption isotherms were generated.

These microcosm studies followed several preliminary studies designed to gain a better understanding of duckweed plants in Cache Valley, their nutrient composition, and their growth rates under a variety of circumstances such as water quality, light intensity, and climate. These factors are summarized in the literature review. Results from the microcosm experiments were combined with existing publications on duckweed growth and actual growth studies from the Wellsville Lagoons to predict the ability of duckweed to remove these contaminants of concern from Wellsville City sewage lagoons.

CHAPTER 2

LITERATURE REVIEW

2.1 Duckweed

Duckweed is one of the smallest macrophytes on the planet. It is a monocot, an angiosperm, floats on water, and has one of the fastest growth rates of any of the macrophytes—contributing to its ability to be high accumulators of nutrients such as phosphorus and trace metals (Mkandawire et al. 2004; Mkandawire and Dudel 2005, 2007; Odjegba and Fasidi 2004; Olguín et al. 2005; Wang et al. 2004). Its location between the air-water interface makes it simple to separate from the water and susceptible to accumulation of hydrophobic chemicals (Brain et al. 2004a; Reinhold et al. 2010).

2.1.1 Characteristics and Common Species in Cache Valley

Lemnacea (common name duckweed) grows naturally in almost every region on earth with a growing season of at least 5 months. Most studies involving duckweed take place in climates with 9 to 10 month growing seasons. Rarer are the duckweed studies taking place in regions like Cache Valley with only 5 to 7 month growing seasons (Culley et al. 1981). Duckweed is a C3 plant—which helps it to grow in the colder climates. Nonetheless, since water freezes in the winter and duckweed floats on water, it does best in warmer climates. Of the four principal duckweed genera, three are found in Cache Valley; the three species from these genera in Cache Valley are all reported to be cold tolerant (Culley et al. 1981; Landolt 1986). The duckweed plants growing in Cache Valley, Utah, include *Lemna turionifera* (or *L. minor*), *Wolffia borealis*, and *Spirodela*

polyrhizza. *L. turionifera* and *L. minor* are difficult to distinguish and both may exist in Wellsville. Landolt (1986) cataloged *L.turionifera* in Cache Valley. Duckweed's native presence in Cache Valley, its tolerance to temperate climates, and its fast growth rates make it promising for nutrient removal in wastewater treatment applications.

The native species in Cache Valley reproduce asexually by growing more daughter fronds (cf. budding fronds) that eventually separate into their own colonies of two to four fronds. *L. turionifera* fronds produce up to 15 daughter fronds throughout their lifetime. The lifespan of *L. turionifera* species is typically 5 to 7 weeks, which can shorten or lengthened depending on water quality, environmental constraints, and crop density (Landolt 1986). *S. Polyrhizza* has a life expectancy of approximately 33 days, which has a tendency to shorten with extended hours of light per day (Landolt 1986).

L. turionifera, *W. borealis* and *S. Polyrhizza* species produce resting buds called turions, or in the case of *L. minor* produce resting fronds. Turions refer to starch-enriched overwintering buds that can sink to the bottom sediment during winter conditions and emerge under warmer conditions, thus enabling them to survive freezing weather (Landolt 1986). Turions can also be produced in warm temperatures (*ca.*, 25ºC) since they were observed in the reactors during this and other studies (Landolt and Kandeler 1987). Resting fronds enable *L. minor* to survive cold temperatures and ice; but unlike turions, they do not sink to the sediment layer, but rather remain suspended in the water column between the sediment and ice. Resting fronds and turions are less buoyant than normal fronds which enables them to exist below the water surface when ice forms (Landolt 1986). Necrotic fronds are less buoyant than living fronds as observed when harvesting duckweed in the early spring (Figure 1) when the duckweed mat was

Figure 1. Duckweed mat in April 2011 about 7 cm thick.

approximately 7 cm thick and only the top layer of the mat at the water surface was composed of living fronds. The floating *Lemnaceae* fronds used in these laboratory experiments had a density of 815 mg-fresh duckweed/mL (specific gravity 0.815).

Lemnaceae species increase in size from *W. borealis, L. turionifera,* and *S. Polyrhizza* species, respectively. Several factors contribute to the size of the fronds. Daughter fronds in laboratory studies are often smaller than the mother fronds (Al-Nozaily 2001), which needs to be considered when basing growth rate on frond count. As plant surface density increases, frond size often decreases. Landolt (1986) listed other factors contributing to an increase in frond size, including: increased light intensity; increased light duration; addition of sugar; increased nitrogen, phosphorus, potassium, calcium, and magnesium concentrations (which can also decrease frond size if too high); and increased temperature (Landolt 1986). Typically, full-size fronds for *W. borealis, L. turionifera,* and *S. Polyrhizza* range from 0.5 to 1 mm, 3 to 5 mm, and 1 to 1.5 cm,

respectively. *L. turionifera* fronds range in sizes depending on the development stage of the frond. Figure 2 shows how *L. turionifera* fronds were characterized depending on development. The figure also shows the utility of using digital imagery to quantify plant density, which can also be correlated with plant mass and growth rates (Eberius 2011; Klassen and Bugbee 2003). Freeware downloaded from the Internet such as Gimp (www.gimp.org) with color indexing and histogram functions make this option very accessible and affordable. Figure 2 and Table 1 show the results from one such analysis that was used to size the microcosms for this study.

The location of the Cache Valley duckweed varies depending on the species. *L. turionifera* and *W. borealis* grow in a mixed culture on the 23 ha (56-acres) of the

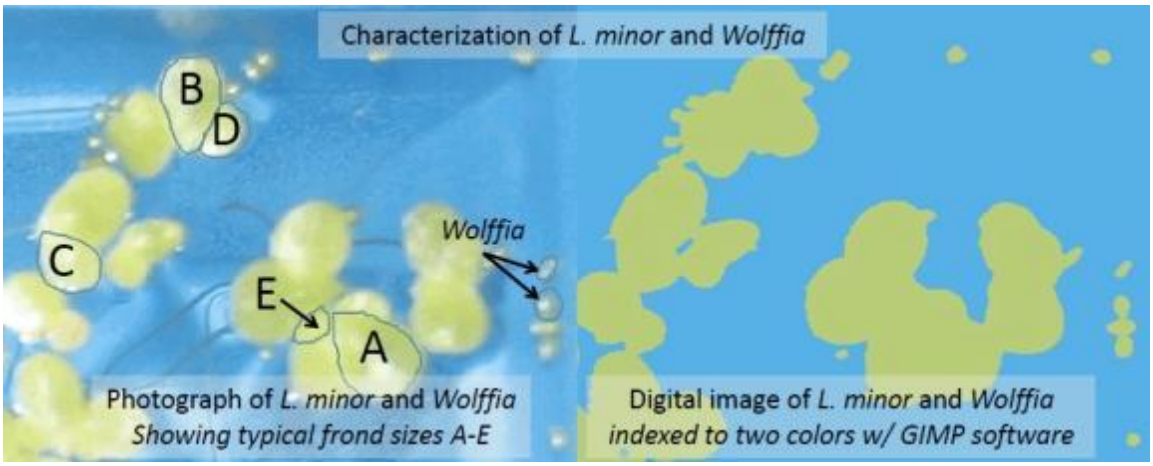

Figure 2. Characterization and digital image of *L. turionifera* and *Wolffia* fronds.

Table 1. Characterization of Figure 2 *L. turionifera* frond by area, mass, and dimensions

Frond physical characteristics	Units	A	B	C	D	E
Avg. area per frond geometry	mm^2	8.3	6.3	4	2.75	0.9
Avg. mass per frond geometry	mg_{fresh}	2.17	2.36	1.15	0.41	0.14
Length (L):Width (W) ratio	L:W	1:0.75	1.67:1	1:1	2:1	2:1
Length	mm	4.8	4	2.4	3	1.6

Wellsville Municipal Sewage lagoons (Figure 3). *L. turionifera* is more predominant in the field than *W. borealis*. The Wellsville lagoons receive some sheltering from wind due to their location in a recessed area bordered by trees along the Little Bear River. Interestingly, these two species are only found in the parts of the nearby Logan Wastewater lagoons protected from wind (*i.e.*, culverts, chlorination basin, and wetlands). The majority of the 186 ha (460 acres) Logan lagoons are not protected from wind and do not contain duckweed (Figure 4). The third Cache Valley species, *S. Polyrhizza*, can be found up Logan Canyon near Third Dam on the north side of the highway.

The growth seasons from 2008 to 2011 saw duckweed coverage on the Wellsville lagoons starting the first part of May and reaching full coverage in June. Coverage continued until about the third week in November. The wastewater lagoons freeze during the winter forcing the duckweed plants into dormancy; however, duckweed fronds appear in the water at the first sign of ice melting off the lagoons in the spring and occasionally on top of the ice in small puddles of water during particularly warm periods in the winter (Figure 5 and Figure 6).

2.1.2 Duckweed Harvesting and Growth

In addition to understanding the distribution of duckweed plants in Cache Valley, a series of preliminary studies conducted in the field and laboratory examined factors influencing duckweed biomass production rates. These factors included: harvesting, crop density with respect to environmental conditions (*i.e.*, light, temperature, and water quality), and growth inhibitors including: wind/movement, algae, fungi, aphids, chlorination, crop density, and light intensity.

Figure 3. Duckweed on 23 ha (56-acres) of Wellsville Municipal Sewage Lagoons, which sit in a hallow. Furthest right lagoon is effluent side. (www.maps.google.com)

Figure 4. Duckweed absent from nearby 186 ha (460 acres) of Logan City Lagoons probably due to algae proliferation or excessive wind movement. (www.maps.google.com)

Figure 5. *L. turionifera* and *Wolffia* duckweed species appearing immediately after the ice melts in Spring.

Figure 6. *L. turionifera* turions floating on 3 cm. water puddle above the ice on Feb. 4, 2011, following unusual rainy winter weather.

2.1.2.1 Harvesting

Harvesting is an essential component of duckweed nutrient removal systems because it physically removes the phosphorus from the system via the duckweed biomass. Without harvesting, the plant tissue would die, settle to the bottom of the lagoon, decompose and then release a significant proportion of the phosphorus and other nutrients back into the water column. This harvested biomass can be used as compost (Don Donahue, personal communication, 6/29/2009), as fodder rich in protein (Culley et al. 1981; Landesman et al. 2011), or to generate fuel like methane (Clark and Hillman 1996; Kesaano 2011).

The frequency of harvesting and the amount of biomass removed per harvest varies from study to study. However, consistent recommendations and observations include: 1) maintenance of 100% coverage should be used to reduce algae growth and 2) harvesting should be carried out at least once every 20 days, and more frequently for improved nutrient removal (Öbek and Hasar 2002). Probably one of the most successful duckweed operations in the world is called the Agriquatics Mirzapur (Bangladesh) System that feeds duckweed harvested from wastewater to cod fish (Dixon 2011; Skillicorn 2008). The system appears to be successful because the harvested biomass is a useful by-product used as fish food. One unsuccessful duckweed system in Boulder City, NV, used duckweed harvested on a 4.5 ha (11-acres) facultative lagoon as a compost amendment at the local landfill. The system had to be abandoned because the four-person crew harvesting the lagoons 40 hours/week with two mechanical harvesters could not keep up with the biomass production (Don Donahue, personal communication, 3/18/2009)—indicating that succesful operations need to have enough labor to harvest and dispose of all the biomass.

Continuous harvesting improves nutrient removal and prevents overcrowding, biomass death, and release of nutrients back into the water column. In Louisiana, Culley et al. (1981) reported that up 50% of the N & P in the biomass gets released if more than 20 days go by between harvests. Several studies support harvesting the entire biomass growth within a 3-week time period for improved nutrient removal. In Bangladesh, Alaerts et al. (1996) harvested every 2 to 3 days at an average of 4.5 g-dry/m^2day to obtain 74-77% total phosphorus removal (90-95% PO_4-P). In Australia, Willet (2005) harvested at 3.5, 5.5, and 10.4-day intervals and concluded that the shorter harvesting intervals correlated to increased biomass production and nutrient concentration in the biomass. In Thailand, Edwards et al. (1992) harvested every 2 to 15 days depending on whether it was the dry (warm) or wet (cool) season, respectively, and found similar conclusions as Willet's while also noting that phosphorus concentrations below 0.3 mg-TP/L did not support normal growth. Another study found that nitrogen and phosphorus concentrations only start to effect duckweed growth below 4.0 mg-N/L and 0.74 mg-P/L, respectively (Frédéric et al. 2006).

While frequent harvesting is recommended for increased plant production and nutrient removal, harvesting frequency also may be limited by the constraint of additional energy and labor costs. With respect to the unsuccessful duckweed system mentioned earlier, Don Donahue, superintendent of a retired Lemna Corporation (www.lemna.com) duckweed covered lagoon in Boulder City, NV, reported harvesting the entire lagoon every week. This required harvesting 4.45 ha (11-acres)/wk at a rate of 37 g/m^2·week for 9 months. This yielded approximately 71 tons (dry duckweed) per year. Two people worked 10 hr shifts 4 days a week and used mechanical harvesters with 4 ft wide

conveyors to remove the fresh duckweed that was then loaded into trucks and composted at the local landfill. Donahue reported that the duckweed system was used for approximately 10 years before being shut down because they could not keep up with the quantity of duckweed produced. Hence, careful solids management programs are necessary to guarantee sustainable and long-lasting duckweed systems.

2.1.2.2 Growth Rates

Most studies recommend starting and maintaining duckweed systems with a monolayer of duckweed sufficient to fully cover the surface area. Full coverage prevents algae proliferation that out competes the duckweed (Al-Nozaily 2001; Edwards et al. 1992; Lemna Corporation 1996). Duckweed mass can double in 2 to 7 days and follows a logarithmic growth trend. Linear growth ranges occur between 10 to 120 g-dry/m^2 for *L. minor* (Reddy and Debusk 1985). Reddy and Debusk (1985) along with Edwards et al. recommended starting with 10 to 11.9 g-dry/m^2 where growth rate is highest without algae; Culley et al. (1981), Chaiprapat et al. (2005), and Zimmo et al. (2002) recommended 30 to 40 g-dry/m^2 where algae competition is minimized; while Willet (2005), Lemna Corporation (1996), and Alaerts et al. (1996) recommended 80 to 132 g-dry/m^2.

Typical seasonal yields ranged from 3 to 9.5 tons-dry/ac-yr. Maximum yields between 17 to 25 tons-dry/ac-yr have also been reported (Alaerts et al. 1996; Edwards et al. 1992). A typical relative growth rate (RGR = $\ln(g_{new}/g_{old})$/day) of duckweed ranges from 0.06 to 0.15 (Chaiprapat et al. 2005; Culley et al. 1981; Willett 2005) while higher growth rates from 0.24 to 0.31, even up to 0.4 (Lasfar et al. 2007), are not uncommon when plant densities are low and other factors such as light, temperature, and nutrients

are optimum. Al-Nozaily (2001) observed that light intensity was the single most important variable controlling RGR, and recommended providing 200 to 300 $\mu mol/m^2 \cdot sec$ (PPF) for highest growth rates indoors. Plant mat density, which is highly dependent on light intensity, is very useful for predicting growth rates and plant production (Driever et al. 2005).

Several factors inhibit duckweed growth rates. Growth rate decreases due to overcrowding as biomass accumulates to the point that fronds start overlapping each other (Al-Nozaily 2001; Chaiprapat et al. 2005; Culley et al. 1981; Reddy and Debusk 1985). Growth rate decreases with nutrient depletion (Edwards et al. 1992). Duckweed prefers ammonium (NH_4^+) to ammonia (NH_3) and other forms of nitrogen (*e.g.*, nitrate NO_3) because the least energy is required to assimilate NH_4^+ into mobile plant amino acids and proteins. Growth decreases when $NH_3 > NH_4^+$ or when pH exceeds the pKa of ammonia of 9.25 (Al-Nozaily 2001; Culley et al. 1981) since NH_3 (aqeous or gas) inhibits cell metabolism and respiration via the electon transport system (Vines and Wedding 1960). Phosphorus precipitation with calcium also occurs at a pH near 9.3, which also leads to nutrient deficiencies and lower growth rates. Several studies indicated that wind or movement decreased growth (Edwards et al. 1992; Willett 2005). Biomass started depleting at temperatures below 17°C, and completely disappeared below 5°C according to Don Donahue and Zimmo et al. (2002). Growth rate also decreased due to competition between species (Clatworthy and Harper 1962; Edwards et al. 1992).

2.1.2.3 Growth Inhibitors

Studies and online comments abound which point out how simple duckweed is to grow and how hard it is to get rid of once it starts growing. Duckweed plants are used for

toxicity studies because they are easy to grow. But in the case of this study, over 2 years were spent developing a nutrient solution and indoor environment suitable for the duckweed growth. As a result of losing several crops, this study demonstrated several factors that inhibit duckweed growth, including: wind/movement, algae, fungi, aphids, chlorination, crop density, and light intensity.

Duckweed prefers growing in quiescent conditions like the hallow holding the 23 ha (56-acres) of the Wellsville Municipal Sewage Lagoons. Early on in this study, it was observed that duckweed mats occur only in areas shielded from the wind and in slow moving water. Several studies have made similar observations (Edwards et al. 1992; Landolt 1986). Two corporations market floating barriers to reduce the impact of wind and water movement (Lemna Corporation 1996; Willett 2005).

Duckweed has the potential to reduce total suspended solids (TSS) in wastewater (Zirschky and Reed 1988), primarily by shading the water column below to prevent algae growth. On the other hand, if the duckweed crop density is much less than 100% cover or 20 to 30 g-dry/m^2 then algae receives enough sunlight to out-compete duckweed (Roijackers et al. 2004; Szabo et al. 1998, 2003, 2005). When algae attaches to duckweed fronds small air bubbles appear below the frond cutting off its contact with the liquid interface (Landolt and Kandeler 1987). Some studies have used sand filters to reduce algae concentrations (Naghavi and Malone 1986). Once algae begin to compete with duckweed then it's difficult to remove (Zirschky and Reed 1988) without chemical treatment (Edwards et al. 1992).

Fungi had a tendency to show up in duckweed crops (see Appendix B.1, Page 117), frequently as a blight of chlorosis and necrosis that, if left unattended, would

quickly multiply and destroy entire duckweed crops (Rejmánková et al. 1986; Wagner 1969). Reports blame pythium fungi for ruining duckweed (Cross 2005). After noticing the fungal infestation in the laboratory, blight patches were observed in the Wellsville Municipal Sewage Lagoon duckweed crops as well. However, it's suspected that the daily climate variation outdoors and the large amount of duckweed were able to prevent fungi from destroying the entire Wellsville duckweed crop. Recommendations to prevent and eliminate fungi infestations in plants include: reduced temperatures, increased silicon in plant tissue (Bugbee 2003, 2004), and the use of fungicides. Lower temperatures and adding potassium silicate to the nutrient solution in the laboratory studies were not able to prevent existing fungi infestations from killing duckweed fronds; it took adding Ridomil Gold EC (fungicide) at a dose of 0.3 μL/L to remove the fungi.

An interesting phenomenon occurred after duckweed plants were inundated with fungi. As chlorosis and tissue damage set in on the duckweed fronds, bacteria colonies and numerous microscopic invertebrates began to decompose the plants, accelerating their decomposition (Suren 1989) (see Appendix A.2).

Aphids (a.k.a. phytophagous-fauna) were observed on top of the duckweed mat when *L. turionifera* and *W. borealis* plants were first brought into the laboratory from the Logan Lagoons. While growth rates in these plants were lower than the rates in plants without aphids, it's undetermined whether or not the aphids played a major role in inhibiting duckweed growth (Cross 2005; Edwards et al. 1992). Aphids living atop duckweed mats in some instances have been associated with decreased growth (Edwards et al. 1992; Zimmo et al. 2002). Aphids were removed by occasionally disturbing the water surface (see Appendix A.3).

Residual chlorine has a tendency to inhibit growth of aquatic vascular plants, like duckweed (Watkins and Hammerschlag 1984). The first batches of duckweed plants taken from the Logan Lagoons were transplanted from the chlorination basin. In addition, the wastewater used for growing the plants was retrieved immediately following the chlorination basin and just prior to the polishing wetlands. When the first studies involving duckweed from the Logan Lagoons showed lower growth rates than other duckweed plants, a bioassay was conducted to determine whether or not chlorine had an effect on duckweed growth. This bioassay showed that duckweed grown on the non-chlorinated wastewater had a significantly higher growth rate than duckweed grown on the chlorinated wastewater (p-value = 0.0485, df = 4). Following these studies, both duckweed and wastewater were retrieved prior to the chlorination basin.

pH values in the Wellsville Municipal Sewage Lagoons varied from 7.8 to 8.3 depending on the sampling location. The uncontrolled pH values in the laboratory reactors sometimes produced pH levels above 9 and occasionally as high as 11. The increased pH was due to algae growth and consumption of CO_2, bicarbonate, and carbonate alkalinity. Alkalinity in the pH 9 to 11 ranges was due to hydroxide, since all the carbonate was removed to supply CO_2 for the algae photosynthesis. Growth on high pH waters affected the duckweed species and the phosphorus concentration. *L. turionifera* species dominated over the *W. borealis* when pH < 9 and also when the water surface was occasionally disturbed (see Appendix B.2); however, this dominance shifted in high pH water as *Wolffia* became the dominant and almost sole species in pH >9 water. Edwards et al. (1992) noticed a decreased biomass yield when *Wolffia* out-competed the *Lemna* species due to its smaller plant size.

Light intensity in the laboratory was only about 10% of the outdoor intensity. Light intensity at noon in Logan in the summer might reach 2,000 PPF. Light intensity in the laboratory under greenhouse 1,000 Watt High Pressure Sodium Lamps (HPSLs) 1 m high only reached 200 PPF in this study. Duckweed growth increased with increasing light intensity and duration. Shop lights containing cool white fluorescent bulbs 25 cm above the water surface produced 85 PPF; aquarium lights about half that, and HPSLs more than double that. Nonetheless all values are only a tenth of the intensity of the sun. Fortunately, duckweed has adapted to growing in shady and cool areas and is able to grow under reduced light intensity.

Crop density has the ability to accelerate or decelerate duckweed growth rates (Driever et al. 2005). Crop densities above 100 g-dry/m^2 cause the duckweed mats to become overcrowded and growth rates to decline. On the other hand, less crowded duckweed mats experience higher growths rates. Crop densities below 20 to 30 g-dry/m^2 lead to lower growth rates due to an increase in algae competition because of the extra light passing through the duckweed mat.

In summary, indoor laboratory experiments are susceptible to decreasing duckweed growth rates due to altering the natural duckweed ecosystem. Indoor conditions have the advantage of controlling some environmental conditions, but the laboratory environment can lead to radical changes in the ecology. For example, pH was more variable in the lab and as pH increased the dominating *L. turionifera* species was overcome by *W. borealis*. Also, it appears that the conditions in the laboratory favored a *pythium*-fungi infestation. Factors affecting duckweed growth in these experiments

included: wind/movement, algae, fungi, aphids, chlorination, crop density, and light intensity.

2.2 Nutrient Solutions and Biomass Composition

Two nutrient solutions for growing duckweed in the laboratory were tested. The main objective of selecting a nutrient solution was to represent the wastewater composition in Cache Valley and also to be relevant to other published studies which frequently use wastewater (Chaiprapat et al. 2005), Hutner's solution (Landolt 1986), and APHA diluted algae medium (Reinhold et al. 2010).

The first nutrient solution consisted of raw Wellsville City and Logan City wastewaters that were collected every other week. The preliminary experiments using raw wastewater lasted just over one year and focused on duckweed growth rates and phosphorus uptake. The second nutrient solution used took into account the ratio of inorganic elements in the duckweed plants, Wellsville City wastewater, and other recommended solutions for growing duckweed. Making this second synthetic nutrient solution proved to be an iterative process following guidelines established by the Crop and Physiology Lab (CPL) at Utah State University (Bugbee 2003, 2004). The CPL has many years of experience using hydroponic solutions. They showed how to develop a custom nutrient solution based on the ratio of elements in the plant.

Preliminary studies of the duckweed plants on the Wellsville Municipal Sewage Lagoons sampled in the summer of 2008 were analyzed for their basic elemental composition. A detailed composition of the inorganic constituents in Wellsville duckweed can be found in a thesis by Maureen Kesaano (2011) who also determined the chemical formula for Wellsville duckweed is $C_{102}H_{159}O_{60}N_9P$. Based on the duckweed

composition and Wellsville wastewater the Wellsville duckweed requires a nitrogen-to-phosphorus molar ratio of 9:1 and mass ratio of 5:1.

Experiments using this second nutrient medium based on the duckweed nutrient composition (Table 2) and Wellsville wastewater composition lasted approximately 8 months. This medium is called Wellsville synthetic nutrient solution (Table 3).

2.3 Uptake of Contaminants of Concern

In addition to understanding the characteristics and growth rates of duckweed plants in Cache Valley, a series of studies examined the plants' ability to uptake several contaminants of concern in Cache Valley, including: phosphorus, Carbamazepine, Sulfamethoxazole, Acetaminophen, Fluoxetine, and Progesterone. Of particular interest are the concentrations of Chemicals of Concern (COCs) in the plant tissue that may result in restricted use of duckweed solids and the concentration of COCs in the water column that shows the performance of duckweed as a treatment option to remove these chemicals.

Table 2. Wellsville duckweed composition

Macro Elements	% Dry wt.	Micro Elements	(mg/kg)	Organic Components	% Fresh wt.	% Dry wt.
N	5.2	Fe	490	Organic Carbon	2	50
P	1	Mn	187	Lipid[a] average	0.25	5
K	3.3	B	736	Protein	1	25
Ca	5.3	Mo	-	Organic Matter	4	80
Mg	0.5	Zn	98.4			
S	0.75	Co	-			
% Water	95	Cu	29.4			

[a] Landolt and Kandeler (1987), p. 20

Table 3. Wellsville synthetic nutrient solution based on elemental composistion of duckweed and Wellsville wastewater

Stock Solution Preparation[b]				Final Nutrient Solution Elemental Composition			
Stock Soln.	Chemical Formula	Concentration	Dillution	Element	Concentration[c]		Molarity	
A	NH_4NO_3	28.6 g/L-soln.A	1:400	N	25	mg/L	1.8	mM
	K_2HPO_4	12.2 g/L-soln.A		P	5.5	mg/L	0.2	mM
B	$FeCl_3x6H_2O$	1.429 g/L-soln.B	1:1000	K	22	mg/L	0.6	mM
	$Na_2(EDTA)x2H_2O$	2.692 g/L-soln.B		Ca[a]	50	mg/L	1.3	mM
	$MnCl_2x4H_2O$	0.714 g/L-soln.B		Mg[a]	20	mg/L	0.8	mM
C	H_3BO_3	1.176 g/L-soln.C	1:1000	S[a]	10	mg/L	0.3	mM
	$Na_2MoO_4x2H_2O$	0.01 g/L-soln.C		Fe	300	µg/L	5.4	µM
	$ZnCl_2$	0.408 g/L-soln.C		EDTA	2100	µg/L	7.2	µM
	$CuCl_2x2H_2O$	0.005 g/L-soln.C		Mn	200	µg/L	3.6	µM
D	K_2SiO_3	15.4 g/L-soln.D	1:1000	B	200	µg/L	18	µM
				Mo	4	µg/L	0.04	µM
				Zn	200	µg/L	3.1	µM
				Cu	2	µg/L	0.03	µM
				Cl	1024	µg/L	29	µM
				Na	325	µg/L	14	µM
				Si	2.8	mg/L	0.10	mM

[a] Ca, Mg, and S frome Logan tap/river water

[b] To prepare the nutrient solution: add 2.5 mL soln.A and 1.0 mL of solutions C, D, and E to 987 mL tap/river water

[c] Nutrient solution concentration based on growth rate of 0.5 g-dry duckweed/L-soln.

2.3.1 Phosphorus

Nutrients such as phosphorus enter the aquatic environment by anthropogenic and natural sources. Anthropogenic sources include agricultural runoff from fertilizers and manure, phosphorus containing detergents in wastewater and human excreta. Non-point agricultural sources are responsible for roughly 42% of the Little Bear River watershed impairments (Utah Division of Water Quality D.o.E.Q. 2006) and can be successfully reduced through best management practices (BMPs) (U.S. Envrionmental Protection Agency O.o.W 2008). Point sources like the Wellsville Municipal Sewage Lagoons are responsible for about 19% of the watershed impairments (Utah Division of Water Quality D.o.E.Q. 2006). Natural, non-anthropogenic, sources originate from phosphorus containing sediment and decomposition of organic material. Naturally occurring duckweed has the potential to remove phosphorus from point sources.

Duckweed plants typically contain more phosphorus in its tissue than other floating plants, which makes them suitable for phosphorus removal. Duckweed systems are usually lagoon based systems that receive weak municipal wastewater containing 1 to 4 mg-P/L; however, duckweed has also been used to treat high strength wastewater like swine lagoon waste containing 62.5 to 135 mg-P/L (Chaiprapat et al. 2005). With respect to phosphorus removal from weak lagoon wastewater duckweed systems are similar to algae systems (Griffiths 2010).

Chaiprapat et al. (2005) reported up to 100% phosphorus removal in bench scale tests; however, 60 to 75% phosphorus removal (Alaerts et al. 1996; Kadlec and Wallace 2009; Zimmo et al. 2002) is more common. These same reports have identified duckweed biomass as contributing 13 to 47% of the total phosphorus removal, and one account

attributes all of it to duckweed. The phosphorus concentration in the effluent coming from duckweed systems almost always falls below 1 mg-TP/L and frequently less than 0.53 mg-P/L down to 0.05 mg-P/L (Alaerts et al. 1996; Edwards et al. 1992; Willett 2005). Edwards et al. (1992) observed that duckweed growth started decreasing when phosphorus levels fall below 0.3 mg/L; however, plants may continue to survive in concentrations as low as 0.03 mg-P/L (1 μmol-P) (Bruce Bugbee, personal communication, 4/12/2012).

A duckweed treatment system would physically remove phosphorus and other assimilated/sorbed compounds via the harvested biomass. Successful wastewater treatment physically removes contaminants from the water and/or converts contaminants into non-harmful constituents. Currently, it's estimated that about 89% of the phosphorus in the Wellsville Municipal Sewage Lagoons is sequestered in the sediments that have never been dredged and/or lost through seepage, while only 11% leaves through the effluent. The 89% removal is based on the assumption that $flow_{in}$ is 0.547 MGD, $flow_{out}$ is 0.252 (54% liquid lost due to evaporation and seepage), phosphorus is 3.88 mg-TP/$L_{influent}$ and 0.95 mg-TP/$L_{effluent}$ (JUB Engineering 2008). Figure 7 shows a mass balance of phosphorus in the Wellsville lagoons and laboratory reactors. Sequestering of phosphorus in the sediment has the potential to re-release phosphorus into the water column as microbes decompose organic matter and as phosphates re-dissolve into the water column, as shown in the phosphorus cycle in Figure 8. Kesaano (2011) reported that 30 to 50% of the phosphorus in the plants gets released by anaerobic digestion.

The degree of treatment by a duckweed system is measured two ways: 1) by the composition of plant solids and 2) by the concentration of the chemical in the effluent.

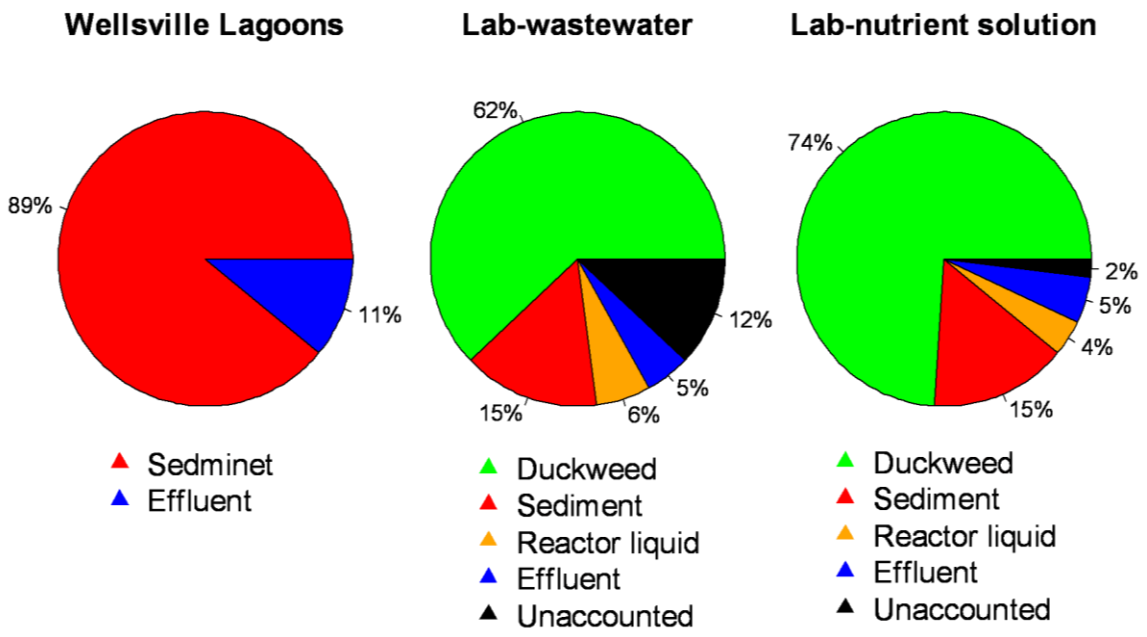

Figure 7. Phosphorus removal and sequestration in Wellsville lagoons and laboratory reactors.

Studies conducted in the laboratory from August to December 2010 (see Appendix Tables C-1 and C-8) show that the phosphorus concentration in the *L. turionifera* tissue increases with increasing phosphorus liquid concentration, as seen in Figure 9 below.

2.3.2 Pharmaceuticals

The pharmaceuticals for this study were selected to represent a broad range of pharmaceutical classes detected in Cache Valley, Utah, specifically and wastewater in general. The compounds selected were: Carbamazepine, Sulfamethoxazole, Acetaminophen, Fluoxetine, and Progesterone. A brief description of these compounds, their characteristics, and unpublished results from preliminary studies at the Utah Water Research Laboratory showing the concentration of these chemicals in various Utah wastewaters is contained in Table 4 and Table 5 (also see Appendix A.1 Table 22).

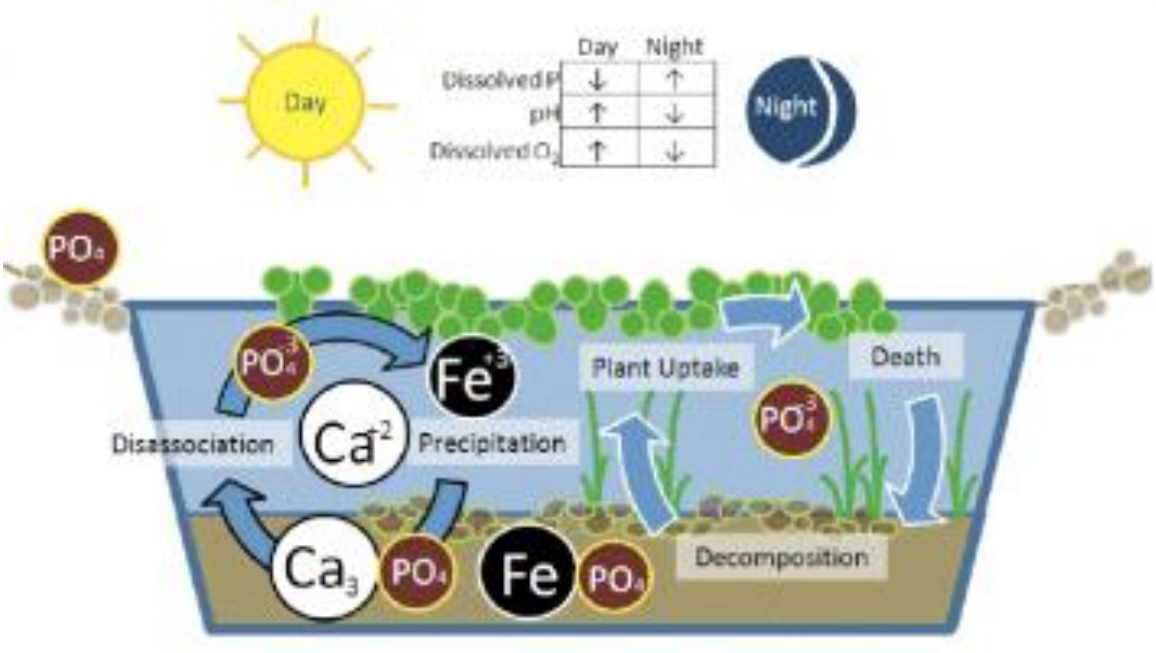

Figure 8. Phosphorus cycle in a typical lagoon system.

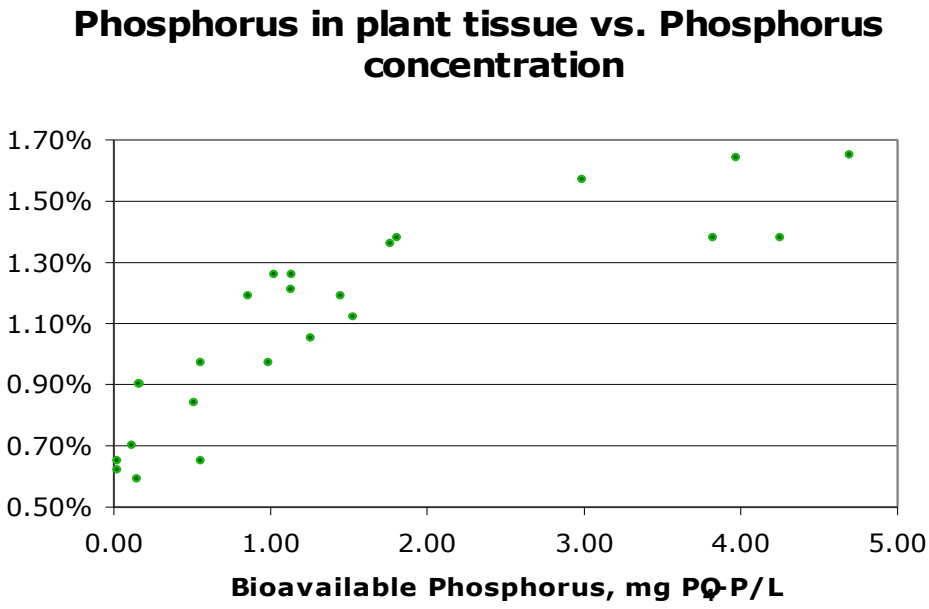

Figure 9. Phosphorus composition in laboratory duckweed tissue as a function of soluble reactive PO_4-P concentration.

Charts showing the physical structure of these molecules, pKa, and isoelectric points were collected online (www.chemaxon.com) and appear at the end of this section. A brief description of each pharmaceutical is described below. Information was obtained from the package inserts submitted to the Food and Drug Administration (FDA) and are available online (www.accessdata.fda.gov/scripts/cder/drugsatfda/). Information regarding their mechanisms of action came from correspondence with a personal acquaintance Dr. Nathan Bay.

Acetaminophen (a.k.a. Tylenol or paracetamol). Acetaminophen (Figure 10) is a fever and pain reducer (analgesic, cf. Salicylic acid). Its mechanism of action inhibits "cyclooxegenase (COX-2) [which] is an enzyme that converts arachadonic acid to prostaglandins which mediate inflammatory and pain responses" (Nathan Bay, personal communication, 4/13/2011). Its plasma concentration is 28 μg/mL with a half-life of 2.4 hours. Its metabolite is a conjugate with glucuronide or sulfate, or formed by oxidation with cytochrome P450. It distribution is <5% parent compound in the urine and > 90% excreted as metabolite within 24 hours (FDA package insert). It has a close to neutral charge at pH 7 (Figure 11).

Sulfamethoxazole (a.k.a. Bactrim). Sulfamethoxazole (Figure 12) is a bacteriostatic sulfonamide antibiotic. Its mechanism of action is to "inhibit DNA replication in bacteria by inhibiting the enzyme that converts para-aminobenzoic acid to folate" (Nathan Bay, personal communication, 4/13/2011). It inhibits the biosynthesis of nucleic acid and proteins essential for bacteria. Its plasma concentrations at steady state are 57.4 μg/mL (free) and 68 μg/mL (total) with 70% bound to plasma proteins and a half-life of 8 to 10 hours. Its principal metabolite is sulfonamide.

Table 4. Five pharmaceuticals and their occurrence, concentrations in wastewater influent/effluent, and chemical characteristics at pH 7 and 25°C

Pharmaceutical Descriptors	ACT	SLF	FLX	CRB	PRG
% occurrence in groundwater	72-81%[a]	23.4%[b]	4.3%[b]	6.4%[b]	unknown
Influent conc. ranges (ng/L)	31.8 x 10^{3}[k], 1.13-201 x 10^{3}[c]	691[k], 354[e]	120-2300[e]	30[k], 6.9[d]	53[k], 64[g], 1.2-108[h]
Effluent conc. ranges (ng/L)	26[k], non-detect[c], 157[d]	135[k], 208[e]	100[d]	33[k], <1[d]	38[k], <1-22[g]
Molecular weight (g)[j]	151.17	253.28	309.33	236.28	314.47
Charge[i] @ pH 7	slightly Anionic	Anionic	Cationic	Neutral	Neutral
Acid/Base/Neutral	Acid	Acid	Base	---	Neutral
pKaw[j]	-10.581	-10.408	-5.439	-8.355	-5.576
pKh[j] (atm-m3/mole)	-12.2	-12.02	-7.05	-9.97	-7.19
BCF[j] (L/kg (fresh))	0.984-3.162	1.472-3.162	---	19.21-19.3	166-450
Solubility[j] (mg/L @ 25°C)	1.4-3.04 x 10^{4}	610-3942	38.35	17.7-112	5.003
Pv[j] (mm Hg @ 25°C)	1.94 x 10^{-6}	1.30 x 10^{-7}	2.52 x 10^{-5}	8.80 x 10^{-8}	3.22 x 10^{-3}
pKow[j]	0.46	0.89	4.05	2.45	3.87
pKa[j]	9.38	5.89[j]	9.62	none	none
Air, Level III Fugacity % Mass	1.29E^{-5}	9.31E^{-6}	0.081	0.00112	0.0487
Water, Level III Fugacity % Mass	22.3	13.8	5.88	11.8	11.6
Soil, Level III Fugacity % Mass	77.6	86	55.6	87.3	79.5
Sediment, Level III Fugacity % Mass	0.0826	0.203	38.5	0.876	8.85

References:
[a](Cunningham et al. 2010)
[b](Focazio et al. 2008; Barnes et al. 2008)
[c](Gracia-Lor et al. 2012)
[d](Fent et al. 2006)
[e](Radjenović et al. 2009)
[f] (Kummerer 2009)
[g] (Snyder et al. 2007)
[h](Chang et al. 2011)

References (continued):
[i](www.chemaxon.com)
[j](EPA Episuite v4.0)
[k](Roth 2012) avgerage concentrations for Brigham City, Hyrum, and Spanish Fork, Utah—measured at the Utah Water Research Laboratory

Abbreviations:
ACT = Acetaminophen
SLF = Sulfamethoxazole
FLX = Fluoxetine
CRB = Carbamazepine
PRG = Progesterone

Table 5. Five pharmaceutical effluent concentrations from seven Utah wastewater treatment plants sampled in September 2010

Wastewater Treatment Plant information		Effluent Wastewater Concentrations (ng/L)				
WWTP Location (& Treatment)	Average Flow (& Design Flow)	Acetaminophen	Sulfamethoxazole	Fluoxetine	Carbamazepine	Progesterone
Brigham City (Oxidation Ditch)	1.4 MGD_{avg} (6.0 MGD_{design})	14.8	137.4	6.2	263	9.4
Price (Trickling Filter)	1.8 MGD_{avg} (4.0 MGD_{design})	17.9	64.8	3.9 (<MDL)	261	4.2 (<MDL)
Wellsville (Lagoons)	0.547 MGD_{avg} (0.3 MGD_{design})	5.3 (<MDL)	18.0	1.7 (<MDL)	32	3.6 (<MDL)
Tremonton (STM Aerotor/Sand Filter)	1.4 MGD_{avg} (1.9 MGD_{design})	2009.2	161.9	3.4 (<MDL)	110	2.9 (<MDL)
Moroni (MBR)	0.6 MGD_{avg} (0.9 MGD_{design})	598.1	34.0	0.8 (<MDL)	20	7.2
Oakley (MBR)	0.1 MGD_{avg} (0.25 MGD_{design})	5.3 (<MDL)	132.4	6.4	499	2.9 (<MDL)
Fairview (MBR)	0.07 MGD_{avg} (0.375 MGD_{design})	3.1 (<MDL)	174.6	2.4 (<MDL)	181	3.8 (<MDL)
Average Concentration	*all locations*	379	103	3.5	195	4.9
Median Concentration	*all locations*	14.8	132.4	3.4	180.6	3.8
Method Detection Limit	*all locations*	7	5.4	4.3	3	4.4

Its distribution is 84.5% in the urine (within 0.72 hours), 30% of which is parent compound (FDA package insert). Its charge is negative at pH 7 (Figure 13).

Fluoxetine (a.k.a. Prozac). Fluoxetine (Figure 14) is an antidepressant, serotonin reuptake inhibitor. It's used to treat depression, obsessive compulsive behavior, bulimia, and panic. Its mechanism of action "...works by inhibiting the reuptake of serotonin (5-hydroxytryptamine), a neurotransmitter, by presynaptic CNS cells and thus allowing more of the neurotransmitter to hang around and exert its effects" (Nathan Bay, personal communication, 4/13/2011). Its plasma concentration is 15 to 55 ng/mL and may reach 91 to 302 ng/mL with continual dosing over 30 days. It is persistent in the body. Its half-life is long, typically 1 to 3 days (acute) and 4 to 6 days (chronic). Its metabolite is norfluoxetine and occurs by demethylation. It binds 94.5% to serum proteins (FDA package insert). At pH 7 its charge is positive (Figure 15).

Carbamazepine (a.k.a. Tegretol). Carbamazepine (Figure 16) is an anti-epileptic, anticonvulsant, mood stabilizer. It is used for treating epilepsy, trigeminal neuralgia, and sometimes bipolar and ADHD. Its mechanism of action occurs "...by stabilizing sodium channels (making depolarization more difficult), and potentiating GABA receptors (which are Chloride channels that hyperpolarize a membrane)" (Nathan Bay, personal communication, 4/13/2011). Its plasma concentration is typically 4 to 12 ng/mL and has a half-life of 25 to 65 hours that reduces to 12 to 17 hours after repeated doses. When concomitant with Fluoxetine its plasma concentrations may increase. It may inhibit the effect of Acetaminophen or hormonal concentrations. Its principal metabolite is Carbamazepine-10, 11-epoxide which is equally potent. It distribution is 72% in the urine and 28% in the feces, with only 3% as parent compounds and the rest as

hydroxylated and conjugated metabolites (FDA package insert). It is practically insoluble. It is non-ionized with zero charge over virtually the entire pH range (Figure 17).

Progesterone (a.k.a. Prometrium). Progesterone (Figure 18) is a steroidal hormone, synthesized in plants, and identical to ovarian progesterone. It assists in the menstrual cycle, pregnancy, and embryogenesis of humans; it may be used to treat skin conditions; and it is sometimes used as a contraceptive. It is an "...agonist for the Progesterone receptor which when bound to Progesterone promotes a host of protein productions which do a host of things" (Nathan Bay, personal communication, 4/13/2011). Its plasma concentration is 17.3 ng/mL (100 mg dose) up to 60.6 ng/mL (300 mg dose). It's first metabolized to pregnanediols and pregnanolones by conjugation to glucuronide or with sulfate. It may be deconjugated and metabolized via reduction, dehydroxylation, and epimerization (FDA package insert). The molecule is non-ionisable (Figure 19).

2.3.2.1 Sources

Symptoms of the excessive inorganic nutrient loading and subsequent eutrophication of rivers, such as algae blooms and fish kills, are often easy to identify. However, biological effects from low concentration pharmaceuticals in a river system are still uncertain and difficult to quantify—although studies have shown both the harmful effects and occurrence of many of these chemicals (Fent et al. 2006). The pharmaceutical contaminants of concern in this study can enter the environment through human disposal, manufacturing, and animal husbandry. Wastewater receives both industrial and household contributions (Ruhoy and Daughton 2008). Household contributions include

the excretion of pharmaceuticals through feces and urine, as well as disposal of unused or expired pharmaceuticals down the toilet. Progesterone and Acetaminophen are sometimes used as veterinary medicines as well.

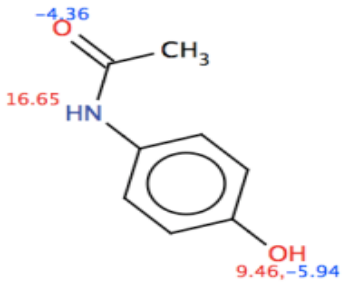

Figure 10. Acetaminophen structure.

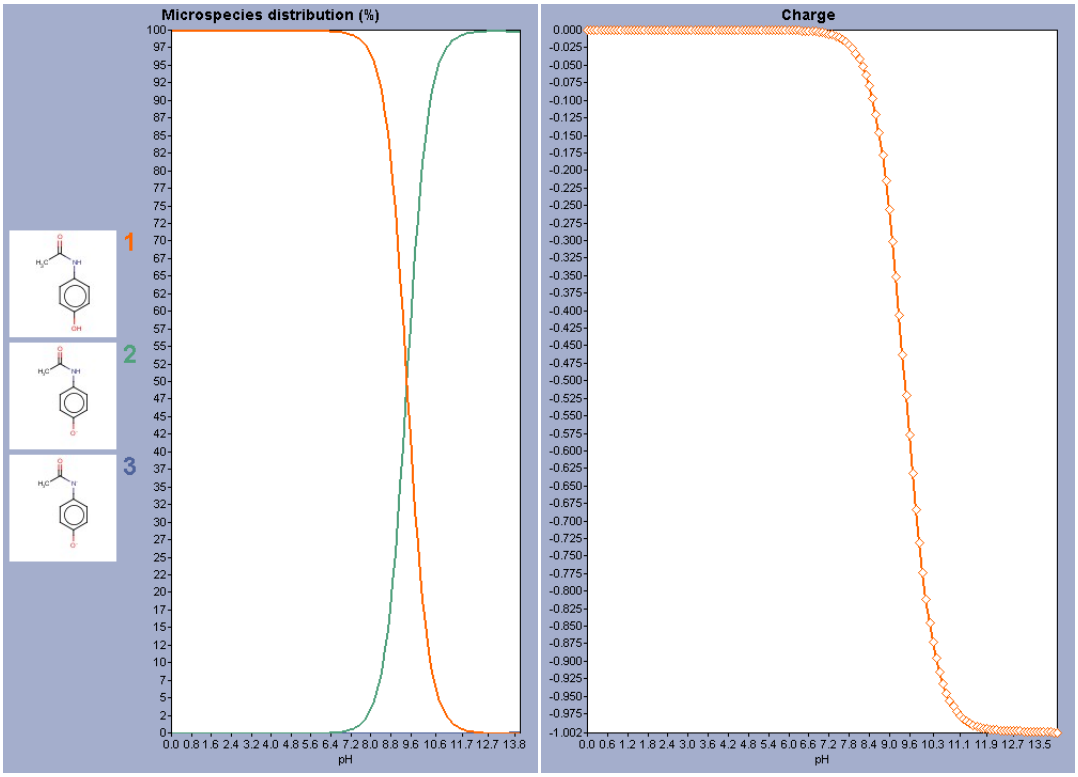

Figure 11. Acetaminophen pKa (left) and isoelectric point (right).

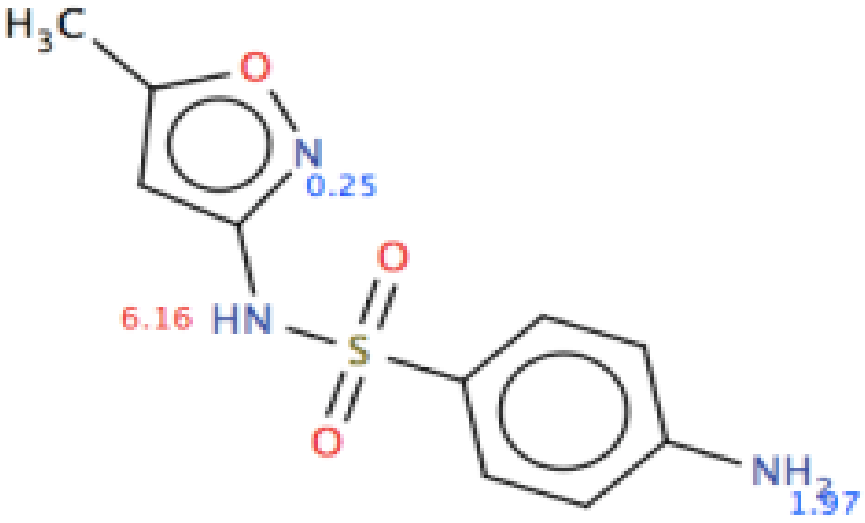

Figure 12. Sulfamethoxazole structure.

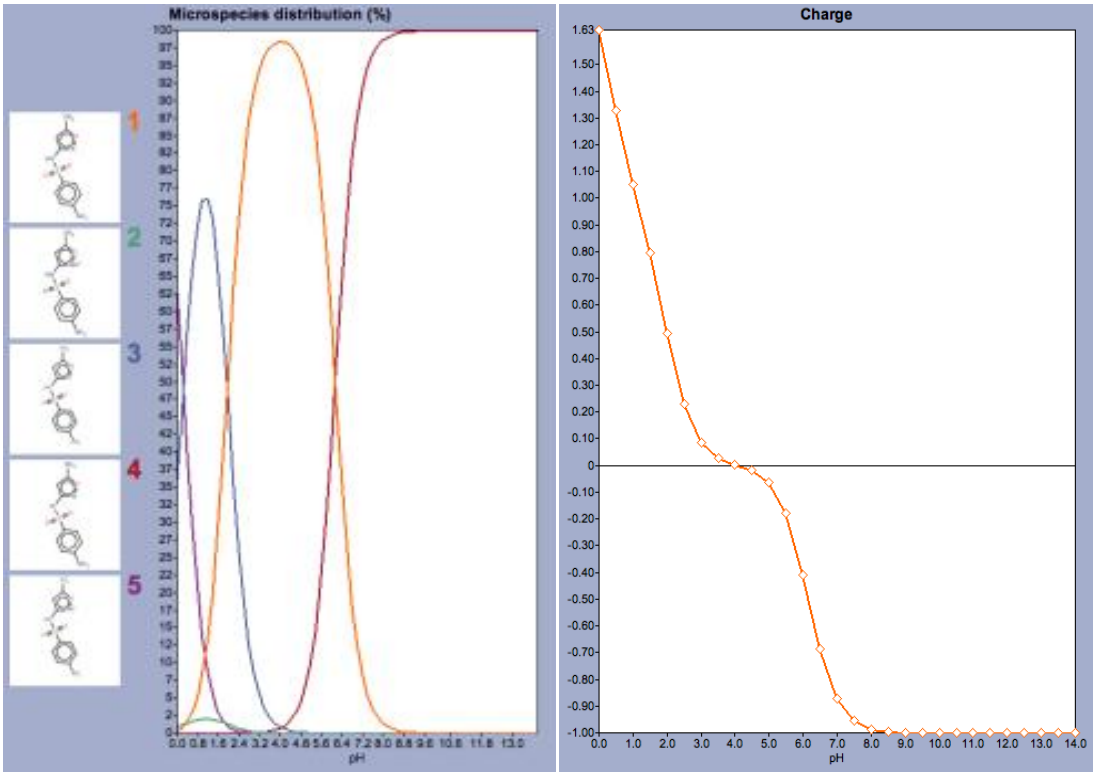

Figure 13. Sulfamethoxazole pKa (left) and isoelectric point (right).

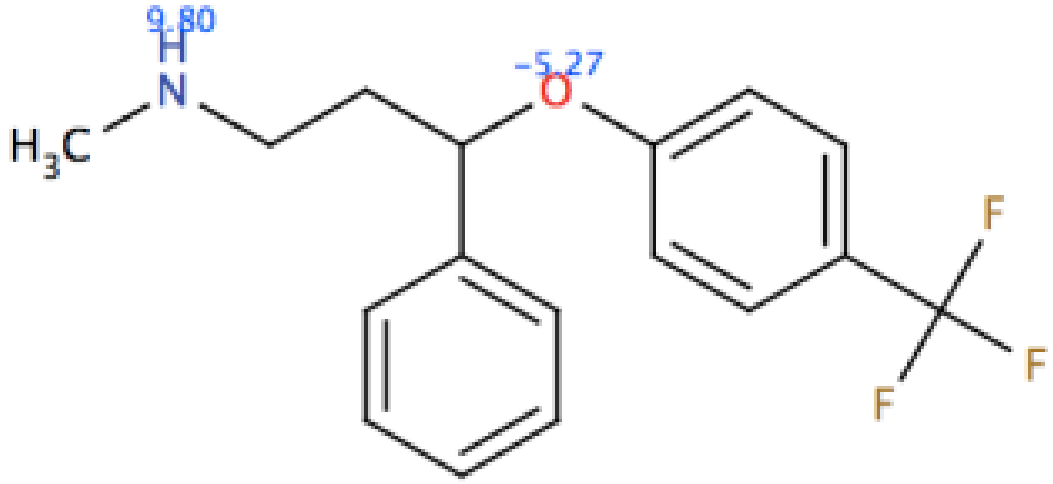

Figure 14. Fluoxetine structure.

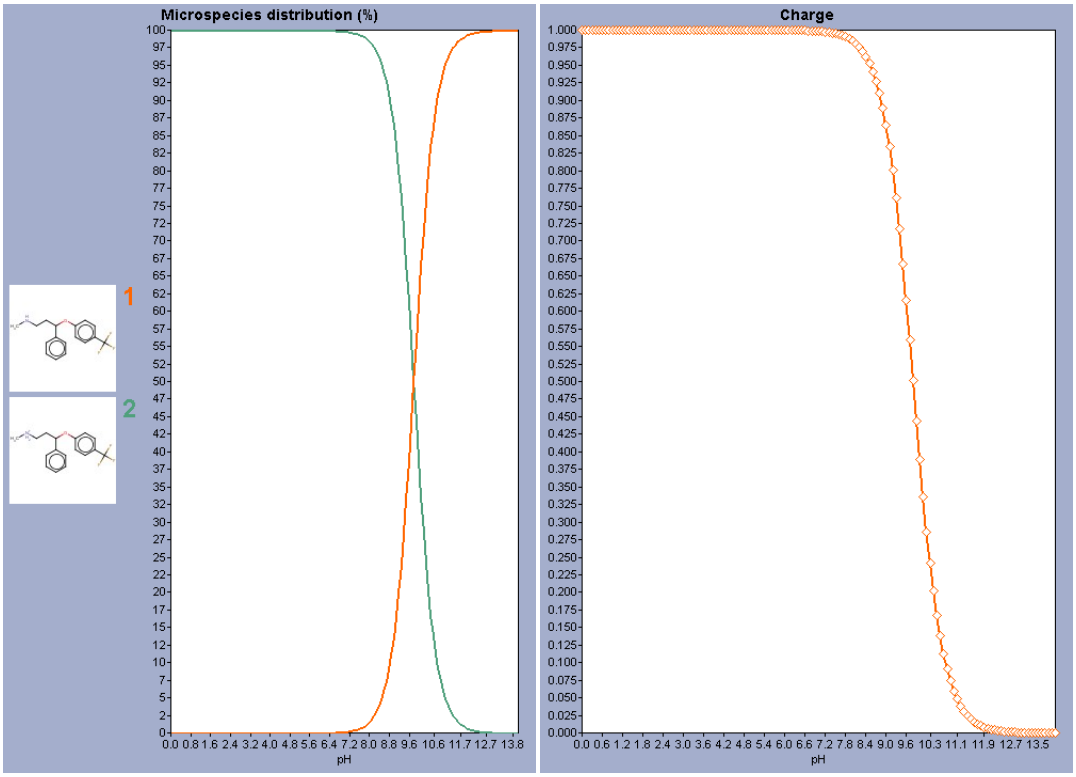

Figure 15. Fluoxetine pKa (left) and isoelectric point (right).

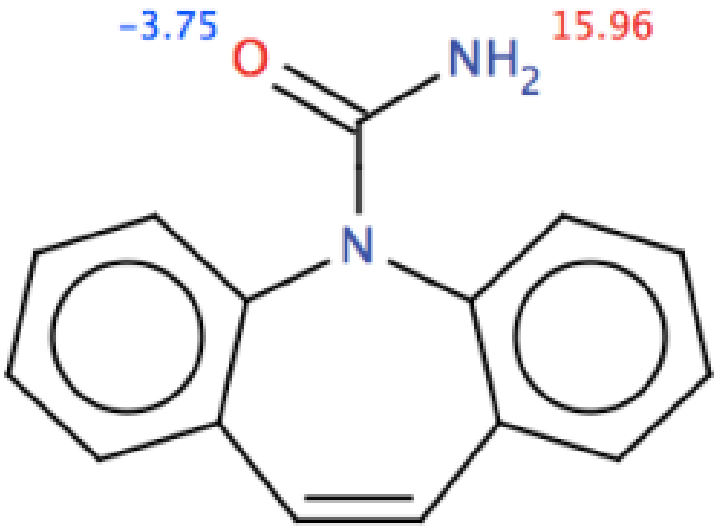

Figure 16. Carbamazepine structure.

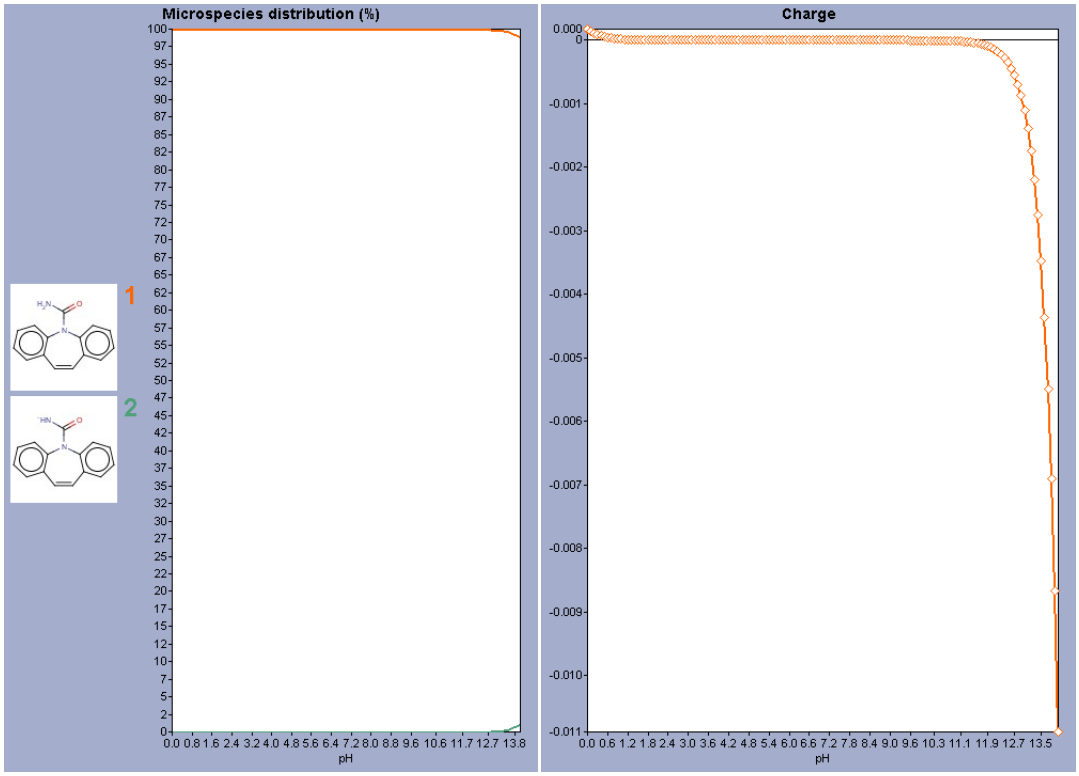

Figure 17. Carbamazepine pKa (left) and isoelectric point (right).

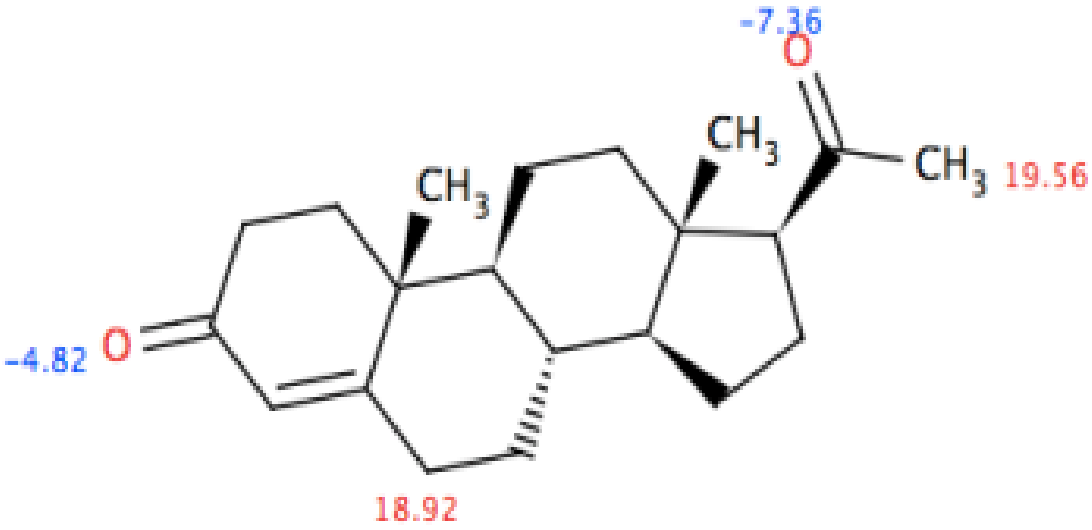

Figure 18. Progesterone structure.

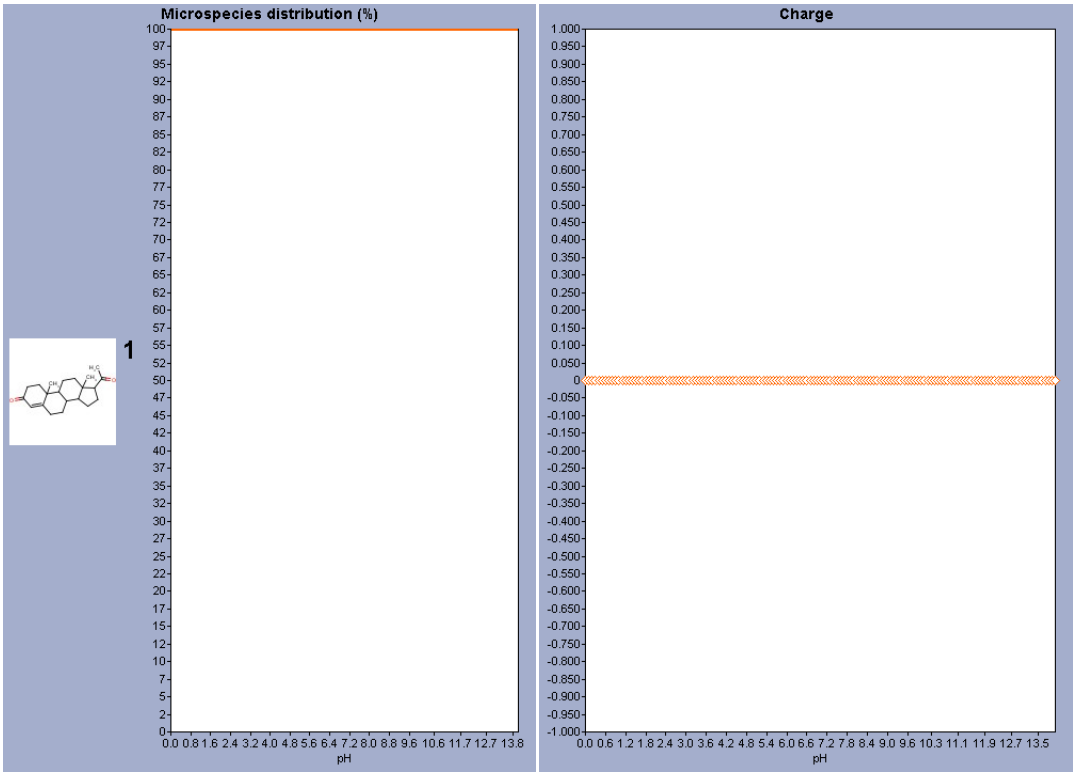

Figure 19. Progesterone structure (top), pKa (left) and isoelectric point (right).

2.3.2.2 Removal/Elimination Mechanisms

Once released into the environment, pharmaceutical compounds will distribute among the air, liquid, or solid phases based on the physical and chemical properties. Proper design of wastewater treatment systems includes not only removal from the water column, but also takes into the account the subsequent fate of the compounds that are removed. Figure 20 summarizes the fate pathways of pharmaceuticals in a duckweed wastewater treatment system.

Air Exposure. The tendency for chemicals in wastewater to volatilize is represented as Henry's law constant (Kaw). The Kaw values for pharmaceutical compounds are typically small (or in the case of pKaw, negative) representing low-volatility. Duckweed may increase the amount of a chemical volatilized by taking it up and discharging it through the transpiration stream. Vapor pressure is essential to plant physiology and phytoremediation. Typically, pressure in the plant decreases below its surrounding pressure as water transpires from the plant, thus drawing more water into the plant through the roots; however, the Lemna species in this study have stomates that never close—even in the dark (Landolt and Kandeler 1987). Water loss with duckweed occurs at the same rate as evaporation without duckweed. Constantly open stomata may result in more water passing through the plant in the dark resulting in more chemical removal. The transpiration stream will carry water and dissolved chemicals unidirectionally from root to shoot by the water potential gradient (Chard 2005). Vapor pressure, and to a greater extent Henry's law constant, also governs whether certain chemicals, like trichloroethylene, will volatilize through plant leaves (Orchard et al. 2000).

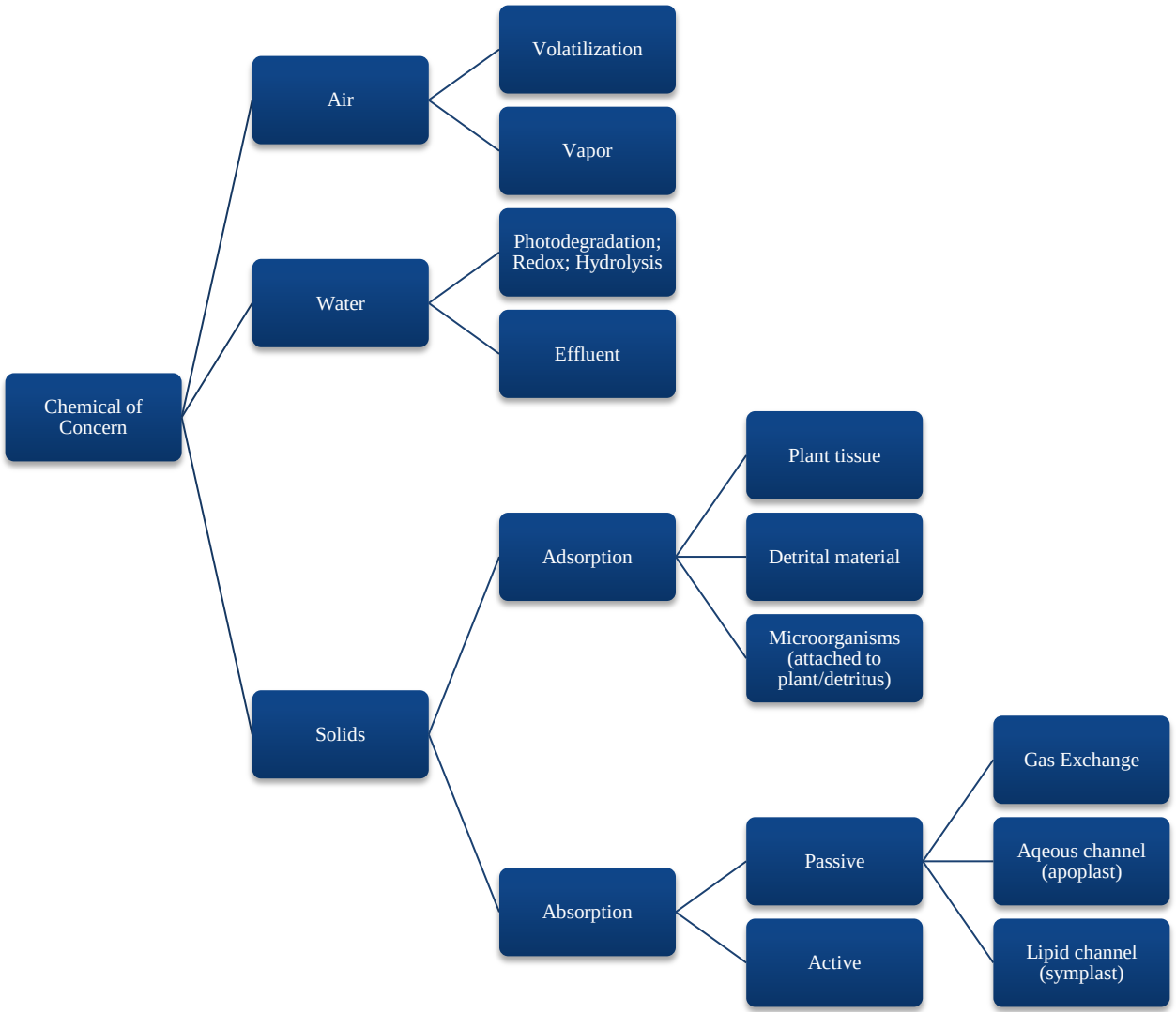

Figure 20. Diagram showing potential pathways for the fate of pharmaceuticals and other chemicals in a duckweed system.

Water column. Pharmaceuticals in water may photodegrade, transform due to pH or redox conditions, or remain unchanged in solution. Medical literature of light sensitivity of various pharmaceuticals can be used to estimate a compounds tendency to degrade in light. Duckweed mats shade the water column and can limit the amount of photodegradation in the water column. High positive redox potentials (*i.e.*, aerobic conditions) and low redox potentials (*i.e.,* less aerobic conditions) affect degradation of some pharmaceutical compounds (Hijosa-Valsero et al. 2010). Redox potential is negative near the facultative sediment-water interface where there is an abundance of organic electron donors. The redox potential at the water surface is positive. If oxygen is transferred to the water by the plants the redox becomes more positive. If oxygen is taken up by the microorganisms attached to the duckweed the redox becomes more negative. pH is an important driver for pharmaceuticals' fate because it affects their ionization which affects their solubility in water and their tendency to adsorb or absorb in duckweed (Tront and Saunders 2006). Pharmaceutical compounds typically remain in the water column when they have low Kow values since they do not sorb to the duckweed (*i.e.*, low reactivity and degradability) (Dettenmaier 2008). For wastewater treatment plants that discharge into nearby river systems, toxicity studies will help evaluate the affects these compounds can have downstream of the WWTP. These compounds have the potential to be taken up into crops irrigated with reused wastewater.

Solids Pathway. Solids in wastewater treatment plants include sediment, floating particulates (*i.e.,* phytoplankton, detritus, and natural sediments), and floating aquatic plants like duckweed. Non-duckweed solids are negligible in this study except for the microbial communities attached to the plants (Zuberer 1984). The microcosms for this

study represent a control volume surrounding the water surface and do not include non-duckweed biota, suspended particles, and sediment.

Pharmaceuticals removed by duckweed plants may be transformed into non-parent compounds, *ad*sorbed/*ab*sorbed, stored/assimilated into plant tissue, and/or transpired and volatilized into the atmosphere or back into the water column.

Duckweed: Phytotransformation. Transformation consists of metabolizing and/or mineralizing the parent compound (Day and Saunders 2004). Plant metabolism occurs in three phases and is compared to a "Green Liver" because plants metabolize many compounds similar to the human liver (Sandermann 1994). Phase I metabolism increases the polarity of the parent compound, which can be further metabolized by glucosidation and amino acids known as Phase II metabolism. Once the polarity is increased, the metabolites can be transferred through aqueous channels to be eliminated or assimilated into the plant, known as Phase III metabolism.

Duckweed: Adsorption. Pharmaceuticals can adsorb to the surface of plant tissue, particulates in the water column (assumed negligible in this study), and microorganisms attached to the duckweed. Acidic compounds have less tendency to adsorb to net-negative sorbates and the opposite is true for basic/zwitterionic compounds. pKa and isoelectric charts showing speciation and charge of pharmaceutical compounds can be used to predict a chemicals' tendency to adsorb to solids. Several studies have used hydrophobicity alone (*i.e.*, high Kow values) to predict adsorption, but this method alone is unreliable based on the variability of results from several studies and adaptations that need to be made depending on the compound (Dettenmaier 2008; Franco and Trapp 2008).

Duckweed: Absorption. Pharmaceuticals can absorb into the duckweed by passive and active mechanisms. Passive mechanisms include gas exchange, aqueous channel uptake, and lipid channel uptake, and are driven by transpiration. Active mechanisms include specific enzymes or routes in the plants and require metabolic energy. Gas exchange involves fixation of gasses into the plants or volatilization of compounds already in the plant transpiration stream.

Some molecules may pass through plant membranes if their size is small enough. Briggs et al. (1982) predicted that plants take up organic chemicals with moderate hydrophobicity (pKow ranging from 0.5 to 3.5) into their tissue following a bell curve with optimal uptake at pKow 1.78. Dettenmeir (2008) predicted that plant uptake follows a sigmoidal curve with highest plant uptake among polar and neutral compounds with pKow < 0. Soluble chemicals (*i.e.*, pKow < 0) are easily transferred through plants via the xylem (Kim et al. 2004) or in some cases not taken up at all if the pKa is too low (Boutonnet et al. 1999). The uptake of hydrophobic and hydrophilic compounds by the plant is illustrated via the root's anatomy.

The plant's roots can be visualized as circular layers of cells, beginning from the outermost layer: epidermis, cortex, endodermis, pericycle, and xylem. Different transport mechanisms exist to transfer chemicals through the different layers. According to Taiz and Zeiger (1998), "Mineral nutrients absorbed by the root are carried to the shoot by the transpiration stream moving through the xylem" via the apoplast. Hydrophobic compounds do not move through the apoplast; rather, they move through a network of interconnected lipid-cells known as the symplast (Taiz and Zeiger 1998). According to Kim et al. (2004), hydrophobic chemicals (*i.e.*, Kow > 0) require symplastic movement

through inner cells, hydrophylic chemicals require apoplastic movement through cell walls, and inorganic nutrients require "specific carrier- and channel-proteins." Once a chemical is taken up by a plant then it can be stored, metabolized (*i.e.,* assimilated), mineralized, or volatilized.

Physiological characteristics of plants (*i.e.,* floating versus rooted) make specific species more adept at removing specific chemicals. Individual laboratory tests frequently concentrate on certain specie's ability to uptake one type of chemical. In reality, ecological systems contain multiple species and chemicals of concern. Some studies have looked into using multiple types of plants, each one with a particular ability to remove a specific chemical (Ornes et al. 1991). Other studies have observed competition among species that promote or inhibit a diversity of species (Clatworthy and Harper 1962; Edwards et al. 1992; Wang et al. 2002) that may affect the types of plants that can be used for phytoremediation in a certain environment.

2.3.2.3 Fate and Fugacity Models

The fate of pharmaceuticals in an aquatic system depends on the characteristics of the chemical relative to environmental conditions, water quality, and solids in the water (*i.e.,* sediments, biota, and floating plants). Fugacity models estimate the equilibrium distribution of chemicals in the environment and several of models (Mackay 1991) are freely available online through Trent University (Trent University 2011). The U.S. EPA also provides a free fugacity model as part of a program called Episuite (www.epa.gov/opptintr/exposure/pubs/episuite.htm). These and other environmental models available online (Nieman 2003) predict the fate of chemicals in the environment by taking into account parameters like: Solid/water distribution coefficient (Kd),

Air/water distribution coefficient (Henry's Law constant (Kaw)), Bio-concentration Factor (BCF), water solubility (S), vapor pressure (P_v), octanol/water coefficient (Kow), and acid disassociation constant (Ka). Examples of results from the Episuite fugacity model for the five pharmaceuticals of interest are summarized above in Table 4.

2.3.2.4 Tests

The ability of duckweed to take up a variety or chemicals has proved advantageous in conducting: toxicity tests to quickly screen for hazardous chemicals (Brain et al. 2004a; Kummerova et al. 2007; Saygieğer and Doğan 2004; Tront and Saunders 2006) and fate/uptake tests to evaluate the potential to remediate polluted water (Day and Saunders 2004; Reinhold and Saunders 2006; Reinhold et al. 2010). Toxicity studies focus on the effects that certain chemicals have on duckweed; whereas, fate/uptake studies focus on the effects that duckweed has on certain chemicals. Toxicity studies are easier to conduct and typically report the lowest observed effective concentration (LOEC), the effective concentration (EC-10/25/50), and lethal dose (LD) values indicating the harmful effect certain concentrations of chemicals have on aquatic plants like duckweed. Fate/uptake studies focusing on phytoremediation and accumulation of pharmaceuticals by duckweed require sophisticated equipment to measure and report chemical concentration changes over time throughout the system and to generate partition coefficients (*i.e.,* K_{PW}) and percent compound removal values over time.

Several organizations have official testing methods for duckweed toxicity testing including both the American Public Health Association (Eaton et al. 2005) and Environment Canada. A review of toxicity studies (Crane et al. 2006) identified 40

toxicity studies that used duckweed as the indicator organism for 22 classes of human pharmaceuticals. The Centre for Toxicology at the University of Guelph, Ontario, Canada, has been involved in evaluating the effects of over 25 pharmaceuticals on the duckweed species *L. gibba* (Brain et al. 2004b). The U.S. EPA, through the Georgia Institute of Technology, has funded several studies looking at the ability of duckweed in wetlands to remediate organic chemicals, including human pharmaceuticals (Reinhold and Saunders 2006).

The majority of these studies investigating the use of duckweed for chemical uptake and toxicity were performed in the laboratory. One advantage of laboratory studies is that they "generate information about the fate of organic chemicals prior to field-scale tests because laboratory tests are less expensive, easier to control, and better enable investigators to elucidate fate mechanisms" (Kim et al. 2004). Due to a variety of different objectives, each study also performed different tests and analyzed the results differently (*i.e.,* EC_{50} versus BCF).

Mass balances of the liquid and solid compartments help determine whether plants transform or store the parent compound. Solid phase extraction (SPE) can enable detection of low chemical concentrations that otherwise might only be detected using radio-labelled compounds. A number of studies used isotope labelled chemicals to show the ratio of chemical in roots, to shoots, to the unaccounted chemical volatilized, degraded, or left unchanged (Böttcher and Schroll 2007).

Many studies report the K_{PW} plant-water partition coefficient (*cf.* BCF) (Mackay 1991) which is the ratio of the amount of chemical in the plant tissue compared to the

amount in the substrate solution. High BCF values (>1000) represents hyperaccumulation (Odjegba and Fasidi 2004).

2.4 Modelling Duckweed Production and Chemical Uptake

Actual performance of a duckweed system to remove phosphorus and accumulate pharmaceuticals in the plant biomass largely depends on the amount of biomass produced. Plant physiologists have developed a theoretical maximum biomass production based on solar radiation capture (*i.e.*, energy units W/m^2) and photosynthetically active radiation (PAR) (*i.e.*, PAR photon units mol/m^2s) (Amthor 2010; Bruce Bugbee, personal communication, 4/20/2012). According to these scientists, duckweed plants may produce up to 1g-dry duckweed/mol PAR photons/m^2day (2.07 g-dry/MJ total solar radiation/m^2day) assuming that duckweed completely covering the water surface absorbs 92% incident PAR (mol photons/m^2day) x 6% quantum yield (mol carbon-assimilated by duckweed/mol photons) x 60% respiration efficiency (mol carbon-sucrose/mol carbon-assimilated) x 30 g dry-duckweed/mol carbon-sucrose.

In the field, 62.2 g-dry duckweed/m^2 is the theoretical maximum duckweed production on a clear summer day in Cache Valley with a peak solar radiation of 1000 W/m^2 (2220 PPF or $\mu mol/m^2s$) and an average daily radiation energy from the sun would be approximately 30 MJ/m^2day, 45% of which is in the PAR range (400-700 nm) available to plants. Using Planck's equation the energy in a photon can be calculated, *e.g.*, $E = hc / \lambda$, where E = energy per photon (J), h = Planck's constant (6.62×10^{-34} J·s), c = speed of light (3×10^8 m/s), and λ = wavelength (m, or the average wavelength of photosynthetic radiation from the sun = 550 nm), so that the energy per photon at 550 nm

= 3.61 x 10^{-19} J. Energy per mole of photons is equal to E x Avogadro's number, or 3.61 x 10^{-19} J/photon x 6.023 x 10^{23} photons/mole = 217,000 J/mole photons or 0.217 MJ/mol PAR photons. Using these values, a summer day with 30 MJ total solar radiation/m^2day x 0.45 PAR photons/total solar radiation = 13.5 MJ PAR photons/m^2day / 0.217MJ/mol PAR photons= 62.2 mol PAR photons/m^2day x 1 g-dry duckweed/mol PAR photons = 62.2 g-dry duckweed/m^2day on a clear summer day in Cache Valley. In the laboratory with 200 PPF light intensity on 16 hr/day, the theoretical maximum production would be 11.52 g-dry duckweed/m^2day.

Engineers and scientists have also created less mechanistic and more empirical models to predict duckweed production (Driever et al. 2005; Frédéric et al. 2006; Landesman et al. 2011; Lasfar et al. 2007) that takes into account several limiting factors. Lasfar et al.'s (2007) model takes into account the most parameters, and includes plant density, light, temperature, phosphorus, and nitrogen. These models were validated against measured duckweed plant production on the Wellsville Municipal Sewage Lagoons during the 2010 and 2011 seasons (May through November) and against laboratory studies at the Utah Water Research Laboratory from 2008-2010.

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection and Maintenance

Two duckweed species *L. turionifera* and *W. borealis*, were collected from the Wellsville City Municipal Sewage Lagoons. Plants were harvested with metal screening and transferred to the laboratory for culturing in nutrient solution indoors. Prior to culturing in the laboratory, the plants were rinsed in tap water which removed the majority of *W. borealis* species resulting in a predominantly a *L. turionifera* culture.

The duckweed plants were seeded into a 110 L acrylic reactor (0.9 m L x 0.6 m W x 0.2 D) divided into three sections simulating three lagoons (Figure 21). The initial plant density was 30 g/m^2 to avoid algae growth that would otherwise inhibit duckweed growth. Following a 1 to 2 week acclimatization period the duckweed were harvested every 3 to 5 days to maintain a 60 g/m^2 cover. The reactors were kept in a 25°C constant temperature room. High-pressure sodium lamps (HPSLs) suspended 1 m above the plants provided 200 $\mu mol/m^2$-sec (PPF) 16 hrs/day. Wellsville synthetic nutrient solution (see previous Table 3) was fed via peristaltic pumps at a rate of approximately 1.77 L/d. Approximately 66% of the influent flow evaporated and so dilution tap water was continuously fed at 0.64 L/d to provide measureable effluent. The effluent was captured in graduated 15 L buckets after leaving the third cell in the reactor and was occasionally measured for nutrients before disposal.

Occasionally, a recirculation airlift pump powered by an aquarium air pump was used to add dissolved oxygen and increase the homogeneity of the liquid. The recirculation pump was connected to a water-detecting motor control to turn the pump off

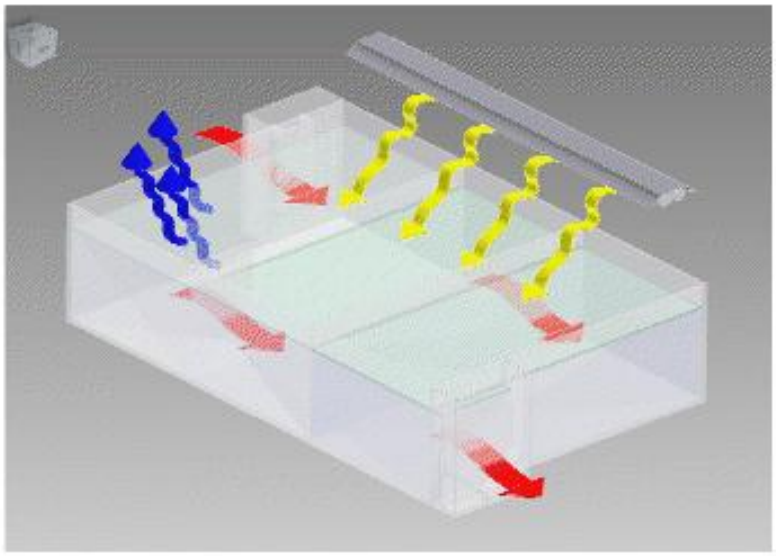

Figure 21. 110 L laboroatory culture reactor.

in case of a leak. A probe shaker disturbed the water surface every 2 minutes in order to remove aphids and prevent *W. borealis* from out-competing *L. turionifera* plants (see Appendix B2).

When fungi appeared, Ridomil Gold EC was applied at a rate of 0.3µL/L-nutrient solution to reduce the harmful effect of fungi. In addition to fungicide, silicon, as potassium silicate, was added to the nutrient solution for "toughening" cell tissue to resist fungal attack and disease. A 28 mg-Si/L as potassium silicate was used in the start-up solution, and then 2.8 mg-Si/L was continuously added via the nutrient solution.

3.2 Microcosm Study

Triplicate samples were collected from the microcosm study over three time periods: 2 hours, 1 day, and 3 (for phosphorus) or 4 (for pharmaceuticals) days of exposure to determine the fractions of pharmaceuticals in the liquid and plant solids. As

explained in the following sections, the experiment was designed to determine the removal of phosphorus and pharmaceuticals from the liquid by several pathways: plant uptake; sorption to plant tissue; photodegradation; volatilization; and sorption to glassware. Freundlich isotherms and plant-water partition coefficients (K_{PW}) were calculated for compounds removed by plant sorption and uptake, respectively. In addition, 50% aqueous depletion times were calculated in order to compare results with other studies.

3.2.1 Experimental Design: Microcosm Study

Duckweed from the 110 L culturing reactor was used for the microcosm studies. Microcosms consisted of 400 mL clear glass jars (8.5 cm I.D. x 8.5 cm height) wrapped with opaque material below the water surface. The microcosms contained no plants in the dark and light (the controls), whole plants in the dark and light, and macerated plants in the light (summarized in Table 6). All jars, except the dark control jars, were placed beneath the same HPS lamps as the culturing reactor. Dark control jars were kept inside a closed box in the 25°C constant-temperature room with vent holes to allow air circulation. A 4-day test interval is recommended by APHA standard methods for duckweed toxicity tests (Eaton et al. 2005) and also coincides with a frequent harvesting interval geared towards nutrient removal. The 2 hours and 1 day test intervals provided information regarding chemical uptake rates by the duckweed.

Approximately 160 mg (dry) or 8 g (fresh) duckweed were added to the plant-containing jars representing 70 g duckweed (dry)/m^2 in order to simulate the lagoons and reduce the potential for algae growth. Each microcosm started with approximately 400 mL Wellsville synthetic nutrient solution that was replenished with deionized water as

evaporation occurred over time. A spiking solution containing all five pharmaceuticals dissolved in methanol was added to each series of jars at environmentally realistic influent wastewater concentrations (except during the isotherm study with 2-3 orders of magnitude greater starting concentrations). The starting pharmaceutical concentrations were approximately two-orders of magnitude greater than the analytical detection limits (see previous Table 5) to allow measurement over a 2-log removal during the experiments.

3.2.2 Experimental Measurements

The method for liquid and solids phosphorus measurements was adapted from the Standard Methods (Eaton et al. 2005) 4500-P.E Ascorbic Acid method and dry ash digestion (see Appendix C). Pharmaceuticals measurements in the liquid and solids were patterned after the EPA Method 1694 "Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS" using HPLC/MS rather than with tandem mass spectrometry. The detailed procedures used for pharmaceutical liquid and solid extractions/measurements are outlined in Appendix C.3 and Farrell (2011). Important changes to the EPA 1694 method included:

a) Ommitted labelled surrogates for every compound due to the added cost
b) Atrazine-D5 used as an internal standard for liquid extractions
c) Liquid and solid extractions performed at pH 5-7 with no pH adjustment
d) Solids stored in methanol for 46 days then extracted with methanol
e) Solid phase extraction ommitted for solids analysis; instead, samples were evaporated to 5 mL followed by vortex, centrifugation, and analysis by HPLC-MS with matrix standards for the calibration curve

Table 6. Experimental design for microcosm duckweed experiments

Microcosm Treatment		Contaminant of Concern		Starting Concentrations (μg/mL)[c]			
Treatment Description	Treatment Abbreviation	Contaminant Name	Contaminant Abbreviation	Low-range[b]	Mid-range	Mid-High range	High range
Dark only[a]	"A"	Acetaminophen	ACT	2.5	50	625	1,000
Dark + Plants	"B"	Sulfamethoxazole	SLF	1.25	50	625	1,000
Light only[a]	"C"	Fluoxetine	FLX	1.25	50	625	1,000
Light + Plants	"D"	Carbamazepine	CRB	1.25	50	625	1,000
Macerated Plants	"E"	Progesterone	PRG	1.25	50	625	1,000
Dark w/ lid[a]	"X"	Phosphorus	P	5,260	NA	NA	NA

[a] Controls
[b] Environmentally relevant wastewater influent concentration
[c] Performed in triplicate over three time periods (2 hrs, 1 d, and 3 days (P) or 4 days (pharmaceuticals)

3.2.3 Experimental Errors

All microcosm treatments were performed in triplicate. Unless otherwise reported, measurements are reported as the mean ± 95% confidence interval. ANOVA, Tukey's test, Student's t-test, and plots were done using the statistical software R using an alpha value of 0.05 (www.cran.R-project.org)

3.2.4 Calculations

The mass balance only accounted for the chemicals partitioned to the liquid and plant compartments, *e.g.*, Total Fraction Recovered = $(M_{Liquid} + M_{Plant}) \div M_{initial}$. Pharmaceuticals unaccounted for in the mass balance may be due to transformation of the parent compound or the inability to extract/analyze mass from the plant tissue.

Due to poor pharmaceutical recoveries from the solids, pharmaceutical removal was calculated based on the mass fraction removed from the liquid only, *e.g.*, Fraction Removed = $(M_{initial} - M_{liquid}) \div M_{initial}$. The different treatments described in Table 6 helped to differentiate pharmaceutical removal into five removal pathways: removal due to the glass reactors ($M_{glassware}$), photolysis ($M_{photolysis}$), plant sorption ($M_{plant\ sorption}$), attached microorganisms ($M_{attached\ microorganisms}$), and plant uptake ($M_{plant\ uptake}$), where:

$M_{glassware} = M_{initial} - M_{dark\ control}$
$M_{photolysis} = M_{dark\ control} - M_{light\ control}$
$M_{plant\ sorption} = (M_{controls} - M_{macerated})$ when $M_{macerated} \geq M_{whole\ plant\ light}$
$M_{attached\ microorganisms} = M_{macerated} - M_{whole\ light\ plant}$ when $M_{macerated@1day} < M_{macerated@4days}$
$M_{plant\ uptake} = M_{whole\ light\ plant} - M_{macerated}$ when $M_{whole\ plant\ light} > M_{macerated}$

Pharmaceutical Removal due to volatilization for all five compounds was assumed negligible due to small K_{AW} values (see previous Table 4).

3.3 Growth Rate Study

Beginning April 29, 2011, duckweed growth rates on the Wellsville City Municipal Sewage Lagoons were measured through October 29, 2011. The measured values along with collected environmental and water quality parameters were entered into four growth rate models to compare the models and determine their limitations.

3.3.1 Experimental Design: Seasonal Growth Rate Study

Three 0.33 m^2 growing cages were constructed from 2 in schedule 40 PVC and lined with metal window screening to reduce in/out flux of duckweed plants (Figure 22 to Figure 23). The framework was anchored near the effluent side of Lagoon #4 from May to October 2011. Plant production was measured every 2 to 23 (average 7) days with starting densities varying from 8 to 142 (average 40) g-dry/m^2 (Figure 24). Continuous monitoring data from the nearby USU Experimental weather station in Wellsville (about 1.5 miles east of the lagoons) determined the radiation and temperature during the study period (www.littlebearriver.usu.edu). Historical monthly influent and effluent nitrogen (NO_3-N and NH_3-N) and total phosphorus levels from 2008 to 2011 were obtained from Wellsville City for input model inputs.

3.3.2 Experimental measurements

Procedures for harvesting duckweed, obtaining fresh/dry weights, and phosphorus measurements in the tissue and liquid are outlined in Appendix B (B.1 through B.3).

Figure 22. PVC frame with three 0.33 m^2cells for duckweed field growth study.

Figure 23. Duckweed inside of metal screened growth cells.

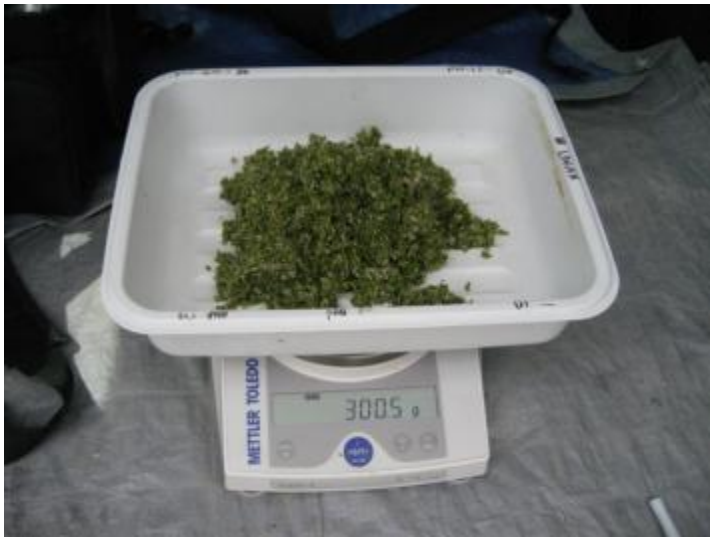

Figure 24. Duckweed starting density 300 g (fresh)/0.33m^2.

3.3.3 Experimental Errors

One of the three cells was occasionally left empty to quantify how much *Wolffia* passed through the screen. Starting June 7, 2011, nylon-mesh bags were placed over the screen to prevent *Wolffia* from entering the growth rate apparatus. The two additional cells provided replicate measurements for calculating the variation and percent error in the measurements. A chicken-wire screen was laid over all three cells to prevent ducks from eating the duckweed.

The predicted values from the models were considered acceptable when $0.5 \leq RGR_{pred}/RGR_{measured} \leq 2$ or in other words, when predicted values were within a factor of two of observed values. Model values were calculated based on daily averages using Microsoft Excel. Tables from Excel (see Appendix C.3 Table 29) were input into R for final analysis and plotting.

3.3.4 Sample Calculations Section

Duckweed plant production is equal to the final mass minus the initial mass. Fresh plant mass was normalized to dry mass by calculating the percent dry matter (%DM), *e.g.*, $\%DM = 1 - (mass_{dry}/mass_{fresh}) \times 100$. The percent difference between air dried duckweed and oven dried duckweed (103°C) masses was typically 1 to 2.5%. The relative growth rate (RGR) was used to normalize growth over time, *e.g.*, $RGR = \ln(\text{Plant mass}_{final}/\text{Plant mass}_{initial})/\text{time}$.

CHAPTER 4

PHOSPHORUS REMOVAL RESULTS AND DISCUSSION

4.1 Phosphorus Microcosm Results

In the phosphorus microcosm study (see raw data in Appendix C.1 Table 23 to Table 24), the reactors in the light reduced phosphorus from 5.57 ± 0.36 mg-P (PO_4)/L to 0.86 ± 0.21 mg-P (PO_4)/L in 3 days (Figure 25). The reactors initially contained 385 ± 7 mL-nutrient solution. Evapo-transpiration occurred at a rate of 26 mL/day (4.58 L/m^2day) in the light and 9 mL/day (1.59 L/m^2day) in the dark. The reactors with plants initially contained 8 g-fresh duckweed (1400 g-fresh/m^2 or 70 g-dry/m^2). In the light, duckweed mass had a relative growth rate (RGR) of 0.14 ± 0.03 at 1 day and 0.06 ± 0.03 at 3 days. In the dark, duckweed mass had a relative growth rate (RGR) of 0.05 ± 0.08 at 24 hours and -0.1 ± 0.05 at 3 days. The initial duckweed contained 1.02% phosphorus (10.2 mg-P/g-dry) that increased to 1.24 ± 0.06% phosphorus by Day 3. All the phosphorus removed from solution could be accounted for in the plant mass, and the total phosphorus mass balance showed that all recoveries were 99 ± 3% (Figure 26).

4.2 Phosphorus Microcosm Discussion

The light microcosms removed 53 ± 4% of the soluble phosphorus in solution within 24 hours when the RGR = 0.14, and 84 ± 8% in 3 days when the RGR = 0.06. The dark microcosms did not remove/release phosphorus within 24 hours (94 ± 10% initial phosphorus) and increased the initial soluble phosphorus mass in solution by 161 ± 37% in 3 days. The initial phosphorus liquid mass was 2.13 mg-phosphorus per reactor

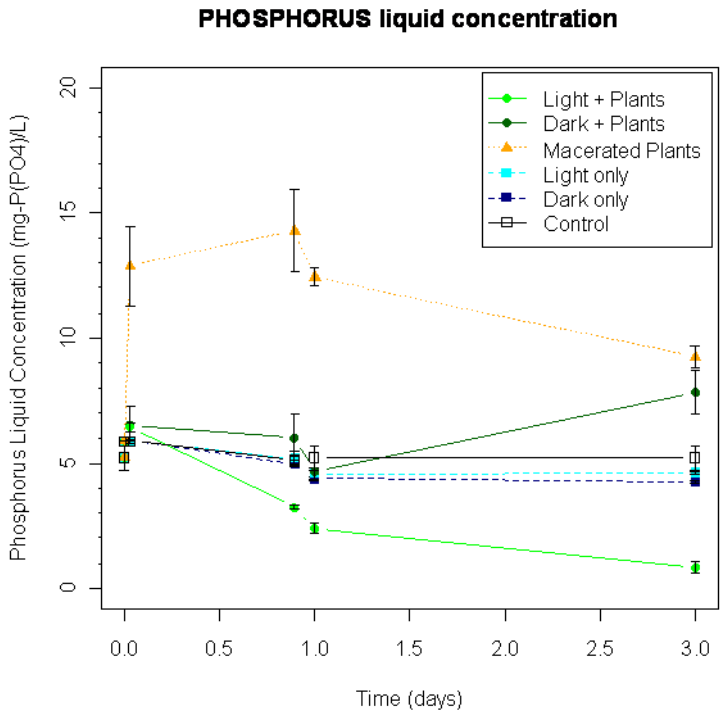

Figure 25. Phosphorus microcosm study showing concentration change over time.

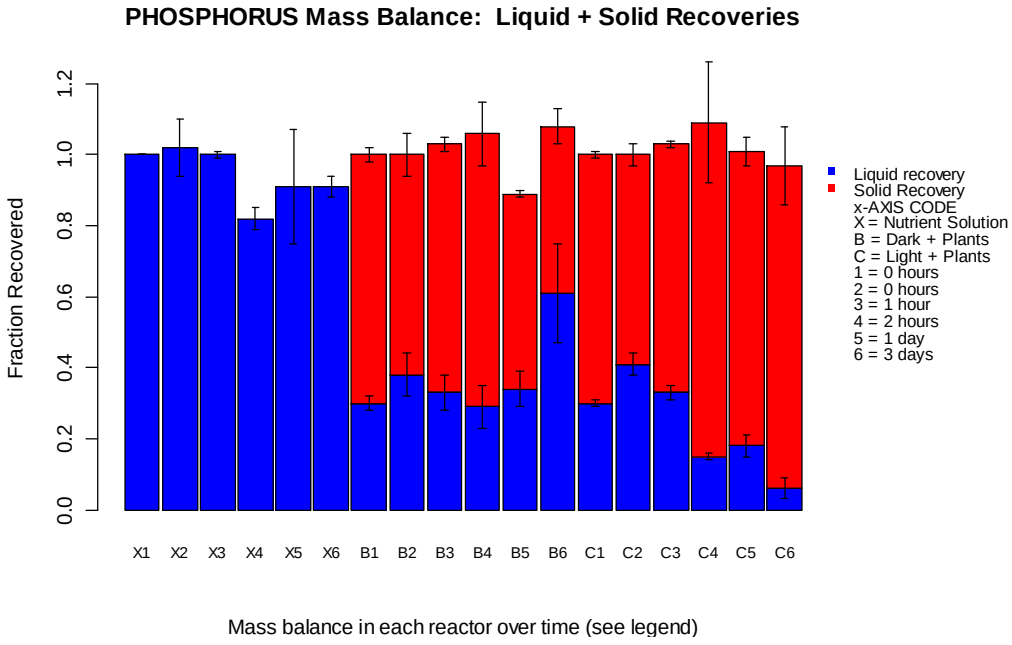

Figure 26. Phosphorus microcosm study mass balance showing total phosphorus recovery and distribution of phosphorus in the liquid and duckweed solids over time.

(57 cm^2 surface area). Removal after 3 days normalized to surface area was approximately 100 mg-P /m^2day in the light and -75 mg-P/m^2day in the dark.

Assuming an average photoperiod of 13.6 hours/season then the combined phosphorus removal for an entire day would be 2.25 g-$P_{removed}$/$m^2season_{light/dark}$, *e.g.,* (0.1 g-P/m^2day_{light}) x (13.6 hrs_{light}/24 hrs) – (0.075 g-P/m^2day_{dark}) x (10.4 hrs_{dark}/24 hrs) ≈ 0.025 g-$P_{removed}$/$m^2day_{light/dark}$ x 90 days/season ≈ 2.25 g-$P_{removed}$/$m^2season_{light/dark}$. These results are similar to those of Al-Nozaily et al. (2000) of 12-35 mg-P/m^2·day but lower than those Kadlec and Wallace (2009) who concluded that duckweed systems can achieve over 20 g-P/m^2·yr.

If overcrowding does not become an issue and phosphorus removal occurs at the rate it did at 24 hours (*i.e.,* 53% of 5.57 mg-P/L in 0.385 L and 0.0057 $m^2_{suface\ area}$) then removal normalized to surface area was approximately 200 mg-P/m^2day in the light and 0 mg-P/m^2day in the dark. In this scenario, the seasonal predicted phosphorus removal would be 10.2 g-$P_{removed}$/$m^2season_{light/dark,}$ still below the range established by Kadlec, *e.g.*, (0.2 g-P/m^2day_{light}) x (13.6 hrs_{light}/24 hrs) - 0 g-P/m^2day_{dark} x (10.4 hrs_{dark}/24 hrs) ≈ 0.113 g-$P_{removed}$/$m^2day_{light/dark}$ x 90 days/season ≈ 10.2 g-$P_{removed}$/$m^2season_{light/dark}$. However, the growing season is only half as long (*cf.,* 3 months to 6 months) in northern Utah which explains the lower results.

All phosphorus removed from solution was accounted for in the duckweed plant tissue. Predicted tissue concentration at 24 hours and 3 days were made using the assumption that all phosphorus removed from the liquid was incorporated into plant tissue, *e.g.,* % P (mg-P/mg-dry)$_{predicted}$ = (mg-$P_{out\ liquid}$ + mg-$P_{plant\ initially}$) / mg-$dry_{plant\ tissue}$. Predicted tissue concentrations were not significantly different (Student's t-test, $\alpha = 0.05$)

than the measured concentrations in all scenarios. This shows that all phosphorus removed *from* or released *into* solution is accounted for in the plant tissue (see Appendix D.1 Table 30).

In the light, duckweed plants had more growth in the first 24 hours than by the third day which was due to over crowding—indicated by a RGR less than 0.1 and a plant density above 60 g-dry/m^2. However, despite the decreasing growth rate the duckweed continued to remove phosphorus by accumulating the phosphorus in its tissue. The 1.24 ± 0.06% phosphorus in the plant tissue on the third day was significantly higher (α = 0.05, p-value = 0.018) than the initial 1.02 ± 0.01% phosphorus. In fact, phosphorus tissue concentration was only significantly different than the initial concentration for day three reactors in the light—indicating that hyper-P-accumulation did not occur until inhibition (*i.e.*, over-crowding) occurred. During the first 24 hours, phosphorus tissue concentrations did not change suggesting that new daughter fronds had the same phosphorus concentrations as the mother fronds during this time period.

The dissolved oxygen concentration decreased in the light reactors indicative of respiration rather than photosynthesis (Figure 27). This suggests that microbial respiration is occurring simultaneously with photosynthesis, *i.e.*, microorganisms in the liquid and attached to plants are consuming oxygen. Dead duckweed plants were seen on the bottom of the reactors in the light (Figure 28). Decomposition of senescing plants releases phosphorus into the water column which can be used to grow more fronds. In the light, the growth rate exceeded the degradation rate.

In the dark, the phosphorus solution concentration had increased by the third day but remained unchanged during the first 24 hours, indicative of decomposition of

duckweed plants if left in the dark for more than 24 hours. The phosphorus tissue concentrations remained constant over the entire 3-day period. By the third day liquid phosphorus concentrations had increased proportionately to the amount of plant mass loss (*i.e.*, 10 mg-P_{loss}/mg-$dry_{plant\ loss}$). This suggests that there was a lag time between when the dark period began and when actual decomposition and phosphorus release started to occur. In some dark reactors, duckweed growth actually increased during the first 24 hours. Heterotrophic growth is possible since some light passed through the vent holes in the box covering the dark reactors.

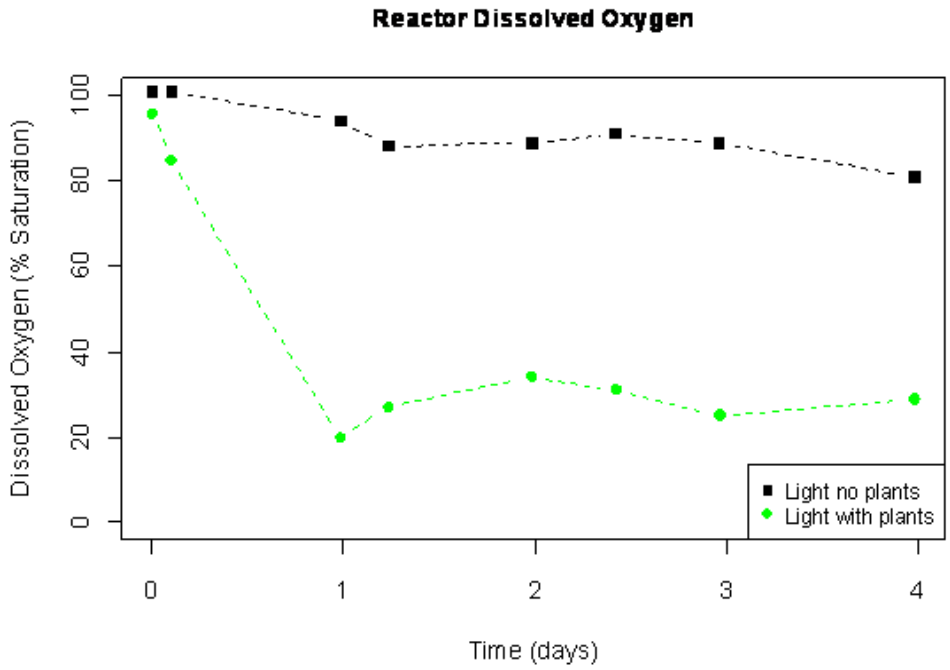

Figure 27. Dissolved oxygen change over time in the light reactors at 25 °C and 200 µmol/m^2sec (PPF) light intensity.

Figure 28. Light reactor with plants showing roots and senescing plants below the water surface—possible contributors to dissolved oxygen consumption.

CHAPTER 5

PHARMACEUTICAL REMOVAL RESULTS AND DISCUSSION

5.1 Liquid Extraction/Analysis Method Development

Extraction efficiencies of 96 ± 16% from the liquid samples with part per trillion (ng/L) concentrations were obtained after discovering, and correcting where possible, losses that occur in the method . In this study, the major loss occurred when the glassware was not properly rinsed nor silanized during the concentration step. Losses due to heating samples to 60 °C, and errors in the calibration curves and instrument drift were also investigated.

Sorption to glassware can be a significant loss during treatment and analysis (Table 7). When evaporating samples after solid phase extraction, rinsing and silanizing were important steps necessary to prevent up to 70% of compounds from remaining on the glassware (Baker 2011). These losses were discovered by spiking 25 mL-methanol samples in silanized receiver flasks with 200 ng-pharmaceuticals, concentrating them to 250 μL (half were rinsed and half were not rinsed), and then bringing the volume up to 1 mL for HPLC/MS analysis. Previous tests had shown that negligible amounts of pharmaceuticals were lost during loading/eluting the solid phase extraction cartridges prior to the concentration steps.

Table 7. Recovery (%) due to not rinsing and rinsing silanized glassware during the liquid Solid Phase Extraction Procedure

% Recovery	ACT	SLF	FLX	CRB	PRG
w/o rinsing	30 ± 5%	34 ± 2%	36 ± 2%	34 ± 2%	34 ± 2%
w/ rinsing	99 ± 16	93 ± 18	92 ± 16	95 ± 14	102 ± 16

Recovery of pharmaceuticals at 60 °C was investigated to determine if thermal degradation during the drying/evaporation of samples affect recovery results. Table 8 shows that recovery of the parent compound after evaporating the compound to dryness at 60 °C and then re-constituting to 1 mL before HPLC/MS analysis gave lower than 80% recovery for sulfamethoxazole and fluoxetine. The reason for the loss is probably more a function of evaporating to dryness than it is thermal degradation since none of the compounds should loose more than 10% mass at 60 °C based on thermal degradation charts (Childs et al. 2004; Fernandes et al. 1999; Fini et al. 2008; Kobayashi et al. 2000; Kogan et al. 2007; Qi et al. 2008). During the concentration step, EPA Method 1694 only recommends evaporating to 1 mL and then bringing the volume up to 4 mL. If some compounds are concentrated below 0.5 mL then their extraction efficiency appears to decrease significantly.

After samples were extracted and then concentrated to 1 mL, the errors due to HPLC/MS accuracy and instrument drift were investigated. The accuracy in the calibration curve's predicted concentrations (5-1000 ng/mL) for each compound is shown in Table 8 and ranges from 97 to 111%. The HPLC/MS analysis was slightly less accurate in the 5-33 ng/mL range than the 50-500 ng/mL range, particularly for acetaminophen analysis in acetonitrile solution (Table 9). Samples with measured values below 33 ng/mL were typically false positive. The majority of liquid samples were made in 0.1% formic acid solution, while solid samples were extracted in methanol. Atrazine-D5 internal standards corrected for instrument drift and matrix effects. All samples were spiked with the same amount of internal standard (250 ng/mL) and then final reported concentrations were corrected depending on the ratio of detected versus

known internal standard. Sensitivity (*i.e.*, peak area from chromatogram) decreased over the length of the sample run and also decreased due to matrix interferences in solutions (*e.g.*, macerated samples contained more interferences than nutrient solution samples without plants) (see Appendix D.2 Figure 53).

The liquid extraction efficiency for the liquid samples using the modified EPA Method 1694 with solid phase extraction (SPE) and rinsing/silanizing glassware showed that all the laboratory control samples (LCSs) produced 100% recovery for each compound (Table 10). LCSs consisted of 25 mL deionized water samples in silanized receiver flasks spiked with 200 ng-pharmaceuticals, concentrated to 250 μL, and then brought up to 1 mL with 0.1% formic acid prior to HPLC/MS analysis.

Table 8. Recovery (%) after evaporating liquid sample to dryness in 60 °C oven

ACT	SLF	FLX	CRB	PRG
110 ± 3	73 ± 3	79 ± 1	104 ± 2	92 ± 1

Table 9. Percent accuracy (mean) and precision (95% confidence interval) of predicted measurements of standards based on HPLC/MS calibration curves

Standards Solution	ACT	SLF	FLX	CRB	PRG	n
0.1% Formic Acid[a]	111 ± 8%	100 ± 3%	100 ± 4%	97 ± 3%	109 ± 7%	94
0.1 % Formic Acid[b]	123 ± 28%	110 ± 12%	114 ± 19%	92 ± 5%	122 ± 20%	17
Acetonitrile[b]	212 ± 14%	84 ± 3%	91 ± 7%	97 ± 5%	104 ± 16%	3
Methanol[b]	121 ± 16%	102 ± 5%	104 ± 4%	100 ± 3%	109 ± 7%	58

[a] Standards ranging from 0-1000 ng/mL [b] Standards ranging from 0-33 ng/mL

Table 10. Liquid Extraction Efficiency (%)

ACT	SLF	FLX	CRB	PRG
99 ± 16	93 ± 18	92 ± 16	95 ± 14	102 ± 16

5.2 Solids Extraction/Analysis Method Development

Extraction efficiency from the solids was poor using the EPA Method 1694 with SPE (Table 11). To determine extraction efficiency, duckweed solids were spiked with 50 to 500 ng-pharmaceuticals before and after 60 °C oven drying. After extracting the solids in acetonitrile and/or methanol, the solution was concentrated to approximately 10 mL which caused some solids to precipitate from solution. Without constant rinsing of the glassware, solids would adhere to the glassware. More solids continued to precipitate from solution with every concentration step. The unaccounted extracted pharmaceuticals likely re-associated with the precipitated solids. Compound loss due to filtering with glass fiber filters was negligible except in the case of fluoxetine and progesterone which retained 6± 5% (n=7) and 8± 6% (n=7) on the filters, respectively.

Solid extractions improved by omitting concentrations steps—included omitting solid phase extraction. Calibration curves using standards made in the extraction matrix accounted for matrix interferences. Solid samples were stored in methanol after drying/crushing. Following storage for 46 days, the 12 mL samples were vortexed, centrifuged, and then evaporated to 5 mL without pH adjustment. Table 12 shows the solids extraction efficiency obtained with the modified method and also the change in extraction efficiency due to storing samples for over a month. Twelve samples representing all three solid treatments at each of the three time periods were spiked with 75 ng/mL pharmaceuticals prior to HPLC/MS analysis. Taking into account that the solid extraction method was not able to recover 100% of the compounds, despite acceptable spike recoveries from the prepared samples (Table 13), a multiplication factor (inverse extraction efficiency$_{=34\ days}$) (Table 14) for each compound was applied to the

Table 11. Solids Extraction Efficiency using EPA Method 1694

ACT	SLF	FLX	CRB	PRG
16%	14%	28%	29%	25%

Table 12. Solids extraction efficiency using the modified method without solid phase extraction step

Extraction Efficiency ε	ACT	SLF	FLX	CRB	PRG
ε @ time = 1 days	56%	58%	62%	82%	70%
ε @ time = 34 days	71%	24%	51%	56%	39%

Table 13. Spike recovery in 12 extracted solid samples spiked with 75 ng-pharmaceuticals/mL

ACT	SLF	FLX	CRB	PRG
109 ± 22%	106 ± 17%	105 ± 10%	100 ± 11%	102 ± 12%

Table 14. Multiplier to apply to final solids pharmaceutical concentration in order to account for samples stored for over 1 month in methanol

ACT	SLF	FLX	CRB	PRG
1.41	4.08	1.96	1.80	2.58

final concentration after HPLC/MS analysis. Interferences due to matrix effects were minimized by making standards in extractions of duckweed not exposed to pharmaceuticals. Matrix interferences were greatest in the macerated samples (see Appendix D.2 Figure 53).

5.3 Pharmaceutical Microcosm results

The microcosm reactors containing environmentally relevant pharmaceutical concentrations were designed to distinguish removal (up to 2-log removal) by several

pathways: photolysis, air exposure, sorption to glassware, bioactivity (*i.e.*, plant uptake and attached microorganisms) and plant sorption (see raw data in Appendix C.2 Table 25 to Table 28).

5.3.1 Photolysis

The control reactors were set up to account for pharmaceutical removal due to photolysis, glassware adsorption, and air exposure. The first sets of control reactors were uncovered without plants in the dark (triplicate reactors). The second sets of control reactors were uncovered without plants in the light (triplicate reactors). The third set of control reactors were covered without plants in the dark (single reactors). ANOVA/Tukey's analysis ($\alpha = 0.05$, df = 3, see Appendix D.2 Table 31) among all treatments and time periods showed that there was no significant difference between the uncovered dark and light reactors. Therefore, photolysis by high pressure sodium lamps (90 W/m^2 and 200 PPF) was considered irrelevant in this experiment. These results disagree with some findings (Lam et al. 2004) that performed a similar experiment in outdoor reactors in actual sunlight and found that photolysis contributed to 50% removal in 1 day for acetaminophen. These findings, however, similarly showed minimal removal after 4 days for carbamazepine (97% $recovery_{t=4days}$) and sulfamethoxazole (87% $recovery_{t=4days}$) similar to this study.

Table 15 summarizes the pharmaceutical removal after 4 days for each treatment. It also shows removal after 4 days at four different concentration ranges for light treatments with and without whole plants in order to compare total removal as a function of concentration. Carbamazepine showed no signs of removal. Fluoxetine and progesterone showed signs of sorption. Sulfamethoxazole showed signs of sorption and

desorption over time. Sulfamethoxazole was the only negatively charged compound tested and was expected to be low sorbing. Acetaminophen and fluoxetine showed signs of plant uptake and attached microorganism removal. Each compound is discussed in more detail below with their corresponding pathways.

Table 15. Percent removal of five pharmaceuticals after four days

Low Range Treatment (Co = 1250 ng/L, Co-ACT = 2500 ng/L)						
	Treatment	ACT	SLF	FLX	CRB	PRG
	Light + Plants	99 ± 0	86 ± 5	85 ± 4	-16 ± 5	93 ± 2
	Dark + Plants	76 ± 6	54 ± 9	68 ± 12	-23 ± 2	92 ± 3
	Macerated Plants	61 ± 4	77 ± 2	90 ± 1	0 ± 2	98 ± 0
	Light only	49 ± 4	30 ± 7	40 ± 7	-10 ± 4	19 ± 4
	Dark only	44 ± 4	22 ± 6	27 ± 4	-10 ± 2	15 ± 5
Mid Range Treatment (Co = 50,000 ng/L)						
	Treatment	ACT	SLF	FLX	CRB	PRG
	Light + Plants	93 ± 3	54 ± 11	92 ± 1	6 ± 8	98 ± 0
	Dark + Plants					
	Macerated Plants					
	Light only					
	Dark only	-4 ± 20	13 ± 7	33 ± 2	30 ± 5	28 ± 5
Mid-High Range Treatment (Co = 650,000 ng/L)						
	Treatment	ACT	SLF	FLX	CRB	PRG
	Light + Plants	70 ± 4	40 ± 14	93 ± 1	38 ± 9	98 ± 0
	Dark + Plants					
	Macerated Plants					
	Light only					
	Dark only	27 ± 5	32 ± 27	26 ± 13	23 ± 15	46 ± 3
High Range Treatment (Co = 1,000,000 ng/L)						
	Treatment	ACT	SLF	FLX	CRB	PRG
	Light + Plants	56 ± 21	45 ± 22	82 ± 17	11 ± 9	82 ± 35
	Dark + Plants	34 ± 4	18 ± 1	65 ± 4	16 ± 3	91 ± 6
	Macerated Plants	46 ± 9	21 ± 8	64 ± 6	8 ± 12	98 ± 4
	Light only	10 ± 6	7 ± 9	9 ± 7	2 ± 9	0 ± 8
	Dark only	14 ± 4	8 ± 4	17 ± 4	11 ± 4	11 ± 8

5.3.2 Sorption to Microcosm Glassware and Air Exposure

Sorption to glassware contributes to pharmaceutical removal in the dark and light control reactors and has the potential to remove up to 70% of the compound in un-silanized glassware (see previous Table 7). In this study, sorption to glassware in the control reactors showed an average of 22 ± 16% removal depending on the compound, with acetaminophen and fluoxetine showing most removal due to glassware sorption, and carbamazepine the least (see Appendix D.2 Figure 58).

Although the covered "X" reactors were single measurements at each of the three time periods, the covered reactors showed an average of 11 ± 2.4% less removal over time (see Appendix D.2 Figure 58) compared to the uncovered reactors. Air exposure is the main difference between the covered and uncovered controls, suggesting *possible* pharmaceutical loss by coevaporation rather than volatilization. However, since the "X" reactors only had single measurements and since loss due to air exposure did not increase with respect to aqueous concentration, results of removal due to air exposure was combined with removal due to glassware sorption for the purpose of further discussion.

5.3.3 Non-reactive Compounds

In the case of **carbamazepine**, removal was not significant in any treatment. Typically, carbamazepine has poor removal and biodegradability from wastewater treatment plants (Behera et al. 2011; Leclercq et al. 2008; Radjenović et al. 2009; Santos et al. 2009). However, another reason for poor removal may be due to concomitant exposure with drugs that inhibit P450 systems such as fluoxetine (Delgado and Zarkowski 2004) since they inhibit metabolism of carbamazepine (www.drugs.com): "Concurrent use [of fluoxetine] with carbamazepine may inhibit the metabolism of

carbamazepine, resulting in increased plasma concentrations and toxicity....” Although this type of inhibition has been studied for human metabolism, plants also contain P450 enzymes and the same type of inhibition may occur in duckweed. No removal pathway, including glassware sorption was significant for carbamazepine and the mass balance accounted for 100% recovery in the liquid over the 4 day reaction period (Figure 29).

5.3.4 Sorbing Compounds

In the case of **progesterone**, the liquid recovery results (Figure 29) show that progesterone levels decreased rapidly in the macerated samples, while the whole plant samples reached the same removal levels within 24 hrs. This trend suggests that removal is achieved primarily by sorption to duckweed cells (Figure 30) as expected given its high pKow and neutral properties. The rapid decrease in the macerated samples is attributed to more sorption sites due to having more intracellular tissue exposed in solution. This study attributed approximately 80% progesterone removal to sorption to duckweed plants, which is also the fraction that is predicted to sorb to sewage sludge (Hörsing et al. 2011). Progesterone is neutral and likely absorbed to lipid in duckweed cells. Duckweed can transfer lipid chemicals through the plant by symplastic movement. If progesterone was absorbed to inter-cellular tissue then extraction from the solids may be more difficult. Recovery from the solids was poor (Figure 31) most likely due to inability to desorb progesterone from duckweed, matrix effects, or metabolism by microorganisms attached to duckweed tissue.

In the case of **sulfamethoxazole**, results consistently showed that sorption and desorption both occurred (Figures 29-30 and Appendix D.2 Figures 54-56). The sorption mechanism taking place here is not well understood since negatively charged compounds

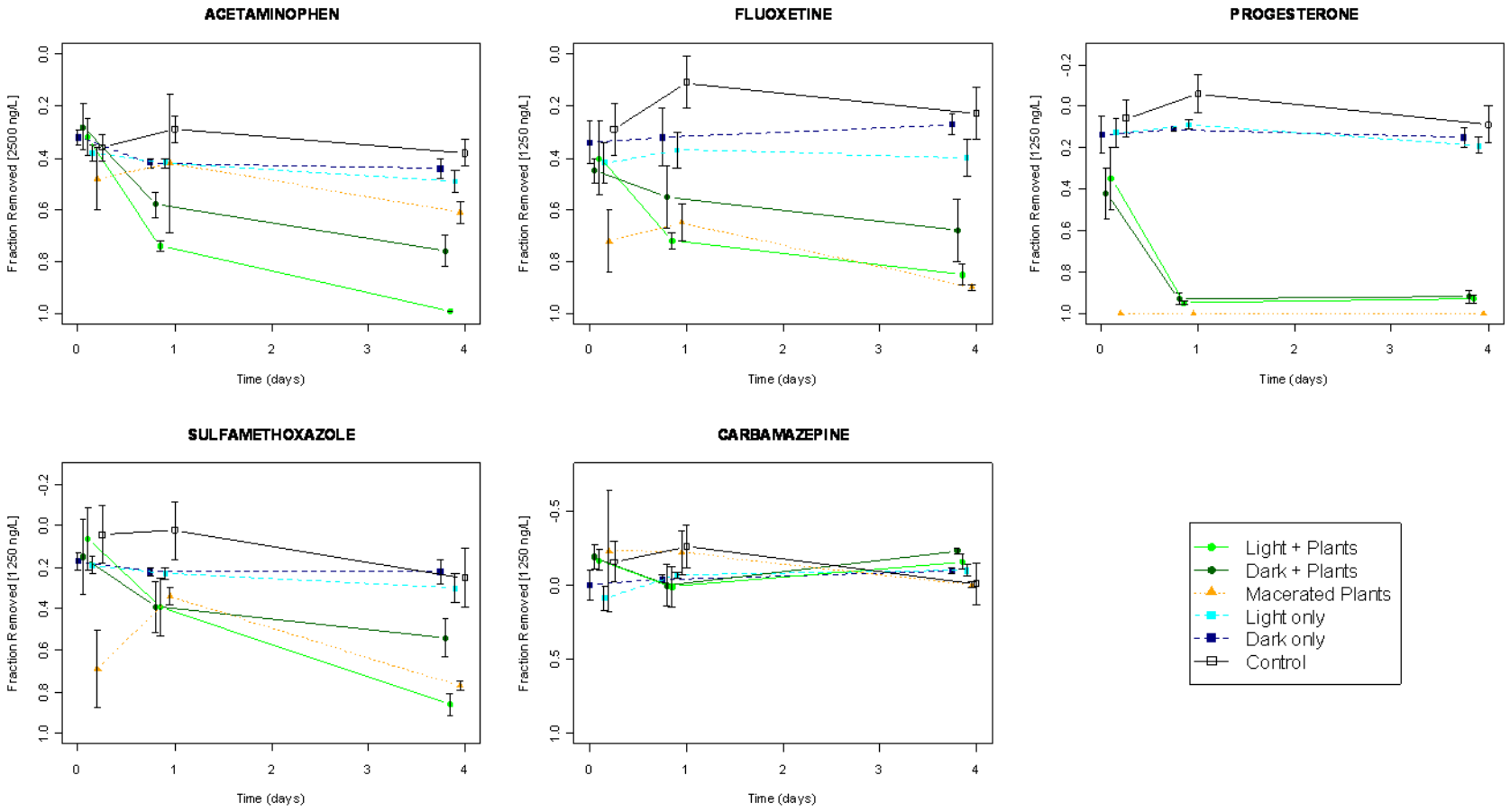

Figure 29. Pharmaceutical removal by treatment over 4 days with 1250 to 2500 ng/L starting concentrations.

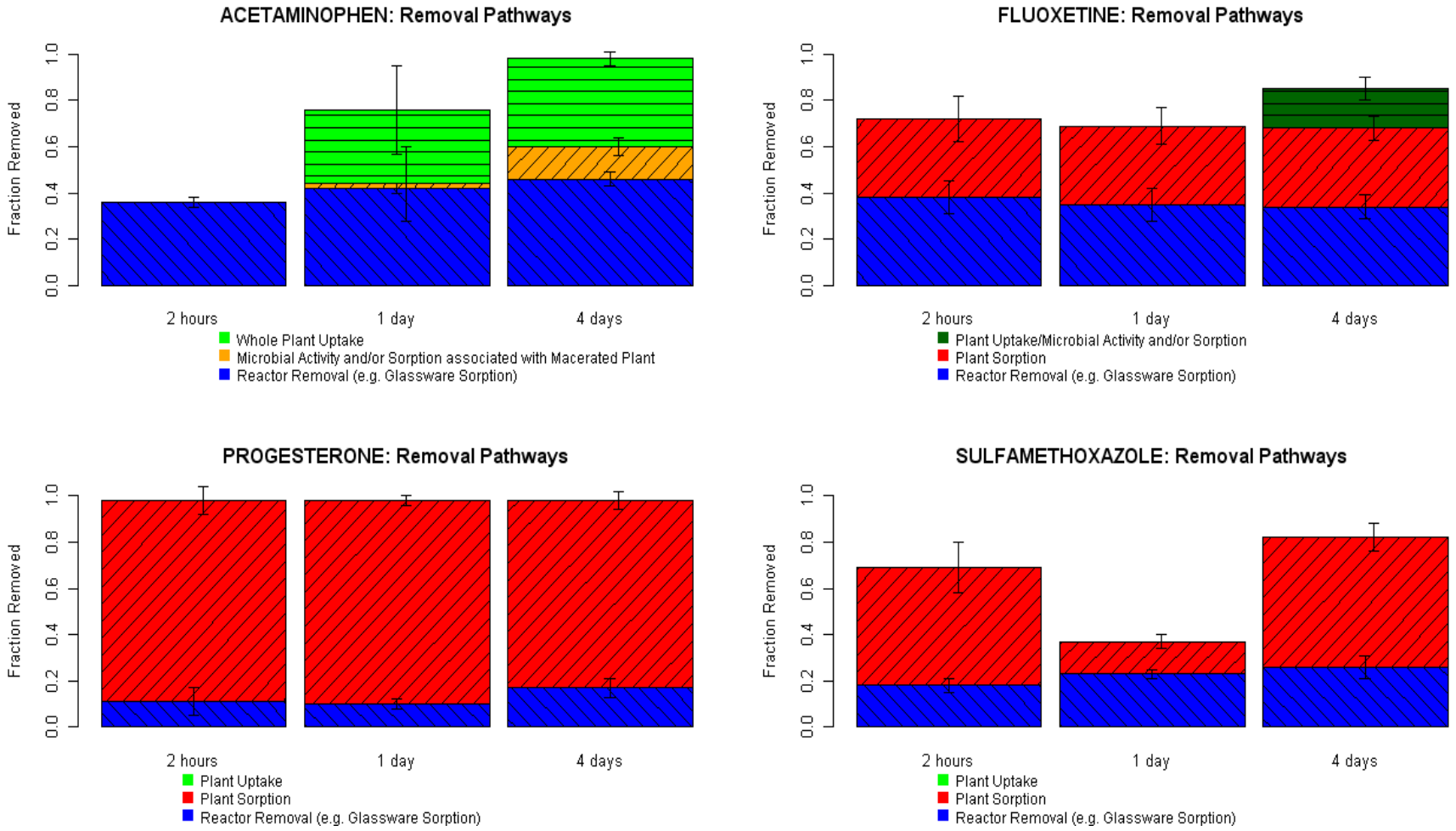

Figure 30. Major pharmaceutical removal pathways by compound (Carbamazepine showed no significant removal).

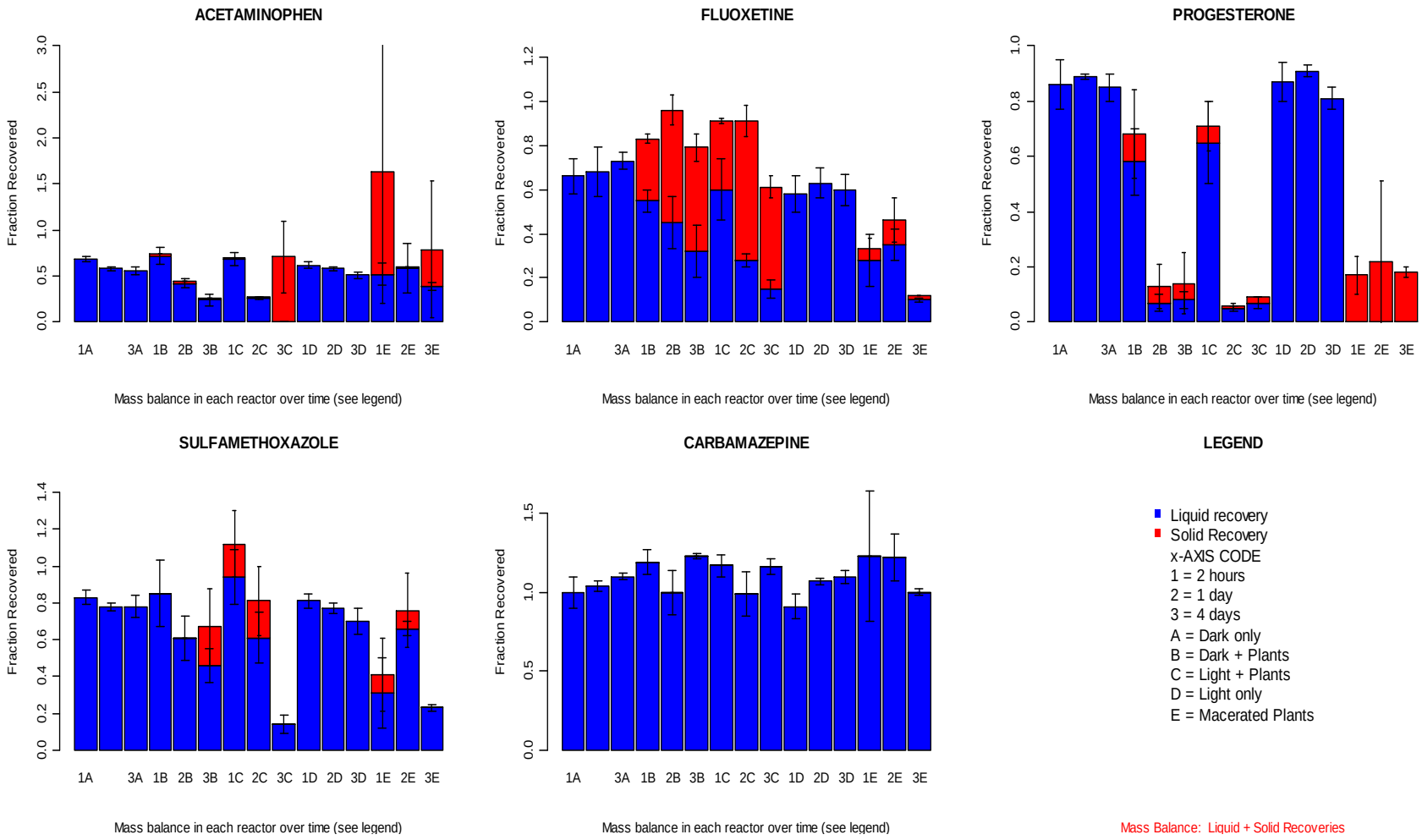

Figure 31. Mass balance showing pharmaceutical fractions recovered from liquids plus solid.

should remain soluble in the water column and not absorb to net-negative surfaces like duckweed unless they have a positive or neutral charge (Fukahori et al. 2011). Sulfamethoxazole was negative in the pH >7.5 range of this study (Figure 32) while neutral and positive charges dominate when the pH is 3.7 and <1.7, respectively. Possible explanations for sulfamethoxazole sorption occurring when not expected include: co-sorption with other organic, anionic, and cationic compounds in solution (*cf.,* positively charged fluoxetine) (Zhang et al. 2011, 2012); lack of repulsion due to weak surface charge of the sorbent; and both hydrophobic and hydrophylic interactions occurring simultaneously (Zhang et al. 2010). As a polar compound, sulfamethoxazole contains a negatively charged hydrophylic nitrogen ion sandwiched between other neutral functional groups. The neutral functional groups would have a stronger affinity for organic compounds like duckweed which may be the reason the sulfamethoxazole appears to sorb and then desorb from duckweed as the bipolar ends compete between the water and organic duckweed. Other studies suggest that sulfamethoxazole may be removed by biodegradation rather than sorption (Nghiem et al. 2009); however, this does not explain the simultaneous sorption and desorption of sulfamethoxazole taking place in this study and so sorption is assumed to be the primary means of removal.

5.3.5 Sorbing and Bioactive Compounds

Similar to progesterone, **fluoxetine** removal from the liquid occurs primarily by sorption, as expected with its positive charge, with some bioactivity. Rapid sorption kinetics accounting for approximately 40% removal occur within the first 2 hours with macerated plants and within 24 hours with whole plants in the light (Figure 29). Additional removal by the fourth day is significant (α=0.05, p-value = 0.01) and

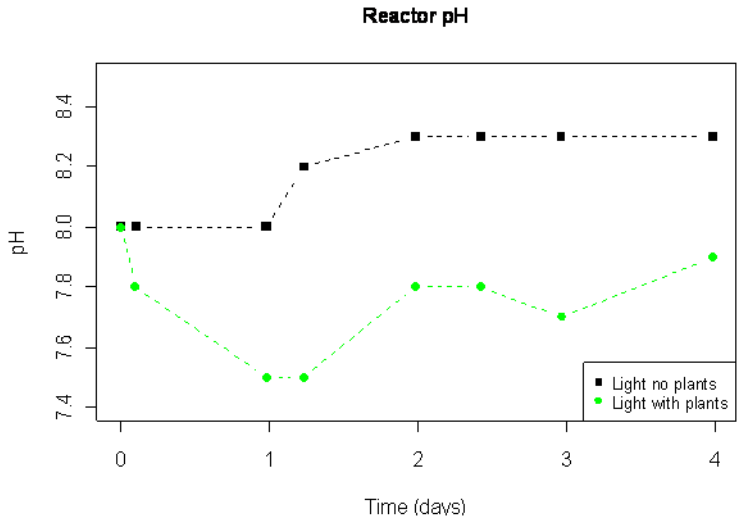

Figure 32. pH change over time in the light reactors at 25 °C and 200 μmol/m^2sec (PPF) light intensity.

contributes to an additional 17 ± 5% removal which may be a function of additional sorption and/or removal by attached microorganisms similar to the phenomenon seen with acetaminophen removal in macerated plants (Figure 30). Removal by sorption and plant uptake was consistent with results by Reinhold et al. (2010). Reinhold et al. (2010) attributed 55.6 ± 3.9% removal of fluoxetine from solution to sorption, with sorption equilibrium not being reached until 12-24 hours. She also observed additional removal of fluoxetine by Day Four, which was attributed to plant uptake rather than microbial degradation. Horsing et al. (2011) reported 100% removal of fluoxetine by sewage sludge as opposed to 40-50% by duckweed.

Fluoxetine recovery from the solids was highest compared to the other compounds (Figure 31). Fluoxetine likely adsorbed to the surface of the duckweed since it is a basic compound with a positive charge. Compounds adsorbed to the surface should require less effort to extract than compounds absorbed to inner cells like progesterone is assumed to have done. Solids recovery from the whole plants in the dark was highest,

followed by whole plants in the light and macerated plants. Improved recovery from whole plants in the dark may be due to decay of duckweed plants over time. Poor recovery from the macerated solids may be due to matrix interferences and/or inability to desorb fluoxetine from the duckweed cells in methanol solution.

5.3.6 Compounds Demonstrating Plant Uptake

The reactors with whole plants in the dark and light were designed to show removal due to plant uptake (including both active and passive uptake). In order to distinguish between removal due to plant sorption and plant uptake, one set of reactors contained macerated plants in the light. Removal due to plant uptake was assumed to occur when removal in the whole plant reactors exceeded removal in the macerated plant reactors. Removal due to attached microorganisms was assumed to occur when removal by the macerated plants increased significantly after the first day assuming that sorption kinetics occur rapidly and reach equilibrium within 12-24 hours.

In the case of **acetaminophen**, the results suggest that uptake by duckweed plays the major role in its removal (Figure 29 and Figure 30). The results also suggest that attached microorganisms play a minor role in compound removal. The liquid removal figures show that whole plants removed significantly more compound than the macerated plants, indicative of plant uptake rather than sorption. The macerated plants did not show rapid uptake, instead uptake increased gradually over time indicative of biological removal by attached microorganisms rather than sorption. Acetaminophen is an acidic compound, which means it is less likely to sorb to net-negative sorbents (*cf.* duckweed) which supports removal by plant uptake rather than sorption. Acetaminophen has the

lowest Kow value of the compounds in this study. Its low pKow of 0.46 supports plant passive uptake according to Dettenmeir (2008).

One study showed that plants are able to metabolize acetaminophen by glucosidation (Huber et al. 2009) supporting the possibility of active acetaminophen removal by plants. The study showed that horseradish root cells metabolized 82% acetaminophen (a.k.a. paracetamol) producing the metabolites: 64% paracetamol-glucoside, 17% paracetamol-glutathione, and 1% of the cysteine conjugate.

The mass balance results and qualitative metabolite searches can help distinguish between plant storage and metabolism of acetaminophen. The mass balance could only account for complete recovery of the parent compound at 2 hours (before significant plant uptake) and at 4 days, but not at 1 day. The metabolites were searched for using Agilent's Qualitative MassHunter program. Some of the metabolites were identified in the liquid samples and increased (with respect to the area of the chromatograms) as the parent compound decreased (see Appendix D.2 Figure 59). Although it is certain that duckweed plants uptake acetaminophen, due to the inconsistent mass balance results over time and the lack of confidence in the metabolite searches, this study was not able to distinguish between plant storage and/or metabolism of acetaminophen by duckweed plants.

5.4 Pharmaceutical Microcosm Discussion

The major pathways discussed previously include sorption and plant uptake. Equations to estimate sorption came from isotherms, while equations to estimate plant uptake came from plant-water partition coefficients (K_{PW}). The depletion of pharmaceuticals from solution over time was also calculated based on the 50% aqueous

depletion time (*cf.* half-life, $\tau_{0.5}$). These equations are useful for constructing fugacity models and estimating treatment removal by duckweed plants.

5.4.1 Sorption Isotherms

Results for progesterone, fluoxetine, and sulfamethoxazole showed that sorption was the major pathway for the removal of these pharmaceuticals in the microcosms. To confirm that sorption occurred, isotherms were created (see Appendix D.2 Figure 57). Approximately 8 g (fresh) duckweed in 400 mL nutrient solution were exposed to several concentrations of pharmaceuticals (low range = 1250 ng/L (2500 ng-ACT/L); mid-range = 50 μg/L; mid-high range = 650 μg/L; high range = 1 mg/L). All compounds were assumed to reach equilibrium by Day Four and the results (mass sorbate/mass sorbent) were calculated and fit to Freundlich and Langmuir isotherm equations. Mass sorbate removed was equal to the total pharmaceutical removal minus the removal in the control reactors. All compounds fit the Freundlich model better than the Langmuir model based on r^2 values—which is typical of an increasing number of sorption sites (*cf.* continuous plant growth).

Progesterone removal by sorption is supported by a good fit to a Freundlich isotherm at 25 °C (r^2 = 0.948, df=12, slope predicted/measured = 0.926, see Figure 33). According to the significance of regression in R an $r^2 > 0.28$ for df=12 and $\alpha = 0.05$ is considered a good fit. Like progesterone, fluoxetine also fits a Freundlich isotherm at 25°C (r^2 = 0.992, n=12, slope predicted/measured = 0.974, see Figure 34). Sulfamethoxazole did not fit an isotherm as well, probably due to weak sorption as discussed previously. Acetaminophen fit the isotherm model well, but its removal is contributed to plant uptake not sorption (Appendix D.2 Figure 57). Table 16 shows

Table 16. Freundlich isotherms coefficients for fresh and dry duckweed mass

Coefficients	ACT	SLF	FLX	CRB	PRG	K_f units
K_f *(fresh)*	0.008	0.007	7.66	NA	100	$ng_{sorbate}/g\text{-}duckweed_{fresh})/(ng_{sorbate}/L)^{1/n}$
n (fresh)	0.872	0.883	1.42	NA	2.05	
K_f *(dry)*	7.52	0.261	554	NA	3720	$ng_{sorbate}/g\text{-}duckweed_{dry})/(ng_{sorbate}/L)^{1/n}$
n (dry)	1.19	0.93	1.72	NA	2.37	

the predicted coefficients for the Freundlich isotherm equation for each compound, *e.g.*, $qe = K_f Ce^{1/n}$ where *qe* = mass of adsorbate adsorbed per unit mass of adsorbent (ng adsorbate/g plant mass), K_f= Freundlich capacity coefficient (ng absorbate/g plant mass)(L nutrient solution/ng adsorbate)$^{1/n}$, and *Ce* = equilibrium concentration of adsorbate in solution at 4 days, ng/L.

5.4.2 Plant-water Partition Coefficients

Partition coefficients explain the amount of chemical distributed between two phases, in this case plants and water (K_{PW}). The K_{PW} values for each of the five pharmaceuticals were computed by dividing the ng-compound removed from solution per gram duckweed by the ng-compound in solution at 4 days per mL solution ($ng/g_{fresh\ duckweed} \div ng/mL_{liquid} = mL_{liquid}/g_{fresh\ duckweed}$). Table 17 summarizes the K_{PW} values for fresh duckweed materials. The K_{PW} for dry duckweed is equal to the fresh K_{PW} value multiplied by 20 g-fresh/g-dry (assuming duckweed is 95% water). Due to the difficulty in extracting/measuring pharmaceuticals from the solids, it was assumed for this study that pharmaceuticals partitioning to the solids will not re-enter the solution, except in the case of sulfamethoxazole, and due to plant decay. Further studies are necessary to determine the mobility of the compounds after partitioning to duckweed.

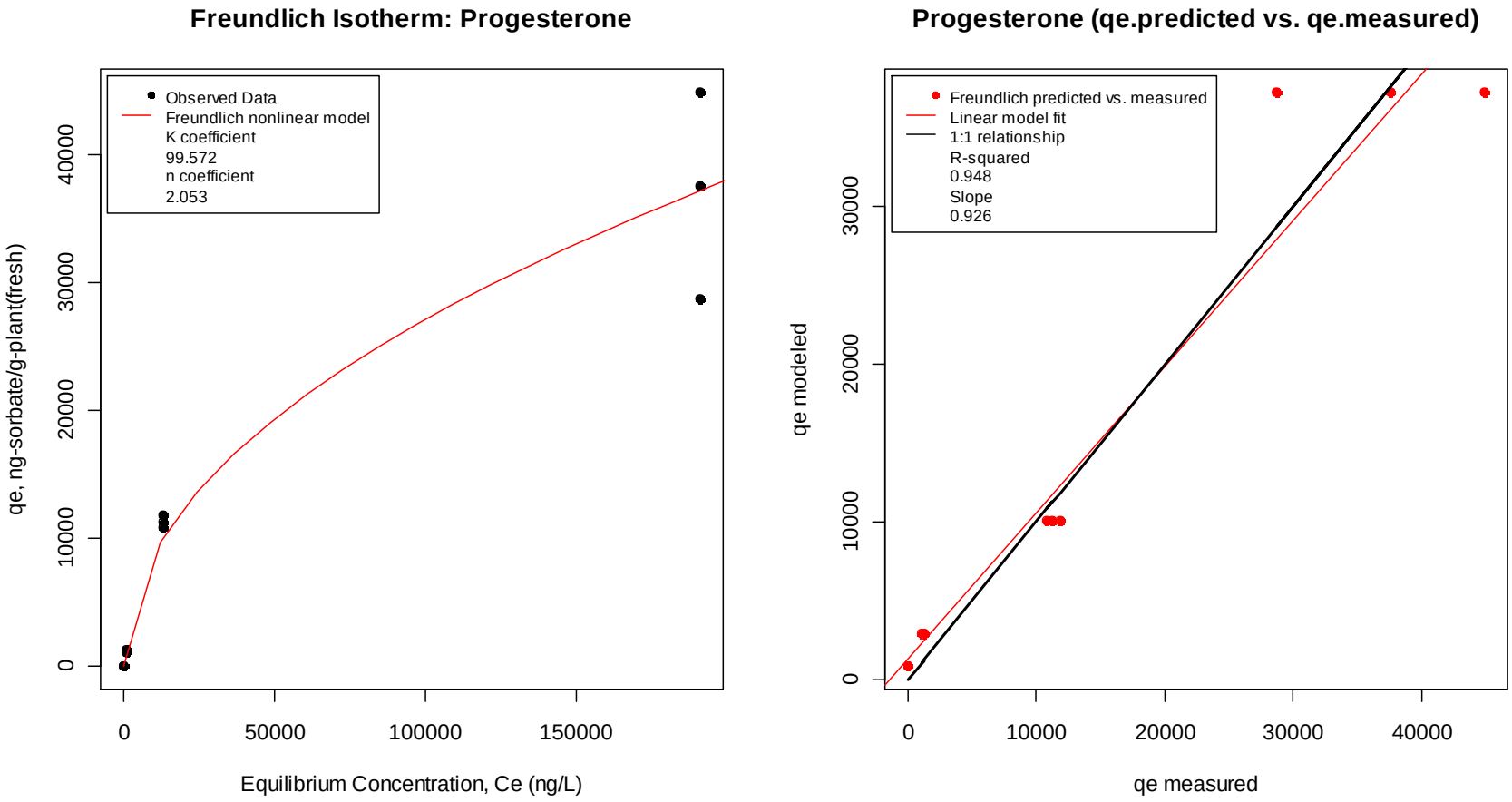

Figure 33. Progesterone Freundlich Isotherm at 25 °C and predicted vs. measured relationship.

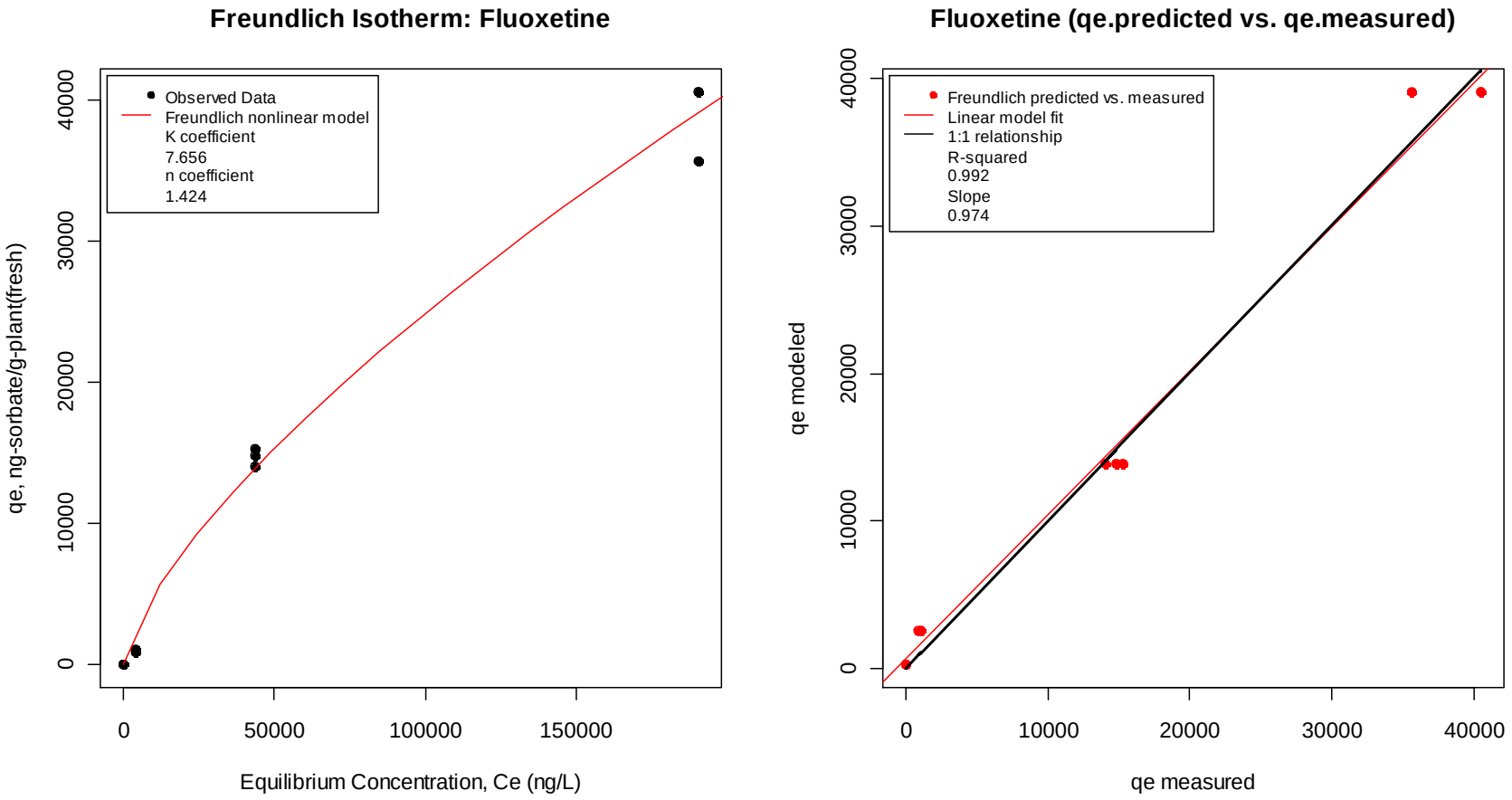

Figure 34. Fluoxetine Freundlich Isotherm at 25 °C and predicted vs. measured relationship.

Table 17. Plant-water partition coefficients (K_{PW} = ng/g-fresh / ng/mL-liquid) of five pharmaceuticals at four concentrations

Initial Mass of Pharmaceuticals	Measure K_{PW}				
	ACT	SLF	FLX	CRB	PRG
LR = 1250 ng/L (2500 ng-ACT/L)	1605 ± 432	156 ± 76	144 ± 48	-4 ± 2	399 ± 104
MR = 50 µg/L	482 ± 357	25 ± 3	224 ± 50	-7 ± 2	1002 ± 130
MHR = 625 µg/L	43 ± 5	5 ± 6	298 ± 31	7 ± 5	760 ± 37
HR = 1,000 µg/L	59 ± 31	56 ± 64	301 ± 208	6 ± 6	20138 ± 4280

The K_{PW} for acetaminophen decreases with increasing concentration. On the other hand, fluoxetine and progesterone K_{PW} values increase with increasing concentration indicative of more available sorption sites than are being utilized at the lower concentrations.

5.4.3 50% Aqueous Depletion Time

The 50% aqueous depletion time is similar to half-life but without the assumption that removal occurs solely by biological means. All 50% depletion times were less than 4 days for all concentrations and compounds except Carbamazepine, which showed no significant removal after 4 days (Table 18 and Appendix D.2 Figure 60). These depletion times generally increase with increasing pharmaceutical concentration, except in the case of Sulfamethoxazole, which had increasing/decreasing liquid concentrations over time indicative of sorption/desorption. Reinhold et al. (2010) reported that fluoxetine had a half-life of 0.41 ± 0.17 days, similar to this study. The half-life values were calculated using nonlinear least-squares analysis to fit the removal over time for each concentration range to a logarithmic function using the statistical software R.

Table 18. 50% Aqueous depletion ($t_{0.5}$) of five pharmaceuticals at four concentrations

Initial Mass of Pharmaceuticals	Measured $t_{0.5}$				
	ACT	SLF	FLX	CRB	PRG
LR = 1250 ng/L (2500 ng-ACT/L)	0.419	1.415	0.411	-19.911	0.129
MR = 50 μg/L	0.689	1.949	0.100	24.196	0.174
MHR = 625 μg/L	1.254	0.217	0.263	5.295	0.120
HR = 1,000 μg/L	2.368	1.846	0.449	22.022	0.661

5.4.4 Toxicity Due to Pharmaceutical Concentrations

Excessive pharmaceutical concentrations have been linked to toxicity to several aquatic organisms including duckweed. This study examined the inhibition and toxicity of a concomitant solution of five pharmaceuticals based on duckweed plant growth; however, the study lacked a positive control. Plant growth by whole duckweed plants in the light decreased with increasing pharmaceutical concentrations. The EC_{50} concentration that inhibits 50% of the potential duckweed growth occurs at 614 μg/L (13 $\mu mol_{5\ pharmaceuticals}/L$) (Figure 35) as determined by the biexp() function for calculating half-life using the Pharmacokinetic (PK) package in R. This concentration is higher than reported by Brain et al. (2004a) who observed an EC50 at 1.15-1.59 μmol/L. The difference is likely due to the fact only three (acetaminophen, sulfamethoxazole, and carbamazepine) of Brain et al.'s eight compounds were used in this study. The limitation of analyzing toxicity due to a mixture of pharmaceuticals is that is cannot isolate the toxic effects of individual compounds. The advantage of measuring toxicity in a mixture of solutions is that it provides a more environmentally relevant situation. Pharmaceutical concentrations are never expected to reach 614 μg/L in the Wellsville lagoons and should not be toxic to the plants. For individual compounds, Brain et al. (2004b) reported that sulfamethoxazole has an EC50 effect on wet weight production at 249 μg/L.

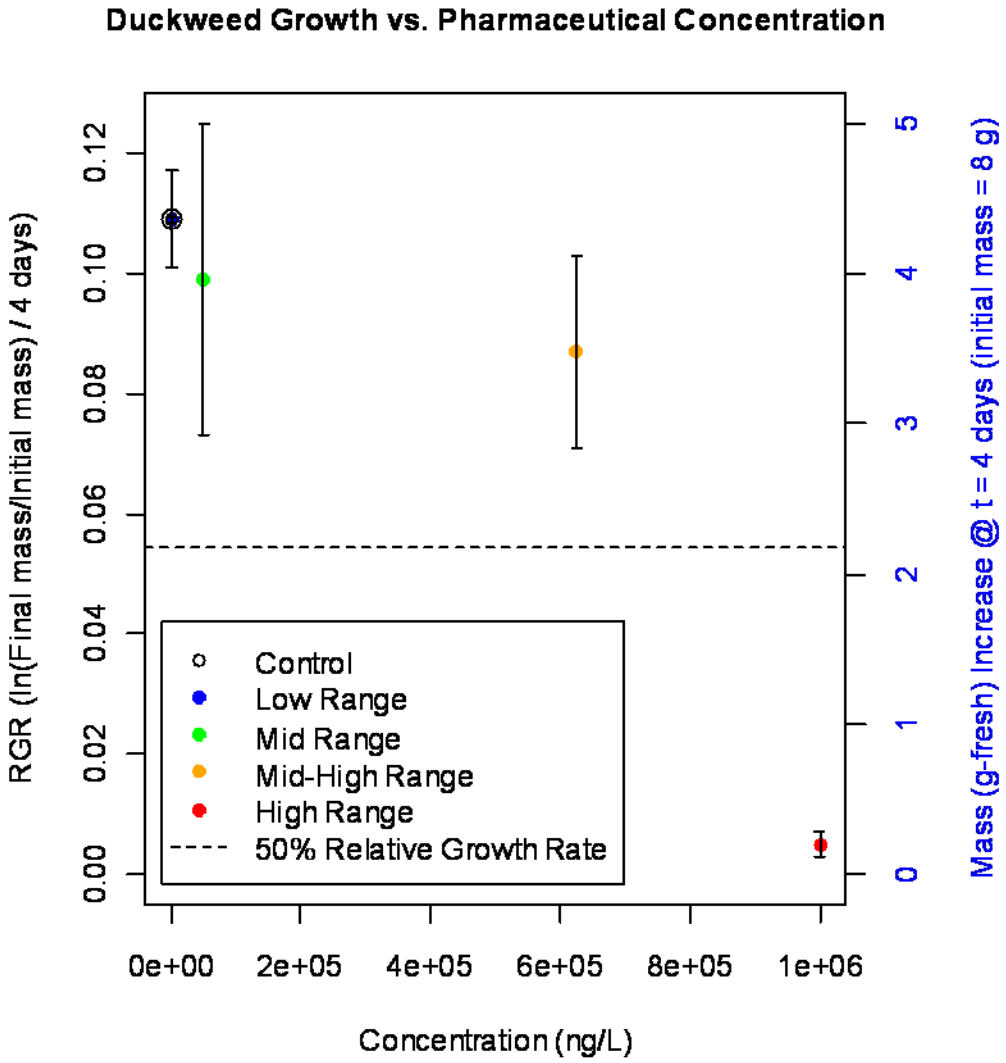

Figure 35. Toxicity effect of five pharmaceuticals on duckweed growth.

CHAPTER 6

DUCKWEED GROWTH RESULTS AND DISCUSSION

6.1 Duckweed Growth Results

Field growth studies took place in 2008 (August 29th to November 11th) and 2011 (May 3rd to October 14th) along with laboratory studies. Appendix C.3 Table 29 shows the raw data from these experiments. Relative growth rates averaged 0.10 ± 0.02 (n = 72) in 2011, averaged 0.06 ± 0.02 (n = 8) in 2008, and 0.10 ± 0.016 (n = 36) in the laboratory. Compared to the theortical duckweed plant production (see § 2.4) the measured growth was lower than expected—field growth in the spring and summer were 0.34 ± 0.1and 0.11 ± 0.03 of the theoretical, respectively; while laboratory results were 0.53 ± 0.09. The results from the growth studies were also compared to the predictions from the Landesman, Lasfar, and Driever models (see Appendix D.3 Figure 61and Figure 62). Predictions within a factor of two of the measured value were considered acceptable. The optimum growth rate coefficient for each model was adjusted using the nonlinear least squares (nls) function in R (Appendix D.3 Table 32). In the laboratory 83%, 67%, and 64% of the predictions were acceptable for the Landesman, Driever, and Lasfar models, respectively. The percent of acceptable predictions in the 2011 field data were significantly lower with values of only 49%, 29%, and 26%, respectively.

To explain the reasons for the poor fit between measured and predicted values in the field, the independent variables were analyzed for multicolinearity (see Appendix D.3 Figure 63). This analysis showed a strong correlation between plant starting density and temperature (see Appendix D.3 Figure 64). There was also a high correlation between

phosphorus and nitrogen concentrations as well as expected correlations between temperature, photoperiod, and solar radiation.

6.2 Duckweed Growth Discussion

The number one finding from the growth study was that the duckweed in Cache Valley experiences some of its highest growth rates in the spring when temperatures were lowest (Figure 36). This high spring growth rate coincides with increasing percent germination of turions (Landolt and Kandeler 1987) and possibly increased CO_2 concentration. Increased CO_2 concentrations increase the duckweed photosynthetic rate (Landolt and Kandeler 1987, p. 72)—CO_2 solubility is higher in colder temperatures; however, this may be offset by increased microbial activity in the summer which respires CO_2 and increased CO_2 demand in the colder seasons (Bruce Bugbee, personal communication, 4/20/2012). According to Landolt, turion germination for *L. turionifera* increases exponentially starting at 17°C and reaches 100% germination around 19°C. In order to adjust the models to replicate this finding, the temperature function in each model had to be modified so that the optimum temperature for growth was lowered to 7.5°C in order to produce acceptable predictions in the spring.

Adjusting models by season is not unique. Other limnology studies find that phytoplankton populations thrive depending on different variables at different times of the year and therefore specific models need to be developed depending on the season:

> [Phytoplankton production results] indicated that the correlations between variables changed significantly with the time of the year, sometimes making the analysis of the entire data set meaningless. The data were therefore split into three seasonal sets: spring (April-June), summer (July-September), and fall (October-December) and factor analysis was carried out on the three sets separately. (Munawar and Burns 1970)

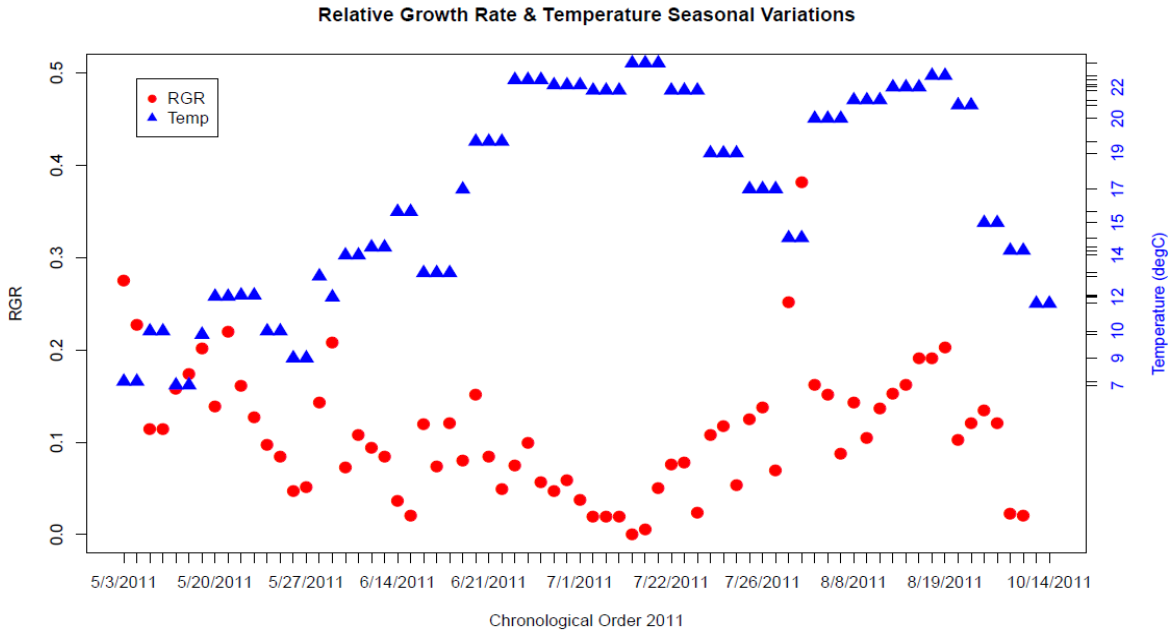

Figure 36. Inverse relationship between relative growth rate (RGR) and temperature and season.

Duckweed most likely uses up nutrient reserves to support its high growth in the Spring.[1] Researchers at North Carolina State University under the direction of Dr. Jay Cheng found that duckweed utilizes its nutrient reserves to maintain growth when growing conditions are not ideal. Dr. Cheng pointed out that duckweed biomass continued to increase despite growing in nitrogen and phosphorus deficient water due to the fact that they were able to use the N and P stored in their biomass (Chaiprapat et al. 2005). Unlike "endogenous decay"

[1] Improved growth in lower temperatures is also seen in Cache Valley turfgrasses which are greenest in the spring and fall, but under stress in the summer--similar to the duckweed plants. Upon closer inspection, growth curves for cold-season turfgrass is the inverse of the curve for warm-season turfgrass. Researchers attribute the grasses ability to grow rapidly in the spring to the plants' ability to utilize its nutrient reserves stored up during the winter.

which decreases cell mass as bacteria utilize their energy and nutrient reserves; "endogenous growth," as seen in duckweed, increases cell mass by using nutrient storage.

The Lasfar model was selected to show the improvement of adjusting the temperature function in the model to predict the higher growth rates in the spring. The Driever model also significantly improved with this adjustment (see Appendix D.3 Figure 62). In the field, the Lasfar model only predicted 26% of the measured values (n = 68). After adjusting for the high growth rates in the spring and removing the events when the starting density was less than 30 g-dry/m^2, the Lasfar model successfully predicted 63% of the measured values (Figure 37).

All three models were also notably unreliable at predicting the growth rates in July. One would expect from the laboratory growth tests that the models would predict more acceptable values in the 20 to 25°C temperature range. The lower growth rates in July may be misleading due to low starting densities, herbicide inputs to the lagoon as discussed below, and/or nutrient limitation. Starting densities in July were below 30 g-dry/m^2 when algae inhibition occurs. Also, the operator of the lagoons had sprayed herbicide (Roundup®) around the perimeter of the lagoons on July 3rd right before the lowest growth rates started occurring. Also, cloth liners were installed over the metal screens starting June 7th, which eventually developed a biofilm that could have reduced nutrient levels for the plants.

The models were also unreliable at predicting values at the very end of the season after temperatures fell below 15°C (the point of highest growth in the spring) due to

formation of turions. The duckweed in October stopped growing. Fronds in October were dark green and only had one or two fronds per colony indicative of turions. It appears from these results that turions begin dormancy when temperatures fall below 15°C in the fall and then start germination after temperatures increase above 15°C in the spring.

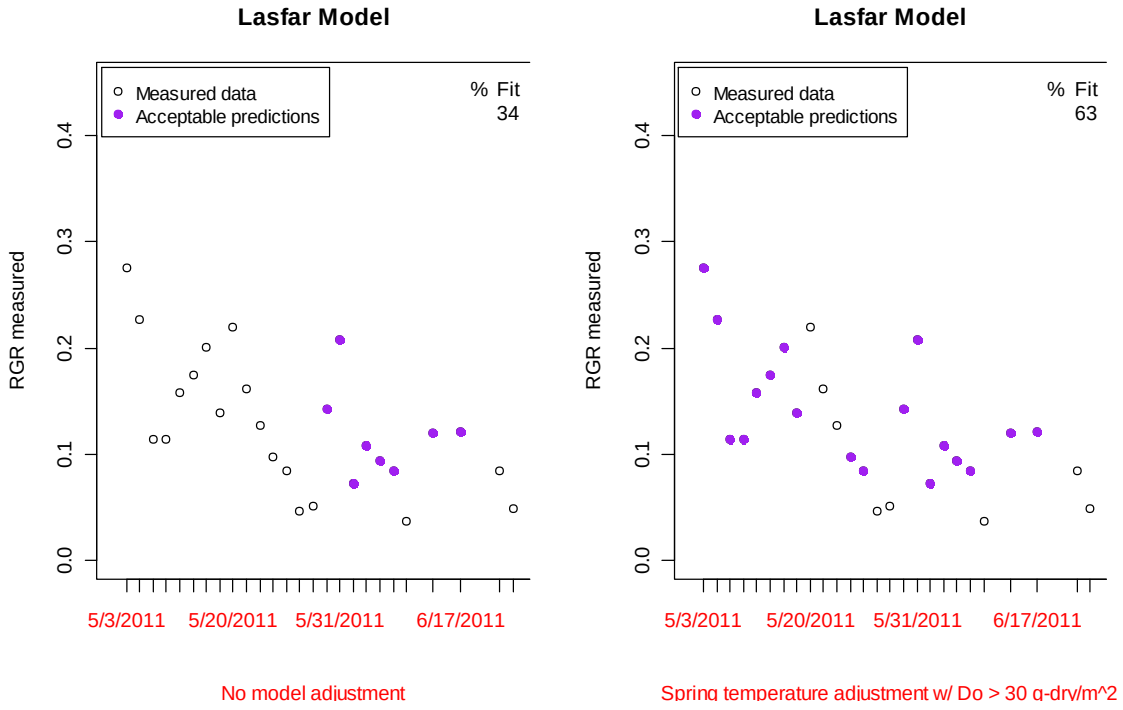

Figure 37. Lasfar model showing improved predictions after lowering the optimum temperature in the spring.

CHAPTER 7

CONCLUSIONS

7.1 Phosphorus

Phosphorus content in duckweed tissue averages 1% dry weight, which may increase due to overcrowding or sustained phosphorus liquid concentrations above 1 mg-P/L. *L. turionifera* and *W. borealis* species used in this study removed phosphorus at a rate of 100-200 mg-P(PO_4)/m^2day in 200 PPF light with the lower removal taking place as the plant density increased. Duckweed growing in the dark released phosphorus at a rate 10 mg-P per g-dry duckweed destroyed. It is estimated that a combined light/dark period would remove phosphorus at a rate of 113 mg-P/m^2day if the plant density is maintained around 70 ± 10 g-dry/m^2.

The phosphorus concentration in the microcosm study decreased by 84% after 3 days from 5.6 to 0.86 mg-P (PO_4)/L, with all of the removed phosphorus accounted for in the duckweed tissue. The larger 100 L reactors reduced the phosphorus concentration from 3.16 mg-P/L to 0.16 mg-P/L (0.32 mg-TP/L) with constant duckweed harvesting and a liquid retention time of 46 days.

7.2 Pharmaceuticals

The five pharmaceuticals tested showed a range of possible removal mechanisms ranging from glass sorption, plant uptake, plant sorption, attached microorganisms, and no removal in the case of **Carbamazepine**. Up to 40% of the pharmaceuticals were removed by sorption to the glassware.

Acetaminophen removal primarily occurred by plant uptake and accounted for 40-50% of the total removal; probably due to its relatively low Kow and neutral charge. The plant-water partition coefficient (K_{PW}) for acetaminophen at low concentrations (2500 ng/L) was 1605.

Progesterone removal occurred by sorption to duckweed tissue and accounted for >80-90% of the total removal; probably absorbed to inner-lipid cells through the symplast. Progesterone sorption to fresh duckweed fit the Freundlich isotherm model well with isotherm coefficients K_f = 100 and n = 2.05. The fresh mass of duckweed required to obtain a certain progesterone liquid concentration at 25 °C can be calculated using the Freundlich isotherm equation, *e.g.*, qe = ng-$PRG_{removed}/g_{fresh\ duckweed}$ = 100(ng-$PRG_{final\ aqueous}$/L)$^{1/2.05}$.

Fluoxetine removal occurred by sorption to duckweed tissue (35% removal), followed by plant uptake (20%) accounting for 55% of the total removal; probably due to adsorption to a net-negative duckweed plant. Fluoxetine sorption to fresh duckweed fit the Freundlich isotherm model well with isotherm coefficients K_f = 0.041 and n = 1.16. The fresh mass of duckweed required to obtain a certain progesterone liquid concentration at 25 °C can be calculated using the Freundlich isotherm equation, *e.g.*, qe = ng-$FLX_{removed}/g_{fresh\ duckweed}$ = 0.041(ng-$FLX_{final\ aqueous}$/L)$^{1/1.16}$. The plant-water partition coefficient (K_{PW}) for fluoxetine at low concentrations (1250 ng/L) was 144.

Sulfamethoxazole removal occurred primarily by sorption and accounted for 20 to 60% of the total removal. During the 4-day test period, sorption and desorption occurred among all four concentrations tested. Despite being negatively charged, sulfamethoxazole still sorbed to the duckweed probably due to the fact that it is a polar

compound with neutral functional groups that have an affinity for organic compounds. The K_{PW} value with fresh duckweed varied between 5 and 156 with no correlation to pharmaceutical concentration. This suggests that sulfamethoxazole is weakly sorbed to the biomass, and the chance for mobility if applied to another system (*i.e.*, land application) is high (Yang et al. 2011).

7.3 Duckweed Growth

Densities should be kept above 30 g-dry/m^2 to prevent inhibition by algae. Duckweed growth decreased with increasing plant density due to overcrowding. Duckweed growth after ice melt in the spring was highest despite low temperatures; probably due to the germination of turions (see §6.2). Harvesting should begin after 100% of the turions have germinated and when duckweed covers 100% of the lagoons (*ca.*, June 17th). Harvesting should finish once temperatures start decreasing below 15°C and duckweed starts forming turions (*ca.*, September 15th) in order to leave enough turions to re-seed the lagoons the following spring. If predicting growth during turion germination in the spring, adjustment needs to be made (*e.g.*, modified temperature function) for the higher growth rates starting when average daily temperature exceed 8°C (April through May).

Harvesting frequency and starting plant density both affect final plant production as seen by comparing the field results with the modelled values (see Appendix D.3 Figure 65). The Landesman model predicted laboratory results best but does not take plant density into consideration. The Driever and Lasfar models are recommended because they account for plant density and produced a similar percentage of acceptable predictions of the Wellsville 2011 field data. The Lasfar model was selected to represent

growth on the Wellsville lagoons because it predicted the highest plant production rates assuming that the Wellsville 2011 results were false-negative growth rates due to the limitations in the summer discussed earlier and due to the fact that duckweed production was less than 50% of the theoretical production.

CHAPTER 8

RECOMMENDATIONS

8.1 Phosphorus

Many studies have already measured phosphorus uptake by duckweed plants; however, excessive phosphorus concentrations may exist in the Wellsville lagoons after the ice melts. Measuring phosphorus in the lagoons by season may help determine whether the high growth rates in the spring are a function of high nutrient concentrations or turion germination.

In addition, this study assumes that phosphorus in duckweed tissue is 1%; however, results have shown that this percentage varies with phosphorus liquid concentration. The total phosphorus removal by duckweed estimates may improve by incorporating a function of phosphorus tissue composition versus phosphorus liquid concentration.

8.2 Pharmaceuticals

Macerated duckweed does not exist in natural lagoons and may give a false impression of compound sorption to duckweed. Other studies have replaced macerated plants with whole gently freeze-dried duckweed plants to preserve as much sorption structure as possible and eliminate matrix interferences.

Preliminary measurements showed lower pharmaceutical effluent concentrations from the Wellsville lagoons compared to more advance treatment methods. In order to verify whether lagoon systems remove more pharmaceuticals, then influent concentrations and effluent concentrations need to be measured.

8.3 Duckweed growth

The field growth rate studies in the summer had several flaws that prevented density and nutrient levels from being independent variables in the models. The starting densities correlated with time and so future studies should use three starting densities (*e.g.*, 30, 75, and 120 g-dry/m^2) at each growth period. Nutrients should be measured rather than relying on sparse historical data. It may be possible to only measure phosphorus or nitrogen since field and laboratory results showed that the two were correlated with each other with a 5:1 nitrogen (as nitrate-N and ammonia-N) to phosphorus concentration ratio. Simple orthophosphate measurements using aquarium quick test strips would be a simple way to estimate nutrients available for duckweed growth. Also, constructing better cages that prevent both Wolffia infiltration and biofilm from occurring would eliminate errors due to possible nutrient deficiencies.

CHAPTER 9

ENGINEERING SIGNIFICANCE

9.1 Duckweed Growth

Higher than 0.05 mg-P/L phosphorus in the Little Bear River is linked to eutrophication within the Cutler Reservoir into which the Little Bear discharges. Phosphorus in the Little Bear River comes from wastewater effluents, agriculture, and natural sources. This study was designed to estimate how much phosphorus a managed duckweed system on the Wellsville Municipal Sewage Lagoons can remove prior to discharge in the Little Bear River. Phosphorus removal by duckweed is a function of harvesting frequency, duckweed mat density, temperature, sunlight, and phosphorus in the liquid and plant tissue.

Although duckweed can be seen growing on ponds in climates like northern Utah for 5 to 6 months, the period between germination and sowing is the time to harvest duckweed and lasts for only 3 months between June 17 and September 15. Germination of turions begins after the ice melts and temperatures increase above 5°C. The percent germination of turions increases exponentially around 17°C and reaches 100 percent around 19°C (Landolt and Kandeler 1987). These temperatures were reached around June 17 in 2011, which was also the time when duckweed started to completely cover the lagoons. Duckweed should not be harvested until after complete coverage in order to minimize algae proliferation. Sowing coincides with turion formation. Turion formation begins when average daily temperatures begin falling below 15 °C and is associated with lower growth rates. These temperature drops and lower growth rates occurred around September 15 according to 2008 nd 2011 field growth studies.

The Lasfar model, which is most sensitive to plant density, was used to predict duckweed plant production per season. Spring model adjustments for germination were unnecessary since harvesting should not begin until summer.

Plant density and harvesting frequency are the two variables controlled by an operator harvesting duckweed-covered lagoons. The objective is to remove as much duckweed as possible in as few harvests as possible. Plant production was plotted versus plant density and harvesting frequency (Figure 38). More frequent harvesting produces more duckweed. Duckweed production is highest with a starting density between 45 to 75 g-dry/m^2 (Frédéric et al. 2006). Without periodic harvesting, the Wellsville lagoons reach a maximum plant density of 267 ± 62 g-dry/m^2 by September 15 based on field results. A starting density of 60 g-dry/m^2 harvested bi-weekly or every 4 days, should produce 1215 g-dry/m^2 to 1515 g-dry/m^2 per season, respectively, based on Lasfar's model. This harvesting scheme would produce 67 tons of dry duckweed per season with one harvest; and up to 300 to 380 tons of dry duckweed if harvested bi-weekly or every 4 days, respectively. Every ton of dry duckweed represents 20 tons-fresh, 30 cubic yards of fresh duckweed, and 181 kg phosphorus.

9.2 Phosphorus Removal

Phosphorus removal from the Wellsville Municipal Sewage Lagoons requires physically removing the plants from the system. The 23 ha (56-acres) lagoons currently receive 0.547 MGD with an average influent of 3.88 to 5 mg-TP/L. Assuming that the lagoons receive 5 mg-TP/L with 15% (570 kg-P) sequestered in the sediments (see previous Figure 7), then the lagoons would have to remove 2780 kg-P/year to meet their 432 kg-P discharge limit. Assuming that all duckweed contains 1% phosphorus (dry

weight) then 1227 g-dry duckweed/m^2 would have to be produced per season. Depending on the harvesting strategy, all models and field results predicted that at least 1227 g-dry duckweed/m^2 can be produced per season (see Figure 38 and Appendix D.3 Figure 66).

The microcosms predicted a phosphorus removal of 113 mg-P/m^2day, which corresponds to 2304 kg-P/90-day season. This predictions falls short of the 2780 kg-P removal requirement. The estimate may be low due to the fact that the microcosm study was performed under 24 hours artificial light (90 W/m^2 = 7.8 MJoules/day and 200 PPF) which produced about 40% of the outdoor radiation (25 to 15 MJoules/day). Plant production and phosphorus removal should increase with increasing light.

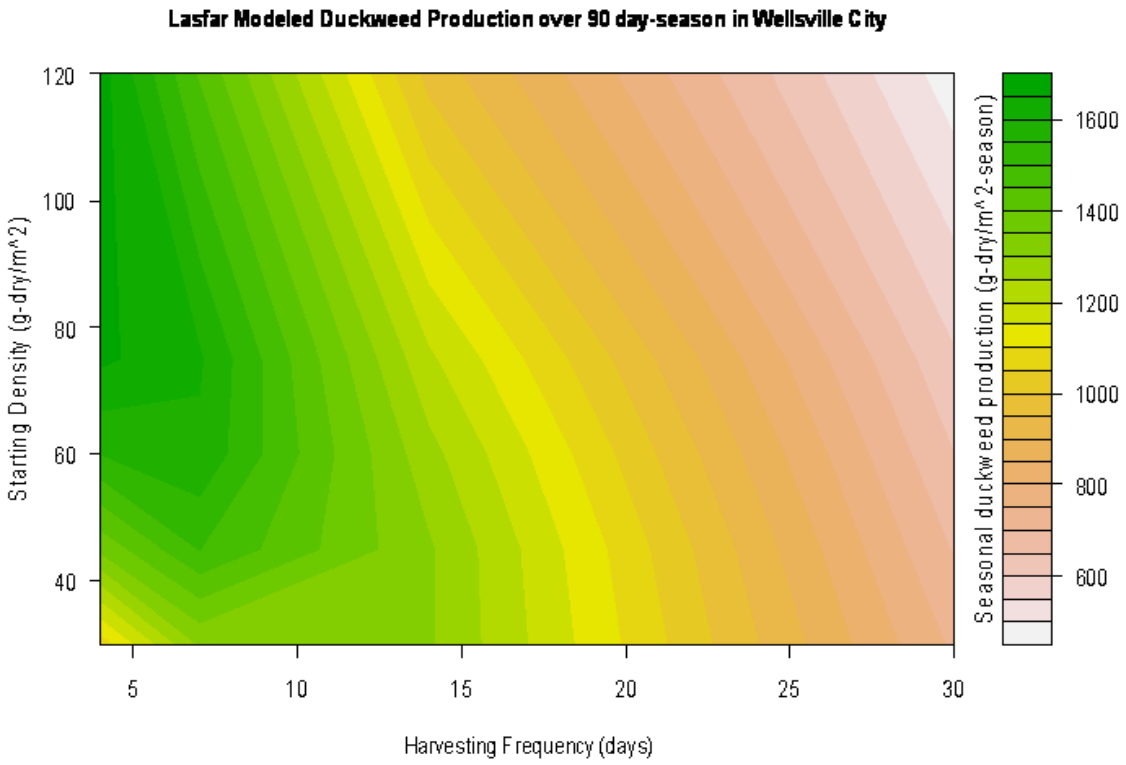

Figure 38. Estimated duckweed production as a function of starting density and harvesting frequency on the Wellsville Municipal Sewage Lagoons between June 17th and September 15th using the Lasfar model.

Based on calculations using Lasfar's model and 2011 Wellsville City environmental conditions, the 23 ha (56-acres) lagoons will produce at least 1227 g-dry duckweed/m^2 if harvested bi-weekly between June 17 to September 15 with starting densities anywhere between 30 to 45 g-dry/m^2. Hence, with Lasfar's predictions, duckweed harvesting is a feasible option for phosphorus removal to meet the 432 kg-P/year discharge limit—floating barriers may need to be installed to ensure that a uniform duckweed cover exists throughout the lagoons. This is considered a sustainable system if the duckweed harvesting stops on September 15 to allow time for the duckweed mat to re-establish and form enough turions to replenish the system the following year.

If the Wellsville City population increases and wastewater flow increase to 0.656 MGD by 2017 as expected (JUB 2008) then 3500 kg-P would have to be removed per harvesting season which amounts to 1544 g-dry duckweed/m^2. Harvesting frequency would have to increase to weekly harvests with starting densities between 60 to 75 g-dry/m^2. If flow increased to 0.931 MGD by 2027 as expected, then the system would have to look into removing phosphorus by means other than duckweed (*i.e.,* land application, effluent filtration, or chemical precipitation). Therefore, a duckweed system in Wellsville is expected to help the city meet their phosphorus limits until wastewater flows start exceeding 0.656 MGD (Table 19).

Table 19: 15 year required phosphorus removal and duckweed production to meet Wellsville City 472 kg-phosphorus discharge per year permit

Wellsville City flow	0.547 MGD	0.656 MGD	0.931 MGD
P loading / yr	3800 kg-P	4500 kg-P	6430 kg-P
Required P-removal with 570 kg-P retained in sediments	73 %	78 %	84 %
Required duckweed	1475 g-dry/m^2	1550 g-dry/m^2	2400 g-dry/m^2

9.3 Pharmaceutical Removal

In addition to phosphorus, pharmaceuticals in water have been linked to environmental problems (Fent et al. 2006). Pharmaceuticals are emerging contaminants of concern and are considered when wastewater treatment plants consider new equipment or processes for plant upgrades. Smaller cities using lagoons may justify replacing their lagoons systems with membrane bio-reactor plants in order to remove the pharmaceuticals because they are more technological (personal communication with citizen of Richmond, UT). However, less complex and less expensive treatment processes, such as wetlands, have also been recommended and shown to remove pharmaceuticals, such as fluorinated pollutants and halogenated phenols (Reinhold et al. 2010).

Assuming pharmaceutical removal results from duckweed microcosms in this study reflect actual removal in the wastewater lagoon, then the duckweed would be capable of achieving comparable removal with MBR and PAC treatment plants (Snyder et al. 2007) for all the pharmaceuticals in this study except carbamazepine (Table 20 and Table 21). Only reverse-osmosis consistently out-performed duckweed; however it was preceded by conventional water treatment and membrane technology and acted as a polishing system receiving lower concentrations of pharmaceuticals than the other tests. Advanced oxidation techniques, such as ozone, added to conventional treatment may be superior to carbon (*i.e.*, activated carbon and duckweed), membrane-based treatments, and even duckweed due to its ability to transform parent compounds (Kummerer 2009; Ten Eyck 2004) whereas the others rely upon size-exclusion and sorption for the majority of removal they achieve.

Reinhold et al.'s (2010) study concluded that duckweed does not uptake every class of pharmaceuticals. Multiple regression stepwise analysis in R was conducted to determine an empirical formula to predict pharmaceutical removal of the five compounds in this study (see Appendix D.4 Table 33 and Figure 67) verified against Reinhold et al.'s (2010) eight compounds. The empirical model to predict total pharmaceutical removal in active duckweed for the pharmaceuticals was constructed with stepwise regression step(lm()) function in R, *e.g.*, Total Removal (%) = 13.44 – 1.01E-5 x Concentration (ng/L) -1.67 x Molecular weight (g/mol) + 2.33 x pKa +149 * pKow – 267 x $charge_{pH=8}$ + 0.054 x mg-dry. This model predicted fluoxetine removal within a factor of two of Reinhold et al.'s results; however, the model was unable to make acceptable predictions for the other seven compounds. Reinhold et al.'s experiments had starting concentrations that were 2 to 3 times greater than the highest concentration used in this study, and up to four orders of magnitude greater than environmentally relevant concentrations which may have caused the poor predictions.

9.4 Fate of Duckweed, Phosphorus, and Five Pharmaceuticals in the Wellsville Lagoons

The removal values and duckweed growth predictions can be combined to predict the mass of phosphorus and pharmaceuticals associated with the duckweed that can be released into the environment if the duckweed were to be land applied to alkaline soil in Wellsville City. This study estimated that weekly harvests between June 17th and September 15th on the Wellsville Municipal Sewage Lagoons would produce 350 Mg-dry duckweed/23 ha (56 acres) and is equivalent to 8620 m^3-fresh duckweed which would cover a hectare with 0.86 m-fresh duckweed (2.13 m-fresh/acre). Assuming a flowrate of

2483 m^3/day (0.656 MGD) with a loading of 5 mg-TP/L; 31.8 μg-ACT/L; 691 ng-SLF/L; 100 ng-FLX/L; 33 ng-CRB/L; and 53 ng-PRG/L (see Table 4) then based on the results from this study (see Table 16 and Table 17) 350 Mg-dry duckweed would contain 3500 kg-P, 28.6 kg-ACT, 0.29 kg-SLF, 0.89 kg-FLX, 0 kg-CRB, and 0.05 kg-PRG.

The potential fate of the compounds associated with duckweed can be predicted, but further desorption studies and degradability studies are necessary to confirm the predictions. According to Kesaano (2011) after duckweed decomposes it may release up to 50% of its stored phosphorus which in this scenario would account for 1750 kg-P. Wellsville City would need to apply the duckweed to 5 ha (12.2 acres) to match the phosphorus loading due to land application of biosolids according to the nearby city of Hyrum that land applies approximately 346 kg-P/ha/yr (140 kg-P/acre/yr). Wellsville City would need to apply 0.17 m-fresh duckweed/ha (7 in/acre) if only 5 ha (12.2 acres) were used for land application.

With respect to pharmaceuticals, this study could not determine whether Acetaminophen or any of the other compounds were metabolized. Therefore, a conservative assumption is that all the compounds associated with the plants will be available to the soil or groundwater after the land applied duckweed solids decompose. Acetaminophen will likely be biodegraded due to its low pKow value and close to neutral charge. According to solid extractions illustrated in Figure 31, Fluoxetine has the greatest tendency to be released from the duckweed solids but then will likely sorb to negatively charged soil. Based on physicochemical properties, Sulfamethoxazole will most likely become mobile due to its negative charge and higher pKow. Progesterone will likely remain sorbed to duckweed or to soil as the duckweed decays. Carbamazepine

will most likely be discharged through the effluent or be removed by a non-duckweed biological process in the complex ecology of the wastewater lagoon.

Table 20. Pharmaceutical removal by duckweed microcosms and pilot/full scale membranes and activated carbon treatments

Treatment	Ultrafiltration[a]		Microfiltration[a]		MBR pilot[a]		MBR Full Scale #1[a]		MBR Full Scale #2[a]		RO[a]	
Pharmaceutical	ng/L	Removal	ng/L	Removal	ng/L	Removal	ng/L	Removal	ng/L	Removal	ng/L	Removal
Acetaminophen	18	5%	16	38%	172000	99.9%	4095	99.9%	400-200	97-98%	11.4	>90%
Sulfamethoxazole	66	5%	340	0%	1110	57%	103	0%	1490-883	0-24%	15.5	>90%
Fluoxetine	45	68%	12	0%	<100	77%	4.4	0%	17-15	0-40%	6.9	>90%
Carbamazepine	191	16%	174	0%	189	96%	138	0%	367-243	0-18%	181	99%
Progesterone	64	98%	no data		no data		22	0%	6.4	84%	1	>90%

Treatment	5 mg/L PAC[a]		5-35 mg/L PAC[a]		Duckweed-LR ≈200 mg-OC[b,c]		Duckweed-MR ≈295 mg-OC[b,c]		Duckweed-MHR ≈270 mg-OC[b,c]		Duckweed-LR ≈250 mg-OC[b,c]	
Pharmaceutical	ng/L	Removal	ng/L	Removal	ng/L	Removal	ng/L	Removal	ng/L	Removal	ng/L	Removal
Acetaminophen	100	55%	100-200	70-85%	2500	99%	50,000	93%	6.5E5	70%	1E6	56%
Sulfamethoxazole	100	25%	100-200	35-55%	1250	86%	50,000	54%	6.5E5	40%	1E6	45%
Fluoxetine	100	33%	100-200	90%	1250	90%	50,000	92%	6.5E5	93%	1E6	82%
Carbamazepine	100	35%	100-200	70-90%	1250	0%	50,000	0%	6.5E5	38%	1E6	11%
Progesterone	100	0%	100-200	85-90%	1250	98%	50,000	98%	6.5E5	98%	1E6	82%

[a] non-duckweed treatment data from Snyder *et al.* (2007) "Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals" *Desalination,* vol 202, pp. 156-181 [b] results from this study [c] OC stands for "organic carbon" and is approx. 50% dry wt.

Table 21. Comparing pharmaceutical removal by duckweed microcosms to pilot/full scale membranes and activated carbon

Treatment	ACT	SLF	FLX	CRB	PRG
Duckweed[b]	**56-99%**	**45-86%**	**82-93%**	**0-38% (poor)**	**82-98**
Ultrafiltration[a]	poor	poor	<	better	comparable
Microfiltration[a]	poor	poor	poor	comparable	no data
MBR[a]	comparable	< varies	comparable	0-96% varies	0-84% varies
RO[a]	comparable	better	better	better	comparable
PAC[a]	<	comparable	comparable	better	comparable

LITERATURE CITED

Alaerts, G.J., Mahbubar, M.R., and Kelderman, P. (1996). "Performance analysis of a full-scale duckweed-covered sewage lagoon." *Water Research*, 30(1), 843-852.

Al-Nozaily, F.A. (2001). *Performance and process analysis of duckweed-covered sewage lagoons for high strength sewage: the case of Sana'a, Yemen, Balkema*, IHE Delft, Rotterdam, the Netherlands.

Al-Nozaily, F.A., Alaerts, G.J., and Veenstra, S. (2000). "Performance of duckweed-covered sewage lagoons—II. Nitrogen and phosphorus balance and plant productivity." *Water Research*, 34(10), 2734-2741.

Amthor, J.S. (2010). "From sunlight to phytomass: on the potential efficiency of converting solar radiation to phyto-energy." *New Phytologist*, 188(4), 939-959.

Baker, D.R. (2011). "Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography–mass spectrometry." *Journal of Chromatography A 1218:44*,(1), 8036-8059.

Barnes, K.K., Kolpin, D.W., Furlong, E.T., Zaugg, S.D., Meyer, M.T., and Barber, L.B. (2008). "A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States - I) Groundwater." *Science of the Total Environment*, 402(1), 192-200.

Behera, S.K., Kim, H.W., Oh, J.E., and Park, H.S. (2011). "Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea." *Science of the Total Environment*, 409(20), 4351-5360.

Böttcher, T. and Schroll, R. (2007). "The fate of isoproturon in a freshwater microcosm with *Lemna minor* as a model organism." *Chemosphere*, 66(1), 684-689.

Boutonnet, J.C., Bingham, P., Calamari, D., de Rooij, C., Franklin, J., Kawano, T., Libre, J.M., McCulloch, A., Malinverno, G., and Odom, J.M. (1999). "Environmental risk assessment of trifluoroacetic acid." *Human and Ecological Risk Assessment*, 5(1), 59-124.

Brain, R.A., Johnson, D.J., Richards, S.M., Hanson, M.L., Sanderson, H., Lam, M.W., Young, C., Mabury, S.A., Sibley, P.K., and Solomon, K.R. (2004a). "Microcosm evaluation of the effects of an eight pharmaceutical mixture to

the aquatic macrophytes *Lemna gibba* and *Myriophyllum sibiricum*." *Aquatic Toxicology*, 70(1), 23-40.

Brain, R.A., Johnson, D.J., Richards, S.M., Sanderson, H., Sibley, P.K., and Solomon, K.R. (2004b). "Effects of 25 pharmaceutical compounds to *Lemna gibba* using a seven-day static-renewal test." *Environmental Toxicology and Chemistry*, 23(1), 371-382.

Briggs, G.G., Bromilow, R.H., and Evans, A.A. (1982). "Relationships between lipophilicity and root uptake and translocation of non-ionized chemicals by barley." *Pesticide Science*, 13(5), 495-504.

Bugbee, B. (2003). "Research: hydroponics and plant nutrition - principles of nutrient management in recirculating hydroponic culture." *Crop Physiology Laboratory*, <http://www.usu.edu/cpl/research_hydroponics3.htm> (March 29, 2012).

Bugbee, B. (2004). "Nutrient management in recirculating hydroponic culture." *Acta Horticulturae*, 648, 199-112.

Chaiprapat, S., Cheng, J.J., Classen, J.J., and Liehr, S.K. (2005). "Role of internal nutrient storage in duckweed growth for swine wastewater treatment." *Transactions of the ASAE*, 48(1), 2247-2258.

Chang, H., Wan, Y., Wu, S., Fan, Z., and Hu, J. (2011). "Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens." *Water Research*, 45(2), 732-740

Chard, B.K. (2005). "Greenhouse study to determine the uptake of trichloroethylene by apple and peach trees and their fruit." *All Graduate Theses and Dissertations*, Utah State University Merrill-Cazier Library, Logan, Utah.

Childs, S.L., Chyall, L.J., Dunlap, J.T., Smolenskaya, V.N., Stahly, B.C., and Stahly, G.P. (2004). "Crystal engineering appraoch to forming cocrystals of amine hydrochlorides with organic acids. Molecular complexes of Fluoxetine Hydrochlorice with Benzoic, Succinic, and Fumaric Acids." *Journal of the American Chemical Society*, 126(41), 13335-13342.

Clark, P.B. and Hillman, P.F. (1996). "Enhancement of anaerobic digestion using duckweed (*Lemna minor*) enriched with iron." *Water and Environment Journal*, 10(1), 92-95.

Clatworthy, J.N. and Harper, J.L. (1962). "Comparative biology of closely related species living in same area: inter- and intraspecific interference within cultures of *Lemna spp.* and *Salvinia natans*." *Journal of Experimental Botany*, 13(1), 307.

Crane, M., Watts, C., and Boucard, T. (2006). "Chronic aquatic environmental risks from exposure to human pharmaceuticals." *Science of the Total Environment*, 367(1), 23-41.

Cross, J.W. (2005). "Duckweed herbivores and pathoges: animals and microorganisms that feed on duckweeds." *The Charms of Duckweed*, <http://www.mobot.org/jwcross/duckweed/duckweed-pests.html> (March 29, 2012).

Culley, D.D., Rejmánková, E., Květ, J., and Frye, J.B. (1981). "Production, chemical quality and use of duckweeds (*Lemnaceas*) in aquaculture, wastewater management, and animal feeds." *Journal of the World Mariculture Society*, 12(1), 27-49.

Cunningham, V.L., Perino, C., D'Aco, V.J., Hartmann, A., and Bechter, R. (2010). "Human health risk assessment of carbamazepine in surface waters of North America and Europe." *Regulatory Toxicology and Pharmacology*, 56(3), 343-351.

Day, J.A. and Saunders, F.M. (2004). "Glycosidation of chlorophenols by *Lemna minor*." *Environmental Toxicology and Chemistry*, 23(1), 613-620.

Delgado, P.L. and Zarkowski, P. (2004). "Treatment of mood disorders." *Textbook of Biological Psychiatry*, Panksepp J., ed., Wiley-Liss, Inc., Hoboken, New Jersey, 231.

Dettenmaier, E. (2008). "Measuring and modeling of plant root uptake of organic chemicals." *All Graduate Theses and Dissertations*, Utah State University Merrill-Cazier Library, Logan, Utah.

Dixon, J.L. (2011). "Duckweed for bio-fuels." *Dixon Laboratory: Nutritional Sciences Department / Rutgers Center for Lipid Research*, <http://www.dixonlab.rutgers.edu/pages/duckweed_introduction> (May 2, 2012).

Driever, S.M., Nes, E.H., and Roijackers, R.M. (2005). "Growth limitation of *Lemna minor* due to high plant density." *Aquatic Botany*, 81(1), 245-251.

Eaton, A.D., Clesceri, L.S., Rice, E.W., and Greenberg, A.E. (2005). *Standard methods for the examination of water and wastewater*, American Public Health Association, Washington, DC.

Eberius, M. (2001). "Oberservation parameters in the duckweed growth inhibition test." *LemnaTec*, <http://www.lemnatec.com/Literatur/LT001.pdf> (May 2, 2012).

Edwards, P., Hassan, M.S., Chao, C.H., and Pacharaprakiti, C. (1992). "Cultivation of duckweeds in septage-loaded earthen ponds." *Bioresource Technology*, 40(1), 109-117.

Farrell, J.B. (2011). "Procedure for testing pharmaceutical removal by duckweed plants via HPLC-MS analysis." *Duckweed Research*, <http://www.duckweedresearch.blogspot.com/2011/11/pharmaceutical-removal-by-duckweed.html> (March 15, 2012).

Fent, K., Weston, A.A., and Caminada, D. (2006). "Ecotoxicology of human pharmaceuticals." *Aquatic Toxicology*, 76(1), 122-159.

Fernandes, N.S., Filho, M.A. d. S. C., Antonio, M.R., and Ionashiro, M. (1999). "Thermal decomposition of some chemotherapic substances." *Journal of the Brazilian Chemical Society*, 10(6).

Fini, A., Ospitali, F., Zoppetti, G., and Puppini, N. (2008). "ATR/Raman and fractal characterization of HPBCD/Progesterone complex solid particles." *Pharmaceutical Research*, 70(1), 409-420.

Focazio, M.J., Kolpin, D.W., Barnes, K.K., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Barber, L.B., and Thurman, M.E. (2008). "A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II) Untreated drinking water sources." *Science of the Total Environment*, 402(1), 201-216.

Franco, A. and Trapp, S. (2008). "Estimation of the soil-water partition coefficient normalized to organic carbon for ionizable organic chemicals." *Environmental Toxiciology and Chemistry*, 27(10), 1995-2004.

Frédéric, M., Samir, L., Louise, M., and Abdelkrim, A. (2006). "Comprehensive modeling of mat density effect on duckweed (*Lemna minor*) growth under controlled eutrophication." *Water Research*, 40(1), 2901-2910.

Fukahori, S., Fujiwara, T., Ito, R., and Funamizu, N. (2011). "pH-dependent adsorption of sulfa drugs on high silica zeolite: modeling and kinetic study." *Desalination*, 275(1), 237-242.

Gracia-Lor, E., Sancho, J.V., Serrano, R., and Hernández, F. (2012)."Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia." *Chemosphere*, (87)5, 453-462.

Griffiths, E.W. (2010). "Removal and utilization of wastewater nutrients for algae biomass and biofuels." *All Graduate Theses and Dissertations*, Utah State University Merrill-Cazier Library, Logan, Utah.

Hijosa-Valsero, M., Matamoros, V., Cardona-Sidrach, R., Martín-Villacorta, J., Bécares, E., and Bayona, J.M. (2010). "Comprehensive assessment of the design configuration of constructed wetlands for the removal of pharmaceuticals and personal care products from urban wastewaters." *Water Research*, 44(12), 3669-3678.

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., Jansen, J.C., and Andersen, H.R. (2011). "Determination of sorption of seventy-five pharmaceuticals in sewage sludge." *Water Research*, 45(1), 4470-4482.

Huber, C., Bartha, B., Harpaintner, R., and Schröder, P. (2009). "Metabolism of acetaminophen (paracetamol) in plants - two independent pathways result in the formation of a glutathione and a glucose conjugate." *Environmental Science and Pollution Research*, 16(1), 206-213.

JUB Engineering I. (2008). "Wellsville City wastewater treatment facilities plan: draft for city review." Logan, Utah.

Kadlec, R.H. and Wallace, S.D. (2009). *Treatment wetlands*, CRC Press, Boca Raton, Florida.

Kesaano, M. (2011). "Sustainable management of duckweed biomass grown for nutrient control in municipal wastewaters." *All Graduate Theses and Dissertations*, Utah State University Merrill-Cazier Library, Logan, Utah.

Kim, J., Drew, M.C., and Corapcioglu, M.Y. (2004). "Uptake and phytotoxicity of TNT in onion plant." *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering*, 39(1), 803-819.

Klassen, S.P. and Bugbee, B. (2003). "Real-time imaging of ground cover: relationships with radiation capture, canopy photosynthesis, and daily growth rate." American Society of Agronomy special publication No. 66, 3-14.

Kobayashi, Y., Ito, S., Itai, S., and Yamamoto, K. (2000). "Physicochemical properties and bioavailability of carbamazepine polymorphs and dihydrate." *International Journal of Pharmaceutics*, 193(2), 137-146.

Kogan, A., Popov, I., Uvarov, V., Cohen, S., Aserin, A., and Garti, N. (2007). "Microemulsion-facilitated crystallization of carbamazepine." *Journal of Dispersion Science and Technology*, 28(1), 1008-1019.

Kummerer, K. (2009). "Antibiotics in the aquatic environment - A review - Part I." *Chemosphere*, 75(1), 417-434.

Kummerova, M., Zezulka, S., Krulova, J., and Triska, J. (2007). "Photoinduced toxicity of fluoranthene on primary processes of photosynthesis in lichens." *Lichenologist*, 39(1), 91-100.

Lam, M.W., Young, C.J., Brain, R.A., Johnson, D.J., Hanson, M.A., Wilson, C.J., Richards, S.M., Solomon, K.R., and Mabury, S.A. (2004). "Aquatic persistence of eight pharmaceuticals in a microcosm study." *Environmental Toxicology and Chemistry*, 23(1), 1431-1440.

Landesman, L., Fedler, C., and Duan, R. (2011). "Plant nutrient phytoremediation using duckweed." *Eutrophication: causes, consequences and control*, Ansari, A.A., Lanza, G.R., Gill, S.S., and Rast W., eds., Springer, New York, 341-354.

Landolt, E. (1986). *Biosystematic investigations in the family of duckweeds (Lemnaceae). Vol. 2. The family of Lemnaceae - A monographic study. Part 1 of the monograph: morphology; karyology; ecology; geographic distribution; systematic position; nomenclature; descriptions.*, Veröffentlichungen des Geobotanischen Institutes der ETH, Zürich, Switzerland.

Landolt, E. and Kandeler, R. (1987). *Biosystematic investigations in the family of duckweeds (Lemnaceae). Vol. 4. The family of Lemnaceae - a monographic study. Part 2 of the monography: phytochemistry; physiology; application; bibliography*, Veröffentlichungen des Geobotanischen Institutes der ETH, Zürich, Switzerland.

Lasfar, S., Monette, F., Millette, L., and Azzouz, A. (2007). "Intrinsic growth rate: a new approach to evaluate the effects of temperature, photoperiod and phosphorus-nitrogen concentrations on duckweed growth under controlled eutrophication." *Water Research*, 41(1), 2333-2340.

Leclercq, M., Mathieu, O., Gomez, E., Casellas, C., Fenet, H., and Hillaire-Buys, D. (2008). "Presence and fate of Carbamazepine, Oxcarbazepine, and seven of their metabolites at wastewater treatment plants." *Archives of Environmental Contamination and Toxicology*, 56(3), 408-415.

Lemna Corporation. (1996). "Operation and maintenance manual for Boulder City, Nevada." Lemna Corporation, St. Paul, Minnesota.

Mackay, D. (1991). *Multimedia environmental models: the fugacity approach*, Lewis Publishers, Inc., Chelsea, Michigan.

Mkandawire, M. and Dudel, E.G. (2005). "Accumulation of arsenic in *Lemna gibba L.* (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany." *Science of The Total Environment*, 336(1), 81-89.

Mkandawire, M. and Dudel, E.G. (2007). "Are *Lemna spp.* effective phytoremediation agents?" *Bioremediation, Biodiversity and Bioavailability*, 1(1), 56-71.

Mkandawire, M., Taubert, B., and Dudel, E.G. (2004). "Capacity of *Lemna gibba L.* (Duckweed) for uranium and arsenic phytoremediation in mine tailing waters." *International Journal of Phytoremediation*, 6(1), 347-362.

Munawar, M. and Burns, N.M. (1970). "Relationships of phytoplankton biomass with soluble nutrients, primary production, and chlorophyll-a in Lake Erie." *Journal of the Fisheries Research Board of Canada*, 33(3), 601-611.

Naghavi, B. and Malone, R.F. (1986). "Algae removal by fine-sand-silt filtration." *Water Research*, 20(1), 377-383.

Nghiem, L.D., Tadkaew, N., and Sivakumar, M. (2009). "Removal of trace organic contaminants by submerged membrane bioreactors." *Desalination*, 236, 127-134.

Nieman, K.C. (2003). "How to use a Level I Fugacity Model to estimate contaminant partiitoning in the subsurface." <http://www.infoclearinghouse.com/files/enviro/fugacity.pdf.html> (March 15, 2012).

Öbek, E. and Hasar, H. (2002). "Role of duckweed (*Lemna minor*) harvesting in biological phosphate removal from secondary treatment effluents." *Fresenius Environmental Bulletin*, 11(1), 27-29.

Odjegba, V. and Fasidi, I. (2004). "Accumulation of Trace Elements by *Pistia stratiotes*: implications for phytoremediation." *Ecotoxicology*, 13(1), 637-646.

Olguín, E.J., Sánchez-Galván, G., Pérez-Pérez, T., and Pérez-Orozco, A. (2005). "Surface adsorption, intracellular accumulation and compartmentalization of Pb(II) in batch-operated lagoons with *Salvinia minima* as affected by environmental conditions, EDTA and nutrients." *Journal of Industrial Microbiology & Biotechnology*, 32(1), 577-586.

Orchard, B.J., Doucette, W.J., Chard, J.K., and Bugbee, B. (2000). "A novel laboratory system for determining fate of volatile organic compounds in planted systems." *Environmental Toxicology and Chemistry*, 19(1), 888-894.

Ornes, W.H., Sajwan, K.S., Dosskey, M.G., and Adriano, D.C. (1991). "Bioaccumulation of selenium by floating aquatic plants." *Water Air and Soil Pollution*, 57-8(1), 53-57.

Qi, S., Avalle, P., Saklatvala, R., and Craig, Q. M. (2008). "An investigation into the effects of thermal history on the crystallisation behaviour of amorphous paracetamol." *European Journal of Pharmaceutics and Biopharmaceutics*, 69(1), 364-371.

Radjenović, J., Petrović, M., and Barceló, D. (2009). "Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment." *Water Research*, 43(3), 831-841.

Reddy, K.R. and Debusk, W.F. (1985). "Growth-characteristics of aquatic macrophytes cultured in nutrient-enriched water. 2. Azolla, Duckweed, and Salvinia." *Economic Botany*, 39(1), 200-208.

Reinhold, D.M. and Saunders, E.M. (2006). "Phytoremediation of fluorinated agrochemicals by duckweed." *Transactions of the Asabe*, 49(1), 2077-2083.

Reinhold, D., Vishwanathan, S., Park, J.J., Oh, D., and Saunders, F.M. (2010). "Assessment of plant-driven removal of emerging organic pollutants by duckweed." *Chemosphere*, 80(1), 687-692.

Rejmánková, E., Blackwell, M., and Culley, D.D. (1986). "Dynamics of fungal infection in duckweeds (*Lemnaceae*)." Veröffentlichungen des Geobotanischen Instituts der ETH, 87(1), 178-189.

Roth, O. (2012). "Evaluating the effectiveness of three wastewater treatment facilities in Utah in removing pharmaceuticals and personal care products." *All Graduate Theses and Dissertations*, Utah State University Merrill-Cazier Library, Logan, Utah.

Roijackers, R., Szabo, S., and Scheffer, M. (2004). "Experimental analysis of the competition between algae and duckweed." *Archiv Fur Hydrobiologie*, 160(1), 401-412.

Ruhoy, I.S. and Daughton, C.G. (2008). "Beyond the medicine cabinet: an analysis of where and why medications accumulate." *Environment International*, 34(1), 1157-1169.

Sandermann, H. (1994). "Higher-plant metabolism of xenobiotics - the green liver concept." *Pharmacogenetics*, 4(1), 225-241.

Santos, J.L., Aparicio, I., Callejón, M., and Alonso, E. (2009). "Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain)." *Journal of Hazardous Materials*, 164(2-3), 1509-1516.

Saygieğer, S. and Doğan, M. (2004). "Lead and cadmium accumulation and toxicity in the presence of EDTA in *Lemna minor* and *Ceratophyllum demersum*." *Bulletin of Environmental Contamination and Toxicology*, 73(1), 182-189.

Skillicorn, P. (2008). "Agriquatics Mirzapur system." *YouTube*, <http://www.youtube.com/watch?v=M93HZDoqhsE&feature=player_embedded> (May 1, 2012).

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Wert, E.C., and Yoon, Y. (2007). "Role of membranes and activated carbon in the removal." *Desalination*, 202(1), 156-181.

Suren, A.M. (1989). "Histological-changes in macrophyte tissue during decompostition." *Aquatic Botany*, 33(1), 27-40.

Szabo, S., Braun, M., Balazsy, S., and Reisinger, O. (1998). "Influences of nine algal species isolated from duckweed-covered sewage miniponds on *Lemna gibbia L*." *Aquatic Botany*, 60(1), 189-195.

Szabo, S., Roijackers, R., and Scheffer, M. (2003). "A simple method for analysing the effects of algae on the growth of *Lemna* and preventing algal growth in duckweed bioassays." *Archiv für Hydrobiologie*, 157(1), 567-575.

Szabo, S., Roijackers, R., Scheffer, M., and Borics, G. (2005). "The strength of limiting factors for duckweed during algal competition." *Archiv für Hydrobiologie*, 164(1), 127-140.

Taiz, L. and Zeiger, E. (1998). *Plant physiology*, Sinauer Associates, Sunderland, Massachusetts.

Ten Eyck, C.H. (2004). "Evaluation of pharmaceutical fate in pilot and bench scale drinking water treatment plants." *All Graduate Theses and Dissertations*, Utah State University Merrill-Cazier Library, Logan, Utah.

Trent University. (2011). "Model index." *The Canadian Centre for Environmental Modelling and Chemistry*, <http://www.trentu.ca/academic/aminss/envmodel/models/models.html> (March 15, 2012).

Tront, J.M. and Saunders, F.M. (2006). "Role of plant activity and contaminant speciation in aquatic plant assimilation of 2,4,5-trichlorophenol." *Chemosphere*, 64(1), 400-407.

U.S. Envrionmental Protection Agency O.o.W. (2008). "Section 319 nonpoint source program success story Utah." *Riparian area grazing management practices reduce phosphorus loads and restore water quality*, Allred M. and Kleeman G., eds., 2.

Utah Division of Water Quality D.o.E.Q. (2000). "Little Bear River Watershed TMDL." Salt Lake City, Utah.

Utah Division of Water Quality D.o.E.Q. (2006). *Utah 2006 intergrated report volume I - 305(b) assessment*, Utah Department of Environmental Quality (UDEQ), Salt Lake City, Utah.

Vines, H.M. and Wedding, R.T. (1960). "Some effects of ammonia on plant metabolism and a possible mechanism for ammonia toxicity." *Plant Physiology*, 35, 820-825.

Wagner, D.T. (1969). "Monocentric holocarpic fungus in *Lemna minor L*." *Nova Hedwigia*, 18(1), 203-208.

Wang, Q., Cui, Y., and Dong, Y. (2002). "Phytoremediation of polluted waters potentials and prospects of wetland plants." *Acta Biotechnologica*, 22(1), 199-208.

Wang, H., Shan, X.Q., Wen, B., Zhang, S., and Wang, Z.j. (2004). "Responses of antioxidative enzymes to accumulation of copper in a copper hyperaccumulator of *Commoelina communis*." *Archives of Environmental Contamination and Toxicology*, 47(1), 185-192.

Watkins, C.H. and Hammerschlag, R.S. (1984). "The toxicity of chlorine to a common vascular aqautic plant." *Water Research*, 18(1), 1037-1043.

Willett, D. (2005). "Duckweed-based wastewater treatment systems: design aspects and integrated reuse options for Queensland conditions." The State of Queensland Department of Primary Industries and Fisheries, Brisbane, Australia.

Yang, S.F., Lin, C.F., Lin, A.Y.C., and Hong, P.K.A. (2011). "Sorption and biodegradation of sulfonamide antibiotics by activated sludge: experimental assessment using batch data botained under aerobic conditions." *Water Research*, 45(11), 3389-3397.

Zhang, D., Pan, B., Zhang, H., Ning, P., and Xing, B. (2010). "Contribution of different sulfamethoxazole species to their overall adsorption on functionalized carbon nanotubes." *Environental Science and Technology*, 44, 3806-3811.

Zhang, D., Pan, B., Wu M., Wang, B., Zhang H., Peng, H., Wu, D., and Ning P. (2011). "Adsorption of sulfamethoxazole on functionalized carbon nanotubes as affected by cations and anions." *Environmental Pollution*, 159(10), 2616-2621.

Zhang, D., Pan, B., Wu M., Zhang H., Peng, H., Ning P., and Xing, B. (2012). "Cosorption of organic chemicals with different properties: their shared and different sorption sites." *Environmental Pollution*, 160(1), 178-184.

Zimmo, O.R., Al-Saed, R.M., Van der Steen, N.P., and Gijzen, H.J. (2002). "Process performance assessment of algae-based and duckweed-based wastewater treatment systems." *Water Science and Technology*, 45(1), 91-101.

Zirschky, J., Reed, S.C. (1988). "The use of duckweed for waste-water treatment." *Journal Water Pollution Control Federation*, 60(1), 1253-1258.

Zuberer, D.A. (1984). "Microbial colonization of some duckweeds (*Lemnacea*): examination by scanning and transmission electron and light microscopy." *Aquatic Botany*, 18(3), 275-285.

APPENDICES

Appendix A Preliminary Studies

A.1 Preliminary: Pharmaceutical Concentrations in Wellsville Wastewater

Table 22. Preliminary results of pharmaceutical concentrations in wastewater effluent discharge locations throughout Utah measured at the Utah Water Research Laboratory

Sample Volume, mL	Sample Name	ACT[a]	SLF[a]	CRB[a]	TDEP[a]	DEET[a]	FLX[a]	β-Estradiol[a]	Estrone[a]	PRG[a]	Units
1001	Brigham City 9/13	14.8	137.4	263	355	17	6.2	<1000	<100	9.4	ng/L
952	Price 9/15	17.9	64.8	261	435	73	3.9	<1000	<100	4.2	ng/L
886	Wellsville 9/17	5.3	18	32	111	2810	1.7	<1000	<100	3.6	ng/L
949	Tremonton 9/13	2009.2	161.9	110	104	4075	3.4	<1000	<100	2.9	ng/L
786	Moroni 9/17	598.1	34	20	36	931	0.8	<1000	<100	7.2	ng/L
999	Oakley 9/11	5.3	132.4	499	53	130	6.4	<1000	<100	2.9	ng/L
1001	Fairview 9/15	3.1	174.6	181	253	3.4	2.4	<1000	<100	3.8	ng/L

ACT = Acetaminophen SLF = Sulfamethoxazole CRB = Carbamazepine FLX = Fluoxetine PRG = Progesterone

Final Concentration (ng/L) of 10/07/2010 Analysis of R2 Municipal Re-extract under acidic conditions with centrifuged clean-up

A.2 Preliminary: Fungi Inhibition of Duckweed Growth in the Laboratory

The succeeding figures show the effect fungi can have on duckweed. The duckweed was transplanted from very healthy outdoor populations; however, once they came indoors the conditions began to favor a certain fungi that within several days destroyed the duckweed crop.

Figure 39 shows the start of the experiment with healthy duckweed from Cache Valley (*Lemna minor* species).Also shown are the Omega pH controller (pH 7.7, later lowered to 6.5), pH probe, temperature probe, 110 L reactors, and HPSLs 4 ft above supplying approximately 175 PPF (16/8 hrs on/off) at a constant temperature of 25°C.

Figure 40 to Figure 43 show the stages of chlorosis/necrosis setting-in causing yellowing and bleaching of some duckweed fronds until the majority of fronds were destroyed. Similar blight patches were seen in actual duckweed covers outdoors on the wastewater lagoons in Wellsville; however, it appears there is enough duckweed to overcome the blight at the field scale. An interesting article titled "Dynamics of fungal infection in duckweeds (*Lemnaceae*)" (Rejmánková et al. 1986) talks about this fungi phenomenon and is referred to on this informative website: (www.mobot.org/jwcross/duckweed.htm).

Figure 43 shows a mat of chlorotic/necrotic (*i.e.,* dead) duckweed which is submerged just below the surface of the water. Perhaps the submersion actually suffocates the plants. Figure 44 shows the culprit. Microorganisms are thriving all over the tissues of the unhealthy duckweed plants. These observations support an interesting article discussing why invertebrates consume decaying macrophytes rather than living

ones (Suren, 1989). Figure 45 shows the stringy organisms that appear to be fungi which cause the duckweed to mat together leading to its death.

Figure 39. Start of experiment demonstrating inhibition by fungi.

Figure 40. Chlorosis/necrosis setting in.

Figure 41. More blight and bleaching of fronds short time after.

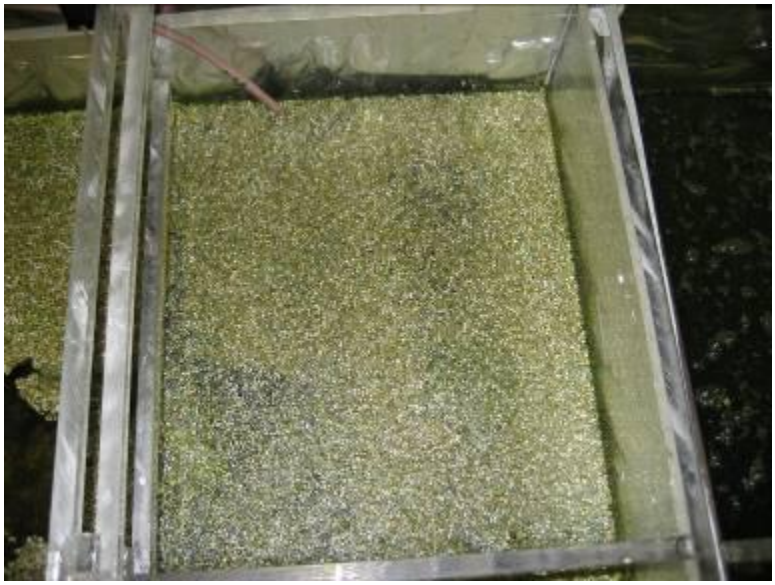

Figure 42. Majority of duckweed is chlorotic within a short period of time.

Figure 43. Mat of duckweed submerged below water surface.

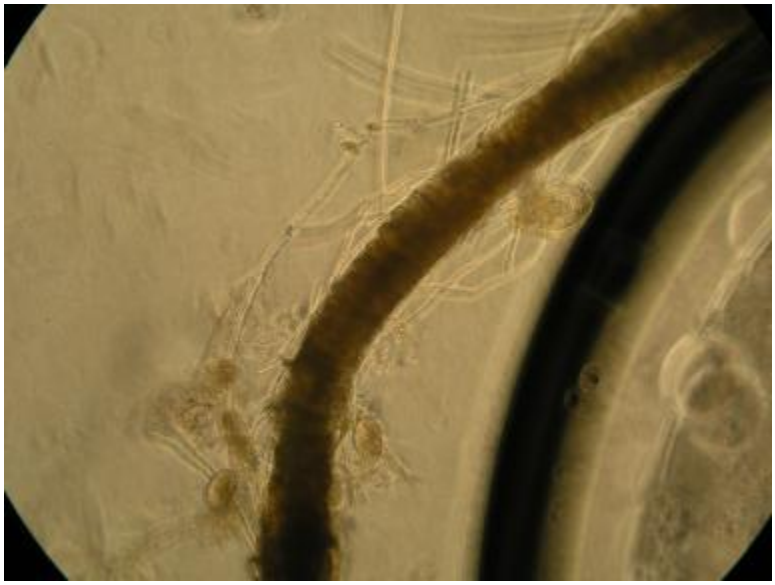

Figure 44. Microorganisms covering duckweed roots.

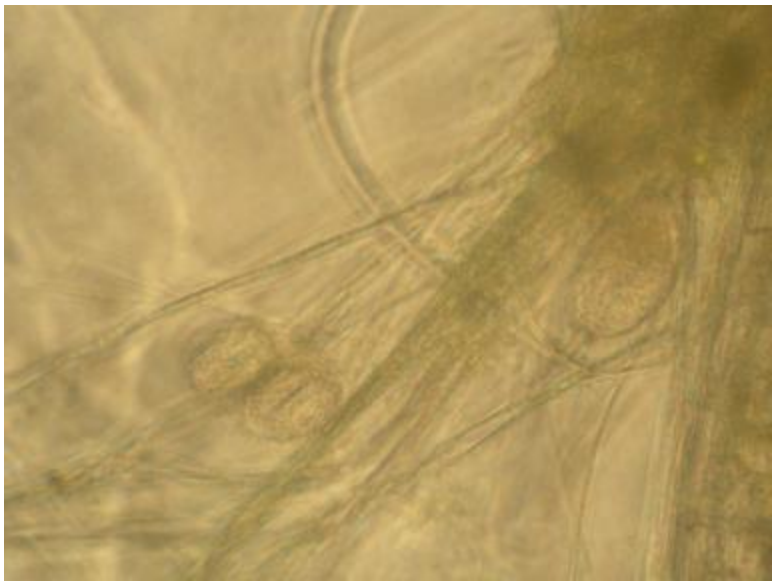

Figure 45. Fungi covering duckweed.

A.3 Preliminary: Aphid Inhibition and *L. turionifera* vs. *W. borealis* Competition

Background: After running the dissolved oxygen probe shaker for several months, the *L. turionifera* duckweed species started accumulating around the probe; unlike *W. borealis* species which out-competed the *L. turionifera* species in un-stirred parts of the reactor (*i.e.*, parts with no probe and no disturbance to the water surface). After the plants became infested with aphids, no aphids appeared on the plants closest to the probes.

Hypothesis: By gently disturbing the water surface every two minutes then: first, *W. borealis* will not out-compete *L. turionifera* species and take over the reactor; and second, aphids were removed from the reactors.

Results: The dissolved oxygen probe(s) were replaced with weighted 250 mL bottles (5 cm O.D. x 13 cm) in order to disturb the entire water surface in two of the three cells (0.167 m^2 each) with no disturbance in the third cell (*i.e.*, the control). Prior to the experiment 90% of the surface area was occupied by *Wolffia* and only 10% by *Lemna.* In less than a week after replacing the dissolved oxygen probes with the bottles, the *L. turionifera* plants re-established themselves and competed with the *W. borealis* species for complete surface coverage (Figure 46).

Prior to the experiment, the quiescent duckweed mat was covered with sedentary aphids. After stirring, the aphids went away. Some aphids migrated to the larger bottles used to disturb the water surface (Figure 47). Aphids remained on the undisturbed control cell and Wolffia dominated (Figure 48).

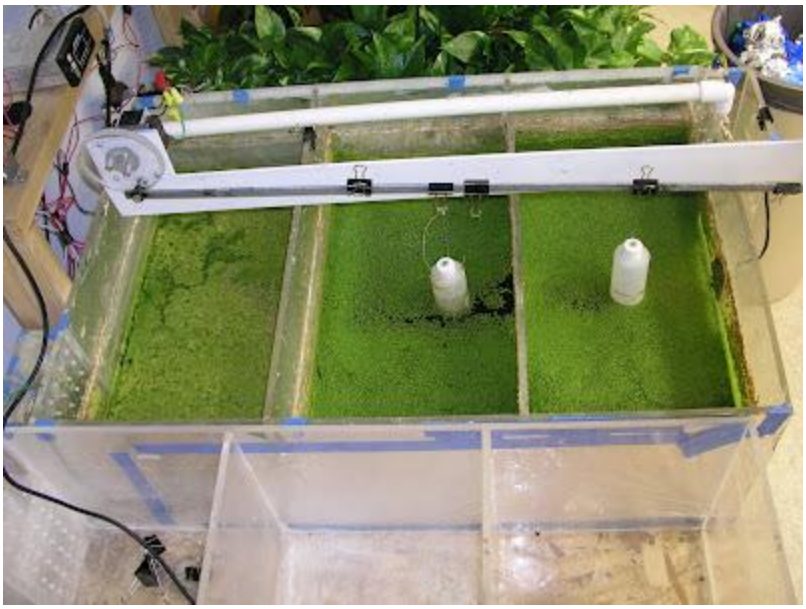

Figure 46. Occasional stirring promotes L. turionifera species and removes aphids.

Figure 47. Occasional stirring caused aphids leave duckweed mat and migrate to stirring probe.

Figure 48. Without stirring, *W. borealis* species out-compete *L. turionifera* and aphids remain on the surface.

Appendix B Analytical Methods

B.1 Method: Phosphorus Measurements in Plant Tissue and Liquids

B.1.1 Plant Tissue Digestion Procedure

Adapted from: Westerman, R.L. "Dry Ashing Procedure," *Soil Testing and Plant Analysis*. Soil Science Society of America, Inc., Madison, WI (1990), page 409.

1. Measure out 120-200mg*(dried/ground) duckweed into 10 mL glass vial(s).
2. Ash plant tissue by heating vial(s) un-capped in 550ºC muffle furnace for at least 30 minutes. Remove and let cool.
3. Prepare *aqua regia* solution with 6:3:1 (DI H_20: conc. HCl: conc. HNO_3).
4. Slowly pipette 10 mL *aqua regia* soln. into each vial with ashed plant tissue**. Mix well and let stand for approx. 15 min.
5. Dilute appropriately.
6. Prepare an acid blank.
7. Measure for %P using one of the following two methods (A or B):

*120-200mg-plant tissue assumes approximately 1%-P and is further diluted 1:50 (for IC analysis) or 1:500 (for colorimetric analysis)

**Tissue samples standardized against grape petiole leaves (0.38%-P) from NAPT guidelines donated from the USU Analytical Labs (USUAL).

B.1.2 Measurement for Phosphorus Content in Liquid Samples and Digestions

Using 890 nm spectrophotometer (adapted from Joan McLean USU CEE Environmental Quality Procedures course: Phosphorus lab handout)

Adapted from Standard Methods (APHA) 4500-P. E Ascorbic Acid Method

1. Prepare Combined reagent and Mix in the following order (stable for 4 hours):

50mL-5 N H_2SO_4 (Dilute 70 mL conc. Sulfuric acid in 500 mL DI water)
5 mL-Potassium antimonyl tartrate soln. (Dissolve 1.3715 g potassium antimonyl tartrate in 400 mL distilled water in a 500 mL volumetric flask and dilute to volume—not $x4H_2O$'s)
15 mL-Ammonium molybdate solution (Dissolve 20g ammonium molybdate in 500 mL distilled water. Store in a glass-stoppered bottle)
30 mL-Ascorbic acid, 0.1 M (Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week @ 4ºC—throw out when it turns yellow)

2. Dilute sample(s) accordingly; method detection range is from approx. 25µg/L to 500µg/L

1:10 = approximately 1-2.5 mg(PO_4-P)/L range; use 1 mL sample/10 mL
1:25 = approximately 1.25-6.2 mg(PO_4-P)/L range; use 400 uL sample/10 mL
1:50 = approximately 2.5-12.5 mg/L range; use 200 uL sample/10 mL
For solids (100-200 mg) use 1:500 for approx. 0.5-2%-P; dilute once with 1mL sample/10 mL and then dilute again at 200 uL/10 mL

3. Prepare at least 3 standards (*i.e.*, 25 μg/L, 250 μg/L, and 500 μg/L)
4. Add 1.6 mL combined reagent to sample and let stand for 12 minutes.
5. Measure @ 890 nm

B.2 Standard Operating Procedure: Pharmaceutical Detection

"A Procedure for Testing Pharmaceutical Removal by Duckweed Plants via HPLC-MS Analysis—using a modified version of the EPA 1694 Method: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS"

More detailed instructions with pictures and video available online at (Farrell 2011):

http://duckweedresearch.blogspot.com/2011/11/pharmaceutical-removal-by-duckweed.html

1. Equipment
    a. Pharmaceuticals
        i. Primary Standards
            1. Acetaminophen (abbreviation "ACT") obtained from: Sigma-Aldrich; 4-Acetamidophenol PN# A7302-5G-A; $16 for 5 grams; CAS# 103-90-2
            2. Sulfamethoxazole (abbreviation "SLF") obtained from: Bioworld; PN# 41910016-1 (762346-6); $41.90 for 25 g; CAS#723-46-6
            3. Carbamazepine (abbreviation "CRB") obtained from: Sigma-Aldrich; PN# C4024-1G; $21 for 1 gram; CAS# 298-46-4
            4. Fluoxetine hydrochloride USP (abbreviation "FLX")obtained from: Spectrum Chemical Mfg. Corp.; PN# F1200; $152.90 for 5 grams; CAS# 59333-67-4
            5. Progesterone (abbreviation "PRG")obtained from: Sigma-Aldrich; PN# 850454-5G; $20 for 5 grams; CAS# 57-83-0
            6. Atrazine-D5 (internal standard) obtained from: Fluka/Sigma-Aldrich; PN# 34053; $229 for 10 mg; CAS# 163165-65-1
            7. [NOT USED] Nicotine (abbreviation "NIC") obtained from: Cambridge Isotope Laboratories, Inc.; PN# N-008; $18 for 1 mg/mL in MeOH; CAS#54-11-5
            8. [NOT USED] Cotinine (abbreviation "COT") obtained from: Cambridge Isotope Laboratories, Inc.; PN# C-016; $25 for 1 mg/mL in MeOH; CAS#486-56-6
        ii. Secondary Standards
            1. Makeup individual solutions for each compound in HPLC grade methanol at 500,000 ng/mL, with the exception of the Atrazine-D5 internal standard make up at 50,000 ng/mL in MeOH.

iii. Intermediate Standards
    1. Makeup combined solution with all seven compounds in HPLC grade methanol at 2500 ng/mL and 5000 ng/mL (acetaminophen only).
    2. Makeup internal standard solution with Atrazine-D5 at 50 or 250 ng/mL.
iv. Working Standards
    1. Makeup HPLC/MS calibration standards in 0.1% Formic Acid (liquid HPLC/MS analysis) or in methanol (solid HPLC/MS analysis) at approximately: 10, 25, 50, 100, 250, 500, and 1000 ng/mL.

b. Microcosm treatments
    i. "X" Control reactor. Dark. No plant. Covered.
    ii. "A" Dark control reactor. Dark. No plant. Uncovered.
    iii. "B" Dark plant reactor. Dark. With plant. Uncovered.
    iv. "C" Light plant reactor. Light. With plant. Uncovered.
    v. "D" Light control reactor. Light. No plant. Uncovered.
    vi. "E" Macerated plant reactor. Light. Macerated plant. Uncovered.

2. Prepare samples
    a. Add liquid/chemicals
        i. Rinse sample jars (wide-mouth pint-size Mason jars) with HCl, MeOH, and then tap water.
        ii. Label sample jars (triplicate jars with treatments: A = dark no plant; B = dark with plant; C = light w/ plant; D = light no plant; E = light w/ macerated plant; X=control w/ lids).
        iii. Record tare weight of jars.
        iv. Fill jars with approximately 400 mL tap water (fill jars for macerated duckweed with only 200 mL tap water.
        v. Spike jars with Wellsville Synthetic nutrient solution (1:400 "A" and 1:1000 "B,C,D" stock solutions).
        vi. Spike jars with 500 ng each pharmaceuticals (except 1000 ng-acetaminophen).
        vii. Wait 1 hr to chemicals to equilibrate in solution
        viii. Weigh jars with solution.
        ix. Cover jars with paper shields that are white on outside and black on the inside.
    b. Add whole plants
        i. Acclimatize duckweed in container with 10+ liters of Wellsville Synthetic nutrient solution (1:400 "A" and 1:1000 "B,C,D") for 24 hours.

ii. Spoon approximately 300 g-fresh duckweed and strain excess water with butterfly-netting.
iii. Place squeezed duckweed into tarred aluminum dish, adjust weight to approximately 300 g-fresh, and record fresh weight.
iv. Pour duckweed into appropriate jar with nutrient solution and pharmaceutical compounds.
v. Set aside an aliquot of fresh material prepared the same way to obtain the dry weight (60 °C).

c. Add macerated plants
i. Prepare fresh duckweed according to instruction above for enough jars that will require macerated duckweed (*i.e.,* triplicate jars x 3 time periods x 300 g-fresh/jar = 2700 g-fresh duckweed).
ii. Pour duckweed into a blender with no more than one-third the required tap water (*i.e.,* 9 jars required x 400 mL/jar x 0.33 = 1200 mL).
iii. Macerate fresh duckweed in the blender at low speed (to reduce foaming) for approximately 2 minutes.
iv. Decant liquid into a glass beaker, rinsed blender to recover all the solids.
v. Mix contents of beaker with glass stir-rod to obtain a uniform suspension of solids.
vi. While mixing, pour the proper amount of solution into a graduated cylinder and then to the proper test jar for macerated duckweed (e.g. 2700 g-fresh/1200 mL per 9 jars = 300 g-fresh/133 mL per jar).
vii. Rinse walls of the graduated cylinder with tap water in order to recover all solids and then bring the volume up to approximately 400 mL like the other test jars.

d. Start Experiment at time = 0 hrs
i. Place all light-treatment jars 40” below 1000 W HPSLs (200 $\mu mol/m^2$-sec).
ii. Place all dark-treatment jars underneath a cardboard box close to the light jars in order to maintain at similar temperatures.
iii. Record the testing start time.

3. Collect samples at time = 2 hrs, 24 hrs, 4 days
a. Separate liquid and solids[2]

[2] Note: macerated plant samples are filtered to separate solids using several glass fiber pre-filter pads (e.g. AP40 or Whatman GF/A). Filter pads are tared, collected in 15 mL centrifuge tubes, dried at 60 °C, weighed after drying (total dry weight - (filter pad tare(s) + centrifuge tare) = dry macerated plant solids), and filled to 15 mL with methanol preparatory to extracting the solids.

i. Record stop time and jar weight.
ii. Cover open top of jar loosely with butterfly netting (leave a little pouch of netting above the jar), hold in place with rubber band around the mouth of the jar.
iii. Invert jar into a 1000 mL beaker.
iv. Squeeze excess liquid from duckweed solids without removing the butterfly netting from the jar.
v. Rinse the inside of the jar and butterfly netting with tap water (waste this liquid) for approximately 10 seconds to rinse solids sticking to the jar and capture as much solids as possible in the bottom of the netting.
vi. Remove the netting from the jar (do not squeeze yet), pour the liquid captured in the 1000 mL beaker back into the testing jar.
vii. Rinse the 1000 mL beaker and hand to dry for the next harvest.
viii. Twist the netting and squeeze the excess liquid from the duckweed back into the testing jar for a count of seven.
ix. Tare an aluminum weighing dish.
x. Put the clump of freshly harvested duckweed solids onto the tarred dish; scrape excess solids from the netting.
xi. Record fresh weight of duckweed and set in 60 °C oven[3] (dry for 10 hours until a stable dry weight is obtained, try not to exceed 24 hours). Once dry, solids are ready for extraction preparation steps.
xii. Record the weight of the test jar plus liquid, place a lid on the jar which is now ready for the cleanup step (perform cleanup step within 6 hours of harvesting).

4. Analyze samples
    a. Liquid cleanup
        i. Filter liquid[4][5] and optional quality control to verify retention by filters

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[3] Note: thermal degradation at 60 °C should not be an issue. Thermal degradation (TD) charts for each compound show that it does not create significant removal below 100 °C. On the other hand, evaporating the compound to dryness at 60 °C and then re-constituting to 1 mL and analyzing by HPLC/MS can lower the recoveries for several of the compounds. During the concentration step, the EPA 1694 method only recommends evaporating to 1 mL and then bringing the volume up to 4 mL. If the sample is concentrated below 0.5 mL then extraction efficiencies may decrease.

[4]Note: If solids are visible in the liquid then it is highly recommended to filter the samples before passing contents
through the Waters Oasis HLB 6cc Solid Phase Extraction cartridge which will speed up extraction times and allow for more liquid to pass through the cartridge

[5]Note: When pre-filtering liquids, glass-fiber filters (AP40 and GF/A 1.6 pore size used for these tests) are recommended; however, perform tests to determine the pharmaceutical retention by the filters

1. Rinse 500 mL Erlenmeyer flask/filter apparatus with methanol and tap water.
2. Place AP40 or GF/A filter pad on the filter apparatus.
3. Slowly pass liquid sample through the filter(s) and the rinse gently with deionized water.
4. Put all used filter pads for each sample into a 15 mL centrifuge tube.
5. Pour Erlenmeyer flask contents back into the test jar, rinse with deionized water, and record liquid weight (to be used in case not all contents pass through the Oasis HLB cartridge).
6. Clean filter apparatus and flask with methanol and then decant into the 15 mL centrifuge tube containing the used filter(s), and bring to the 15 mL mark with methanol.
7. Liquid now ready for cleanup. Vortex (5 min) and sonicate (30 min) in an ultrasonic bath before evaporating and HPLC/MS analysis.

ii. Load solid phase extraction cartridge

1. Clean vacuum tubes by rinsing with methanol, blowing air through the tubing, and rinsing again with deionized water.
2. Precondition Oasis HLB 6cc (Waters PN# 106202) solid phase extraction cartridges: **Note: due to the nature of the pharmaceuticals selected for this study, all liquid samples were extracted under neutral conditions (*i.e.*,no pH change) in order to obtain the highest recoveries for all seven compounds.
3. Rinse cartridges with at least 3 cartridge volumes of methanol (approximately 6 mL per cartridge), do not let it go dry.
4. Rinse cartridges with at least 2x the methanol rinse volume (approximately 30 mL), do not let it go dry.
5. Attach vacuum tubes to cartridges and sample bottles.
6. Load cartridge with sample(s) at a rate of 5-10 mL/min (approximately 7 mmHg)--watching constantly to prevent cartridge from going dry.
7. Dry cartridge under vacuum for approximately 5 minutes.
8. Elute cartridge with 10 mL of methanol at a slow rate (approximately 3 mmHg), capture in borosilicate vial.
9. Re-condition cartridges with 20-30 mL methanol and save for future use.

iii. Concentration
    1. Clean receiver flask by soaking in soapy water for at least 3 hours, rinsing with deionized water, followed by rinsing with methanol. To improve recoveries by approximately 10% then silanize the receiver flask.
        a. Silanize receiver flask (Baker 2011) by pouring dichloromethyltoluene (DCMT) solution into the receiver flask and then decanting back into the DCMT bottle.
        b. Rinse receiver flask with methanol, fill with methanol, and let stand for at least 1 hour.
    2. Pour 10 mL eluted volume into the receiver flask and dry to 0.25 mL (minimal precipitation) or 1 mL (visible precipitation/floc) under a gentle stream of nitrogen gas (10-15 psi) at 55 °C (TurboVap).
        a. While drying, thoroughly rinse the receiver flask sidewalls (increases percent recoveries by more than 50% for several compounds) as the solution evaporates. Rinse with the solution in the flask, adding more methanol is not typically necessary.
        b. As solids precipitate to the glassware, rinse and sonicate the receiver flask.
        c. When the solution reaches approximately 2-5 mL then rinse the receiver flask using a disposable Pasteur pipette.
    3. Bring volume up to 1 mL (minimal precipitation) or 4 mL (visible precipitation/floc) with 0.1% formic acid.
        a. If the diluted solution still appears cloudy then it was necessary to centrifuge the solution.
        b. Pipette diluted solution into a polypropylene micro centrifuge tube.
        c. Centrifuge for 3 min at 10,000-11,000 rpm.
    4. Pipette sample into 2 mL HPLC glass vials with Teflon septum. Add spikes and/or internal standards, if necessary. Samples are now ready for HPLC/MS analysis.

iv. HPLC/MS configuration/analysis
    1. Devices: Agilent 1200 Series High Performance Autosampler SL (PN# G1367C); Agilent 1260 Series Binary Pump (PN# G1312B); Agilent 1200 Series

Thermostatted Column compartment SL (PN# G1316B); Agilent G6220A Time of Flight Detector (PN# G6220A)

2. TOF/Q-TOF Mass Spectrometer: Ion Source: Dual ESI, Ion Polarity: Positive Mode; Gas Temp. 350 °C; Gas flow 12 L/min; Nebulizer 25 psi; Scan Vcap = 3500 V; Reference masses 121.050873 to 922.009798; Chromatagram TIC type; 9.2 min stop time; 10 min run time w/ 3 min runtime between runs. Retention times shown in Figure 49.
3. Binary Pump: Flow 0.35 mL/min; Pmax = 500 bar; Solvent "A" 0.1% Formic Acid and 0.1% MeOH; Solvent "B" 90/10 Acetonitrile/H2O + 0.1% Formic Acid; Pump Time Table: 0 min. @ 3% "B", 1 min. @ 3% "B", 5 min. @ 100% "B", 8 min. @ 100% "B", and 10 min. @ 3% "B"
4. Column: Agilent Eclipse Plus C18 (2.1 x 55 mm, 1.8 um) PN# 959741-902; Column-SL temperature 30 °C
5. Autosampler: Injection Volume 5 uL w/ 3 sec. needle wash; Draw position 3 mm; Draw/Eject speed 200 uL/min; Equilibration time 0-2 sec. ***Custom Injection Program for Internal Standard Runs: 1st Eject, 2nd Draw 5 uL Atrazine-d5 (instd), 3rd Needle wash 3 sec., 4th Draw 5 uL sample, 5th Needle wash 3 sec, 6th Wait 2 sec., 7th Inject 10 uL

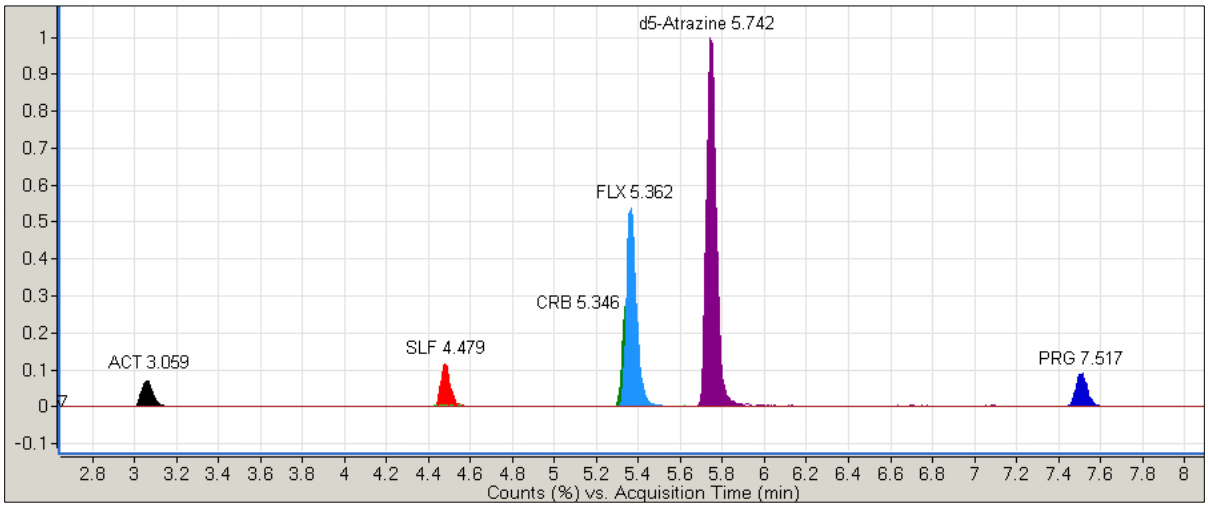

Figure 49. Pharmaceutical compounds HPLS/MS retention times.

b. Solid extractions
    i. with solid phase extraction (lower extraction efficiency)
        1. Place 60 °C dried duckweed into mortar/pestle.
        2. Crush duckweed.
        3. Pour crushed duckweed into weighing dish, record mass, and then pour into 15 mL centrifuge tube.
        4. Rinse aluminum weighing dish (used for drying) and mortar/pestle with methanol. Save methanol and add to the 15 mL centrifuge tube. Repeat rinsing until centrifuge tube is full.
        5. Allow solids to sit in methanol for >24 hours.
        6. Vortex sample 5 min.
        7. Ultrasonic bath for 30 min.
        8. Centrifuge 5000 rpm for 5 min.
        9. Decant solution into receiver flask.
        10. Add 10 mL methanol + 3 mL deionized water.
        11. Repeat vortex/ultrasonic bath/centrifuge/decant steps.
        12. Add 10 mL methanol and repeats vortex/ultrasonic steps.
        13. Pour all contents through a filter into the receiver flask.
        14. Rinse solids/filter with methanol.
        15. Evaporate solution to 5-10 mL under a gentle stream of nitrogen gas (10-15 psi) and 55 °C water bath (TurboVap). Constantly rinse and ultrasonicate the glassware as the volume decreases and more solids precipitate from solution.
        16. Bring the volume to 200-500 mL with deionized water, the solution is now ready for the cleanup/elution/concentration steps.
    ii. without solid phase extraction (higher extraction efficiency)[6]
        1. Follow steps outlined previously to dry/crush solids. Store crushed solids (approx. 500 mg-dry) in a 15 mL centrifuge tube with 12 mL-methanol plus 2 mL-deionized water
        2. After storage in MeOH for 24 hours, vortex for 5 min, centrifuge, and then:

[6] Note: previous attempts to extract the solids using the TurboVap received poor extraction efficiencies due to matrix effects and solids precipitating from solution as the evaporation volume decreased. Running samples in Methanol without severe evaporation steps provided as good or better results.

a. if the expected ng/mL concentration is more than 100 ng/mL, run 1 mL sample on HPLC/MS (approximately 1:14 dilution)[7]
b. If the expected concentration is less than 100 ng/mL, decant supernatant from 15 mL centrifuge tube into 15 mL disposable glass culture tube. Place in 60 °C oven and evaporate to 4 mL. Add 1 mL-methanol without formic acid[8], vortex in order to clean the sidewalls of the vials, and then micro-centrifuge sample. Analyze 1 mL sample on HPLC/MS (approximately 1:5 dilution)

iii. HPLC/MS analysis with matrix standards

1. Add 500 mg (dry) duckweed--that has not been exposed to pharmaceuticals--to a 15 mL centrifuge tube. Add 12 mL-methanol plus 2 mL-deionized water. Store for 24 hours.
2. After storage, vortex 5 min. and centrifuge samples.
3. Evaporate to 5 mL if extracted solids are also being evaporated, vortex, and centrifuge.
4. Prepare at least five 1-mL aliquots of solution from which to make the matrix standards. Spike in the appropriate amount of secondary pharmaceutical standards to make the working standards.[9]
5. Run the matrix-standards with the extracted solids samples to create a matrix-calibration curve which takes into account interferences that reduce the signal sensitivity of the HPLC/MS. The matrix-sample signals are typically 2-10x's less than matrix-free signals.
6. If necessary, multiply the results by the dilution factor, the Extraction Efficiency multiplier (*i.e.*,the inverse of the extraction efficiency), and correct for losses due to long storage time, if applicable.

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[7] Note: even though the instrument can detect concentrations around 5 ng/mL +/- 10%, the matrix effects produce readings that are 5-10x's less than equivalent concentrations samples without matrix interferences.
[8] Note: formic acid causes solids to precipitate from solution therefore was not added to the solids extracted samples.
[9] Note: also prepare one 250 ng/mL matrix-free methanol standard in order to obtain the correct retention times. This is recommended because the matrix standards have more interferences and it's harder to be certain of the retention times without running a matrix-free methanol standard). This matrix-free methanol standard will not be used to construct the actual calibration curve.

B.3 Standard Operating Procedure: Plant Harvesting and Weighing Procedure

B.3.1 Harvesting by Percent Removal

1. Each reactor contains 3 cells and each cell is harvested individually as follows:
2. Place divider at 25%, 50%, or 75% mark in the cell.
3. Using the screening apparatus shown below (Figure 50), remove all the duckweed from within the divided area (Figure 51).
4. Allow the liquid to drain until there is only a slow drip coming from the screen—it may be helpful to gently press some liquid out with a spoon to expedite the process.
5. Place the fresh duckweed into a tarred container to determine the fresh weight (g).
6. Place the fresh measured duckweed into a glass beaker and oven dry at 103°C for at least 24 hours to determine the dry weight (g).
7. Record the % harvested/cell fresh weight and dry weight.

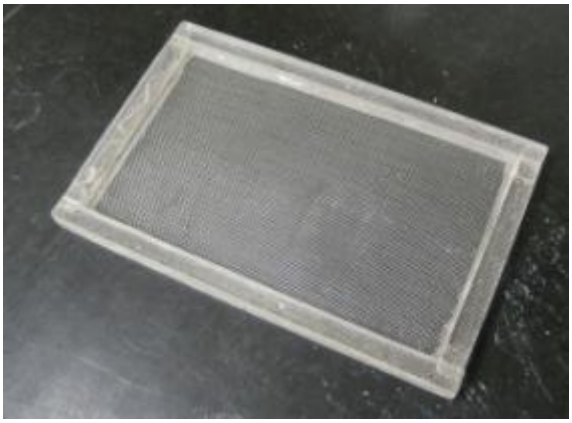


Figure 50. Screening apparatus (12 in x 7 in) made with metal window screening sealed with acrylic cement between 3/8” acrylic pieces.

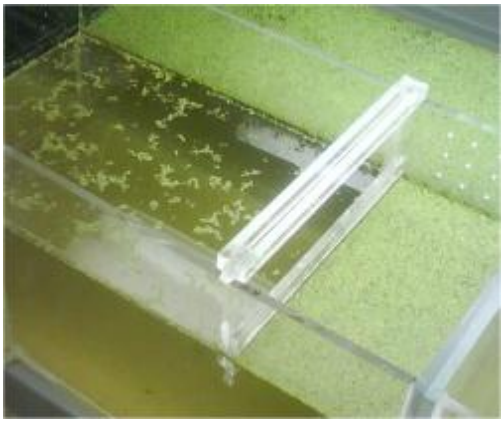


Figure 51. Physical barrier separating harvested duckweed and non-harvested duckweed.

B.3.2 Harvesting by Restoring to a Known Initial Plant Density

Each reactor contains 3 cells and each cell is harvested individually as follows:

1. Using the screening apparatus shown in Figures 50 and 51, remove all the duckweed from within the cell (Figure 52).
2. Allow the liquid to drain until there is only a slow drip coming from the screen—it may be helpful to gently press some liquid out with a spoon to expedite the process.
3. Place the fresh duckweed into a tarred container to determine the fresh weight (g).
4. Set aside the desired amount of fresh duckweed to be put back into the cell (assume approximately 20 g (fresh) / g (dry) until calculated precisely later).
5. Replace the desired amount of fresh duckweed back into the cell—it may be necessary to gently distribute the duckweed with a spoon to keep it evenly distributed.
6. Place the harvested amount of fresh duckweed into a glass beaker and oven dry at 103°C for at least 24 hours to determine the dry weight (g).
7. Record the fresh weight (*i.e.*,final wt.), growth (final wt. – initial wt.), re-planted weight (*i.e.*,initial wt.), dry weight (g), and fresh:dry ratio (g (fresh) / g (dry)).

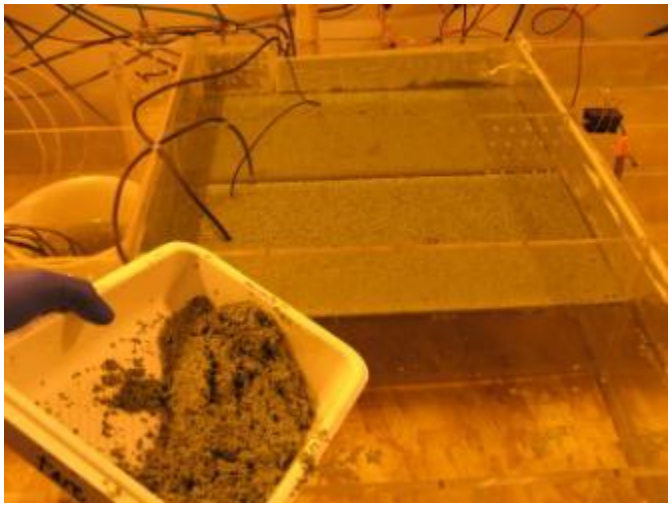


Figure 52. Fresh duckweed after screening, ready to be seeded into the third cell of the reactor. Orange hue in the photograph due to the HPLS lights overhead.

Appendix C Raw Data Tables

C.1 Phosphorus Removal Appendix Raw Data

Table 23. Raw Data: Phosphorus removal by duckweed in laboratory over four days

Tag	Vol (mL)	Time (days)	mg-P/L	mg-P liquid	g-solids (fresh)	mg-solids (dry)	%P-solids	mg-P solids	Total mg-P/reactor	*Evapo-Transpiration (g-H20/day/50cm^2)
r1A1	386	0.00	5.18	2.00	NA	NA	NA	NA	2.00	-9.0
r1A2	388	0.00	5.18	2.01	NA	NA	NA	NA	2.01	-8.9
r1A3	388	0.00	5.18	2.01	NA	NA	NA	NA	2.01	-7.2
r1B1	382	0.00	5.18	1.98	10.2	400	1.02%	4.09	6.07	-9.2
r1B2	385	0.00	5.18	1.99	6.9	271	1.02%	2.77	4.76	-8.8
r1B3	388	0.00	5.18	2.01	7.2	283	1.02%	2.89	4.90	-8.0
r1C1	391	0.00	5.18	2.02	6.7	263	1.02%	2.69	4.71	-26.1
r1C2	385	0.00	5.18	1.99	7.3	287	1.02%	2.93	4.92	-26.2
r1C3	403	0.00	5.18	2.08	8.4	330	1.02%	3.37	5.46	-26.7
r1D1	384	0.00	5.18	1.99	NA	NA	NA	NA	1.99	-26.8
r1D2	382	0.00	5.18	1.98	NA	NA	NA	NA	1.98	-26.3
r1D3	386	0.00	5.18	2.00	NA	NA	NA	NA	2.00	-26.1
r1E1	389	0.00	5.18	2.01	29.6	337	0.93%	3.15	5.16	-28.7
r1E2	383	0.00	5.18	1.98	34.7	395	0.93%	3.69	5.67	-25.2
r1E3	387	0.00	5.18	2.00	34.6	394	0.93%	3.68	5.68	-25.8
r2A1	372	1.30	4.47	1.66	NA	NA	NA	NA	1.66	-9.0
r2A2	349	1.30	4.41	1.54	NA	NA	NA	NA	1.54	-8.9
r2A3	372	1.30	4.30	1.60	NA	NA	NA	NA	1.60	-7.2
r2B1	374	1.30	4.99	1.87	---	*314*	*1.06%*	*3.33*	5.20	-9.2
r2B2	376	1.30	4.01	1.51	---	*220*	*1.16%*	*2.55*	4.06	-8.8
r2B3	378	1.30	5.08	1.92	---	*261*	*1.04%*	*2.71*	4.63	-8.0
r2C1	378	1.30	2.00	0.76	---	*338*	*1.12%*	*3.77*	4.53	-26.1
r2C2	384	1.30	2.61	1.00	---	*368*	*1.12%*	*4.11*	5.12	-26.2
r2C3	362	1.30	2.56	0.92	---	*423*	*1.12%*	*4.73*	5.66	-26.7
r2D1	357	1.30	4.24	1.51	NA	NA	NA	NA	1.51	-26.8
r2D2	374	1.30	5.11	1.91	NA	NA	NA	NA	1.91	-26.3
r2D3	348	1.30	4.36	1.51	NA	NA	NA	NA	1.51	-26.1
r2E1	346	1.30	11.70	4.05	---	---	---	---	4.05	-28.7
r2E2	351	1.30	12.92	4.53	---	---	---	---	4.53	-25.2
r2E3	375	1.30	12.69	4.75	---	---	---	---	4.75	-25.8
r3A1	407	4.00	4.36	1.77	NA	NA	NA	NA	1.77	-9.0
r3A2	401	4.00	4.27	1.71	NA	NA	NA	NA	1.71	-8.9
r3A3	395	4.00	4.18	1.65	NA	NA	NA	NA	1.65	-7.2
r3B1	412	4.00	8.45	3.48	8.12	234	1.10%	2.58	6.06	-9.2
r3B2	407	4.00	6.07	2.47	5.51	172	1.29%	2.22	4.69	-8.8
r3B3	413	4.00	8.91	3.68	7.63	237	1.05%	2.50	6.18	-8.0
r3C1	375	4.00	0.51	0.19	8.38	372	1.23%	4.58	4.78	-26.1
r3C2	371	4.00	0.86	0.32	7.45	333	1.19%	3.95	4.27	-26.2
r3C3	392	4.00	1.22	0.48	9.81	401	1.30%	5.21	5.68	-26.7
r3D1	389	4.00	4.73	1.84	NA	NA	NA	NA	1.84	-26.8
r3D2	384	4.00	4.56	1.75	NA	NA	NA	NA	1.75	-26.3
r3D3	376	4.00	4.53	1.70	NA	NA	NA	NA	1.70	-26.1
r3E1	381	4.00	8.45	3.22	---	263	0.90%	2.37	5.59	-28.7
r3E2	371	4.00	9.42	3.49	---	233	0.80%	1.85	5.35	-25.2
r3E3	380	4.00	9.89	3.76	---	235	0.55%	1.29	5.05	-25.8
Ctrl1	NA	4.00	7.06	NA	NA	NA	0.23%	NA	NA	NA
Ctrl2	NA	4.00	5.12	NA	NA	NA	0.21%	NA	NA	NA
Ctrl3	NA	4.00	5.27	NA	NA	NA	0.22%	NA	NA	NA
Ctrl4	NA	4.00	5.23	NA	NA	NA	0.37%	NA	NA	NA
Ctrl5	NA	4.00	5.12	NA	NA	NA	0.32%	NA	NA	NA

Table 24. Raw Data: Phosphorus removal by duckweed in laboratory over 1day

Time (days):	0.00	0.03	0.13	0.31[a]	0.58	0.90	3.17	0.00	0.03	0.13	0.31	0.58	0.90	3.17	0.00	0.03	0.13	0.31	0.58	0.90	3.17
Tag	Volume (mL)							Phosphorus Concentration (mg-PO_4-P/L)							mg-P liquid						
X1	384	384	383	382	380	378	374	*5.90*	5.93	5.63	---	5.14	5.06	4.52	2.27	2.28	2.16	---	1.95	1.91	1.69
X2	376	376	375	374	372	370	367	*5.90*	5.82	6.56	5.39	5.19	5.04	4.52	2.22	2.19	2.46	2.02	1.93	1.86	1.66
X3	384	384	383	382	380	378	376	*5.90*	5.96	5.75	---	5.31	5.29	4.52	2.27	2.29	2.20	---	2.02	2.00	1.70
A1	387	387	385	382	379	374	341	*5.90*	*5.90*	5.59	---	5.24	5.04	---	2.28	2.28	2.15	---	1.98	1.88	---
A2	382	382	380	377	374	369	336	*5.90*	*5.90*	5.66	5.25	5.05	4.87	---	2.26	2.25	2.15	1.98	1.89	1.80	---
A3	373	373	371	368	365	360	327	*5.90*	*5.90*	5.87	---	5.10	4.99	---	2.20	2.20	2.18	---	1.86	1.80	---
B1	391	391	389	386	383	378	345	*5.90*	6.46	6.63	---	6.02	5.71	---	2.31	2.52	2.58	---	2.30	2.16	---
B2	358	358	356	353	350	345	312	*5.90*	5.93	6.11	6.02	5.69	5.43	---	2.11	2.12	2.18	2.13	1.99	1.87	---
B3	408	408	406	403	400	395	362	*5.90*	7.20	7.53	---	7.05	6.96	---	2.41	2.94	3.06	---	2.82	2.75	---
C1	399	398	394	387	376	364	275	*5.90*	6.48	5.59	---	4.26	3.23	---	2.36	2.58	2.20	---	1.60	1.18	---
C2	397	396	392	385	374	362	273	*5.90*	6.61	5.85	6.16	4.36	3.31	---	2.34	2.62	2.29	2.37	1.63	1.20	---
C3	375	374	370	363	352	340	251	*5.90*	6.27	5.75	---	4.55	3.17	---	2.21	2.35	2.13	---	1.60	1.08	---
D1	357	356	352	345	334	322	237	*5.90*	*5.90*	5.89	---	5.40	5.39	4.66	2.11	2.10	2.08	---	1.81	1.73	1.10
D2	382	381	377	370	359	347	237	*5.90*	*5.90*	5.66	5.25	5.17	5.27	4.97	2.26	2.25	2.13	1.94	1.86	1.83	1.18
D3	390	389	385	378	367	355	263	*5.90*	*5.90*	5.42	---	5.31	4.92	4.64	2.30	2.30	2.09	---	1.95	1.75	1.22
E1	369	368	364	357	346	334	236	*5.90*	14.47	14.08	---	14.24	15.78	14.36	2.18	5.32	5.13	---	4.93	5.27	3.39
E2	389	388	384	377	366	354	261	*5.90*	12.16	12.57	13.11	12.48	14.24	12.21	2.30	4.72	4.83	4.94	4.57	5.04	3.19
E3	420	419	415	408	397	385	282	*5.90*	11.96	11.91	---	11.95	12.88	11.82	2.48	5.01	4.94	---	4.75	4.96	3.33
E1[b]	369	368	364	357	346	334	236	*5.90*	13.27	13.40	---	---	14.92	13.03	2.18	4.88	4.88	---	---	4.98	3.08
E2[b]	389	388	384	377	366	354	261	*5.90*	11.91	11.50	---	---	13.00	12.00	2.30	4.62	4.42	---	---	4.60	3.13
E3[b]	420	419	415	408	397	385	282	*5.90*	11.17	11.12	---	---	11.99	10.49	2.48	4.68	4.62	---	---	4.62	2.96

Table 24 (continued)

Time (days):	0.03	0.90	0.03	0.90	0.03	0.90	0.90	0.90	0.00	0.03	0.90
Tag	Plant (g-fresh)		Plant (g-dry)		%DM		RGR	%P solids[c]	Total mg-P/reactor (solids)		
X1	NA	NA	NA	NA	NA	NA	NA	NA	2.27	NA	NA
X2	NA	NA	NA	NA	NA	NA	NA	NA	2.22	NA	NA
X3	NA	NA	NA	NA	NA	NA	NA	NA	2.27	NA	NA
A1	NA	NA	NA	NA	NA	NA	NA	NA	2.28	NA	NA
A2	NA	NA	NA	NA	NA	NA	NA	NA	2.26	NA	NA
A3	NA	NA	NA	NA	NA	NA	NA	NA	2.20	NA	NA
B1	8.18	8.9	*0.42*	0.440	*94.8%*	95.1%	0.047	1.27%	7.76	5.45	5.59
B2	8.18	10.08	*0.42*	0.470	*94.8%*	95.3%	0.120	1.39%	7.57	5.45	6.53
B3	8.09	8.44	*0.42*	0.410	*94.8%*	95.1%	-0.020	1.38%	7.80	5.39	5.66
C1	8	8.84	*0.41*	0.470	*94.8%*	94.7%	0.145	1.54%	7.69	5.33	7.25
C2	8.06	9.17	*0.42*	0.480	*94.8%*	94.8%	0.160	1.25%	7.72	5.37	6.02
C3	8.06	8.85	*0.42*	0.460	*94.8%*	94.8%	0.113	1.79%	7.59	5.37	8.23
D1	NA	NA	NA	NA	NA	NA	NA	NA	2.11	NA	NA
D2	NA	NA	NA	NA	NA	NA	NA	NA	2.26	NA	NA
D3	NA	NA	NA	NA	NA	NA	NA	NA	2.30	NA	NA
E1	8	---	*0.41*	---	*94.8%*	---	---	---	7.51	5.33	---
E2	8	---	*0.41*	---	*94.8%*	---	---	---	7.63	5.33	---
E3	8	---	*0.41*	---	*94.8%*	---	---	---	7.81	5.33	---
E1**	8	---	*0.41*	---	*94.8%*	---	---	---	2.18	5.33	---
E2**	8	---	*0.41*	---	*94.8%*	---	---	---	2.30	5.33	---
E3**	8	---	*0.41*	---	*94.8%*	---	---	---	2.48	5.33	---

No**tes:** [a] time period 0.31 days preceeded by 2 hour dark period in the light reactors [b] represents centrifuged samples

[a] Controls Phosphorus Tissue Composition (%-P/g-dry)

Std. 0.38%-P GrapePetiole1	0.34%
Std. 0.38%-P GrapePetiole2	0.30%
Std. 0.38%-P GrapePetiole3	0.31%
Plant Control1@t=0	1.25%
Plant Control2@t=0	1.30%
Plant Control3@t=0	1.32%

C.2 Pharmaceutical Removal Appendix Raw Data

Table 25. Raw data: Low range pharmaceutical removal by duckweed

Low Range Samples										Initial Mass (ng-COC/reactor)[a,b]							Final Liquid Mass (ng-COC liquid/reactor)						
Dose	Time (days)	Tag	Vol (mL)	Initial Plant (g-fresh)	Final Plant (g-fresh)	Initial Plant (g-dry)	Final Plant (g-dry)	RGR (fresh)	RGR (dry)	NIC	COT	ACT	SLF	FLX	CRB	PRG	NIC	COT	ACT	SLF	FLX	CRB	PRG
LR	0.083	r1A1	397	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	415	406	650	396	293	449	392
LR	0.083	r1A2	413	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	486	490	674	430	325	530	470
LR	0.083	r1A3	407	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	518	479	706	413	366	522	435
LR	0.083	r1B1	424	6.14	5.66	0.327	0.268	-0.982	-2.639	500	500	1000	500	500	500	500	391	496	637	444	263	565	308
LR	0.083	r1B2	409	5.63	4.41	0.300	0.232	-2.943	-3.355	500	500	1000	500	500	500	500	758	573	796	490	302	586	330
LR	0.083	r1B3	377	7.00	6.60	0.373	0.320	-0.704	-2.010	500	500	1000	500	500	500	500	528	650	716	339	262	638	233
LR	0.083	r1C1	418	7.57	5.98	0.403	0.278	-2.840	-4.676	500	500	1000	500	500	500	500	402	510	677	504	323	578	332
LR	0.083	r1C2	419	5.61	5.57	0.299	0.236	-0.086	-2.988	500	500	1000	500	500	500	500	400	576	626	508	348	620	387
LR	0.083	r1C3	403	7.06	5.72	0.376	0.327	-2.531	-1.744	500	500	1000	500	500	500	500	370	575	750	395	230	560	257
LR	0.083	r1D1	382	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	411	452	592	394	259	478	403
LR	0.083	r1D2	412	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	454	468	621	427	286	414	464
LR	0.083	r1D3	382	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	367	415	637	400	330	468	434
LR	0.083	r1E1	374	12.66	12.66	0.674	0.640	0.000	-0.944	500	500	1000	500	500	500	500	354	327	642	57	78	415	10
LR	0.083	r1E2	378	12.40	12.40	0.661	0.730	0.000	1.799	500	500	1000	500	500	500	500	160	117	485	191	185	764	10
LR	0.083	r1E3	412	12.13	12.13	0.647	0.540	0.000	-3.244	500	500	1000	500	500	500	500	132	139	441	213	152	663	10
LR	1	r2A1	403	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	447	463	567	393	370	534	444
LR	1	r2A2	415	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	476	478	606	395	362	527	453
LR	1	r2A3	381	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	425	462	579	376	284	505	442
LR	1	r2B1	402	6.33	5.30	0.337	0.229	-0.178	-0.386	500	500	1000	500	500	500	500	303	508	440	322	222	521	41
LR	1	r2B2	415	5.94	5.14	0.317	0.235	-0.145	-0.296	500	500	1000	500	500	500	500	353	566	453	345	277	549	44
LR	1	r2B3	398	6.68	4.01	0.356	0.270	-0.510	-0.275	500	500	1000	500	500	500	500	387	312	371	242	171	430	19
LR	1	r2C1	373	6.37	6.88	0.340	0.568	0.076	0.498	500	500	1000	500	500	500	500	283	593	273	345	149	513	28
LR	1	r2C2	391	5.64	4.16	0.300	0.468	-0.304	0.429	500	500	1000	500	500	500	500	263	551	244	338	148	544	25
LR	1	r2C3	355	6.69	7.96	0.356	0.678	0.174	0.624	500	500	1000	500	500	500	500	297	295	268	236	123	424	19
LR	1	r2D1	374	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	465	492	597	397	350	522	452
LR	1	r2D2	395	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	471	511	582	390	296	542	466
LR	1	r2D3	399	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	517	527	564	369	297	537	446

			Low Range Samples							Initial Mass (ng-COC/reactor)[a,b]							Final Liquid Mass (ng-COC liquid/reactor)						
Dose	Time (days)	Tag	Vol (mL)	Initial Plant (g-fresh)	Final Plant (g-fresh)	Initial Plant (g-dry)	Final Plant (g-dry)	RGR (fresh)	RGR (dry)	NIC	COT	ACT	SLF	FLX	CRB	PRG	NIC	COT	ACT	SLF	FLX	CRB	PRG
LR	1	r2E1	349	12.40	12.40	0.661	0.730	0.000	0.092	500	500	1000	500	500	500	500	214	441	860	346	183	652	10
LR	1	r2E2	341	12.13	12.13	0.647	0.040	0.000	-2.552	500	500	1000	500	500	500	500	189	387	475	312	141	645	10
LR	1	r2E3	354	12.40	12.40	0.661	0.747	0.000	0.112	500	500	1000	500	500	500	500	474	498	418	332	201	534	10
LR	4	r3A1	399	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	264	368	536	401	376	547	447
LR	4	r3A2	378	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	291	296	602	406	369	556	423
LR	4	r3A3	382	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	237	232	548	358	342	543	404
LR	4	r3B1	398	6.00	4.60	0.320	0.220	-0.066	-0.094	500	500	1000	500	500	500	500	335	650	272	265	202	622	46
LR	4	r3B2	371	7.01	6.01	0.374	0.271	-0.039	-0.081	500	500	1000	500	500	500	500	280	640	271	238	176	606	50
LR	4	r3B3	363	6.82	6.43	0.364	0.256	-0.015	-0.088	500	500	1000	500	500	500	500	187	568	184	190	97	623	26
LR	4	r3C1	381	6.91	10.51	0.368	0.481	0.105	0.067	500	500	1000	500	500	500	500	101	517	12	88	89	598	43
LR	4	r3C2	345	6.94	11.07	0.370	0.501	0.117	0.076	500	500	1000	500	500	500	500	109	498	16	80	80	554	38
LR	4	r3C3	355	6.95	10.57	0.371	0.521	0.105	0.085	500	500	1000	500	500	500	500	74	481	11	47	57	581	28
LR	4	r3D1	368	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	197	282	528	381	306	563	422
LR	4	r3D2	375	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	265	249	532	356	328	549	408
LR	4	r3D3	362	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	247	312	465	317	266	530	385
LR	4	r3E1	391	12.66	12.66	0.674	0.553	0.000	-0.052	500	500	1000	500	500	500	500	191	617	376	113	55	509	10
LR	4	r3E2	362	12.40	12.40	0.661	0.116	0.000	-0.451	500	500	1000	500	500	500	500	200	570	368	104	46	489	10
LR	4	r3E3	365	12.66	12.66	0.674	0.162	0.000	-0.371	500	500	1000	500	500	500	500	226	618	430	122	48	500	10
LR	0.083	r1X1	353	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	468	527	643	481	357	581	472
LR	1	r2X2	405	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	527	544	711	488	444	629	530
LR	4	r3X3	416	NA	NA	NA	NA	NA	NA	500	500	1000	500	500	500	500	408	464	619	377	384	506	453

[a] NIC = Nicotine[b] COT = Cotinine[b] ACT = Acetaminophen SLF = Sulfmaethoxazole FLX = Fluoxetine CRB = Carbamazepine PRG = Progesterone

[b] ommitted from report due to early elution from HPLC/MS which caused unreliable measurements and split-chromatographic peaks

Low Range Samples (continued)			Liquid Percent Recovery								Solid Percent Recovery						
Dose	Time (days)	Tag	NIC	COT	ACT	SLF	FLX	CRB	PRG	Tag	NIC	COT	ACT	SLF	FLX	CRB	PRG
LR	0.083	r1A1	83%	81%	65%	79%	59%	90%	78%	r1A1	NA	NA	NA	NA	NA	NA	NA
LR	0.083	r1A2	97%	98%	67%	86%	65%	106%	94%	r1A2	NA	NA	NA	NA	NA	NA	NA
LR	0.083	r1A3	104%	96%	71%	83%	73%	104%	87%	r1A3	NA	NA	NA	NA	NA	NA	NA
LR	0.083	r1B1	78%	99%	64%	89%	53%	113%	62%	r1B1	no data	no data	no data	no data	no data	no data	no data
LR	0.083	r1B2	152%	115%	80%	98%	60%	117%	66%	r1B2	67%	0%	2%	0%	27%	0%	2%
LR	0.083	r1B3	106%	130%	72%	68%	52%	128%	47%	r1B3	50%	0%	2%	0%	29%	0%	18%
LR	0.083	r1C1	80%	102%	68%	101%	65%	116%	66%	r1C1	52%	2%	2%	0%	31%	0%	0%
LR	0.083	r1C2	80%	115%	63%	102%	70%	124%	77%	r1C2	49%	0%	2%	25%	31%	0%	2%
LR	0.083	r1C3	74%	115%	75%	79%	46%	112%	51%	r1C3	140%	2%	2%	28%	32%	0%	15%
LR	0.083	r1D1	82%	90%	59%	79%	52%	96%	81%	r1D1	NA	NA	NA	NA	NA	NA	NA
LR	0.083	r1D2	91%	94%	62%	85%	57%	83%	93%	r1D2	NA	NA	NA	NA	NA	NA	NA
LR	0.083	r1D3	73%	83%	64%	80%	66%	94%	87%	r1D3	NA	NA	NA	NA	NA	NA	NA
LR	0.083	r1E1	71%	65%	64%	11%	16%	83%	*2%*	r1E1	0%	2%	252%	0%	2%	0%	12%
LR	0.083	r1E2	32%	23%	49%	38%	37%	153%	*2%*	r1E2	2%	2%	82%	0%	2%	0%	14%
LR	0.083	r1E3	26%	28%	44%	43%	30%	133%	*2%*	r1E3	2%	0%	2%	30%	10%	0%	24%
LR	1	r2A1	89%	93%	57%	79%	74%	107%	89%	r2A1	NA	NA	NA	NA	NA	NA	NA
LR	1	r2A2	95%	96%	61%	79%	72%	105%	91%	r2A2	NA	NA	NA	NA	NA	NA	NA
LR	1	r2A3	85%	92%	58%	75%	57%	101%	88%	r2A3	NA	NA	NA	NA	NA	NA	NA
LR	1	r2B1	61%	102%	44%	64%	44%	104%	8%	r2B1	110%	2%	2%	0%	58%	0%	2%
LR	1	r2B2	71%	113%	45%	69%	55%	110%	9%	r2B2	63%	0%	2%	0%	50%	0%	2%
LR	1	r2B3	77%	62%	37%	48%	34%	86%	4%	r2B3	110%	0%	2%	0%	45%	0%	14%
LR	1	r2C1	57%	119%	27%	69%	30%	103%	6%	r2C1	175%	2%	2%	0%	65%	0%	2%
LR	1	r2C2	53%	110%	24%	68%	30%	109%	5%	r2C2	176%	0%	2%	28%	68%	0%	0%
LR	1	r2C3	59%	59%	27%	47%	25%	85%	4%	r2C3	143%	2%	2%	30%	56%	0%	2%
LR	1	r2D1	93%	98%	60%	79%	70%	104%	90%	r2D1	NA	NA	NA	NA	NA	NA	NA
LR	1	r2D2	94%	102%	58%	78%	59%	108%	93%	r2D2	NA	NA	NA	NA	NA	NA	NA
LR	1	r2D3	103%	105%	56%	74%	59%	107%	89%	r2D3	NA	NA	NA	NA	NA	NA	NA
LR	1	r2E1	43%	88%	86%	69%	37%	130%	*2%*	r2E1	2%	0%	2%	31%	19%	0%	17%
LR	1	r2E2	38%	77%	48%	62%	28%	129%	*2%*	r2E2	2%	2%	2%	0%	2%	0%	50%
LR	1	r2E3	95%	100%	42%	66%	40%	107%	*2%*	r2E3	2%	2%	2%	0%	14%	0%	0%
LR	4	r3A1	53%	74%	54%	80%	75%	109%	89%	r3A1	NA	NA	NA	NA	NA	NA	NA
LR	4	r3A2	58%	59%	60%	81%	74%	111%	85%	r3A2	NA	NA	NA	NA	NA	NA	NA

Low Range Samples (continued)			Liquid Percent Recovery								Solid Percent Recovery						
Dose	Time (days)	Tag	NIC	COT	ACT	SLF	FLX	CRB	PRG	Tag	NIC	COT	ACT	SLF	FLX	CRB	PRG
LR	4	r3A3	47%	46%	55%	72%	68%	109%	81%	r3A3	NA	NA	NA	NA	NA	NA	NA
LR	4	r3B1	67%	130%	27%	53%	40%	124%	9%	r3B1	34%	2%	2%	0%	41%	0%	17%
LR	4	r3B2	56%	128%	27%	48%	35%	121%	10%	r3B2	57%	2%	2%	33%	50%	0%	0%
LR	4	r3B3	37%	114%	18%	38%	19%	125%	5%	r3B3	43%	2%	2%	30%	51%	0%	0%
LR	4	r3C1	20%	103%	1%	18%	18%	120%	9%	r3C1	77%	2%	109%	0%	51%	0%	2%
LR	4	r3C2	22%	100%	2%	16%	16%	111%	8%	r3C2	87%	2%	58%	0%	46%	0%	2%
LR	4	r3C3	15%	96%	1%	9%	11%	116%	6%	r3C3	99%	0%	43%	0%	42%	0%	2%
LR	4	r3D1	39%	56%	53%	76%	61%	113%	84%	r3D1	NA	NA	NA	NA	NA	NA	NA
LR	4	r3D2	53%	50%	53%	71%	66%	110%	82%	r3D2	NA	NA	NA	NA	NA	NA	NA
LR	4	r3D3	49%	62%	47%	63%	53%	106%	77%	r3D3	NA	NA	NA	NA	NA	NA	NA
LR	4	r3E1	38%	123%	38%	23%	11%	102%	*2%*	r3E1	no data	no data	no data	no data	no data	no data	no data
LR	4	r3E2	40%	114%	37%	21%	9%	98%	*2%*	r3E2	2%	0%	78%	0%	2%	0%	18%
LR	4	r3E3	45%	124%	43%	24%	10%	100%	*2%*	r3E3	0%	2%	2%	0%	2%	0%	19%
LR	0.083	r1X1	94%	105%	64%	96%	71%	116%	94%	r1P1	50%	77%	46%	41%	54%	71%	47%
LR	1	r2X2	105%	109%	71%	98%	89%	126%	106%	r2P2	53%	76%	47%	37%	53%	74%	47%
LR	4	r3X3	82%	93%	62%	75%	77%	101%	91%	r3P3	57%	63%	46%	42%	52%	70%	46%

Table 26. Raw data: Mid range pharmaceutical removal by duckweed

				Mid Range Samples						Initial Mass (µg-COC/reactor)							Final Liquid Mass (µg-COC liquid/reactor)						
Dose	Time (days)	Tag	Vol (mL)	Initial Plant (g-fresh)	Final Plant (g-fresh)	Initial Plant (g-dry)	Final Plant (g-dry)	RGR (fresh)	RGR (dry)	NIC	COT	ACT	SLF	FLX	CRB	PRG	NIC	COT	ACT	SLF	FLX	CRB	PRG
MR	0.08	r1C1	375	8.06	8.21	0.45	0.45	0.222	0.00	20	20	20	20	20	20	20	8	2	17	12	11	16	14
MR	0.08	r1C2	380	8.00	8.15	0.45	0.45	0.224	0.00	20	20	20	20	20	20	20	8	2	18	20	13	17	15
MR	0.08	r1C3	332	8.15	8.29	0.45	0.45	0.205	0.00	20	20	20	20	20	20	20	7	2	19	14	10	15	14
MR	0.08	r1D1	NA	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	10	0	22	20	20	22	20
MR	0.08	r1D2	NA	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	10	2	19	20	16	18	16
MR	0.08	r1D3	NA	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	10	2	22	23	19	20	17
MR	1	r2C1	357	8.01	10.06	0.45	0.51	0.228	0.13	20	20	20	20	20	20	20	3	0	7	7	3	20	1
MR	1	r2C2	348	7.91	9.36	0.44	0.51	0.168	0.15	20	20	20	20	20	20	20	3	0	7	10	3	15	1
MR	1	r2C3	356	7.93	8.68	0.44	0.46	0.090	0.04	20	20	20	20	20	20	20	2	0	7	8	3	14	1
MR	1	r2D1	NA	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	7	2	19	19	15	17	16
MR	1	r2D2	NA	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	8	2	20	20	16	17	15
MR	1	r2D3	NA	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	8	0	19	16	16	17	16
MR	4	r3C1	330	8.01	11.51	0.45	0.58	0.091	0.07	20	20	20	20	20	20	20	2	0	2	10	2	20	0
MR	4	r3C2	330	7.95	13.13	0.44	0.62	0.125	0.09	20	20	20	20	20	20	20	1	0	1	9	2	17	0
MR	4	r3C3	356	8.11	11.25	0.45	0.58	0.082	0.07	20	20	20	20	20	20	20	1	0	2	9	1	19	0
MR	4	r3D1	352	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	7	0	21	18	14	15	15
MR	4	r3D2	384	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	7	2	21	16	13	14	14
MR	4	r3D3	390	NA	NA	NA	NA	NA	NA	20	20	20	20	20	20	20	8	0	20	18	13	13	14

Mid Range Samples (continued)			Liquid Percent Recovery						
Dose	Time (days)	Tag	NIC	COT	ACT	SLF	FLX	CRB	PRG
MR	0.083	r1C1	38%	11%	87%	61%	54%	80%	71%
MR	0.083	r1C2	40%	11%	89%	98%	66%	83%	74%
MR	0.083	r1C3	33%	10%	93%	70%	51%	76%	71%
MR	0.083	r1D1	51%	2%	110%	98%	98%	108%	101%
MR	0.083	r1D2	48%	11%	97%	98%	81%	92%	79%
MR	0.083	r1D3	48%	12%	110%	113%	95%	98%	86%
MR	1	r2C1	17%	2%	35%	37%	17%	98%	6%
MR	1	r2C2	13%	2%	36%	49%	17%	77%	3%
MR	1	r2C3	10%	2%	37%	38%	13%	72%	3%
MR	1	r2D1	36%	10%	96%	96%	76%	83%	80%
MR	1	r2D2	39%	11%	100%	101%	82%	84%	76%
MR	1	r2D3	41%	2%	96%	82%	82%	84%	82%
MR	4	r3C1	8%	2%	9%	50%	8%	101%	2%
MR	4	r3C2	7%	2%	3%	43%	8%	87%	2%
MR	4	r3C3	7%	2%	10%	47%	7%	93%	2%
MR	4	r3D1	35%	2%	107%	92%	69%	75%	77%
MR	4	r3D2	33%	11%	107%	80%	67%	69%	71%
MR	4	r3D3	38%	2%	98%	89%	66%	67%	69%

Table 27. Raw data: Mid-High range pharmaceutical removal by duckweed

Mid-High Range Samples										Initial Mass (µg-COC/reactor)							Final Liquid Mass (µg-COC liquid/reactor)						
Dose	Time (days)	Tag	Vol (mL)	Initial Plant (g-fresh)	Final Plant (g-fresh)	Initial Plant (g-dry)	Final Plant (g-dry)	RGR (fresh)	RGR (dry)	NIC	COT	ACT	SLF	FLX	CRB	PRG	NIC	COT	ACT	SLF	FLX	CRB	PRG
MHR	0.08	r1C1	373	8.15	8.28	0.45	0.45	0.191	0.00	250	250	250	250	250	250	250	5	5	205	180	198	263	158
MHR	0.08	r1C2	393	7.94	8.07	0.44	0.44	0.196	0.00	250	250	250	250	250	250	250	5	5	189	239	205	250	158
MHR	0.08	r1C3	382	8.27	8.40	0.46	0.46	0.188	0.00	250	250	250	250	250	250	250	5	5	193	169	198	260	155
MHR	0.08	r1D1	386	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	203	205	198	208	138
MHR	0.08	r1D2	405	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	223	99	305	310	203
MHR	0.08	r1D3	396	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	223	96	363	350	233
MHR	1	r2C1	343	8.15	9.86	0.45	0.48	0.190	0.07	250	250	250	250	250	250	250	5	5	101	5	18	183	8
MHR	1	r2C2	363	7.94	9.60	0.44	0.47	0.190	0.07	250	250	250	250	250	250	250	5	5	113	6	23	203	18
MHR	1	r2C3	351	8.27	10.00	0.46	0.49	0.190	0.07	250	250	250	250	250	250	250	5	5	125	5	20	208	18
MHR	1	r2D1	385	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	185	254	228	238	153
MHR	1	r2D2	404	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	208	230	230	245	163
MHR	1	r2D3	395	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	145	160	175	160	138
MHR	4	r3C1	337	8.15	10.99	0.45	0.54	0.075	0.05	250	250	250	250	250	250	250	5	5	73	131	18	148	5
MHR	4	r3C2	339	7.94	11.97	0.44	0.52	0.103	0.04	250	250	250	250	250	250	250	5	5	78	179	18	178	5
MHR	4	r3C3	331	8.27	11.53	0.46	0.55	0.083	0.05	250	250	250	250	250	250	250	5	5	74	139	15	143	5
MHR	4	r3D1	380	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	190	108	220	220	143
MHR	4	r3D2	399	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	188	221	170	203	135
MHR	4	r3D3	390	NA	NA	NA	NA	NA	NA	250	250	250	250	250	250	250	5	5	173	183	168	158	128

Mid-High Range Samples (continued)			Liquid Percent Recovery						
Dose	Time (days)	Tag	NIC	COT	ACT	SLF	FLX	CRB	PRG
MHR	0.083	r1C1	2%	2%	82%	72%	79%	105%	63%
MHR	0.083	r1C2	2%	2%	76%	96%	82%	100%	63%
MHR	0.083	r1C3	2%	2%	77%	68%	79%	104%	62%
MHR	0.083	r1D1	2%	2%	81%	82%	79%	83%	55%
MHR	0.083	r1D2	2%	2%	89%	40%	122%	124%	81%
MHR	0.083	r1D3	2%	2%	89%	39%	145%	140%	93%
MHR	1	r2C1	2%	2%	41%	2%	7%	73%	3%
MHR	1	r2C2	2%	2%	45%	3%	9%	81%	7%
MHR	1	r2C3	2%	2%	50%	2%	8%	83%	7%
MHR	1	r2D1	2%	2%	74%	102%	91%	95%	61%
MHR	1	r2D2	2%	2%	83%	92%	92%	98%	65%
MHR	1	r2D3	2%	2%	58%	64%	70%	64%	55%
MHR	4	r3C1	2%	2%	29%	53%	7%	59%	2%
MHR	4	r3C2	2%	2%	31%	72%	7%	71%	2%
MHR	4	r3C3	2%	2%	30%	56%	6%	57%	2%
MHR	4	r3D1	2%	2%	76%	43%	88%	88%	57%
MHR	4	r3D2	2%	2%	75%	89%	68%	81%	54%
MHR	4	r3D3	2%	2%	69%	73%	67%	63%	51%

Table 28. Raw data: High range pharmaceutical removal by duckweed

High Range Samples										Initial Mass (µg-COC/reactor)					Final Liquid Mass (µg-COC/reactor)				
Dose	Time (days)	Tag	Vol (mL)	Initial Plant (g-fresh)	Final Plant (g-fresh)	Initial Plant (g-dry)	Final Plant (g-dry)	RGR (fresh)	RGR (dry)	ACT	SLF	FLX	CRB	PRG	ACT	SLF	FLX	CRB	PRG
HR	0.083	r1A1	339	NA	NA	NA	NA	NA	NA	386	355	356	368	350	422	341	338	357	337
HR	0.083	r1A2	381	NA	NA	NA	NA	NA	NA	433	398	399	413	393	397	378	379	390	388
HR	0.083	r1A3	379	NA	NA	NA	NA	NA	NA	432	397	398	412	391	389	367	377	388	366
HR	0.083	r1B1	391	10.50	10.50	0.569	0.569	0.000	0.000	445	409	410	424	403	410	390	366	399	385
HR	0.083	r1B2	378	5.60	5.60	0.254	0.254	0.000	0.000	431	396	397	411	390	373	386	332	372	342
HR	0.083	r1B3	382	6.35	6.35	0.352	0.352	0.000	0.000	434	399	400	414	394	386	378	325	391	350
HR	0.083	r1C1	386	6.50	6.50	0.352	0.352	0.000	0.000	439	404	405	419	398	461	396	377	408	416
HR	0.083	r1C2	386	8.60	8.60	0.390	0.390	0.000	0.000	439	403	404	418	398	360	375	326	354	342
HR	0.083	r1C3	394	10.40	10.40	0.577	0.577	0.000	0.000	449	412	414	428	407	396	368	338	400	362
HR	0.083	r1D1	388	NA	NA	NA	NA	NA	NA	441	406	407	421	400	393	388	387	401	402
HR	0.083	r1D2	352	NA	NA	NA	NA	NA	NA	400	368	369	382	363	353	353	333	341	329
HR	0.083	r1D3	400	NA	NA	NA	NA	NA	NA	455	418	419	434	412	440	409	389	422	395
HR	0.083	r1E1	364	7.32	7.32	0.428	0.428	0.000	0.000	414	381	382	395	376	296	282	261	362	274
HR	0.083	r1E2	371	9.03	9.03	0.528	0.528	0.000	0.000	422	388	389	403	383	302	303	232	354	240
HR	0.083	r1E3	417	7.90	7.90	0.462	0.462	0.000	0.000	474	436	437	452	430	369	372	318	415	322
HR	1	r2A1	334	NA	NA	NA	NA	NA	NA	380	349	350	363	345	408	359	354	374	345
HR	1	r2A2	375	NA	NA	NA	NA	NA	NA	427	392	394	407	387	385	392	361	385	393
HR	1	r2A3	373	NA	NA	NA	NA	NA	NA	425	390	391	405	385	412	392	371	401	386
HR	1	r2B1	384	10.50	9.92	0.569	0.526	-0.057	-0.078	436	401	402	416	396	48	1	184	402	248
HR	1	r2B2	374	5.60	5.52	0.254	0.248	-0.014	-0.025	426	391	392	406	386	162	1	197	369	242
HR	1	r2B3	378	6.35	6.34	0.352	0.344	-0.002	-0.023	430	395	396	410	390	264	2	167	380	258
HR	1	r2C1	369	6.50	6.61	0.352	0.358	0.017	0.018	420	386	387	401	381	194	298	77	374	17
HR	1	r2C2	370	8.60	8.81	0.390	0.408	0.024	0.044	420	386	388	401	381	212	4	96	363	149
HR	1	r2C3	377	10.40	10.54	0.577	0.586	0.013	0.017	429	394	396	409	389	278	8	78	394	202
HR	1	r2D1	368	NA	NA	NA	NA	NA	NA	419	385	386	400	380	390	391	380	402	394
HR	1	r2D2	333	NA	NA	NA	NA	NA	NA	379	348	349	361	343	374	399	354	375	359
HR	1	r2D3	383	NA	NA	NA	NA	NA	NA	436	401	402	416	395	437	424	381	420	398
HR	1	r2E1	345	7.32	*7.32*	0.428	0.428	0.000	no data	392	361	362	374	356	1	1	210	407	248
HR	1	r2E2	356	9.03	*9.03*	0.528	0.528	0.000	no data	405	372	374	387	367	2	1	161	347	187
HR	1	r2E3	400	7.90	*7.90*	0.462	0.462	0.000	no data	455	418	419	434	412	241	1	194	416	273
HR	4	r3A1	377	NA	NA	NA	NA	NA	NA	428	394	395	409	388	368	345	318	360	315

High Range Samples										Initial Mass (µg-COC/reactor)					Final Liquid Mass (µg-COC/reactor)				
Dose	Time (days)	Tag	Vol (mL)	Initial Plant (g-fresh)	Final Plant (g-fresh)	Initial Plant (g-dry)	Final Plant (g-dry)	RGR (fresh)	RGR (dry)	ACT	SLF	FLX	CRB	PRG	ACT	SLF	FLX	CRB	PRG
HR	4	r3A2	398	NA	NA	NA	NA	NA	NA	453	416	418	432	411	374	386	340	369	376
HR	4	r3A3	373	NA	NA	NA	NA	NA	NA	425	390	391	405	385	378	372	339	375	361
HR	4	r3B1	407	10.50	9.92	0.569	0.347	-0.014	-0.124	462	425	426	441	419	319	349	159	380	13
HR	4	r3B2	397	5.60	5.52	0.254	0.217	-0.004	-0.039	452	415	416	431	410	280	341	150	347	47
HR	4	r3B3	398	6.35	6.34	0.352	0.305	0.000	-0.036	453	417	418	432	411	302	337	130	371	47
HR	4	r3C1	370	6.50	6.61	0.352	0.394	0.004	0.028	421	387	388	402	382	274	128	136	385	207
HR	4	r3C2	360	8.60	8.81	0.390	0.517	0.006	0.070	409	376	377	390	371	146	240	46	312	1
HR	4	r3C3	367	10.40	10.54	0.577	0.642	0.003	0.027	417	383	385	398	378	128	262	27	367	1
HR	4	r3D1	332	NA	NA	NA	NA	NA	NA	377	347	348	360	342	349	320	336	371	361
HR	4	r3D2	350	NA	NA	NA	NA	NA	NA	398	366	367	380	361	334	309	308	338	331
HR	4	r3D3	349	NA	NA	NA	NA	NA	NA	398	365	367	379	360	373	370	338	389	373
HR	4	r3E1	347	7.32	*7.32*	0.428	0.428	0.000	no data	394	363	364	376	358	237	309	133	352	25
HR	4	r3E2	369	9.03	*9.03*	0.528	0.528	0.000	no data	419	386	387	400	380	186	275	114	322	1
HR	4	r3E3	373	7.90	*7.90*	0.462	0.462	0.000	no data	424	390	391	405	385	241	310	160	408	1
HR	0.083	r1X1	NA	NA	NA	NA	NA	NA	NA	400	400	400	400	400	384	411	412	412	424
HR	1	r2X2	NA	NA	NA	NA	NA	NA	NA	400	400	400	400	400	408	387	384	384	379
HR	4	r3X3	NA	NA	NA	NA	NA	NA	NA	400	400	400	400	400	408	402	403	404	397

High Range Samples (continued)			Liquid Percent Recovery				
Dose	Time (days)	Tag	ACT	SLF	FLX	CRB	PRG
HR	0.083	r1A1	109%	96%	95%	97%	96%
HR	0.083	r1A2	92%	95%	95%	94%	99%
HR	0.083	r1A3	90%	93%	95%	94%	94%
HR	0.083	r1B1	92%	95%	89%	94%	95%
HR	0.083	r1B2	87%	98%	84%	90%	88%
HR	0.083	r1B3	89%	95%	81%	94%	89%
HR	0.083	r1C1	105%	98%	93%	97%	104%
HR	0.083	r1C2	82%	93%	81%	85%	86%
HR	0.083	r1C3	88%	89%	82%	93%	89%
HR	0.083	r1D1	89%	96%	95%	95%	101%
HR	0.083	r1D2	88%	96%	90%	89%	91%
HR	0.083	r1D3	97%	98%	93%	97%	96%
HR	0.083	r1E1	71%	74%	68%	92%	73%
HR	0.083	r1E2	72%	78%	60%	88%	63%
HR	0.083	r1E3	78%	85%	73%	92%	75%
HR	1	r2A1	107%	103%	101%	103%	100%
HR	1	r2A2	90%	100%	92%	95%	102%
HR	1	r2A3	97%	100%	95%	99%	100%
HR	1	r2B1	11%	0%	46%	97%	63%
HR	1	r2B2	38%	0%	50%	91%	63%
HR	1	r2B3	61%	1%	42%	93%	66%
HR	1	r2C1	46%	77%	20%	93%	5%
HR	1	r2C2	50%	1%	25%	91%	39%
HR	1	r2C3	65%	2%	20%	96%	52%
HR	1	r2D1	93%	102%	98%	101%	104%
HR	1	r2D2	99%	115%	101%	104%	105%
HR	1	r2D3	100%	106%	95%	101%	101%
HR	1	r2E1	0%	0%	58%	109%	70%
HR	1	r2E2	0%	0%	43%	90%	51%
HR	1	r2E3	53%	0%	46%	96%	66%
HR	4	r3A1	86%	88%	81%	88%	81%
HR	4	r3A2	83%	93%	81%	85%	91%
HR	4	r3A3	89%	95%	87%	93%	94%
HR	4	r3B1	69%	82%	37%	86%	3%
HR	4	r3B2	62%	82%	36%	81%	12%

High Range Samples (continued)			Liquid Percent Recovery				
Dose	Time (days)	Tag	ACT	SLF	FLX	CRB	PRG
HR	4	r3B3	67%	81%	31%	86%	12%
HR	4	r3C1	65%	33%	35%	96%	54%
HR	4	r3C2	36%	64%	12%	80%	0%
HR	4	r3C3	31%	68%	7%	92%	0%
HR	4	r3D1	92%	92%	97%	103%	105%
HR	4	r3D2	84%	84%	84%	89%	92%
HR	4	r3D3	94%	101%	92%	102%	103%
HR	4	r3E1	60%	85%	37%	94%	7%
HR	4	r3E2	44%	71%	30%	80%	0%
HR	4	r3E3	57%	80%	41%	101%	0%
HR	0.083	r1X1	96%	103%	103%	103%	106%
HR	1	r2X2	102%	97%	96%	96%	95%
HR	4	r3X3	102%	101%	101%	101%	99%

C.3 Growth Study Appendix Raw Data

Table 29. Raw Data: Wellsville 2011/2008 field and laboratory growth studies

Year[a]	Sample	Start	Finish	Days	D_o	Prod	±Prod	RGR	±RGR	T	SR	E	P	N
2011	1	5/3	5/6	2.88	46	55	6.5	0.275	0.02	7.46	208	14.1	1.39	4.58
2011	2	5/3	5/6	2.88	46	42	5.0	0.227	0.02	7.46	208	14.1	1.39	4.58
2011	3	5/6	5/13	6.91	54	65	9.8	0.114	0.01	9.91	152	14.3	1.39	4.58
2011	4	5/6	5/13	6.91	54	64	9.8	0.114	0.01	9.91	152	14.3	1.39	4.58
2011	5	5/17	5/20	2.77	63	35	4.7	0.158	0.02	7.28	144	14.6	1.39	4.58
2011	6	5/17	5/20	2.77	63	39	4.8	0.174	0.02	7.28	144	14.6	1.39	4.58
2011	7	5/17	5/24	6.77	63	182	20.9	0.201	0.01	9.75	156	14.7	1.39	4.58
2011	8	5/20	5/24	4.00	70	52	16.3	0.139	0.03	11.60	165	14.7	1.39	4.58
2011	9	5/20	5/24	4.00	102	144	16.4	0.220	0.02	11.60	165	14.7	1.39	4.58
2011	10	5/24	5/27	3.06	81	52	18.5	0.161	0.05	11.66	193	14.8	1.39	4.58
2011	11	5/24	5/27	3.06	69	33	18.5	0.127	0.06	11.66	193	14.8	1.39	4.58
2011	12	5/24	5/31	7.02	81	79	9.0	0.097	0.01	9.91	175	14.9	1.39	4.58
2011	13	5/24	5/31	7.02	69	56	6.4	0.084	0.01	9.91	175	14.9	1.39	4.58
2011	14	5/27	5/31	3.96	133	27	8.6	0.047	0.01	8.60	161	14.9	1.39	4.58
2011	15	5/27	5/31	3.96	102	23	6.6	0.051	0.01	8.60	161	14.9	1.39	4.58
2011	16	5/31	6/3	3.00	49	26	17.3	0.143	0.08	12.59	210	15.0	1.68	6.77
2011	17	5/31	6/3	3.00	49	42	17.3	0.208	0.06	11.57	204	15.0	1.66	6.55
2011	18	6/3	6/7	4.00	56	19	9.5	0.073	0.03	13.61	262	15.1	1.83	7.86
2011	19	6/3	6/7	4.00	56	30	9.5	0.108	0.03	13.61	262	15.1	1.83	7.86
2011	20	6/7	6/14	7.23	43	42	4.8	0.094	0.01	14.00	212	15.1	1.83	7.86
2011	21	6/7	6/14	7.23	43	36	4.1	0.084	0.01	14.00	212	15.1	1.83	7.86
2011	22	6/14	6/17	2.75	58	6	0.7	0.037	0.00	15.73	232	15.2	1.83	7.86
2011	23	6/14	6/17	2.75	29	2	0.2	0.020	0.00	15.73	232	15.2	1.83	7.86

Year[a]	Sample	Start	Finish	Days	D_o	Prod	±Prod	RGR	±RGR	T	SR	E	P	N
2011	24	6/17	6/21	4.13	35	22	2.7	0.120	0.01	12.75	210	15.2	1.83	7.86
2011	25	6/17	6/21	4.13	28	10	1.9	0.074	0.01	12.75	210	15.2	1.83	7.86
2011	26	6/17	6/21	4.13	35	23	2.7	0.121	0.01	12.75	210	15.2	1.83	7.86
2011	27	6/17	6/28	11.02	28	39	4.5	0.080	0.01	16.82	253	15.2	1.83	7.86
2011	28	6/21	6/28	6.90	20	37	4.2	0.152	0.01	19.15	277	15.2	1.83	7.86
2011	29	6/21	6/28	6.90	38	29	3.4	0.084	0.01	19.15	277	15.2	1.83	7.86
2011	30	6/21	6/28	6.90	60	24	4.2	0.049	0.01	19.15	277	15.2	1.83	7.86
2011	31	7/1	7/8	7.00	19	13	3.3	0.075	0.01	22.16	238	15.1	1.39	4.82
2011	32	7/1	7/8	7.00	20	20	4.9	0.099	0.02	22.16	238	15.1	1.39	4.82
2011	33	7/1	7/8	7.00	37	18	2.7	0.057	0.01	22.16	238	15.1	1.39	4.82
2011	34	7/1	7/15	14.06	19	18	3.8	0.047	0.01	21.90	223	15.0	1.39	4.82
2011	35	7/1	7/15	14.06	20	25	5.4	0.059	0.01	21.90	223	15.0	1.39	4.82
2011	36	7/1	7/15	14.06	37	26	3.0	0.038	0.00	21.90	223	15.0	1.39	4.82
2011	37	7/8	7/15	7.06	33	5	0.5	0.019	0.00	21.64	208	15.0	1.39	4.82
2011	38	7/8	7/15	7.06	40	6	0.6	0.019	0.00	21.64	208	15.0	1.39	4.82
2011	39	7/8	7/15	7.06	56	8	0.9	0.019	0.00	21.64	208	15.0	1.39	4.82
2011	40	7/15	7/22	7.17	11	0	0.0	0.000	0.00	22.97	244	14.8	1.39	4.82
2011	41	7/15	7/22	7.17	8	0	0.5	0.006	0.01	22.97	244	14.8	1.39	4.82
2011	42	7/15	7/22	7.17	10	4	5.8	0.050	0.06	22.97	244	14.8	1.39	4.82
2011	43	7/22	7/26	3.73	16	5	1.1	0.076	0.01	21.64	258	14.7	1.39	4.82
2011	44	7/22	7/26	3.73	12	4	0.8	0.078	0.01	21.64	258	14.7	1.39	4.82
2011	45	7/22	7/26	3.73	18	2	1.1	0.024	0.01	21.64	258	14.7	1.39	4.82
2011	46	7/22	8/2	10.73	16	36	4.1	0.108	0.01	18.58	218	14.6	1.42	4.81
2011	47	7/22	8/2	10.73	12	30	3.4	0.117	0.01	18.58	218	14.6	1.42	4.81
2011	48	7/22	8/2	10.73	18	15	1.7	0.054	0.01	18.58	218	14.6	1.42	4.81
2011	49	7/26	8/2	7.00	22	31	3.5	0.125	0.02	16.84	195	14.5	1.43	4.81
2011	50	7/26	8/2	7.00	16	26	3.0	0.138	0.02	16.84	195	14.5	1.43	4.81

Year[a]	Sample	Start	Finish	Days	D_o	Prod	±Prod	RGR	±RGR	T	SR	E	P	N
2011	51	7/26	8/2	7.00	20	13	1.6	0.070	0.02	16.84	195	14.5	1.43	4.81
2011	52	8/2	8/4	2.00	15	10	1.2	0.252	0.06	14.45	167	14.3	1.72	4.73
2011	53	8/2	8/4	2.00	12	14	1.6	0.382	0.05	14.45	167	14.3	1.72	4.73
2011	54	8/4	8/8	3.96	18	16	1.8	0.162	0.03	20.27	235	14.2	1.72	4.73
2011	55	8/4	8/8	3.96	14	12	1.3	0.151	0.03	20.27	235	14.2	1.72	4.73
2011	56	8/4	8/8	3.96	16	7	1.2	0.088	0.04	20.27	235	14.2	1.72	4.73
2011	57	8/8	8/11	3.00	14	8	1.1	0.143	0.04	21.17	240	14.1	1.72	4.73
2011	58	8/8	8/11	3.00	26	10	1.8	0.105	0.06	21.17	240	14.1	1.72	4.73
2011	59	8/8	8/11	3.00	23	12	1.7	0.137	0.04	21.17	240	14.1	1.72	4.73
2011	60	8/11	8/19	8.11	25	62	7.1	0.153	0.03	21.80	226	13.9	1.72	4.73
2011	61	8/11	8/19	8.11	15	40	4.5	0.162	0.03	21.80	226	13.9	1.72	4.73
2011	62	8/11	8/19	8.11	12	45	5.2	0.191	0.04	21.80	226	13.9	1.72	4.73
2011	63	8/19	8/23	4.18	39	48	5.4	0.191	0.04	22.36	195	13.6	1.72	4.73
2011	64	8/19	8/23	4.18	26	35	4.0	0.203	0.05	22.36	195	13.6	1.72	4.73
2011	65	8/23	9/15	22.96	36	339	38.8	0.102	0.01	20.92	179	13.0	1.22	4.52
2011	66	8/23	9/15	22.96	19	280	32.0	0.121	0.02	20.92	179	13.0	1.22	4.52
2011	67	9/15	9/21	5.96	30	36	4.1	0.134	0.02	15.19	132	12.4	0.91	4.39
2011	68	9/15	9/21	5.96	40	43	4.9	0.121	0.02	15.19	132	12.4	0.91	4.39
2011	69	9/21	10/14	23.02	37	25	2.9	0.023	0.00	13.84	119	11.7	0.96	4.77
2011	70	9/21	10/14	23.02	65	37	4.3	0.020	0.00	13.84	119	11.7	0.96	4.77
2011	71	10/14	10/19	5.10	142	-69	-7.9	-0.129	0.01	11.26	104	11.1	1.01	5.07
2011	72	10/14	10/19	5.10	127	-64	-7.3	-0.136	0.02	11.26	104	11.1	1.01	5.07
2008	73	8/29	9/2	4.00	42	8	0.9	0.044	0.00	19.34	259	13.1	1.52	4.65
2008	74	8/29	9/2	4.00	24	9	0.1	0.083	0.01	19.34	259	13.1	1.52	4.65
2008	75	8/29	9/5	7.00	42	20	0.4	0.056	0.00	16.26	250	13.1	1.25	4.54
2008	76	9/2	9/5	3.00	24	2	0.0	0.023	0.00	12.16	238	13.0	0.91	4.39
2008	77	9/2	9/5	3.00	42	9	0.5	0.062	0.01	12.16	238	13.0	0.91	4.39

Year[a]	Sample	Start	Finish	Days	D_o	Prod	±Prod	RGR	±RGR	T	SR	E	P	N
2008	78	9/5	9/15	10.00	24	9	0.1	0.033	0.00	15.34	223	12.7	0.91	4.39
2008	79	9/5	9/15	10.00	42	54	4.0	0.082	0.01	15.34	223	12.7	0.91	4.39
2008	80	9/15	11/11	57.00	24	555	307.8	0.056	0.00	9.77	153	11.2	0.95	4.92
Lab	81	8/4	8/23	19.00	87	8	0.8	0.005	0.00	20.00	85	7.4	2.39	7.25
Lab	82	8/23	8/25	2.00	77	13	1.3	0.076	0.01	20.00	85	7.4	0.50	0.15
Lab	83	8/25	8/27	2.00	84	14	1.5	0.079	0.01	21.00	85	7.4	0.17	0.15
Lab	84	8/27	8/30	3.00	90	16	1.7	0.056	0.00	22.00	85	7.4	0.60	0.70
Lab	85	9/1	9/6	5.00	90	23	2.4	0.045	0.00	22.00	85	7.4	1.00	3.00
Lab	86	9/6	9/10	4.00	91	8	0.9	0.022	0.00	22.00	85	7.4	1.50	5.00
Lab	87	9/10	9/13	3.00	83	1	0.1	0.003	0.00	22.00	85	7.4	1.40	1.90
Lab	88	9/13	9/14	1.00	81	3	0.3	0.039	0.01	22.00	85	7.4	1.40	1.90
Lab	89	9/14	9/15	1.00	81	3	0.4	0.041	0.01	22.00	85	7.4	1.40	1.90
Lab	90	9/15	9/17	2.00	80	26	2.8	0.142	0.03	22.00	85	7.4	1.12	4.70
Lab	91	9/17	9/18	1.00	94	12	1.3	0.121	0.03	22.00	85	7.4	1.12	4.70
Lab	92	9/18	9/20	2.00	82	2	0.2	0.010	0.00	22.00	85	7.4	1.12	4.70
Lab	93	9/20	9/24	4.00	76	28	2.9	0.077	0.01	22.00	85	7.4	1.05	3.50
Lab	94	9/24	9/27	3.00	48	17	1.8	0.102	0.01	22.00	85	7.4	1.08	3.50
Lab	95	9/27	9/29	2.00	45	11	1.2	0.113	0.02	22.00	85	7.4	0.85	3.00
Lab	96	9/29	10/1	2.00	39	12	1.2	0.132	0.02	22.00	85	7.4	0.85	3.00
Lab	97	10/1	10/4	3.00	40	20	2.1	0.137	0.02	22.00	85	7.4	0.85	3.00
Lab	98	10/4	10/6	2.00	43	12	1.3	0.124	0.02	23.75	85	7.4	1.80	5.00
Lab	99	10/6	10/8	2.00	43	23	2.4	0.218	0.06	25.50	85	7.4	1.80	5.00
Lab	100	10/8	10/11	3.00	39	16	1.7	0.117	0.02	25.50	85	7.4	4.00	15.00
Lab	101	10/11	10/15	4.00	37	25	2.6	0.129	0.02	25.50	85	7.4	4.00	15.00
Lab	102	10/15	10/18	3.00	40	15	1.6	0.109	0.02	25.50	85	7.4	4.25	21.00
Lab	103	10/18	10/20	2.00	38	10	1.1	0.118	0.02	25.50	85	7.4	4.25	21.00
Lab	104	10/20	10/22	2.00	40	11	1.1	0.116	0.02	25.50	85	7.4	4.67	25.00

Year[a]	Sample	Start	Finish	Days	D_o	Prod	±Prod	RGR	±RGR	T	SR	E	P	N
Lab	105	10/22	10/27	5.00	38	31	3.3	0.120	0.02	25.50	85	7.4	4.67	25.00
Lab	106	10/27	11/3	7.00	37	40	4.2	0.105	0.01	25.50	85	7.4	3.59	19.26
Lab	107	11/3	11/6	3.00	21	10	1.1	0.133	0.02	25.50	85	7.4	3.40	18.70
Lab	108	11/6	11/8	2.00	24	8	0.8	0.139	0.03	25.50	85	7.4	3.40	18.70
Lab	109	11/8	11/13	5.00	16	19	2.0	0.156	0.03	25.50	85	7.4	2.34	11.08
Lab	110	11/13	11/19	6.00	25	18	1.9	0.091	0.01	25.50	85	7.4	1.64	6.00
Lab	111	11/19	11/22	3.00	22	20	2.1	0.212	0.05	25.50	85	7.4	0.08	0.34
Lab	112	11/22	11/23	1.00	21	3	0.3	0.125	0.03	25.50	85	7.4	0.05	0.02
Lab	113	11/23	11/29	6.00	24	27	2.8	0.126	0.02	25.50	85	7.4	0.05	0.02
Lab	114	11/29	12/6	7.00	45	35	3.7	0.082	0.01	25.50	85	7.4	0.20	0.02
Lab	115	12/6	12/13	7.00	40	41	4.3	0.100	0.01	25.50	85	7.4	0.05	0.14
Lab	116	12/13	12/20	7.00	51	45	4.7	0.090	0.01	25.50	85	7.4	0.54	0.50

[a] Column descriptions: Start/Finish = mm/dd; Days = growth period; D_o = starting density (g-dry/m^2); Prod = duckweed production (g-dry/m^2); RGR = relative growth rate; ± = standard deviation; T = average daily temperature (°C); SR = average daily solar radiation (W/m^2); E = photoperiod (hrs); P = average phosphorus concentration; N = average nitrogen concentration

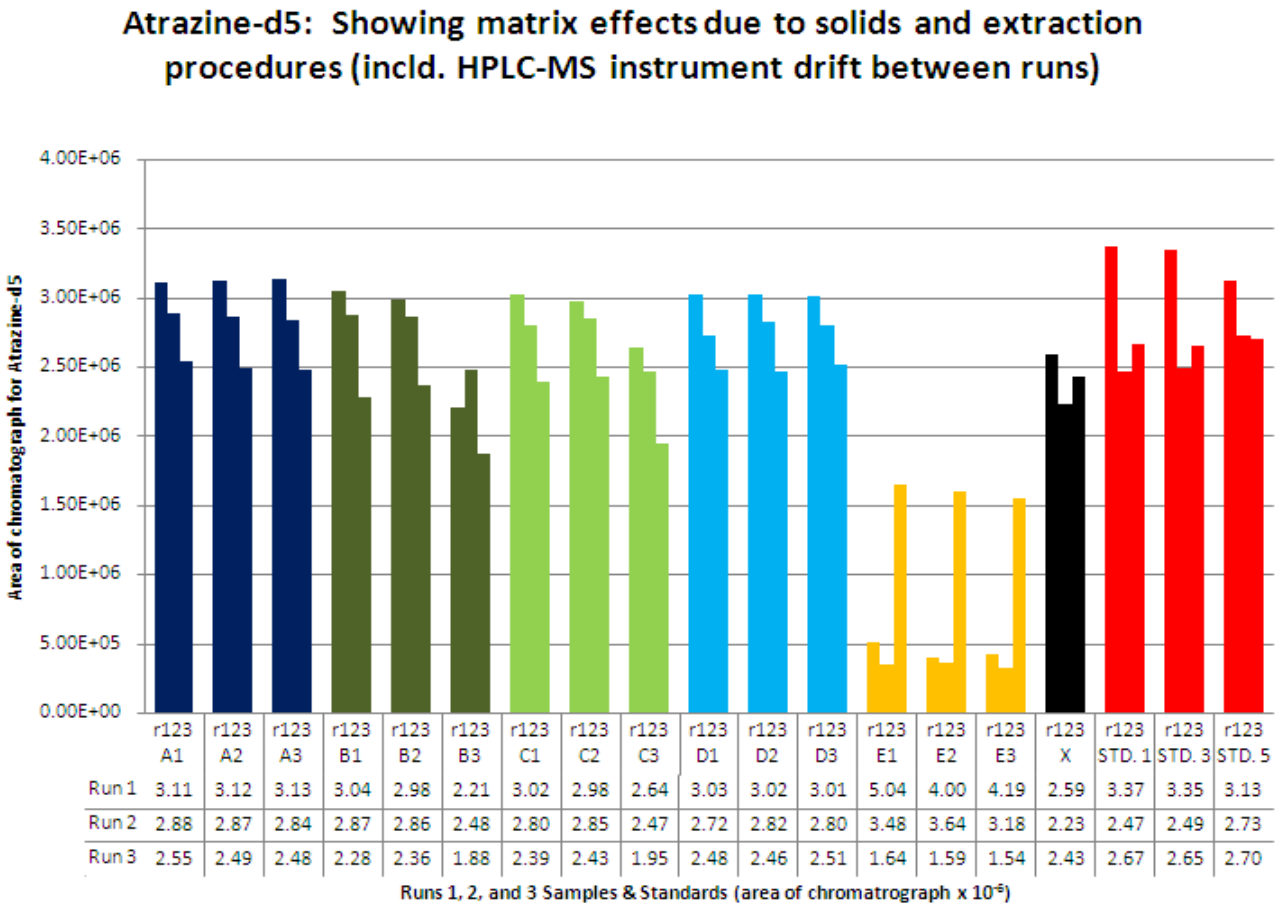
Appendix D Miscellaneous Results and Discussion

D.1 Results and Dicussion: Phosphorus

Table 30. Phosphorus accounted for in plant tissue at 24 hours and 3 days

Reactor	Time (days)	mg-$P_{out\ liquid}$	mg-$P_{out\ liquid}$ + mg-$P_{plant\ initial}$	*%P_predicted*	%P_meas	$mg_{dry\ duckweed}$	RGR
B	3	-1.51	2.59	*1.10*	1.10	234	-0.179
B	3	-0.48	2.29	*1.33*	1.29	172	-0.151
B	3	-1.67	1.22	*0.51*	1.05	237	-0.058
C	3	1.83	4.52	*1.22*	1.23	372	0.115
C	3	1.68	4.61	*1.38*	1.19	333	0.050
C	3	1.61	4.98	*1.24*	1.30	401	0.065
B	1	0.15	5.60	*1.27*	1.27	440	0.042
B	1	0.24	5.69	*1.21*	1.39	470	0.108
B	1	-0.34	5.05	*1.23*	1.38	410	-0.018
C	1	1.18	6.51	*1.39*	1.54	470	0.130
C	1	1.15	6.52	*1.36*	1.25	480	0.144
C	1	1.14	6.51	*1.41*	1.79	460	0.101
X	1	NA	NA	NA	1.25	NA	NA
X	1	NA	NA	NA	1.30	NA	NA
X	1	NA	NA	NA	1.32	NA	NA
X	3	NA	NA	NA	1.02	NA	NA
X	3	NA	NA	NA	1.01	NA	NA
X	3	NA	NA	NA	1.03	NA	NA
Reactors: B = whole plants kept in the dark; C = whole plants kept in the light; X = nutrient solutions without plants							

Table 30 summarizes results from two microcosm studies—one ending when time = 1 day and the other when time = 3 days. Initial and final phosphorus tissue concentrations were measured in each study, along with phosphorus liquid concentrations. From these measurements a predicted and measured %P-tissue was calculated. Student's t-test (alpha = 0.05) showed that there was not difference between the predicted/measured values. Concluding non-aqeuous phosphorus was accounted for in the plant tissue.



	r123 A1	r123 A2	r123 A3	r123 B1	r123 B2	r123 B3	r123 C1	r123 C2	r123 C3	r123 D1	r123 D2	r123 D3	r123 E1	r123 E2	r123 E3	r123 X	r123 STD. 1	r123 STD. 3	r123 STD. 5
Run 1	3.11	3.12	3.13	3.04	2.98	2.21	3.02	2.98	2.64	3.03	3.02	3.01	5.04	4.00	4.19	2.59	3.37	3.35	3.13
Run 2	2.88	2.87	2.84	2.87	2.86	2.48	2.80	2.85	2.47	2.72	2.82	2.80	3.48	3.64	3.18	2.23	2.47	2.49	2.73
Run 3	2.55	2.49	2.48	2.28	2.36	1.88	2.39	2.43	1.95	2.48	2.46	2.51	1.64	1.59	1.54	2.43	2.67	2.65	2.70

Figure 53. Matrix intereferences compensated for by internal standards and matrix standards.

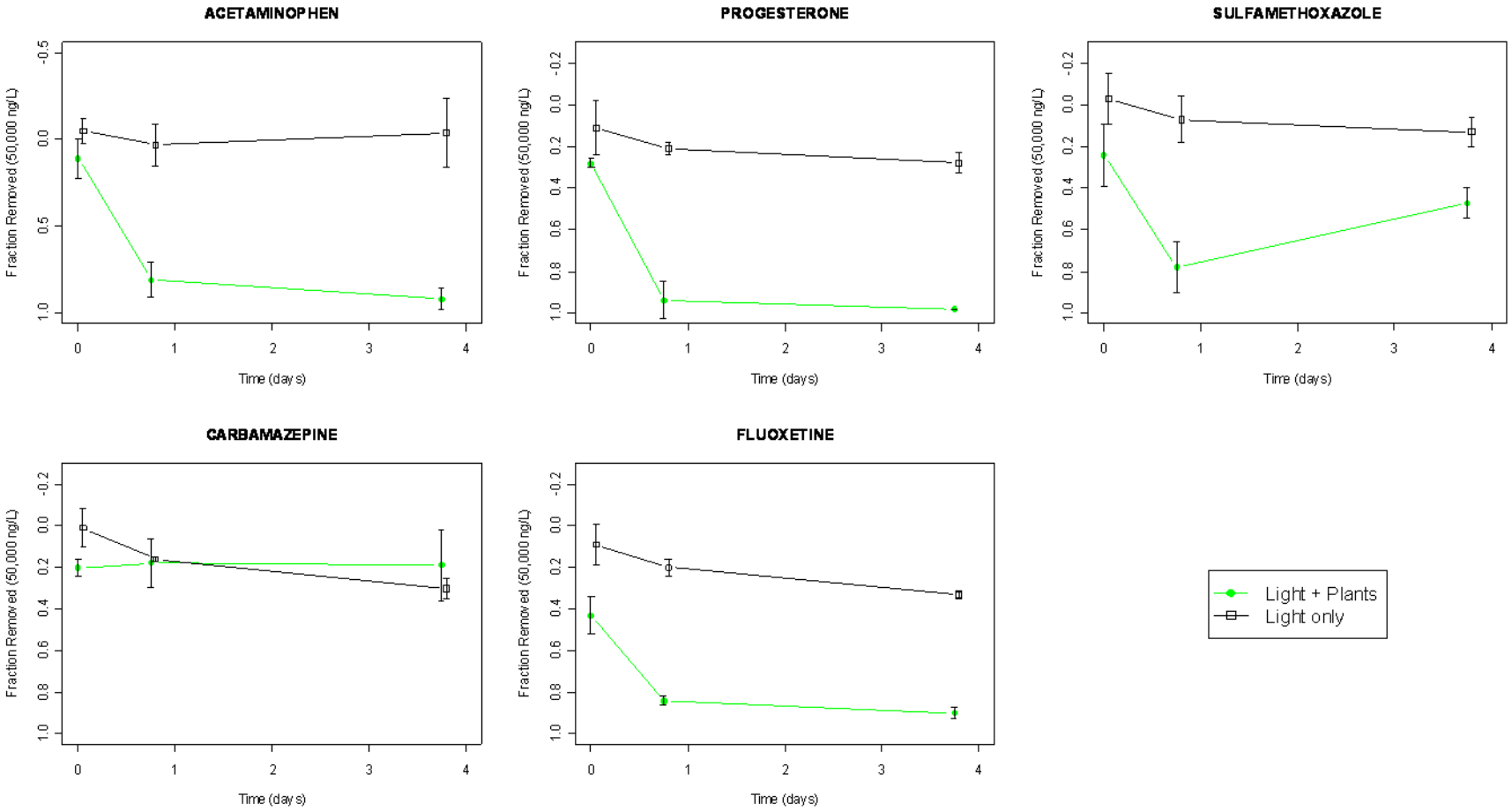


Figure 54. Medium range (50,000 ng/L) liquid removal of five pharmaceuticals.

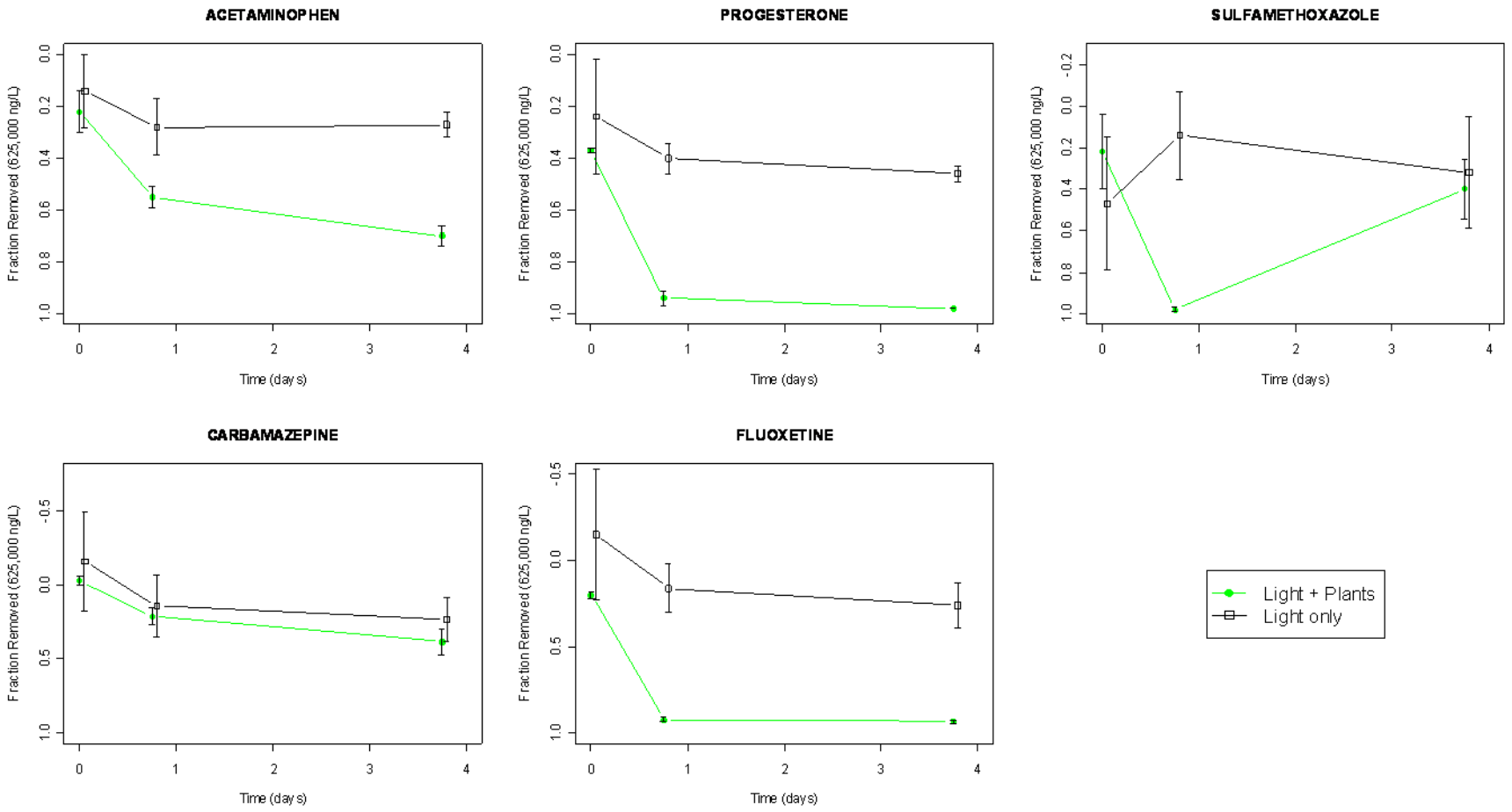


Figure 55. Medium-High range (625,000 ng/L) liquid removal of five pharmaceuticals.

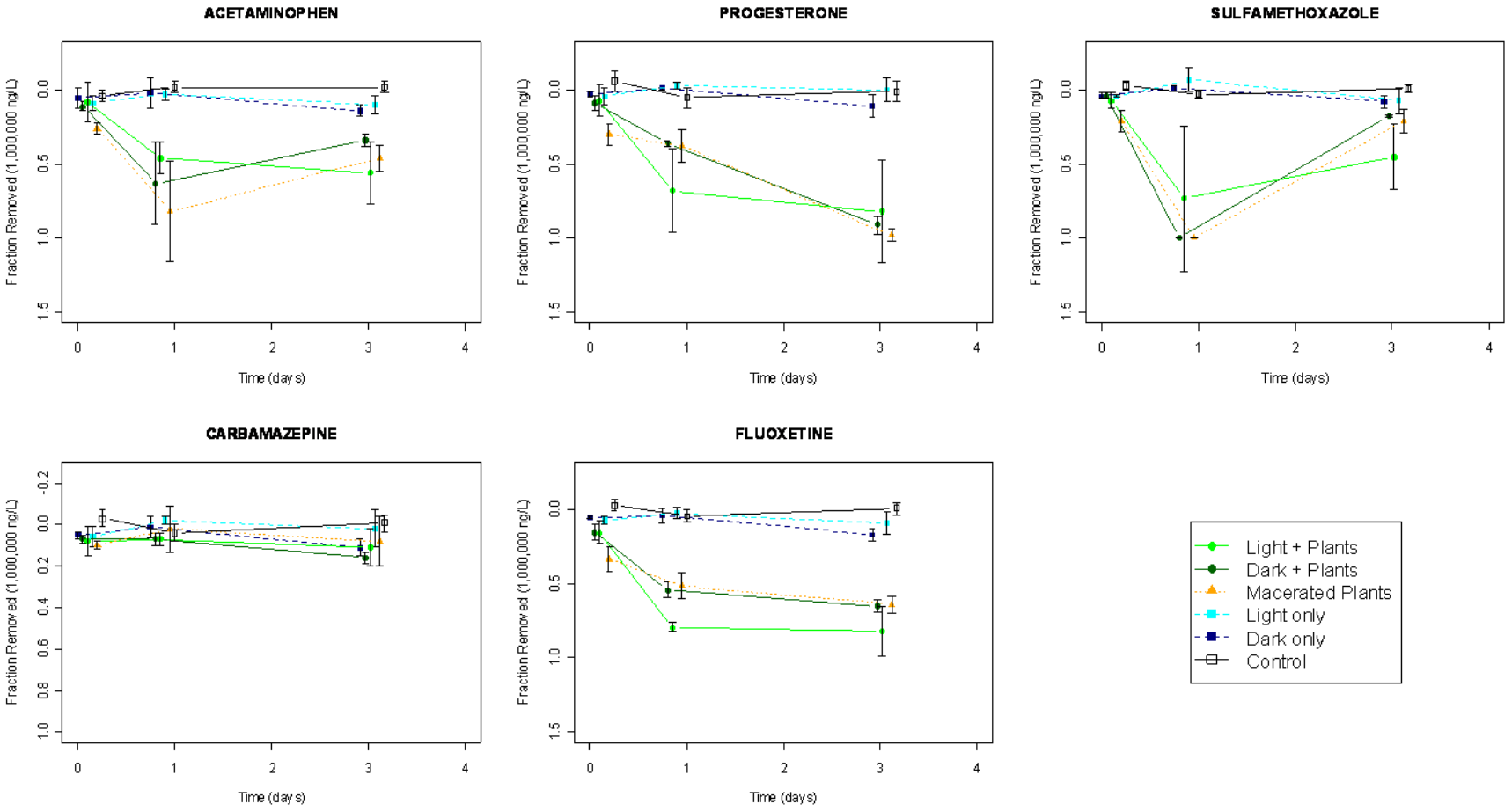


Figure 56. High Range (1,000,000 ng/L) liquid removal of five pharmaceuticals.

Table 31. Comparison between treatments using Tukey's honest significant difference (TukeyHSD) multiple comparison of means with 95% family-wise confidence level

		ACT	PRG	SLF	CRB	FLX
***cf.* treatments**	**Time**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
B-A	0.08	0.927	0.017	0.999	0.665	0.600
C-A	0.08	1.000	0.069	0.776	0.743	0.943
D-A	0.08	0.804	1.000	1.000	0.966	0.844
E-A	0.08	0.106	0.000	0.003	0.521	0.003
C-B	0.08	0.970	0.890	0.885	1.000	0.943
D-B	0.08	0.381	0.015	0.995	0.337	0.989
E-B	0.08	0.031	0.000	0.002	0.999	0.024
D-C	0.08	0.708	0.060	0.704	0.400	0.998
E-C	0.08	0.080	0.000	0.001	0.994	0.008
E-D	0.08	0.472	0.000	0.003	0.239	0.012
B-A	1	0.393	0.000	0.116	0.982	0.023
C-A	1	0.029	0.000	0.147	0.961	0.001
D-A	1	1.000	0.869	1.000	0.999	0.921
E-A	1	1.000	0.000	0.376	0.263	0.002
C-B	1	0.429	0.624	1.000	1.000	0.144
D-B	1	0.429	0.000	0.136	0.931	0.081
E-B	1	0.393	0.032	0.910	0.122	0.576
D-C	1	0.032	0.000	0.171	0.889	0.002
E-C	1	0.029	0.274	0.955	0.101	0.811
E-D	1	1.000	0.000	0.427	0.363	0.008
B-A	4	0.000	0.000	0.000	0.001	0.000
C-A	4	0.000	0.000	0.000	0.160	0.000
D-A	4	0.383	0.439	0.469	1.000	0.182
E-A	4	0.001	0.000	0.000	0.015	0.000
C-B	4	0.000	1.000	0.000	0.055	0.055
D-B	4	0.000	0.000	0.003	0.001	0.001
E-B	4	0.002	0.133	0.003	0.000	0.012
D-C	4	0.000	0.000	0.000	0.160	0.000
E-C	4	0.000	0.165	0.394	0.000	0.854
E-D	4	0.014	0.000	0.000	0.015	0.000

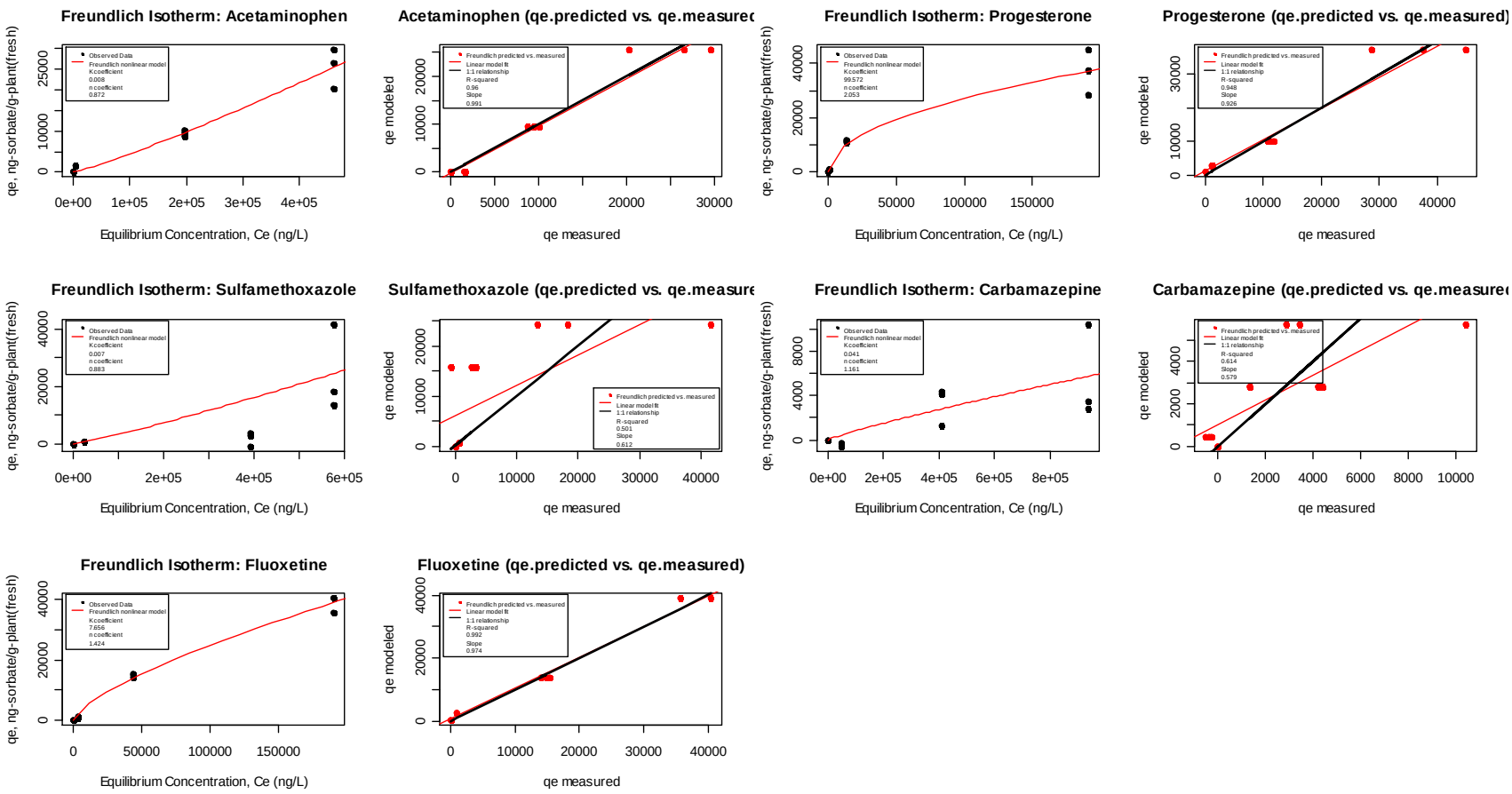


Figure 57. Isotherms at 25 °C for five pharmaceuticals particularly fluoxetine and progesterone.

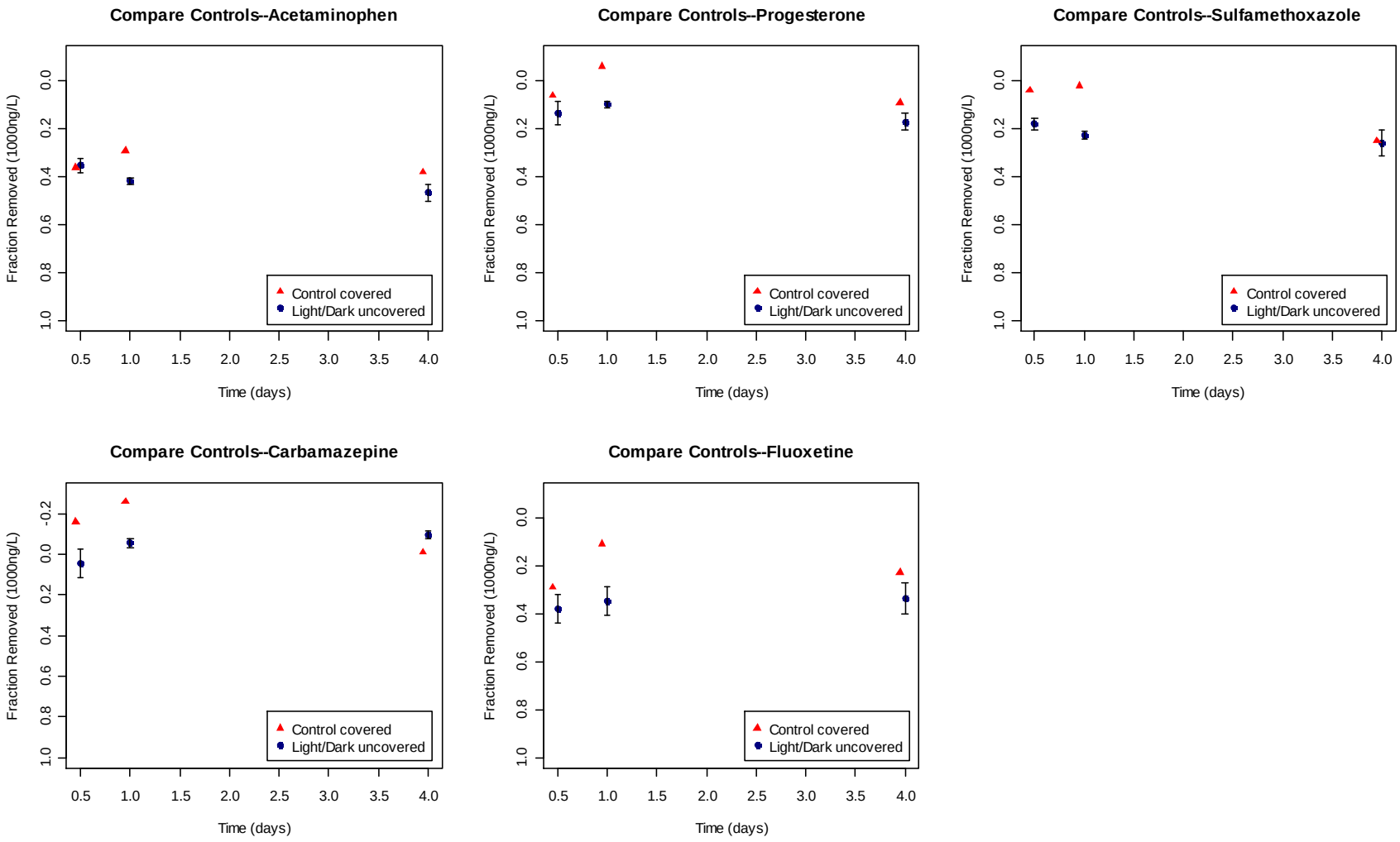


Figure 58. Pharmaceutical losses in covered and uncovered control reactors.

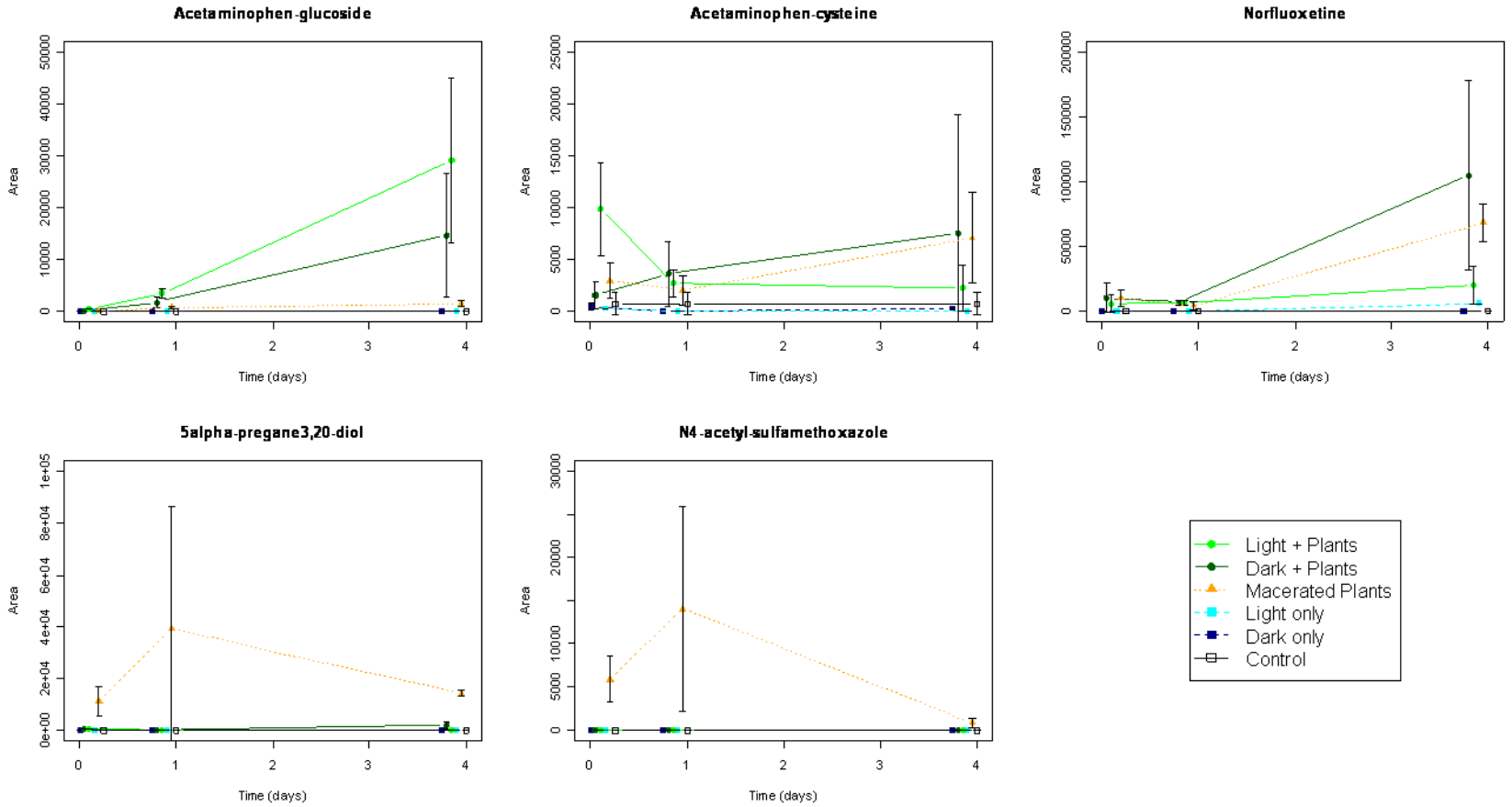


Figure 59. Qualitative search for potential metabolites in liquid solution.
Acetaminophen metabolites: acetaminophen-glucoside (molecular formula C14H19NO7, m/z = 314.1234) and acetaminophen-cysteine (molecular formula C11H14N2O4S, m/z = 271.0747); Fluoxetine metabolite: Norfluoxetine (molecular formula C16H16F3NO, m/z = 296.1257); Progesterone metabolite: 5-alpha-pregnane3,20-diol (molecular formula C21H32O2, m/z = 317.2475); Sulfamethoxazole metabolite: N4-acetyl-sulfamethoxazole (molecular formula: C12H13N3O4S, m/z = 296.0700), and Carbamazepine metabolite Carbamazepine-10,11-epoxide (C15H12N2O2).

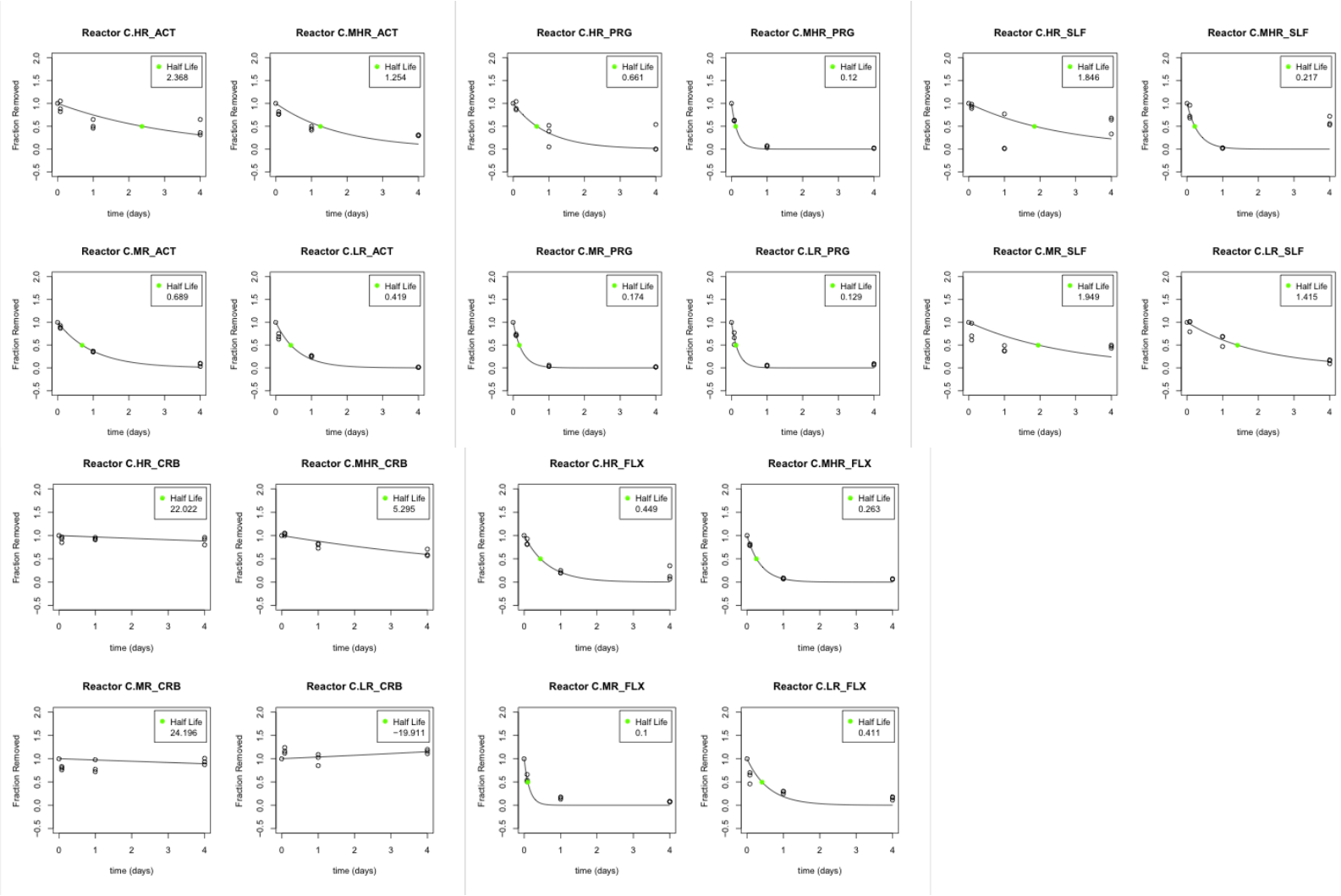


Figure 60. 50% Aqeuous depletion (a.k.a. half-life) of five pharmaceutical in whole plant + light reactors at four concentrations.

D.3 Results and Discussion: Duckweed Growth

Table 32. Field and laboratory coefficients to estimate duckweed growth using Landesman, Driever, and Lasfar models

Field coefficients to estimate duckweed growth								
Landesman model coefficients			Driever model coefficients			Lasfar model coefficients		
A1	0.308		T_{min}	5	°C	R	0.62	1/d
A2	7.18		T_{opt}	26	°C	θ1	0.0025	Temp
A3	0.201		hN	0.04	mg-N/L	θ2	0.66	Temp
A4	7.01		hP	0.05	mg-P/L	θ3	0.0073	Photo
T_{opt}	26	°C	hB	26	g-dry/m^2	θ4	0.65	Photo
SR_{opt}	138	W/m2	r_{max}	1.15	1/d	T_{opt}	26	°C
$A1_{spring}$	0.34		L	0.01	decay	E_{opt}	13	Photo
T_{opt_spring}	7.5	°C	T_{min_spring}	26	°C	K_P	0.31	Phos
			T_{opt_spring}	7.5	°C	K_{iP}	101	max P
			$r_{\_spring}$	0.70	1/d	K_N	0.95	Nitrogen
						K_{iN}	604	max N
						DL	277	g-dry/m^2
						T_{op_spring}	7.5	°C
						$R_{\_spring}$	0.62	1/d

Laboratory coefficients to estimate duckweed growth								
Landesman model coefficients			Driever model coefficients			Lasfar model coefficients		
A1	0.308		T_{min}	5	°C	R	0.62	1/d
A2	7.18		T_{opt}	26	°C	θ1	0.0025	Temp
A3	0.201		hN	0.04	mg-N/L	θ2	0.66	Temp
A4	7.01		hP	0.05	mg-P/L	θ3	0.0073	Photo
T_{opt}	26	°C	hB	26	g-dry/m^2	θ4	0.65	Photo
SR_{opt}	138	W/m2	r_{max}	1.15	1/d	T_{opt}	26	°C
$A1_{spring}$	0.34		L	0.01	decay	E_{opt}	13	Photo
T_{opt_spring}	7.5	°C	T_{min_spring}	26	°C	K_P	0.31	Phos
			T_{opt_spring}	7.5	°C	K_{iP}	101	max P
			$r_{\_spring}$	0.704	1/d	K_N	0.95	Nitrogen
						K_{iN}	604	max N
						DL	277	g-dry/m^2
						T_{op_spring}	7.5	°C
						$R_{\_spring}$	0.62	1/d

coefficients to model post-turion germination

coefficients to model turion-germination

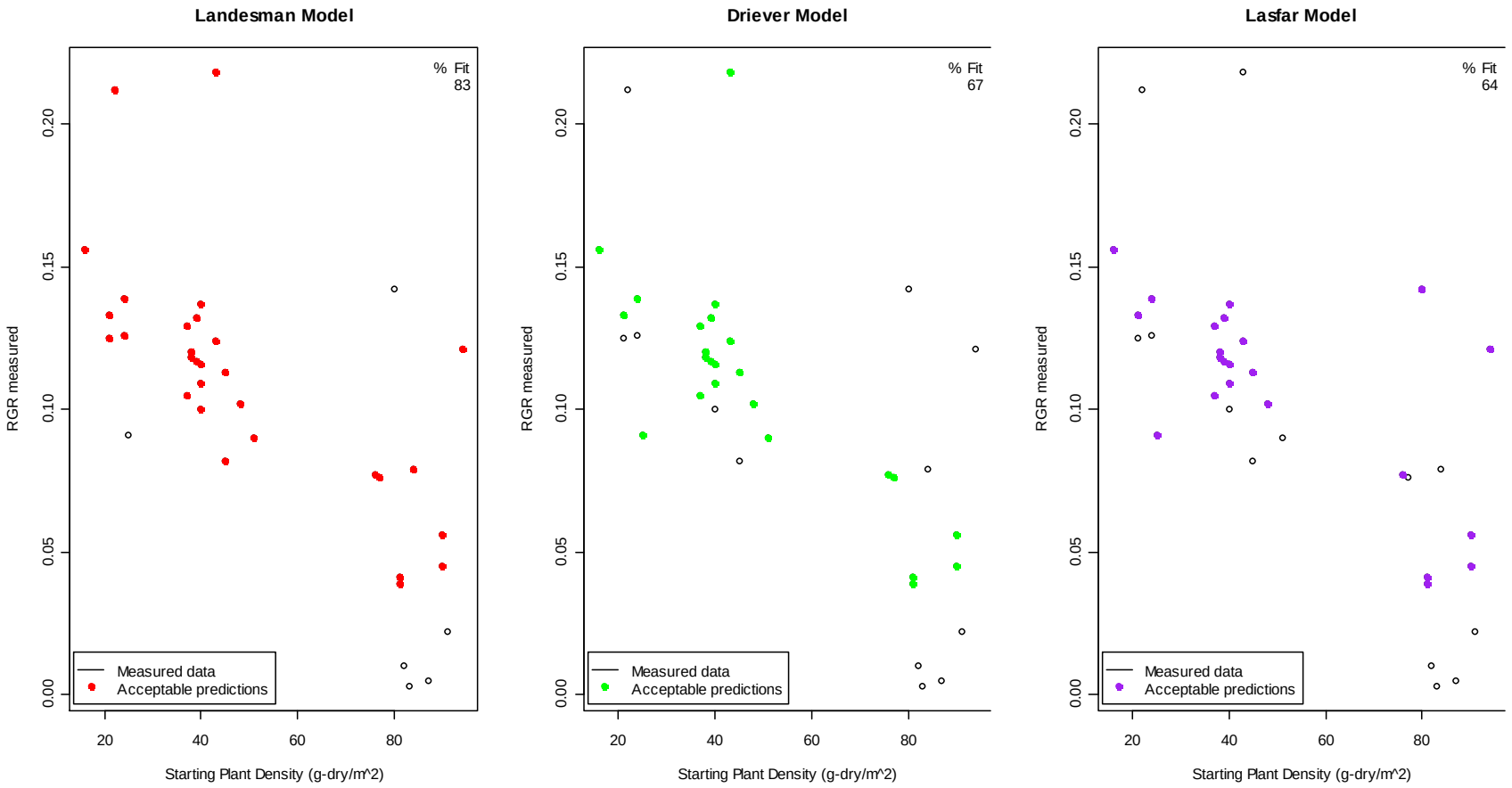


Figure 61. Laboratory measured and acceptable predictions of relative growth rate (RGR) versus starting plant density.

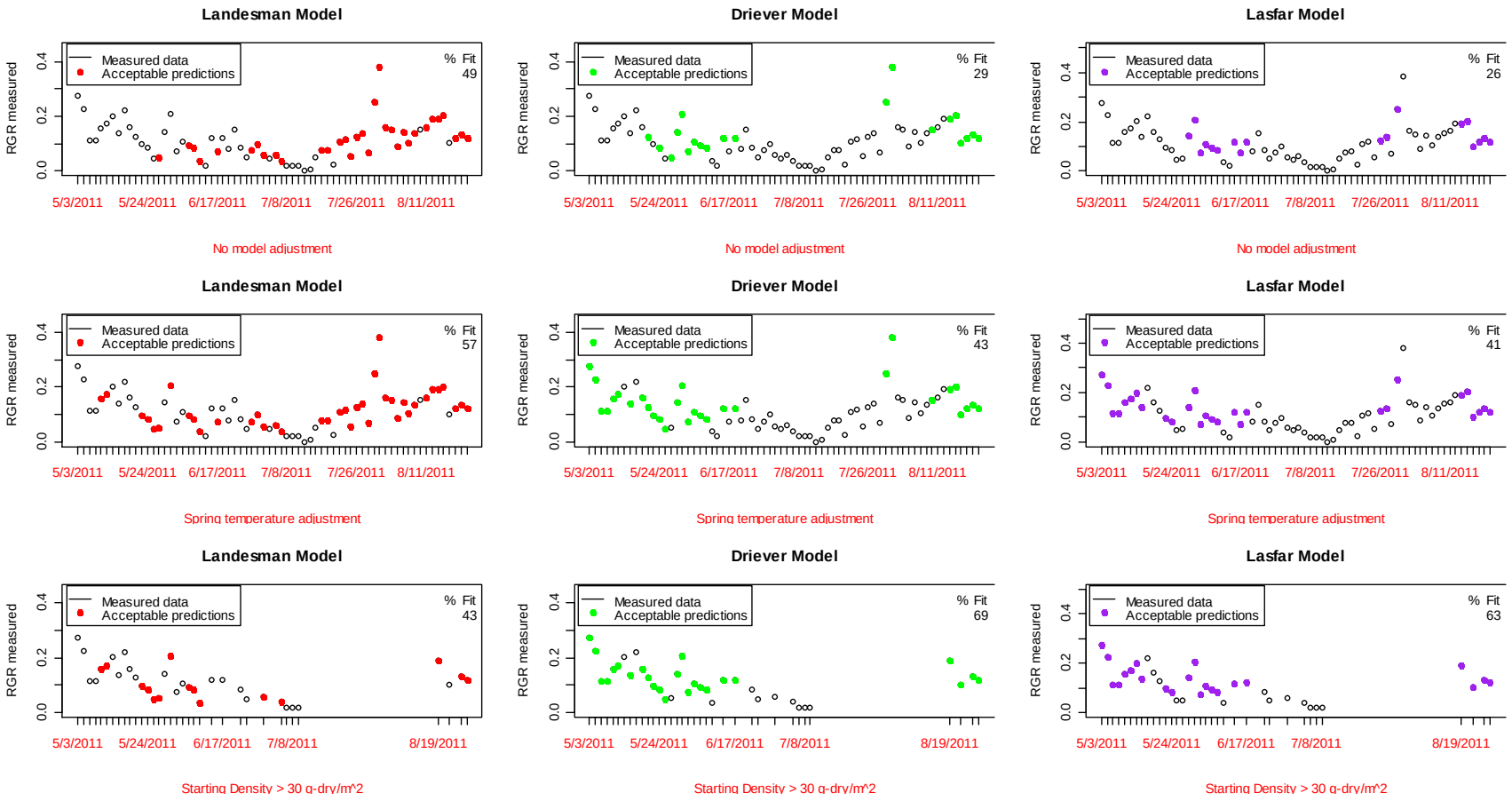


Figure 62. 2011 field predictions vs. measured with and without inverting temperature function and with selecting starting densities above 30 g-dry/m^2.

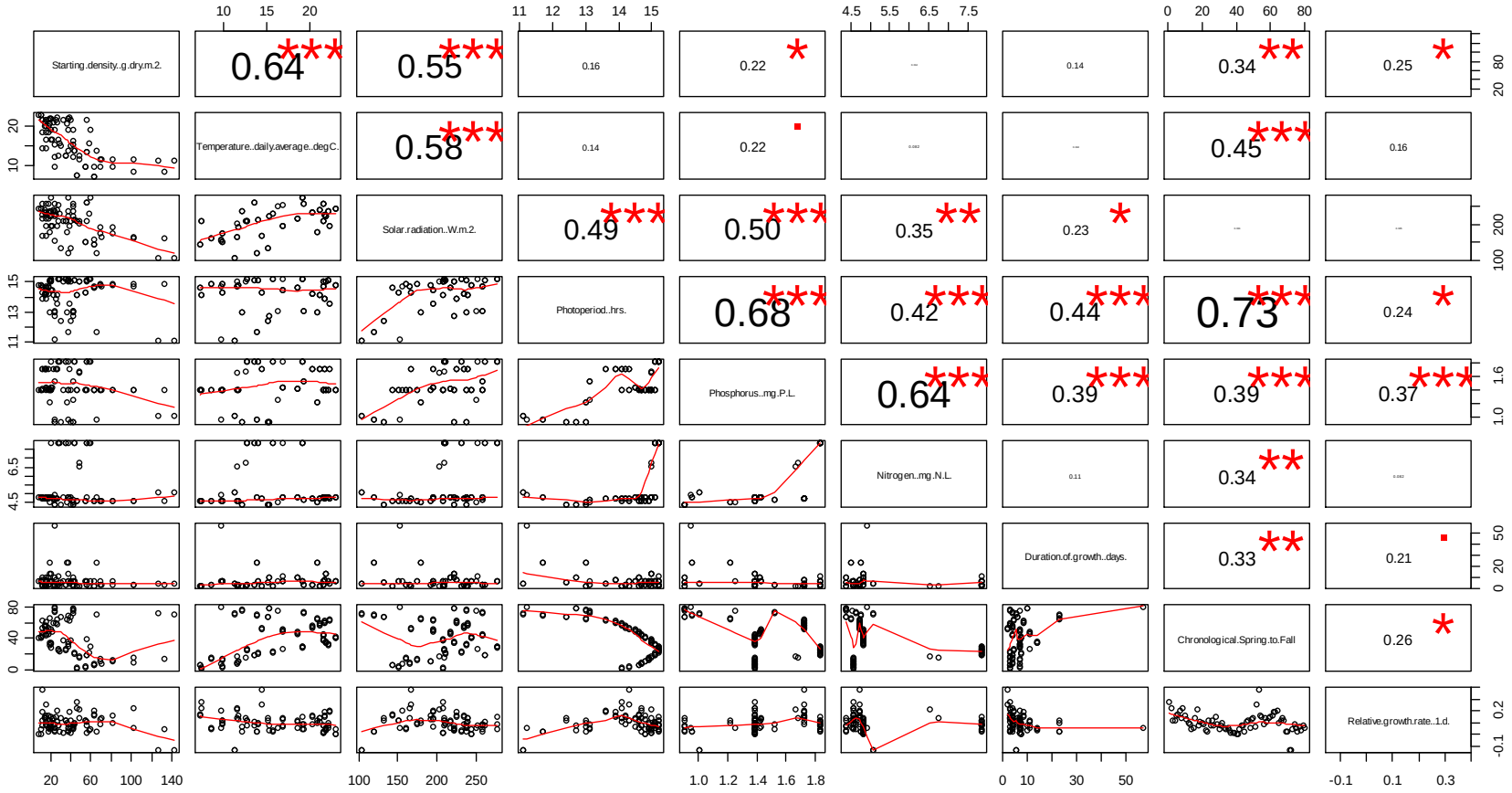


Figure 63. Checking multicolinearity between independent variables used for estimating 2011 growth rates with significance p-values (high correlation "***" = higher number while lower correlation "*" = lower value).

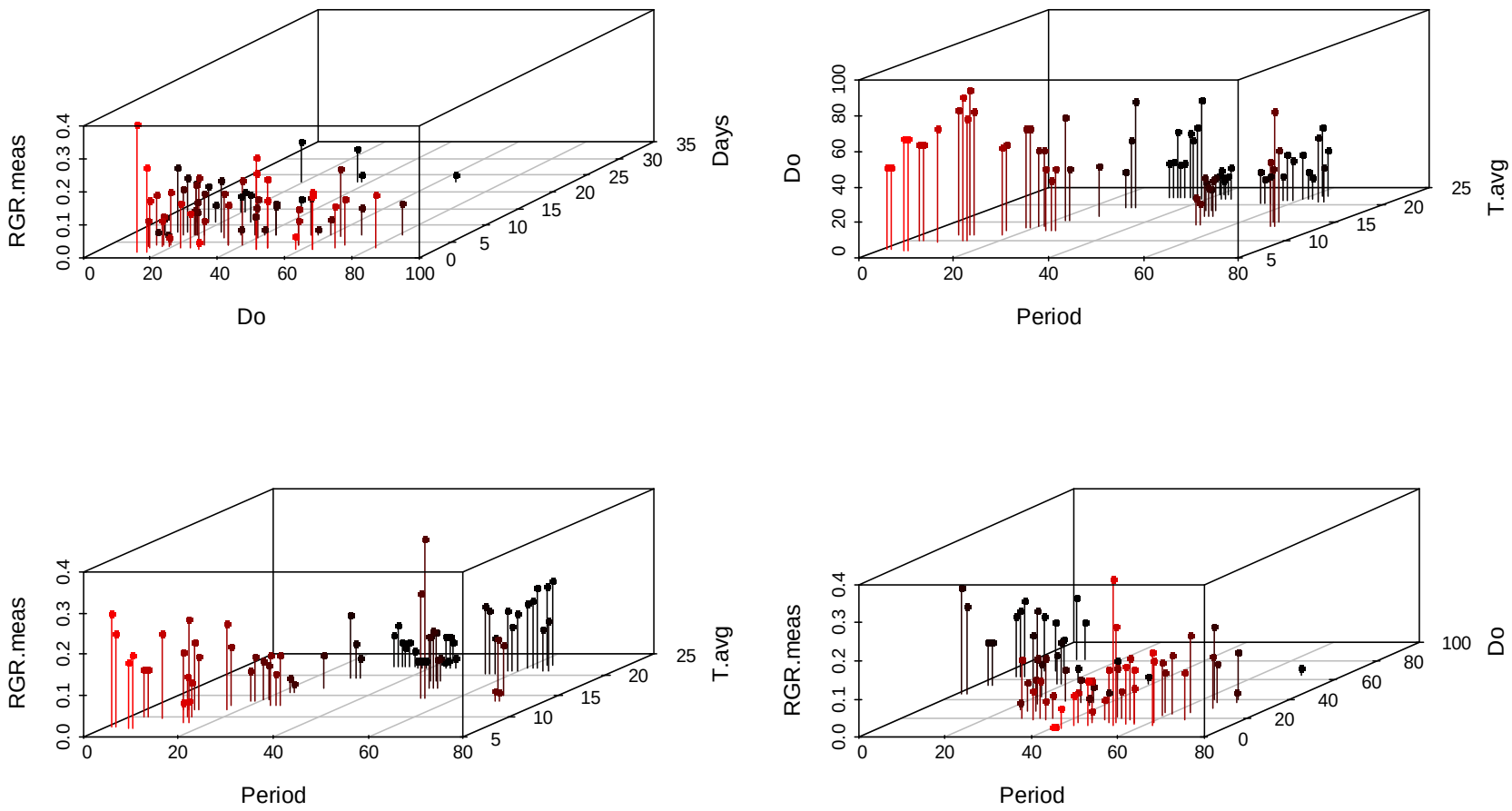


Figure 64. Correlation between variables in the field growth study between May to October 2011 on the Wellsville lagoons.

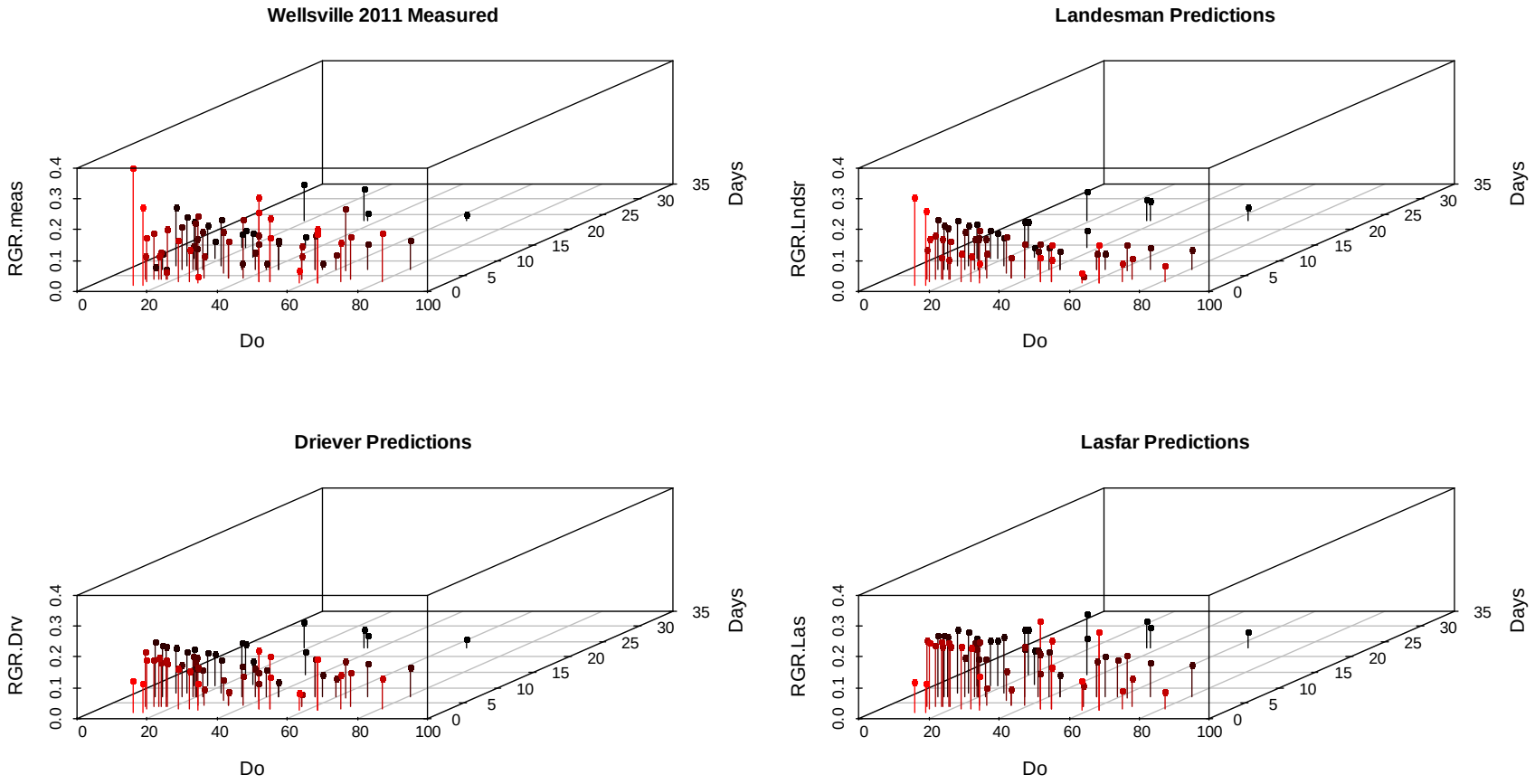


Figure 65. Duckweed growth affected by starting density (Do) and harvesting frequency (Days) in the field versus predicted.

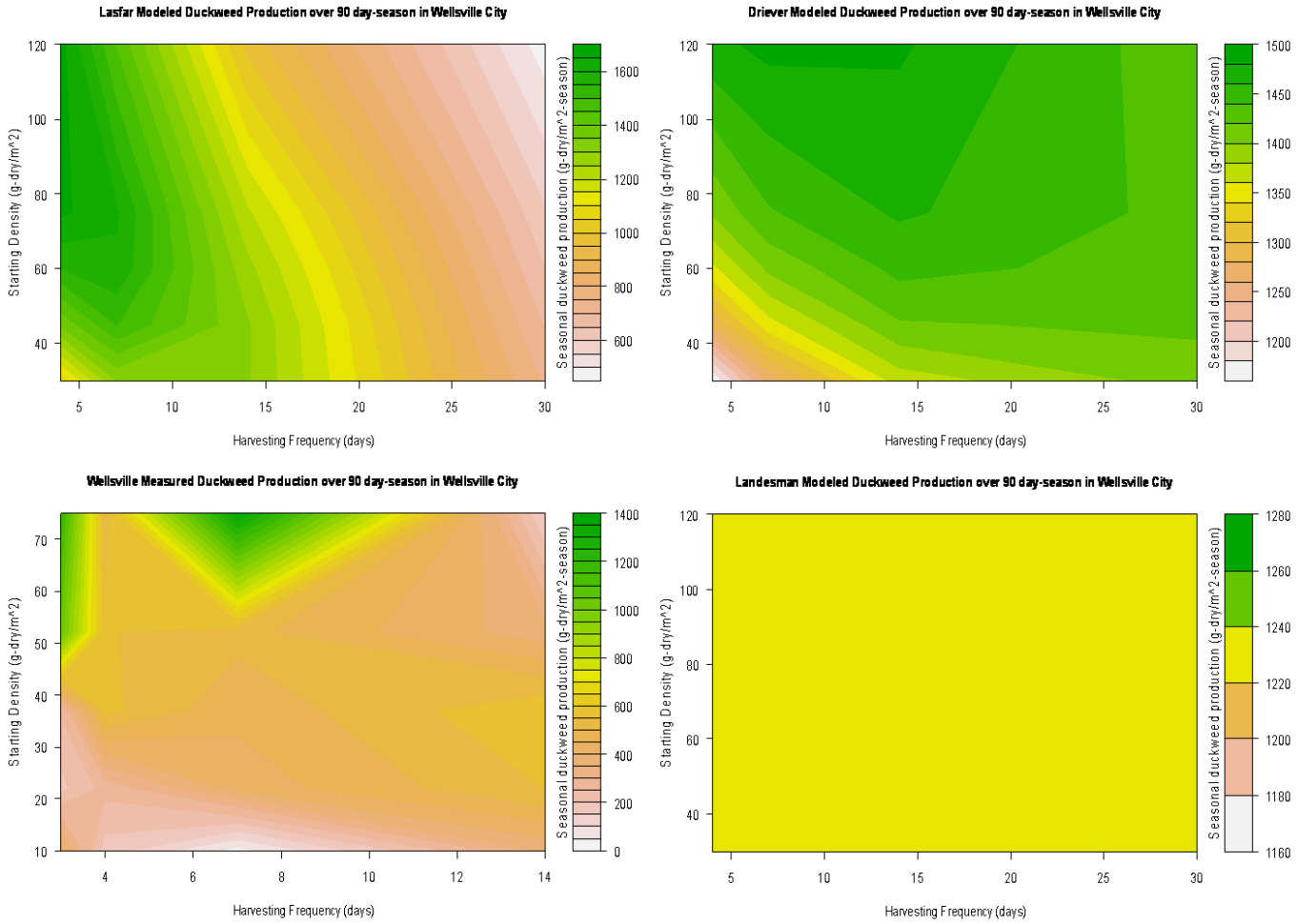


Figure 66. Contour plots demonstrating modeled and measured duckweed production over 90 days on the Wellsville Municipal Sewage Lagoons as a function of starting plant density and harvesting frequency.

D.4 Engineering Significance: Pharmaceuticals

Table 33. Pharmaceutical properties, removal, and plant-water partition coefficients (K_{PW}) for this study and Reinhold et al. (2010)

Compound[a,b]	C (ng/L)	MW	pKa	pKow	charge@pH=8	S (mg/L)	Pv (mm Hg)	mg-dry	RGR	K_{PW}	% Removal	% Predicted Removal
ACT	2500	152	9.38	0.46	-0.027	30400	1.94E-06	481	0.07	1833	99	81
ACT	2500	152	9.38	0.46	-0.027	30400	1.94E-06	501	0.08	1165	98	82
ACT	2500	152	9.38	0.46	-0.027	30400	1.94E-06	521	0.09	1817	99	83
SLF	1250	253	5.89	0.89	-0.634	3942	1.30E-07	481	0.07	119	82	57
SLF	1250	253	5.89	0.89	-0.634	3942	1.30E-07	501	0.08	116	84	58
SLF	1250	253	5.89	0.89	-0.634	3942	1.30E-07	521	0.09	233	91	59
FLX	1250	309	9.62	4.05	0.988	38.35	2.52E-05	481	0.07	121	82	89
FLX	1250	309	9.62	4.05	0.988	38.35	2.52E-05	501	0.08	118	84	90
FLX	1250	309	9.62	4.05	0.988	38.35	2.52E-05	521	0.09	193	89	92
CRB	1250	236	0	2.45	0	112	8.80E-08	481	0.07	-6	-20	12
CRB	1250	236	0	2.45	0	112	8.80E-08	501	0.08	-3	-11	13
CRB	1250	236	0	2.45	0	112	8.80E-08	521	0.09	-4	-16	14
PRG	1250	314	0	3.87	0	5	3.22E-03	481	0.07	349	91	94
PRG	1250	314	0	3.87	0	5	3.22E-03	501	0.08	343	92	95
PRG	1250	314	0	3.87	0	5	3.22E-03	521	0.09	505	94	96
ACT	5.00E+04	152	9.38	0.46	-0.027	30400	1.94E-06	580	0.07	303	91	85
ACT	5.00E+04	152	9.38	0.46	-0.027	30400	1.94E-06	620	0.09	846	97	88
ACT	5.00E+04	152	9.38	0.46	-0.027	30400	1.94E-06	580	0.07	297	90	85
SLF	5.00E+04	253	5.89	0.89	-0.634	3942	1.30E-07	580	0.07	21	50	62
SLF	5.00E+04	253	5.89	0.89	-0.634	3942	1.30E-07	620	0.09	26	57	64
SLF	5.00E+04	253	5.89	0.89	-0.634	3942	1.30E-07	580	0.07	27	53	62
FLX	5.00E+04	309	9.62	4.05	0.988	38.35	2.52E-05	580	0.07	213	92	94
FLX	5.00E+04	309	9.62	4.05	0.988	38.35	2.52E-05	620	0.09	186	92	96

Compound[a,b]	C (ng/L)	MW	pKa	pKow	charge@pH=8	S (mg/L)	Pv (mm Hg)	mg-dry	RGR	K_{PW}	% Removal	% Predicted Removal
FLX	5.00E+04	309	9.62	4.05	0.988	38.35	2.52E-05	580	0.07	273	93	94
CRB	5.00E+04	236	0	2.45	0	112	8.80E-08	580	0.07	-9	-1	17
CRB	5.00E+04	236	0	2.45	0	112	8.80E-08	620	0.09	-5	13	19
CRB	5.00E+04	236	0	2.45	0	112	8.80E-08	580	0.07	-8	7	17
PRG	5.00E+04	314	0	3.87	0	5	3.22E-03	580	0.07	1008	98	99
PRG	5.00E+04	314	0	3.87	0	5	3.22E-03	620	0.09	884	98	101
PRG	5.00E+04	314	0	3.87	0	5	3.22E-03	580	0.07	113	98	99
ACT	6.50E+05	152	9.38	0.46	-0.027	30400	1.94E-06	540	0.05	46	71	77
ACT	6.50E+05	152	9.38	0.46	-0.027	30400	1.94E-06	520	0.04	39	69	76
ACT	6.50E+05	152	9.38	0.46	-0.027	30400	1.94E-06	550	0.05	43	70	78
SLF	6.50E+05	253	5.89	0.89	-0.634	3942	1.30E-07	540	0.05	9	47	54
SLF	6.50E+05	253	5.89	0.89	-0.634	3942	1.30E-07	520	0.04	-1	28	53
SLF	6.50E+05	253	5.89	0.89	-0.634	3942	1.30E-07	550	0.05	7	44	54
FLX	6.50E+05	309	9.62	4.05	0.988	38.35	2.52E-05	540	0.05	295	93	86
FLX	6.50E+05	309	9.62	4.05	0.988	38.35	2.52E-05	520	0.04	272	93	85
FLX	6.50E+05	309	9.62	4.05	0.988	38.35	2.52E-05	550	0.05	327	94	87
CRB	6.50E+05	236	0	2.45	0	112	8.80E-08	540	0.05	10	41	8
CRB	6.50E+05	236	0	2.45	0	112	8.80E-08	520	0.04	3	39	7
CRB	6.50E+05	236	0	2.45	0	112	8.80E-08	550	0.05	10	43	9
PRG	6.50E+05	314	0	3.87	0	5	3.22E-03	540	0.05	797	98	90
PRG	6.50E+05	314	0	3.87	0	5	3.22E-03	520	0.04	736	98	89
PRG	6.50E+05	314	0	3.87	0	5	3.22E-03	550	0.05	746	98	91
ACT	1.00E+06	152	9.38	0.46	-0.027	30400	1.94E-06	394	0.03	27	35	66
ACT	1.00E+06	152	9.38	0.46	-0.027	30400	1.94E-06	517	0.07	73	64	72
ACT	1.00E+06	152	9.38	0.46	-0.027	30400	1.94E-06	642	0.03	76	69	79
SLF	1.00E+06	253	5.89	0.89	-0.634	3942	1.30E-07	394	0.03	121	67	42

Compound[a,b]	C (ng/L)	MW	pKa	pKow	charge@pH=8	S (mg/L)	Pv (mm Hg)	mg-dry	RGR	K_{PW}	% Removal	% Predicted Removal
SLF	1.00E+06	253	5.89	0.89	-0.634	3942	1.30E-07	517	0.07	28	36	49
SLF	1.00E+06	253	5.89	0.89	-0.634	3942	1.30E-07	642	0.03	19	32	56
FLX	1.00E+06	309	9.62	4.05	0.988	38.35	2.52E-05	394	0.03	110	65	75
FLX	1.00E+06	309	9.62	4.05	0.988	38.35	2.52E-05	517	0.07	315	88	81
FLX	1.00E+06	309	9.62	4.05	0.988	38.35	2.52E-05	642	0.03	477	93	88
CRB	1.00E+06	236	0	2.45	0	112	8.80E-08	394	0.03	3	4	-3
CRB	1.00E+06	236	0	2.45	0	112	8.80E-08	517	0.07	12	20	4
CRB	1.00E+06	236	0	2.45	0	112	8.80E-08	642	0.03	3	8	10
PRG	1.00E+06	314	0	3.87	0	5	3.22E-03	394	0.03	51	46	79
PRG	1.00E+06	314	0	3.87	0	5	3.22E-03	517	0.07	22322	100	86
PRG	1.00E+06	314	0	3.87	0	5	3.22E-03	642	0.03	17954	100	92
IBU-R	2.06E+06	206	4.5	3.97	-1	41.05	3.43E-04	100	na	21	30	619
CFA-R	2.15E+06	215	3.5	2.57	0	582.5	1.46E-04	100	na	0	0	104
FLX-R	1.24E+06	309	9.62	4.05	0.988	38.35	2.52E-05	100	na	117	70	56
FLX-R	1.55E+06	309	9.62	4.05	0.988	38.35	2.52E-05	100	na	950	95	53
FLX-R	3.09E+06	309	9.62	4.05	0.988	38.35	2.52E-05	100	na	450	90	38
TRI-R	2.90E+06	290	7.9	4.76	-0.676	4.621	3.76E-05	100	na	228	82	582
DEET-R	1.91E+06	191	2	2.19	0	670	6.18E-03	100	na	0	0	55
2,4-D-R	1.99E+06	221	3.4	2.81	-1	336	5.72E-05	100	na	2450	98	396
PIC-R	2.42E+06	242	2.7	1.36	-1	627	9.13E-07	100	na	0	0	124
ATZ-R	2.16E+06	216	0	2.61	0	214	6.39E-05	100	na	0	0	27

[a] Compounds in this study: ACT = Acetaminophen SLF = Sulfamethoxazole FLX = Fluoxetine CRB = Carbamazepine PRG = Progesterone
[b] Compounds in Reinhold et al. (2010): IBU = Ibuprofen CFA-R = Clofibric Acid FLX-R = Fluoxetine TRI-R = Triclosan DEET-R = DEET 2,4-D-R = 2,4-D PIC-R = Picloram ATZ-R = Atrazine

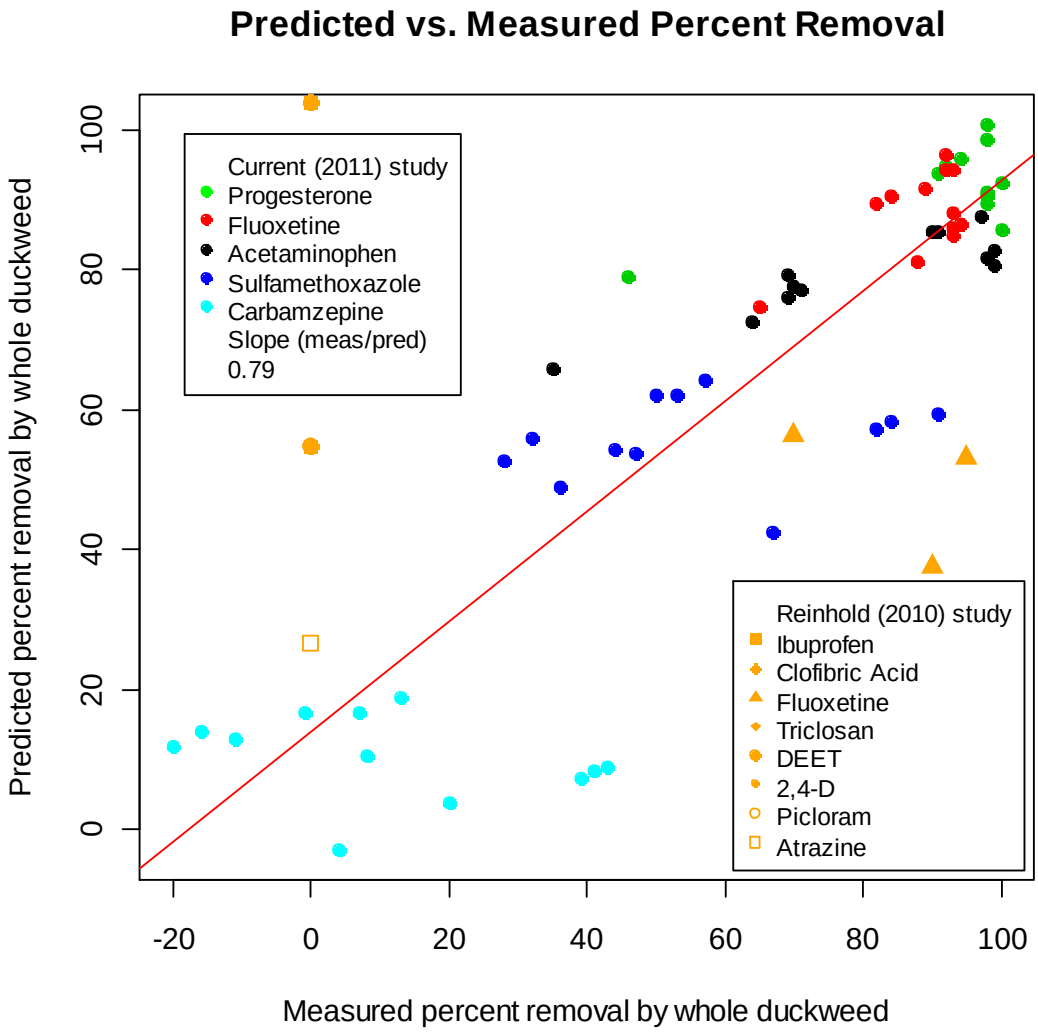


Figure 67. Predicting pharmaceutical percent removal by multiple regression stepwise analysis with verfication of empirical model against Reinhold et al. (2010) study.