To the Graduate Council:

I am submitting herewith a thesis written by Catherine R. Mayhew entitled "Mosquito Control and Pollutant Removal in Constructed Wetlands: A Mesocosm Study Using Dairy Wastewater." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biosystems Engineering Technology.

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Mosquito Control and Pollutant Removal in Constructed Wetlands: A Mesocosm Study Using Dairy Wastewater.

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Luna, Ellie, and Larry.

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ABSTRACT

Both subsurface flow (SSF) and free water surface (FWS) constructed wetland (CW) systems have been used for partial treatment of wastewater from animal production facilities. SSF systems are considerably more expensive, especially with regard to capital costs. However, the less expensive FWS systems have tremendous mosquito production potential; a factor which cannot be ignored by design engineers, especially given increasing public concern about emerging and reemerging vector borne diseases.

One promising method of reducing mosquito production in FWS CW systems is periodic drying. By disrupting the development of larval mosquitoes, this strategy may prevent the emergence of adult mosquito species associated with FWS CW systems. To test the efficacy of this method, experiments were conducted on 12 CW mesocosms ($0.7m^2$ surface area each), divided into three groups. Each group received dairy wastewater, pretreated by a high-rate anaerobic filter (AF), and all mesocosms were populated by cattail (*Typha latifolia*). Group 1 consisted of FWS cells filled with plants that had been established for three years, Group 2 consisted of newly established FWS cells, and Group 3 consisted of newly established SSF cells. The SSF cells were loaded continuously with wastewater, while the FWS cells received wastewater periodically: 7-d on, 7-d off. While receiving flow, each mesocosm was operated at a 7-d hydraulic retention time (HRT); two mesocosms in series thus had a net HRT of 14 d.

Emergence of adult mosquitoes from FWS cells was monitored during the drying phase. The removal rates of organic matter (measured as chemical oxygen demand) (COD), total nitrogen (TN), ammonia nitrogen (NH_3-N) and total phosphorous (TP)

were monitored in all cells, to compare pollutant removal between groups. Periodic drying of FWS CW systems successfully killed mosquito larvae and pupae, thereby preventing adult mosquito emergence, as long as there was no significant rainfall within the first two days of the drying phase. Water quality data indicated that FWS CW cells with established plants do not achieve significantly greater or less pollutant removal rates than newly established FWS cells for COD, TP, NH_3-N, or TP at a 7-d HRT.

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Chapter 1

INTRODUCTION

Livestock production in the U.S. has evolved in such a way that there are fewer producers, larger average herd sizes, and a higher concentration of herds and production facilities on less land area (The Ohio State University, 1992). While this evolution has certain benefits, it has also changed the distribution and characteristics of production byproducts, such as animal waste. For example, the confinement of dairy cattle has concentrated dairy waste. To avoid environmental problems, these concentrated wastes must be handled, treated, and recycled—or disposed of—appropriately.

With increasing pressure for ownership of environmental pollution and contaminants, the challenge facing many agricultural producers today is to install and operate a reliable, effective, and economical waste management system. One relatively new technology being applied to agricultural wastewater is that of constructed wetland (CW) systems (Ancell et al., 1998; Cheng et al., 1998; Moore et al., 1995).

Hammer (1993) defines constructed wetlands as consisting "of former terrestrial environments that have been modified to create poorly drained soils and wetlands flora and fauna for the primary purpose of contaminant or pollutant removal from wastewater." This definition primarily serves to distinguish constructed wetlands from natural, created, or restored wetlands. However, it should be noted that constructed wetlands have many of the same ancillary benefits and drawbacks associated with these other types of wetlands. In recent years, as more large scale CW systems have been built and brought on-line, it has become apparent that the potential for mosquito production – not the effectiveness of the waste treatment – may determine their feasibility (Mortenson, 1982; Martin and Eldridge, 1989; Stowell et al., 1985). This is because wastewater in a CW, with its high nutrient concentrations, can serve as an excellent habitat for the immature stages of certain mosquito species. To further complicate the problem, location of a CW near human, equine, and fowl species increases the chances of mosquito problems, both as disease organism vectors and as pests (Mortenson, 1982). The severity of the problem was illustrated by Martin and Eldridge (1989), who found that five out of nine pilot CW plants built in California between 1974 and 1989 were taken out of operation due to mosquito problems. Stowell et al. (1985) named mosquito production problems as the major drawback of CW systems and called for further research in design, operation, and mosquito control measures as they relate to mosquito production.

One solution to the problem of mosquito production in CW systems is to use subsurface flow (SSF) instead of free water surface (FWS) cells. In SSF CW systems, wastewater flows through a porous media planted with aquatic macrophytes. The water remains below the level of the media, and SSF cells therefore lack the free water surface habitat required for mosquito larval and pupal development. In the case of domestic wastewater, SSF CW systems are reported to achieve the same treatment efficiencies in one-forth the space that a FWS CW system requires (Tchobanoglous, 1997). However, this advantage is neutralized by the mean capital cost for SSF systems, which is roughly four times more per acre than that of FWS systems (Tchobanoglous, 1997). In agricultural applications, where producers typically have ready access to earth-moving equipment, the cost differential between FWS and SSF systems may be even greater.

Various control methods have been attempted to suppress mosquito production in FWS systems. The use of mosquitofish (*Gambusia affinis*) is frequently suggested (Kadlec and Knight, 1996; Knight, 1993; Martin and Eldridge, 1989; Hammer, 1993). However, use of mosquitofish is unrealistic in habitats with excessively low dissolved oxygen (DO), where there are dense stands of emergent macrophytes restricting fish movement and sheltering larvae, or where hot weather elevates water temperatures or dries the cells (Kadlec and Knight, 1996; Knight, 1993; Martin and Eldridge, 1989; Hammer, 1993; Dill,1989).

Organophosphate insecticides and larvicides, particularly the bacterial larvicides *Bacillus thuringiensis israelensis* H-14 (*Bti*) and *Bacillus sphaericus* (*Bs*), have also been studied for their effects on mosquito populations in CW systems, but with limited success (Tennessen, 1993). For example, Tennessen (1993) reported disappointing results from insecticide treatments. Typically, mosquito larvicides are considered effective if a 95% or better reduction rate is achieved. However, the high mean larval densities associated with CW systems do not approach acceptable threshold densities even after a 95% reduction is achieved. Furthermore, owing to the adsorption of toxicants by organic matter, diluting and degrading action by the CW treatment process, and the short life cycle of mosquitoes, insecticides are not a satisfactory long-term control for mosquito production in treatment wetlands (Tennessen, 1993).

In contrast to these biological and chemical approaches, some investigators have considered hydraulic means of limiting mosquito production. For example, Steiner and Freeman (1989) suggest that mosquito control may be one of the benefits of having a CW configuration of multiple parallel cells wherein individual cells are periodically drained and allowed to dry. To date, not much information is available regarding the effectiveness of this method. Toward this end, we have investigated the feasibility of periodically drying FWS CW systems for mosquito control, with the ultimate goal of determining the costs and benefits of such an operation.

In addition to mosquito control, periodic draining of FWS CW systems will presumably have an impact on pollutant removal. To address this issue, we examined the pollutant removal performance of the FWS and SSF CW cells in our experimental system. Because of other ongoing research in our lab (Hawkins & Raman, 1999) we were interested in the feasibility of discharging effluent from a high-rate anaerobic digester to CW cells. We were particularly interested in the impact of an anaerobic effluent on plant health, and on nitrogen removal. Because nitrification rates limit denitrification rates, an aerobic pretreatment is generally advised to promote the oxidizing conditions needed by nitrification (Jenssen et.al, 1997). The periodic draining of FWS CW cells might actually enhance oxygen availability and thus nitrification (Qiu and McComb, 1996).

Chapter 2

LITERATURE REVIEW

Kadlec and Knight (1996) report that the intentional discharge of municipal sewage and wastewater into wetlands has occurred since the beginning of the 20th century. Until the 1960's, it was done as a matter of convenience. German researchers in the 1950's, followed by Americans in the late 1960's, began documenting the inherent capacity of wetlands as wastewater filters. Research on the topic of treatment wetlands exploded during the 1970's, as did confidence in the practice as a viable low-technology solution to wastewater management (Kadlec and Knight, 1996).

Constructed Wetlands

Concurrent to the increase in research exploring the use of wetlands as a wastewater treatment alternative, the United States (U.S.) government passed the Clean Water Act (CWA). The federal CWA's objective, outlined in Section 10(a), is "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters." Included in the Act's provisions are Sections 401 and 402 regulating point source discharges of waterborne pollutants into "waters of the U.S."; of which natural, restored, and created wetlands are included (Hammer, 1993; Fields, 1993). However, constructed wetlands, designed and built specifically for point source and nonpoint source pollutant removal, are not considered to be "waters of the U.S." and consequently are not regulated with regard to influent streams (Fields, 1993; Bastian et al., 1989). Fields (1993) emphasizes that those effluents leaving a constructed wetland and flowing into "waters of the U.S." are subject to the CWA. Discharge into a receiving body is unlikely with agricultural CWs. However, understanding the performance potential and/or limitations of these treatments systems continues to be important. In addition to applicable regulatory requirements, a variety of management issues are associated with constructed wetlands. Among the most important of these is mosquito control (Tchobanoglous, 1993).

Mosquitoes

Oviposition

Clements (1963) described a variety of egg laying strategies by female mosquitoes. The common factor in site selection by ovipositing females is proximity to water—eggs are deposited either on existing water or in moist soil prone to flooding. Beyond this, females of different species are highly selective to both the environment (e.g., shaded/open/stream/rice-field/pond/tree hole/artificial container) and the water conditions (e.g., moving/still/polluted/saline/fresh) of oviposition sites.

There are a number of mosquito species that are associated with the thickly vegetated and nutrient-rich conditions typical of constructed wetlands. Species most frequently found include those from the genera *Culex*, *Culiseta*, *Anopheles*, *Coquillettidia*, and *Aedes* (Tennessen, 1993; Stowell et al., 1985; Walton et al., 1999).

Clements (1963) also reported that while eggs are oviposited in water or moist soil conditions, they are able to maintain their viability out of water. The implication is that while upon oviposition a particular site was favorable for larval development, if that site should become dry, those eggs may arrest their development by way of quiescence or diapause and resume development upon submergence later. Therefore, if a constructed wetland is drained to kill larvae and pupae, upon reinundation larval and pupal densities could recover quickly resulting in emergence. This would necessitate frequently repeated drains to prevent emergence.

Larval and Pupal Development

Mosquito larval food is microplankton including algae, rotifers, protozoa, bacteria, and fungal spores. Studies examining gut contents reported that they are comprised mostly of organic matter ingested as detritus or as colloidal particles (Clement, 1963). Standing water, high nutrient levels, vegetative cover, and the anaerobic and bacteria-laden water conditions found in constructed wetlands provide an ideal medium for the immature growth stages of some mosquito species (Tennessen, 1993).

During their development, all mosquito species pass through four stages of larval development, referred to as instars. At the end of each instar development the larvae shed the outer cuticle in a process referred to as ecdysis. Upon completion of the 4th instar, the mosquito enters its pupal stage. Males tend to pupate faster than females and spend less time in the pupal stage than females. This means that from a given egg batch, males tend to emerge as adult mosquitoes a day earlier than females (Clement, 1963).

Adult Mosquitoes

Clement (1963) reported that adult mosquitoes can fly within a few minutes of emergence. While this is true, the mosquito is still developing and will not be sexually mature for another 24 to 48-hrs. The spermatozoa of the males are mature at emergence; however, they are physically unable to copulate until their terminal segments have rotated 180° during the first 24 to 48-hrs after emergence. The ovaries of emerged females must develop to a resting stage before they are further stimulated to produce eggs by taking a blood meal. However, autogeny—where a female may lay a single first batch of eggs before requiring a blood meal for subsequent oviposition—has been observed. This phenomena was first recorded in *Culex pipiens* but is now known to occur widely among the different mosquito genera (Celment, 1963).

A female mosquito is able to produce a batch of eggs after each blood meal, however it is not necessary to mate more than once to fertilize subsequent batches of eggs. Clement (1963) demonstrated that a female laid 15 batches of fertilized eggs over a 62-day period after a single copulation. The number of eggs in a given batch varies among species. *Culex pipiens* is reported to lay 250-400 in its first batch, *Culiseta* spp. up to 300, and *Aedes detritus* up to 260 (Celment, 1963). Given that the development period from oviposition through adult emergence may occur in seven days or less, it is evident that mosquito populations may explode exponentially in favorable conditions, over a course of weeks.

Mosquito Control in Constructed Wetlands

One frequently suggested method to control mosquito production in constructed wetlands is by stocking with one of the natural predators of the insects larval and pupal stages—the mosquitofish (*Gambusia affinis*) (Kadlec and Knight, 1996; Knight, 1993; Martin and Eldridge, 1989; Hammer, 1993). However, use of mosquitofish is unsuccessful in habitats with excessively low dissolved oxygen (DO), where there are dense stands of emergent macrophytes restricting fish movement and sheltering larvae, or where hot weather elevates water temperatures or dries the cells (Kadlec and Knight, 1996; Knight, 1993; Martin and Eldridge, 1989; Hammer, 1993; Dill, 1989). Such conditions can frequently occur in CW systems treating agricultural wastewaters, where extensive primary and secondary treatment have typically not occurred prior to wetland treatment. Therefore, the use of *G. affinis* as a control is not indicated for such systems.

Tennessen (1993) reported that the Vector Control Program of the Tennessee Valley Authority (TVA) established a threshold correlating mean larval densities in reservoirs with complaints of adult annoyance and requests for insecticide application. TVA determined that when mean larval densities meet or exceed 0.25 mosquito larvae per standard dip sample (237 mL) there is a high incidence of complaints and requests for insecticide treatment from residents near the area.

Tennessen (1993) further reported mean larval densities of 0.1 to 5.0 in natural wetlands of western Kentucky in 1988 and 1989. Between March 1988 and February 1989, a study conducted at two full-scale constructed wetland sites in western Kentucky yielded mean larval densities ranging from 6.1 to 272 and from 1.7 to 37 larvae per dip sample. During 1989 and 1990 the effects of the organophosphate Abate and bacterial larvacides *Bacillus thuringiensis israelensis* H-14 (*Bti*) and *Bacillus sphaericus* (*Bs*) were studied at these same sites. Results of the study showed little promise for the control of mosquito populations below threshold densities with these compounds. Larvacides are considered effective if a 95% or better reduction rate is achieved. However, the high mean larval densities associated with CW systems do not approach acceptable threshold densities even after a 95% reduction is achieved. Furthermore, owing to the adsorption of toxicants by organic matter, diluting and degrading action by the CW treatment process, and the short life cycle of mosquitoes, insecticides are not a satisfactory long-term control for mosquito production in treatment wetlands (Tennessen, 1993).

Vector-borne Disease

Mosquitoes are more than a nuisance to humans. Emerging and reemerging vector-borne disease is a concern throughout the world. Gubler (1998) blamed the global emergence/resurgence of vector-borne disease on changes in public health policy, insecticide and drug resistance, a shift in emphasis from prevention to emergency response, demographic and societal changes, and genetic changes in pathogens. The Director of the Centers for Disease Control and Prevention (CDC) agreed with this caution in a 1999 CDC press release and stated that the CDC has responded with a plan for joining individuals, communities, and nations in a global effort for control and prevention of emerging disease in the 21st century (Hughes, 1999).

This growing concern makes critical design of constructed wetlands very important if the technology is to survive. Eight out of fifteen mosquito species collected in a constructed wetlands study described by Tennessen (1993) are known or suspected vectors for five different types of viral encephalitis (Table 1). As recently as September 1999 the CDC confirmed that West Nile virus—an arbovirus previously unrecognized in the Western Hemisphere—killed and infected individuals in an outbreak in New York and New Jersey (CDC, 1999a). Mosquitoes of the genera *Culex* are the most frequent vectors of this disease (CDC, 1999b). At the time of this writing, Calisher (1999)—formerly of the CDC—reports *Culex pipiens* complex to be the putative vector of the recent West Nile virus outbreak in the U.S. Public health concerns could have devastating effects on the use of constructed wetlands due to *Culex* spp. being the principal mosquito genera associated with these habitats (Hayes, 1975; Stowell, 1985; Tennessen, 1993; Walton et al., 1999).

Table 1. Known or Suspected Vector Capabilities of Eight Mosquito Species Collected in the Benton and Pembroke Constructed Wetlands. Taken from Tennessen (1993).

Species	**Viruses**	**Other**
Culex pipiens	SLE, EEE, WEE	Dog heartworm, fowl pox, bird malaria
Culex restuans	SLE, EEE	Dog heartworm
Culex salinarius	SLE, EEE	
Culex tarsalis	SLE, EEE, CE	
Culex territans	EEE	
Culiseta inornata	SLE, WEE, JBE	
Copquillettidia perturbans	EEE	
Aedes vexans	EEE,WEE, CE	Dog heartworm

Note: SLE, St. Luis encephalitis; EEE, Eastern equine encephalitis; WEE, Western equine encephalitis; CE, California group encephalitis; and JBE, Japanese B encephalitis.

Unfortunately, in their authoritative work on the subject of CW design, Kadlec and Knight (1996) downplayed the mosquito problem in constructed wetlands by generalizing that many mosquito species do not travel far from their hatching points and that disease organisms transmitted by mosquitoes have become rare in many developed nations of the world. They erroneously ignored the fact that avian hosts of vector-transmitted viruses, including the various kinds of encephalitis, have large habitat ranges in addition to annual interstate and intercontinental migration patterns (Morrison et al., 1992; Bolen and Robinson, 1995). Walton et al. (1999) reported a study of dispersal in *Culex erythrothorax* where mean dispersal from a release site was found to be ~0.5 km per night but was recorded as high 2 km per night. Clement (1963) reported that mosquitoes are known to disperse considerable distances from their larval habitats and quotes distances up to 53 km in still air conditions. These factors increase the chances of the U.S. and other developed countries experiencing outbreaks of emerging and reemerging diseases near areas with high mosquito activity.

Walton et al. (1999) stated that while agricultural constructed wetlands are typically built in rural areas, human development will continue to encroach on these areas with suburban sprawl. Furthermore, it is in precisely land-limited situations that CWs are most likely to be built; facilities with a large land base are unlikely to need the high areal removal capacities offered by CWs. Finally, while our work focused on CWs to treat agricultural wastewater, the majority of constructed wetlands currently in existence have been built for municipal or industrial service and are in close proximity to human populations, rendering low dispersal distances irrelevant.

Pollutant Removal

Chemical Oxygen Demand

With respect to the negative effects organic wastes can have on receiving waters, Gray (1989) reported the reduction in dissolved oxygen concentration (DO) to be the most important. The DO in a water system (stream, lake, lagoon, wetland, etc.) is consumed as aerobic or facultative microbes break down organic matter in the system. There are two common tests for quantifying the amount of oxygen that would be needed to support the decomposition of a waste: biological oxygen demand (BOD) and chemical oxygen demand (COD). Testing for BOD reveals the biodegradable fraction of a sample easily metabolized by aerobic bacteria. COD is a measure of the total organic material, both biodegradable and non-biodegradable, which may eventually be oxidized (Hobson and Robertson, 1977; Gray, 1989).

By definition, COD values will always be higher than BOD values for the same sample. Gray (1989) reported that wastewater COD to BOD ratios vary from 1.25 to 2.5 (COD:BOD). The ratio is further reported to be dependent on the type of waste analyzed, and the ratio increases with each stage of biological treatment performed on the liquid.

Assessment of BOD typically involves a standard test where the microbially mediated oxidation of organic matter in the sample is monitored over five days. The resultant value is known as the five-day BOD (or BOD_5). In contrast, the COD test typically used is a two-hour rapid oxidation using potassium dichromate, concentrated sulfuric acid, and a catalyst (Hobson and Robertson, 1977; Gray, 1989). COD testing was used in our study to characterize organic content of influent and effluent wastewater streams.

The assessment of BOD and COD removal is important in water treatment systems discharging to natural watercourses and, particularly in the case of agriculture, effluents destined to be land applied. While organic material may form the basis of the food chain in natural streams and reservoirs, excessive BOD or COD can overwhelm the DO recharge rate in receiving waters. Even if the wastewater is land applied, it may impact a stream or other natural reservoir by leaving a field as runoff. If a receiving stream is unable to compensate for oxygen depletion due to a high strength influent, the results may include hypolimnetic oxygen depletion and/or fish kills (Summerfelt, 1993).

Nitrogen

Nitrogen (N) in wastewater occurs in various organic and inorganic forms. Burks and Minnis (1994) stated that those of greatest interest include organic N (proteins and amino acids), nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3/ NH_4^+), and N gas (N_2). Nitrogen discharges into the environment are a concern due to its role in eutrophication, its effect on the DO in receiving streams, and its toxicity to aquatic species (Kadlec and Knight, 1996).

The different forms of N are interconvertable in a complex chemical and biological cycle referred to as the nitrogen cycle (Burks and Minnis, 1994; Kadlec and Knight, 1996; Metcalf and Eddy, 1991). Figure 1 illustrates selected pathways of the nitrogen cycle relevant to a CW treating animal wastewater. Hobson and Robertson (1977) stated that in aerated systems with adequate DO, ammonia is oxidized to NO_2^- and NO_3^- by nitrification. Burks and Minnis (1994) reported that NO_2^- is not typically observed in natural waters due to being readily converted into NO_3^- by bacterial action. However, if the system becomes anaerobic—a condition common in CWs, NO_3^- will be

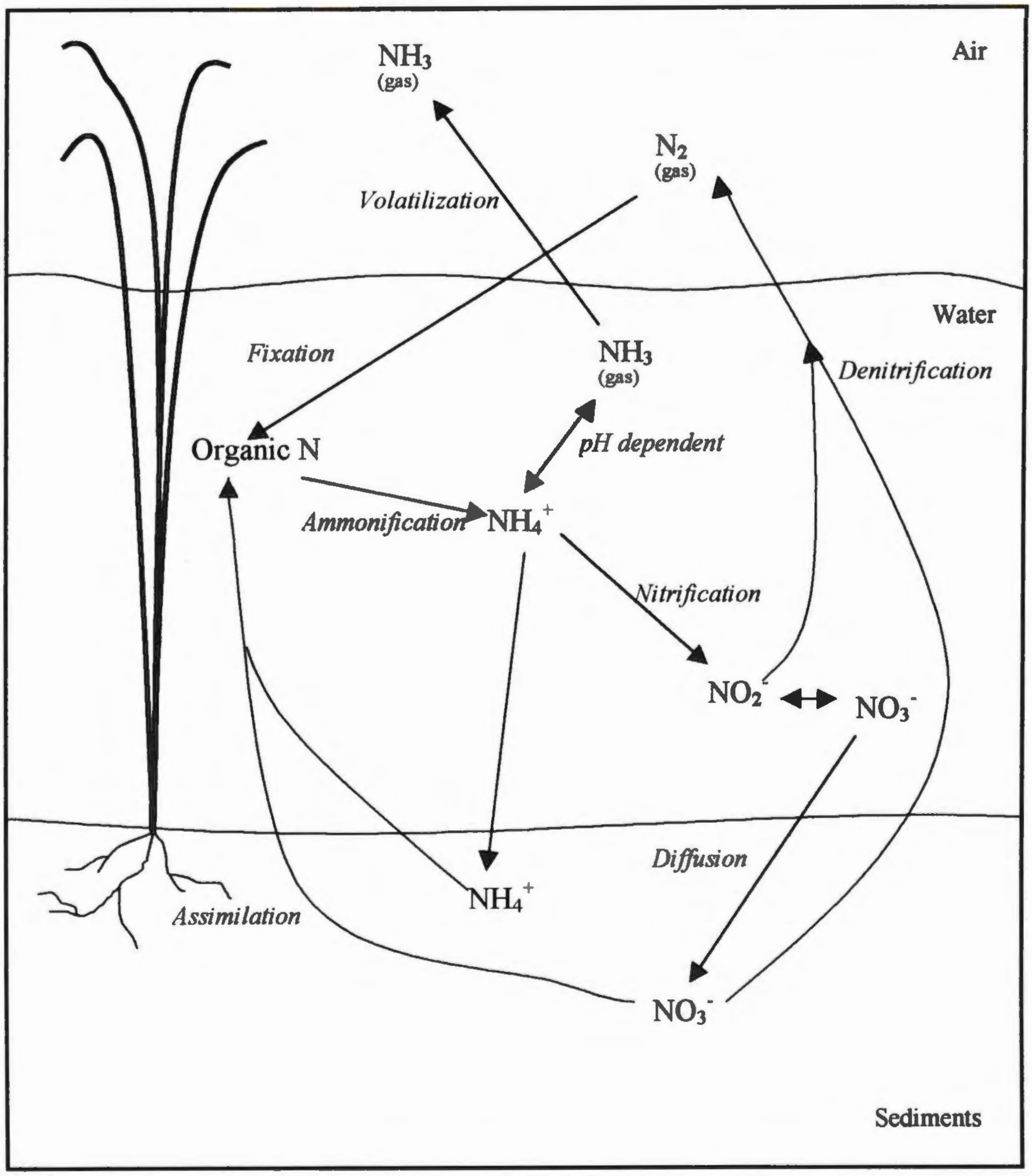


Figure 1. *Illustration of simplified nitrogen cycle in CWs developed from Kadlec and Knight (1996) and Metcalf and Eddy (1991).*

reduced to N_2 or ammonia by denitrification. In this scenario, N is lost to the atmosphere as N_2 or ammonia. Preliminary ion chromatography confirmed negligible levels of NO_2^- and NO_3^- in both the wastewater source and effluents of this study.

Burks and Minnis (1994) identified NO_3^- to be the limiting nutrient for primary production in salt waters. Logan (1995) reported its toxicity to humans and animals is due to its reduction to NO_2^- in the digestive tract, which then binds to hemoglobin and restricts oxygen transfer from the lungs. These concerns have resulted in regulatory agencies emphasizing the reduction of NO_3^- in wastewater treatment system effluents.

In addition to volatilization, assimilation is another major N-removal process in CWs. The two forms of N readily assimilated by plants are ammonia and NO_3^-, with the more reduced ammonia being the more favored source by wetland plants (Kadlec and Knight, 1996).

Ammonia nitrogen occurs in two forms: ionized ammonium (NH_4^+) and unionized—or free ammonia (NH_3). While NH_4^+ is nontoxic to fish, NH_3 is reported to be toxic to aquatic life at concentrations greater than 0.2 mg/L (Kadlec and Knight, 1996; Sawyer and McCarty, 1978). Kadlec and Knight (1996) reported NH_3 to be a small fraction of TN in CWs (<1% of total ammonia). However, at any given time, the amount present in each form is pH dependent and—to a lesser degree—temperature dependent. In general, if pH increases from 7.0 to 8.0, not only does the hydrogen ion concentration decrease by a factor of ten, the percentage of NH_3 increases by a factor of ten. Sawyer and McCarty (1978) concluded that NH_3 should not be a concern in systems where pH is below 8.0 if ammonia nitrogen concentrations are $\leq$ 1 mg/L.

In this study N was evaluated by testing for total nitrogen—organic and inorganic N—and for ammonia nitrogen (expressed as NH_3-N) in the influent and effluent discharges. NO_2^- and NO_3^- were not regularly tested due to the anaerobic nature of the wastestream in this study.

Phosphorous

Phosphorous (P) is reported to be the limiting nutrient in fresh water habitats. Its occurrence in water and wastewater is reported to be in the form of soluble orthophosphate ion (PO_4^{-3}), organically bound phosphate, and other oxidized forms. Excessive influx of P to freshwater systems results in a phenomenon known as eutrophication (Summerfelt, 1993; Burks and Minnis, 1994; Logan, 1995; Kadlec and Knight, 1996). This accelerated growth of photosynthetic phytoplankton can have numerous negative impacts. Summerfelt (1993) stated that dense algal blooms can limit or completely shade-out sunlight needed by aquatic macrophytes, which in turn die and consume DO during decomposition. Similarly, death and decomposition of the algal bloom itself consumes DO. Burks and Minnis (1994) stated that death of the phytoplankton also releases P back into the water, resulting in a persistent chemical reservoir of P.

Because P discharges can be so deleterious, regulations for reducing the amounts of P in effluent discharges are being considered (Burk and Minnis, 1994). Kadlec and Knight (1996) reported treatment wetlands to be inefficient for P removal—so inefficient that the land required for P removal tends to be the largest of all design considerations when planning a CW.

Physical and biological processes are involved in P removal (Burk and Minnis, 1994). Biological removal of P occurs through uptake of P by plants and bacteria in the system. Burk and Minnis (1994) described a process referred to as luxury uptake, where plants and bacteria exposed to P rich water uptake or consume more P than nutritionally needed. However, the plants and bacteria must be removed from the system to prevent P release back into the water upon death. Kadlec and Knight (1996) stated that P removal through harvest of biomass is yet to be proven feasible. Problems cited include difficulty in harvesting rooted emergent macrophytes and the low removal rates attributed to harvest (~3% of total P removal). Floating macrophytes are less difficult to harvest but removal rates remain low (20% of total P removal). Finally, harvesting is potentially labor intensive and costly.

Two physical processes by which P is removed from water in a CW are precipitation (or sedimentation) of particulate P, and sorption of soluble P into the soil (in FWS systems) or media (in SSF systems) (Burks and Minnis, 1994; Kadlec and Knight, 1996). Kadlec and Knight (1996) reported the capacity of CWs to sorb P as variable. It is suggested that the storage potential of soils in FWS CWs may be quickly filled to capacity, while the critical selection of media in SSF CWs can be made to optimize their P storage potential. The principal compounds favoring P precipitation are iron, aluminum, and calcium salts (Burks and Minnis, 1994). Selection of materials that incorporate these salts may increase per area storage potential of SSF CW and increase removal rates (Burks and Minnis, 1994; Kadlec and Knight, 1996).

Because comprehensive nutrient plans on farms are becoming more concerned with P management this study evaluated P removal by testing for total P in the influent and effluent discharges.

Chapter 3

MATERIALS AND METHODS

This study used outdoor laboratory-scale components consisting of a 42-L downflow anaerobic filter (DFAF), eight 0.7-m^2 free water surface flow constructed wetland cells (FWS CW), and four 0.7-m^2 subsurface flow constructed wetland cells (SSF CW). Dairy wastewater was pumped into the anaerobic filter for primary biological treatment, and then pumped into the CWs for additional treatment. FWS cells were periodically drained and allowed to dry by shifting wastewater between FWS cells and rerouting effluent from the DFAF.

Site Description

The DFAF/CW facility was located at the Tennessee Agricultural Experiment Station, Knoxville Station, on an easterly-facing slope with a grade of approximately 10%. The area is in USDA climate zone 7 at 35.97° latitude and –83.88° longitude. For safety and convenience, the experimental CW cells were situated on a level 4 x 10-m gravel pad. A 2.5 x 3-m aluminum shed was constructed adjacent to the west edge of the pad to house the wastewater storage tank, the anaerobic digester, and electronic equipment.

Wastewater Source

Liquid dairy wastewater was collected from a local dairy in Blount County, TN from 03/05/99 through 08/18/99. A 1900-L tank trailer was used to collect liquid waste after it was screen-separated from solids. Obtaining wastewater in this way ensured a realistic wastestream, with components – including milk, cleaning solutions, and antibiotics – that an artificially generated wastewater would not have contained. Disadvantages of this approach were the natural treatment that occurred prior to waste collection, and the inherent variability of the feedstock. Once collected, the wastewater was stored at ambient temperature in a 750-L tank within the shed. Stored wastewater was transferred by submersible centrifugal pump to the inlet port on top of the DFAF.

Pollutant concentrations in the wastewater source decreased noticeably between 07/28/99 and 08/11/99 and an alternate source was found in Loudon County, TN. This wastewater was obtained after solid separation via a screw press. Due to the relatively high COD concentration of this waste compared to the previous source (~15x), an on-site dilution was performed at the DFAF/CW facility by adding tap water to the wastewater in the 750-L feed tank. Use of this feed stock began 08/18/99 and continued until 09/08/99.

Scheduling conflicts and time constraints necessitated the use of an artificially generated wastewater for the influent period spanning 09/08/99 through 09/15/99. Based on prior work (Raman et al., 1997) approximately 30-L of fresh manure obtained from the Knoxville Experiment Station Dairy Unit was mixed with 720-L of tap water in the feed tank to achieve a wastewater similar to that in the lagoon.

Beginning 09/15/99, a high strength dairy waste became available from the Dairy Experiment Station in Marshall County, TN. This wastewater was obtained immediately downstream of a screw press solid separator, and prior to lagoon treatment. Due to the high COD concentration of this waste (~85,000 mg/L COD), it was diluted with tap water in the 750-L feed tank. Use of this feed stock began 09/15/99 and continued until the end of the project (10/14/99).

Downflow Anaerobic Filter (DFAF)

The DFAF received wastewater from the storage tank within the shed. The reactor vessel was constructed of 30.5-cm (12 in.) diameter schedule 40 PVC pipe, with a liquid level height of 104 cm. The reactor was filled to a height of 94 cm with rock lava media (60% porosity) to serve as microbial attachment sites (Hawkins and Raman, 1999). At the design flow of 72 L d^{-1}, the 42-L working volume DFAF operated on a 0.58 d hydraulic retention time (HRT).

Influent to the DFAF occurred via a centrifugal pump controlled by a micrologger (Campbell Scientific, Model 21X, Logan, UT). Pressure changes upstream of the pump—caused by fluctuating liquid levels in the feed tank—required that the influent rate be monitored and subsequent adjustments made to the control program to maintain HRT at the desired value. A small-scale enclosed dosing siphon was constructed and installed between the influent tank and the DFAF to overcome this problem. However, shortly after installation (08/24/99) the dosing siphon had multiple failures of operation over a 48-hr period. Inspection revealed a heavy accumulation of solids and microbial growth/sludge on the level switch arm preventing its operation. This solids accumulation was attributed to a substantial increase in solids content of the new waste stream. Solids were removed and the level switch was cleaned; however, the unit failed again between 09/03/99 and 09/06/99. On 09/06/99 the unit was removed and the influent rate was again monitored manually.

Reactor temperature was monitored with a type-T thermocouple placed in the center of the DFAF. Three heating blankets (Model 764-3, Sunbeam, Schaumburg, IL) placed on the exterior of the DFAF were controlled by the datalogger to switch on if internal temperature fell below 30°C. The entire unit was insulated with Doublesure Fiberglas® Pipe Insulation (Owens Corning™, Toledo, OH).

DFAF effluent was routed to a sump where it accumulated until a level switch activated a submerged pump (Dayton Electric Mfg. Co., Teel Model 3P611E, Niles, IL) to drain the sump (accumulation time was approximately 1 h.). Sump effluent was routed to a flow divider that split the flow into four equal parts (aliquots). The four aliquots then flowed by gravity through open PVC channels into the appropriate CW series.

Constructed Wetlands

The 12 CW mesocosms used in this experiment had been used in past thesis projects and experiments at UTK (Benham, 1995; Bowling, 1996; Raman et al., 1997). They consisted of rectangular boxes constructed from exterior grade plywood with 5 x 10 cm (2" x 4") bracing around the sides, reinforced and waterproofed with resin coated fiberglass. Interior dimensions were 150 x 47 x 60 cm, giving each mesocosm cell a 0.7- m^2 surface area.

The 12 cells were divided into three groups consisting of four mesocosms each (Figure 2). Group 1 consisted of FWS CW mesocosms planted with cattails (*Typhia latifolia*). At the start of the experiment, these cells had been in use for three years receiving concentrated manure wastewater on a weekly basis. Nitrogen loading rate (NLR) for these CWs ranged from 0-5000 kg ha^{-1} yr^{-1} in the first year to 5000-10,000 kg ha^{-1} yr^{-1} in the second and third year (Bowling, 1996; Raman et al., 1997). Group 2 consisted of newly established FWS CW mesocosms, also planted with *T. latifolia*. Soil for Group 2 cells was obtained from the UTK Experiment Station Plant Science Farm along the bank of the Tennessee River at the same site where soil was collected when

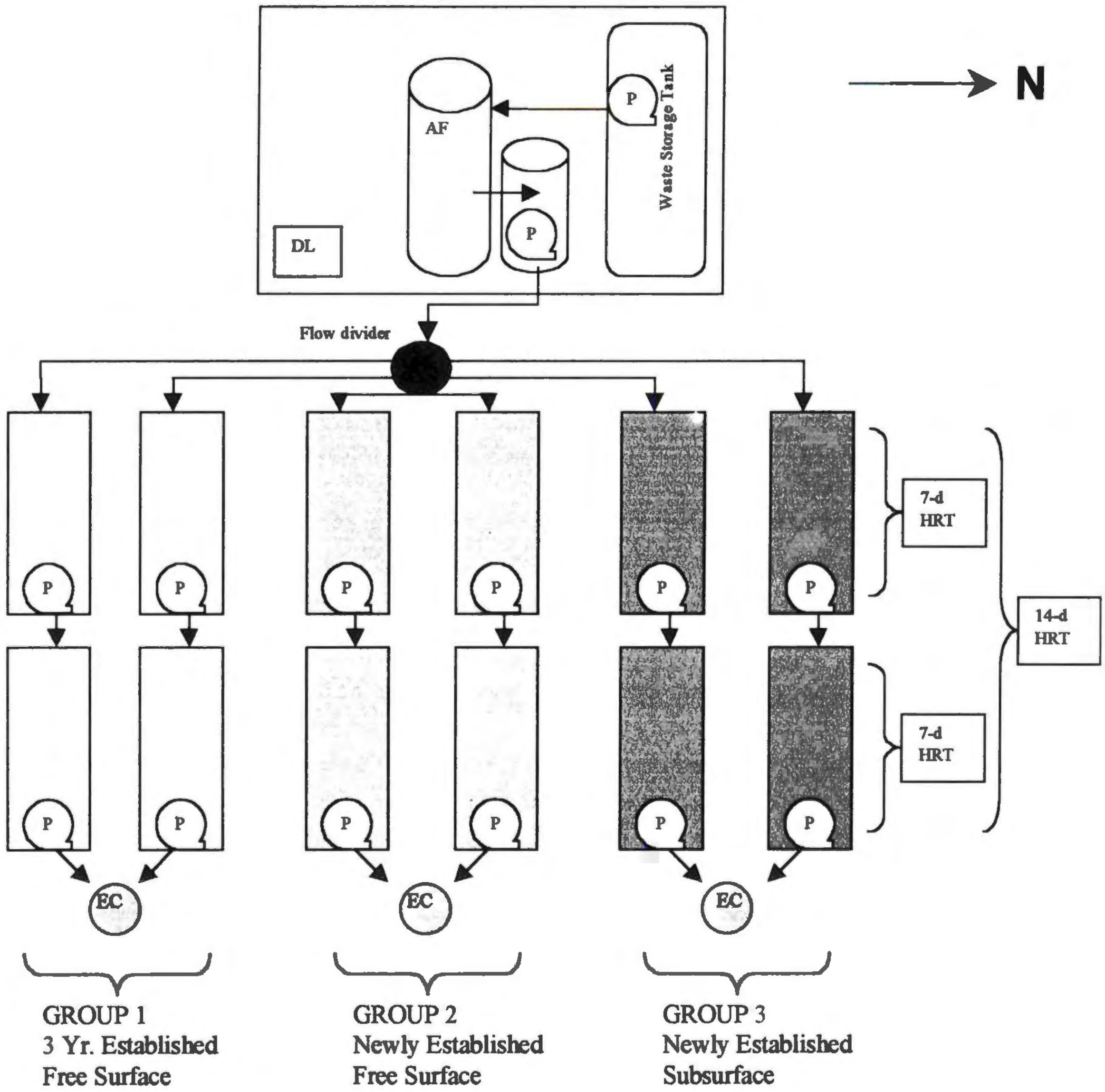


Figure 2. *Overview of experimental setup, showing all three groups of mesocosms. P = pump, DL = datalogger, AF = anaerobic filter, EC = effluent collection.*

Group 1 was established. Cattails were transplanted into these cells in September 1998 by using 10 x 10-cm cuttings from colonies used in a previous study (Raman et al., 1997). The cuttings were planted on 15-cm centers resulting in approximately nine cuttings per CW mesocosm. Figure 3 illustrates the configuration of the FWS CWs in this study. Group 3 consisted of newly established SSF CW mesocosms with 2.5-cm river rock (porosity = 0.40) as the substrate and cattail transplants planted at the same time and in the same fashion as Group 2. All of the wetlands had a 15-cm PVC standpipe for effluent collection, surrounded by a 5-cm layer of river rock.

The four cells in each group were divided into two sets of two cells in series (Figure 4). The FWS cells operated at 18-cm water depth, while the SSF cells operated at a 44.5-cm water depth, giving each cell a 7-d HRT, and two cells in series a 14-d total HRT. Wastewater flowed along the long axis of each cell to the standpipe at the effluent end. A siphon between the two CWs in a particular series equalized liquid levels in each series. A drain siphon placed at the end of each series controlled overall liquid height. Pumps were used to engage each siphon (Dayton Electric Mfg. Co., Teel Model 2P873A, Niles, IL) and activated every three hours for 5 seconds to engage the final siphon in each series and to ensure the integrity of siphons between CWs. Each pump had a floss filter over its inlet to prevent the transfer of mosquito larvae between CW cells. Pumps were wired to three position switches, allowing continuous 120 V power (manual-override: on), datalogger controlled 120-V power, or off (manual-override: off).

Effluent from each series was routed to individual 200-L drums with closures to reduce evaporation from each drum. At the end of each treatment period (draining of a

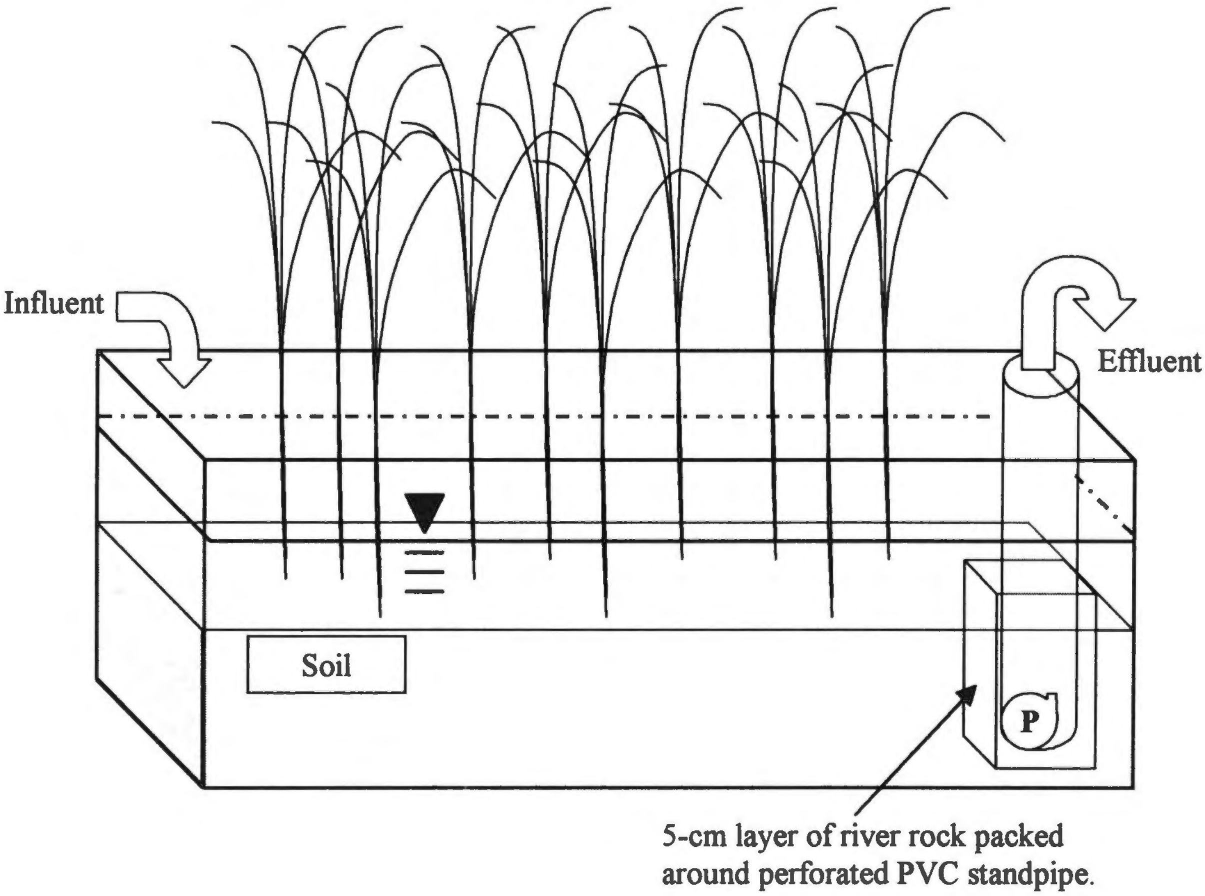


Figure 3. *Illustration of FWS CW mesocosms used for this study. The SSF CW mesocosms differed in that the substrate is river rock instead of soil, and that the liquid level was below the surface of the substrate. Pump (P) was used to engage effluent siphon. See text for details.*

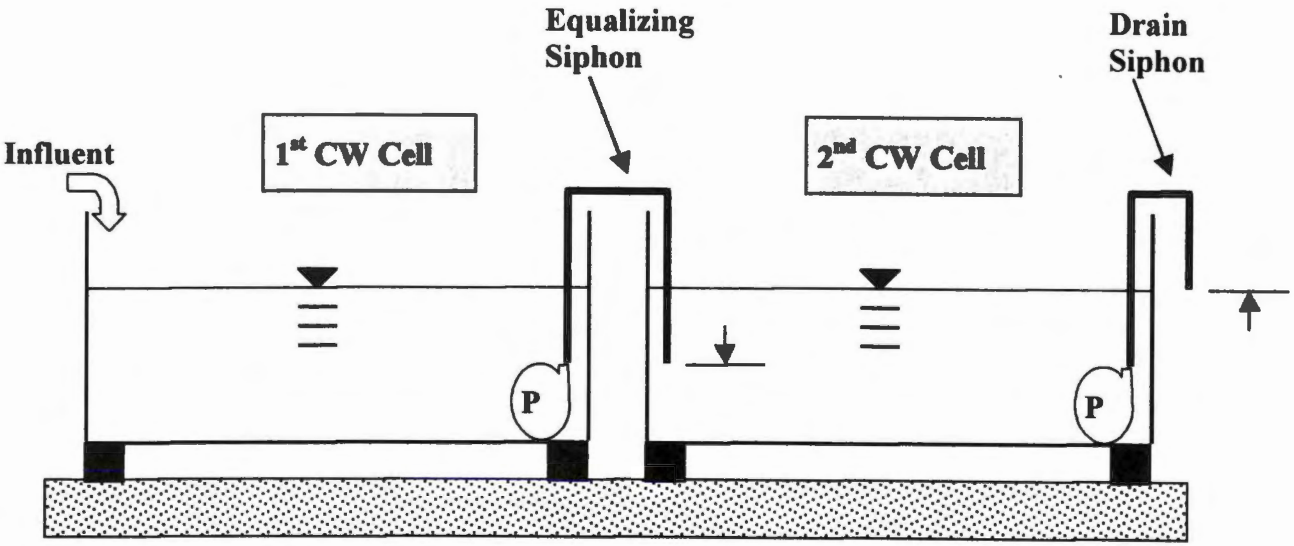


Figure 4. Profile of a CW series (applies to both FWS and SSF) illustrating siphon mechanisms for liquid leveling between two CWs in a series, and overall liquid leveling with a drain siphon.

CW series) the contents of each drum was transferred via centrifugal pump through a flow meter (FTB-4110, Omega Engineering, Stamford, CT) to a sanitary sewer for disposal. The amount transferred was recorded as the volume of effluent from a given series and reflected net rainfall and evapotranspiration (ET).

Weather Data

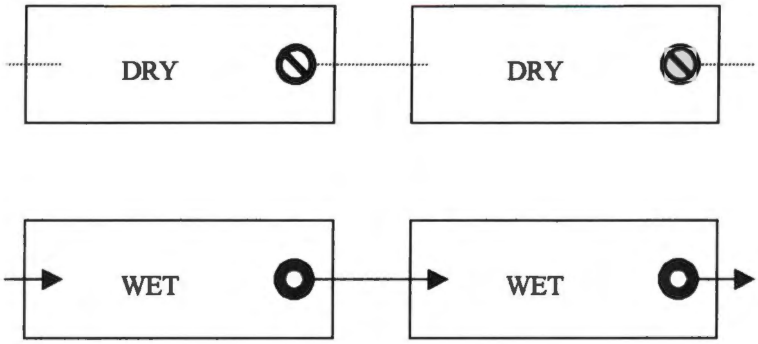
Precipitation data from an existing weather monitoring station situated approximately 30 m east of the CWs was used to characterize site rainfall amounts (mm). The station contained a Campbell Scientific CR10 datalogger (Logan, UT) and a tipping bucket rain gauge (Weathertronics, Model 6011-A, Sacramento, CA).

Drying Schedule

In each group of FWS cells, one set was full while the other set was drying. When a set was to be drained, the operator routed tubing from the full cell to the dry cell next to it. Pumps were then manually activated to drain the cells. Upon completion of draining, the pumps were switched back to computer-controlled operation, and the tubing was routed accordingly (See Figure 5).

Mosquito Surveillance

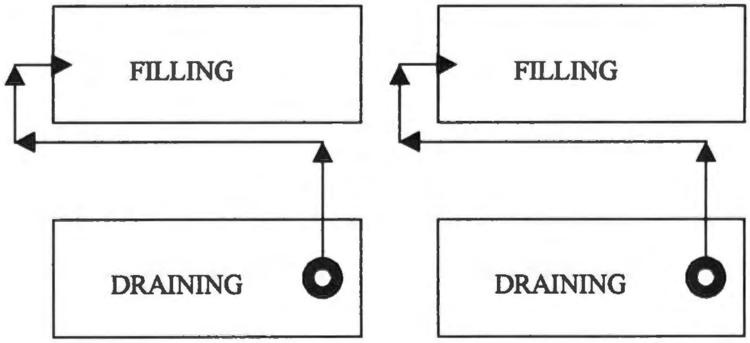
Free surface wetlands were monitored for mosquito larvae and pupae using the dip method. A standard mosquito dip sampler (Clark Mosquito Control Products, Inc., Roselle, IL) was used to dip a water sample from the water surface of each full wetland. A standard operating procedure (Service, 1976) ensured consistency of sampling efforts between sampling events. Samples were quantified and categorized by quantity of each instar (1^{st}, 2^{nd}, 3^{rd}, and 4^{th}) and quantity of pupae.



Flow Pattern Normal

= *Manual-Override: Pump Off*

= *Datalogger Controlled Pump*



Flow Pattern During Cell Switch

= *Manual-Override: Pump On*

Figure 5. *Overhead diagram of liquid flow patterns within CW series during normal flow and between CW sister series during liquid transfer.*

Drained CW cells were monitored to capture adults that had emerged from pupae left to desiccate in the CW. Two emergence traps were placed into each of the drained mesocosms and checked daily for adults. Traditional floating emergence traps made from solid sheets of plastic (Service, 1976) were unsuitable for our work, because they prevent ventilation of the soil surface. This necessitated the construction and use of a novel emergence trap (Figure 6), with a design based loosely on traditional floating emergence traps. Specifically, a cylindrical PVC frame surrounded by screen mesh was employed to allow evaporation from the CW surface. A 1-L widemouth jar with an inverted funnel entry was attached at the top of the frame to direct flying mosquitoes into the collection container. When adults were collected, they were counted and identified to species. In addition, soil surface moisture (i.e. moist, moderately dry, very dry) was also noted whenever adults were observed.

The drying schedule was selected so that few, if any, mosquitoes emerged as adults. To prevent emergence, the wetland surface must have dried sufficiently to desiccate and kill larvae and pupae before the cell was refilled. Failure to kill the larvae and pupae during the drying period resulted in their continued development when the CW was refilled. In preliminary laboratory experiments larvae and pupae survived for more than two days, and some pupae emerged as adults, when kept on a damp paper towel. Therefore, it was reasoned that a high-degree of drying of the wetland surface must occur to successfully control the production of adult mosquitoes.

Prior experience with the CW cells suggests that the principal mosquito species in the mesocosms was the so-called "filth breeder" *Culex pipiens* complex. During the summer of 1998, larvae were collected from the established FWS CW cells, reared to

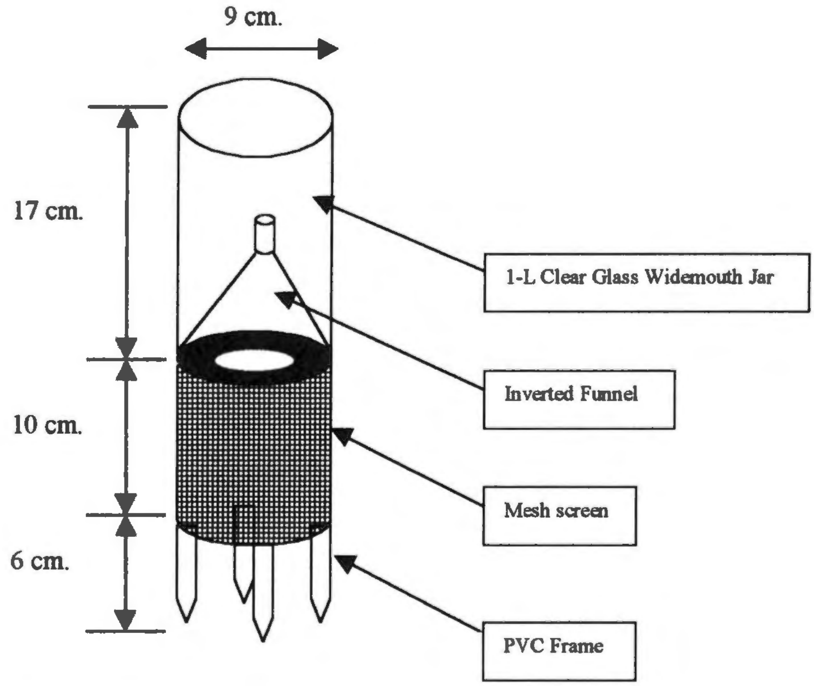


Figure 6. *Mosquito emergence trap designed to collect flying adults while allowing surface ventilation. Frame was constructed from PVC wrapped with screen mesh. A 1-L widemouth glass jar lid with 6-cm dia. hole was attached (inverted) to the top of the frame. An inverted funnel was fastened over the hole, and the glass jar was screwed into the attached lid.*

adults and identified as *Cx. pipiens* complex. Additionally, adults collected from a standpipe in the spring of 1999 were identified as *Cx. pipiens* complex.

Completion of the 4th instar was selected as the critical point for starting a drying phase due to observed hardiness of pupae in semi-moist conditions in preliminary laboratory experiments. Rueda et al. (1990) reported temperature dependent development rates for egg hatch to time to complete the 4th instar as 25.35, 9.53, 7.95, 5.38, and 5.31 days at constant temperatures of 15, 20, 25, 27, and 30 °C, respectively. A simple two-parameter model was fit to this data, to allow computation of time-to-develop (TD) from knowledge of temperature (T), as follows:

$$TD = TD_{max}(e^{-k(T-15)}) \quad (1)$$

where TD_{max} = 25 d, k = 0.16 °C^{-1}, and T is temperature (°C). Equation (1) was used to estimate TD during summer months when water temperature varies diurnally from an estimated 15°C to 27°C, arriving at an average TD value of 7 d from time of egg hatch to completion of the 4th instar.

Water Quality Monitoring

A water quality monitoring schedule allowed assessment of the CW treatment efficiencies. Sample points included system influent, AF effluent, and each CW effluent. Grab samples were collected from the sample points on a routine schedule for laboratory testing. For the first three months, sampling events occurred twice weekly. Based on the low variability observed between same-week samples, this schedule was changed to once each cycle for the FWS cells, and weekly for the SSF cells.

All samples were analyzed for total nitrogen (TN), ammonia nitrogen (NH_3-N), total phosphorous (TP), chemical oxygen demand (COD), pH, temperature, and DO. TN,

NH_3-N, TP and COD were analyzed using digestion-spectrophotometric methods (DR/2000 Direct Reading Spectrophotometer, HACH, Loveland, CO). Handheld DO and pH meters (Model HI 9142, Hanna Instruments Limited, Bedfordshire, UK, and Model 63, YSI, Yellow Springs, OH, respectively), were used to obtain DO and pH at the standpipe of each mesocosm. Type-T thermocouples, placed in the first CW of each series, were used to record average hourly temperatures of the wastewater.

Volumetric Flow Calculations

Volumetric flow in a CW during a given sampling period was calculated through the following generalized equation:

$$VI_i + VT_{INi} - VT_{OUTi} + VR_i - VET_i - VE_i = 0 \qquad (2)$$

where VI_i is the volume of influent entering CW_i, VT_{INi} is the volume transferred into CW_i from its sister CW, VT_{OUTi} is the volume transferred out of CW_i to its sister CW, VR_i is volume of rainfall entering CW_i, VET_i is the volume evapotranspiring CW_i, and VE_i is the volume of effluent leaving CW_i. Subscript *i* specifies the first CW in a series ($_A$), the second CW in a series ($_B$), or both combined ($_{Series}$). Transfer of liquid for the purpose of mosquito control did not occur in SSF CWs; therefore in SSF CWs VT_{INi} and VT_{OUTi} were always equal to zero. Figure 7 defines the flows used in Equation 2.

During this study, three distinct scenarios were observed in the FWS CWs. Scenario 1 occurred when the liquid level in each CW in a series was maintained at the proper siphon-controlled level. Scenario 2 occurred when the liquid level fell below the siphon-controlled level in each CW in a series. Scenario 3 occurred when the liquid level in the first CW in a series fell below the siphon-controlled liquid level and the second CW in a series was dry. This scenario arose due to such low liquid levels that the siphon

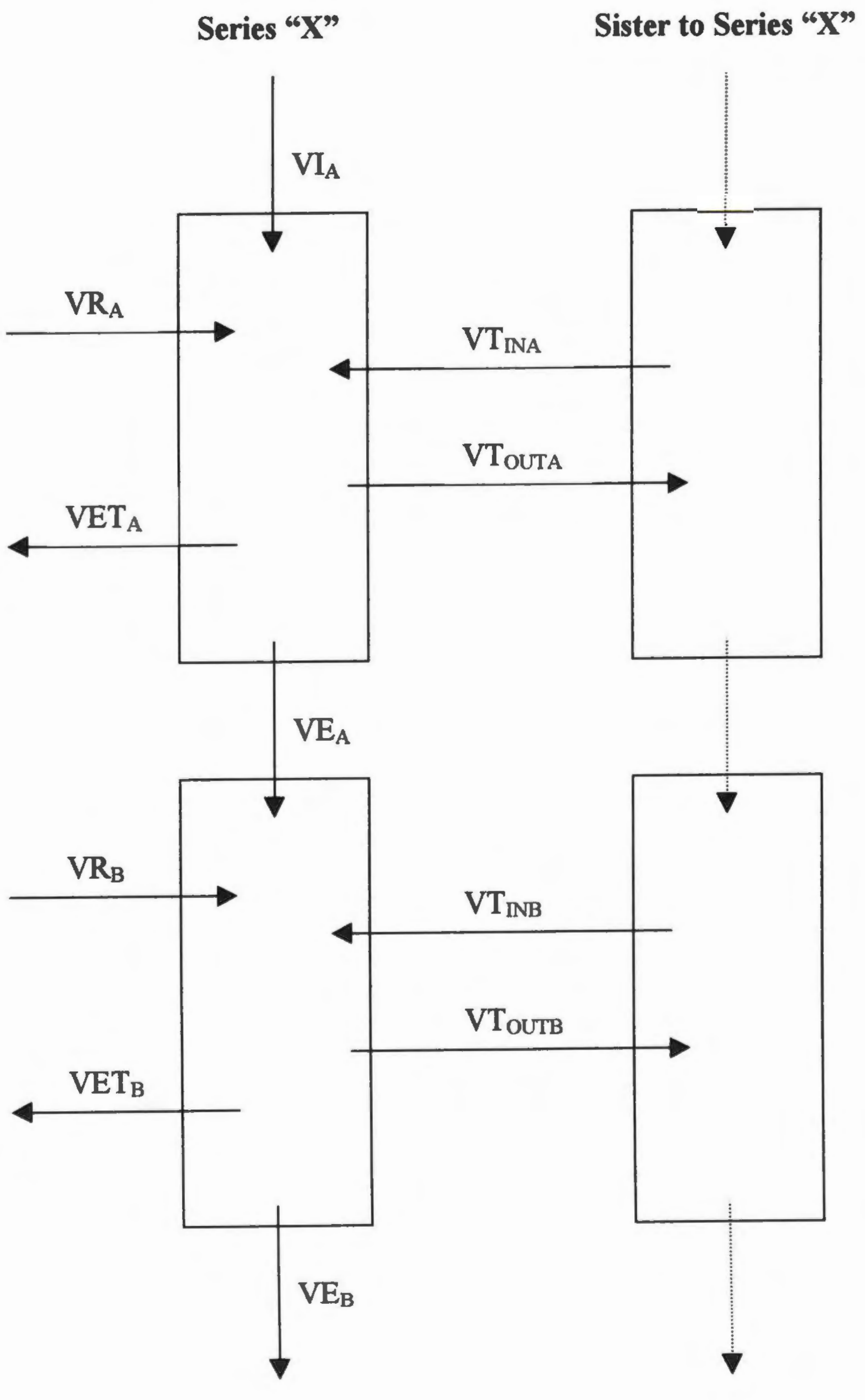


Figure 7. *Illustration of volumetric flow patterns in a CW series during a given treatment period.*

between CWs could not maintain itself between activations. The SSF CWs remained at the designed liquid depth throughout this study (equivalent to Scenario 1 in FWS CWs).

For Scenarios 1 and 2, with the volume (L) from the series collection barrel, referred to as VE_B, one can rearrange Equation 2 and solve for overall VET_{Series} in a CW series:

$$VET_{Series} = VI_{Series} + VT_{INA} - VT_{OUTA} + VT_{INB} - VT_{OUTB} + VR_A + VR_B - VE_B \quad (3)$$

Due to comparable plant densities between the first and second CW in each series, it was assumed that $VET_A = VET_B$. Therefore, one can use the equation:

$$VET_B = VET_{Series}/2 = VET_A \quad (4)$$

With VET_A, Equation 2 can be rearranged to solve for VE_A:

$$VE_A = VI + VT_{INA} - VT_{OUTA} + VR_A - VET_A \quad (5)$$

A different approach had to be taken to solve for VET and VE for scenario 3. Initially, using $VE_B = 0$ one could solve the equation for VET_{Series}. However, due to the second CW cell in a series being dry during the treatment period—the assumption $VET_A = VET_B$ cannot be justified. Since the siphon between CWs was broken between activations VE_A is assumed to be the volume of liquid (L) moved during activations. The pumps were activated eight times a day for 5 seconds, resulting in roughly 2.4 L d^{-1} effluent. This volume multiplied by the number of days in the period results in VE_A. Then VET_A and VET_B were found separately by the equations:

$$VET_A = VI_A + VT_{INA} - VT_{OUTA} + VR_A - VE_A \quad (6)$$

and

$$VET_B = VE_A + VT_{INB} - VT_{OUTB} + VR_B - VE_B \quad (7)$$

Mass Removal Calculations

Mass removal rates for COD, TN, NH_3-N, and TP in individual CWs were calculated as follows:

$$\frac{VI_i * CI_i - VE_i * CE_i}{D} \qquad (8)$$

where CI_i is the influent concentration (mg/L) of the analyte of interest, VE_i is the volumetric effluent (L) for that CW, CE_i is the effluent concentration (mg/L) of the analyte of interest, and D is the number of days in the treatment period.

Data Analysis

After six months of system operation, the results of water quality testing were used to compare pollutant removal between the different groups of CWs. Specifically, comparisons were made between SSF and periodically dried FWS cells, and between newly planted vs. established FWS cells.

SPSS for Windows, Release 8.0.0 (Statistical Product and Service Solutions, Chicago, IL) was used to perform GLM Type III analysis giving partial tests on each factor while adjusting for others in the model. A completely randomized design model was used, as follows: $y_{ijk} = \mu + C_i + H_j + CH_{ij} + e_{ijk}$, with dependent variable y (pollutant removal rates), fixed factors C (CW type) and H (HRT level), the interaction between C and H, and error. Custom contrasts were performed to compare mean effects. Statistical hypotheses tested were H_o: $\mu_1 - \mu_2 = 0$ for each of the following comparisons:

- Group 1 vs. Group 2 @ 7-d.
- Group 1 vs. Group 3 @ 7-d.
- Group 2 vs. Group 3 @ 7-d.

- Group 1 vs. Group 2 @ 14-d.
- Group 1 vs. Group 3 @ 14-d.
- Group 2 vs. Group 3 @ 14-d.

Chapter 4

RESULTS AND DISCUSSION

For clarity, in this chapter the 3-yr free water surface (FWS) constructed wetlands (CW) will be referred to as Group 1, the new free water surface constructed wetlands will be referred to as Group 2, and the new subsurface flow (SSF) constructed wetlands will be referred to as Group 3.

Mosquito Surveillance

Emergence

Dip sampling for mosquito larvae and pupae began in early spring (mid March, 1999). The first appearance of larvae occurred in mid-April. Results from the first trap-week were promising with no adult mosquitoes caught in the emergence traps from either of the FWS groups. However, during the second trap-week, flying adults were found in the emergence traps of the Group 2 cells. Emergence of adult mosquitoes from the CW surface was thought to be due to the differences in rainfall during the two trap-weeks. Figure 8 shows the number of emerged mosquitoes trapped during each drying period plotted with the amount of rainfall (cm) that occurred in the 24-hours immediately following the drain. During first trap week, total rainfall was 1 cm, the majority of which fell on the sixth day of the drying phase. During the second trap-week, 3 cm of rain fell in the first two days. When rainfall stopped, standing water in Group 2 cells was removed to enhance drying. (Interestingly, Group 1 cells were drier, probably because the denser vegetation caused higher ET in these cells.) Despite the draining, adults were

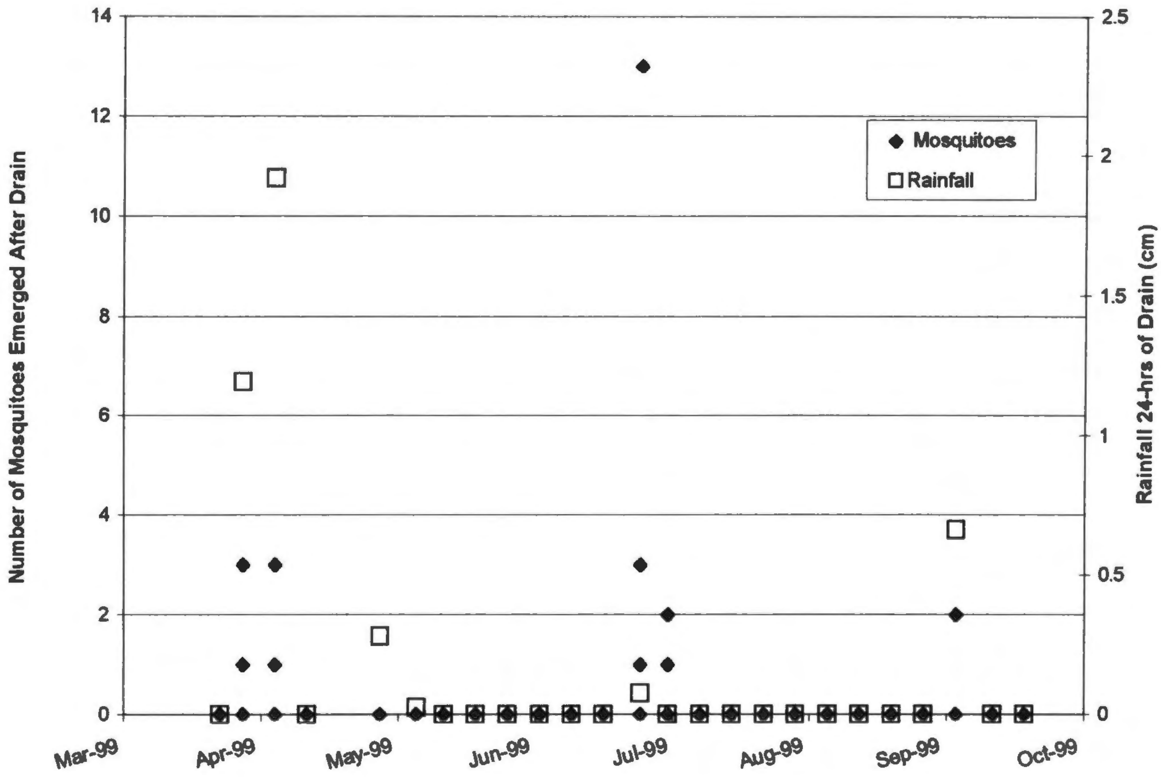


Figure 8. *Number of mosquitoes collected in emergence traps during each drying period plotted with the amount of rainfall (cm) that occurred within 24 hours of initiation of the drying period.*

collected in the Group 2 cells two days later. No additional adults were collected after this time.

The third trap-week was similar to the second trap-week, with 2 cm of rain within the first 24 h of the drying phase (5 cm over 48-hrs). Standing rainwater was then removed to enhance drying. Despite this effort, adults were collected in both Group 1 and Group 2 cells.

This trend occurred two more times during the study, where emergence of mosquitoes was observed to coincide with rainfall within 24 hours of the initiation of a drying period (Refer to Figure 8). This may indicate a need for additional methods of mosquito control such as aerial application of insecticide. Mosquitoes were not observed in the emergence traps for 20 out of 25 drying cycles. This indicates that—overall—draining CWs has the desirable negative effect on the immature stages of mosquitoes and thus prevents adult emergence.

Larval/Pupal Development

Larvae were not observed in either group of FWS CWs until mean water temperature exceeded 15°C in mid-April (refer to Figures 9 and 10). From Figures 9 and 10, one observes that the number of larvae/pupae per dip tended to both increase and fluctuate with mean water temperatures over the course of the study. Due to the large variability of dip sample results a variance-stabilizing transformation was performed on number of larvae/pupae per dip sample. Specifically, a transformed variable, y′ was defined based on y (the larval/pupal count per sample), as follows: $y' = \log_{10}(y + 1)$. The addition of 1 prior to taking the logarithm was to avoid problems with $y = 0$ data. A plot

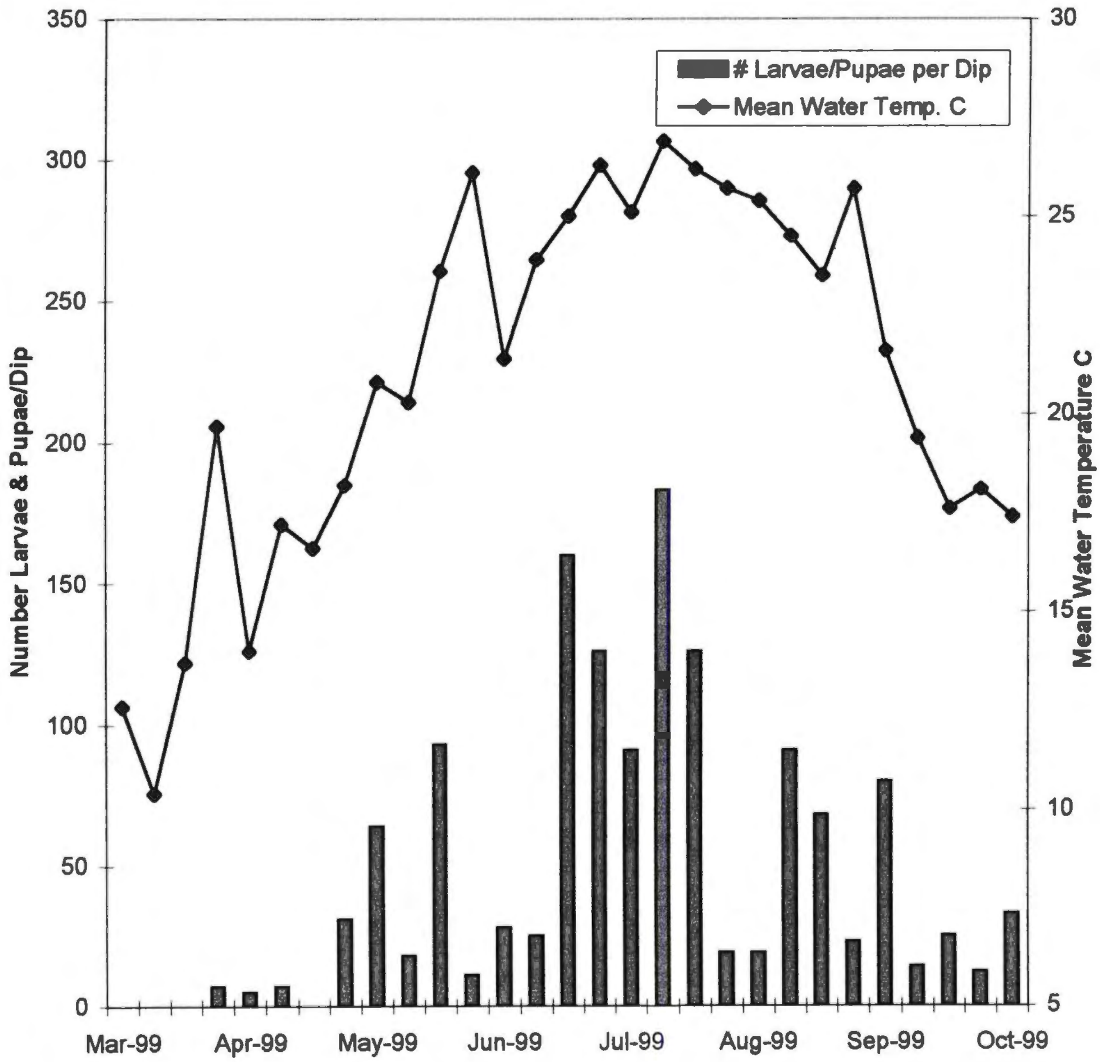


Figure 9. *3-yr FWS: Total number of larvae/pupae per dip sample plotted with weekly mean water temperature (℃).*

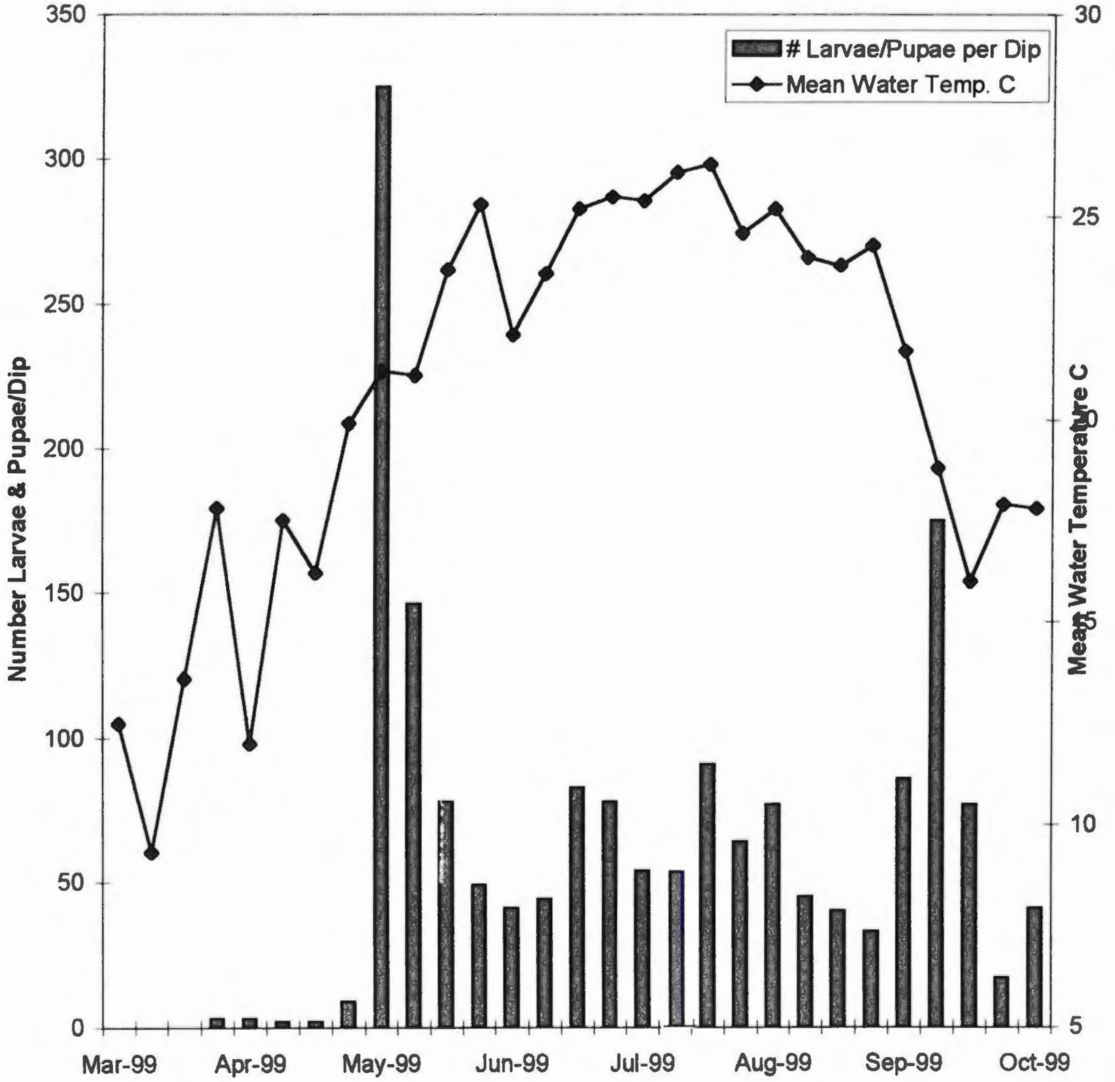


Figure 10. *New FWS: Total number of larvae/pupae per dip sample plotted with weekly mean water temperature (℃).*

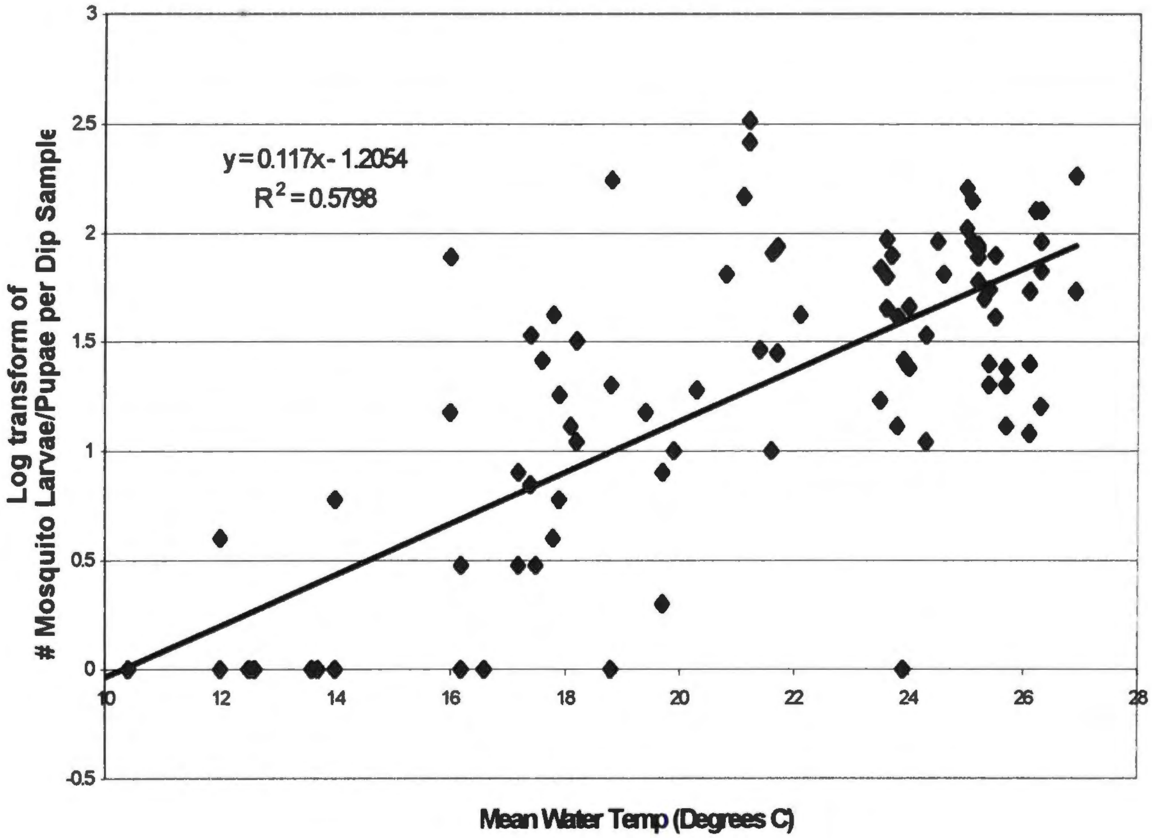


Figure 11. *Mean water temperature (℃) for period preceding drain plotted against log transformation of number of mosquito larvae/pupae per dip sample. 3-yr FWS and New FWS data plotted together. Trendline shows best linear fit for the data.*

Pump inlet filters prevented the transfer of larvae and pupae between CWs. However, populations of larvae appeared and progressed through the stages of instar development during a CWs wet cycle. Figure 12 shows the mean composition of mosquito larvae (1^{st} – 4^{th} instar) and pupae in dip samples immediately preceding drain cycles. From these figures, it is observed that a seven-day cycle did not entirely prevent pupation. The lack of observed emergence in the presence of large numbers of pupae further indicates the negative impact drying a CW has on adult mosquito emergence.

Pollutant Removal

One-hundred sixty-nine days of water quality data (representing between 27 and 54 sampling events per CW type by HRT) were used for statistical evaluation of pollutant removal.

Initially, this discussion will compare pollutant removal expressed as mass removed per unit area per day (i.e., an instantaneous value) while loaded. This approach allows for a comparison of FWS CWs (without a mosquito control drain plan) and SSF CWs, and assumes that periodic drying did not effect pollutant removal rates. At the end of this chapter, a discussion of the time-averaged pollutant removal rates is included to account for land requirement doubling when alternating wet and dry periods are used for mosquito control in the FWS cells.

Mean influent mass loading rates of 423 kg ha^{-1} d^{-1} COD, 80 kg ha^{-1} d^{-1} TN, 67 kg ha^{-1} d^{-1} NH_3-N, and 10 kg ha^{-1} d^{-1} TP to the primary cell of each series were observed. Series effluent removal rates are summarized on pages 47, 48, 50 and 52, with error bars representing standard errors. Also included in each figure is a dotted line, representing the mean influent mass loading rate of the pollutant in question. Results of custom

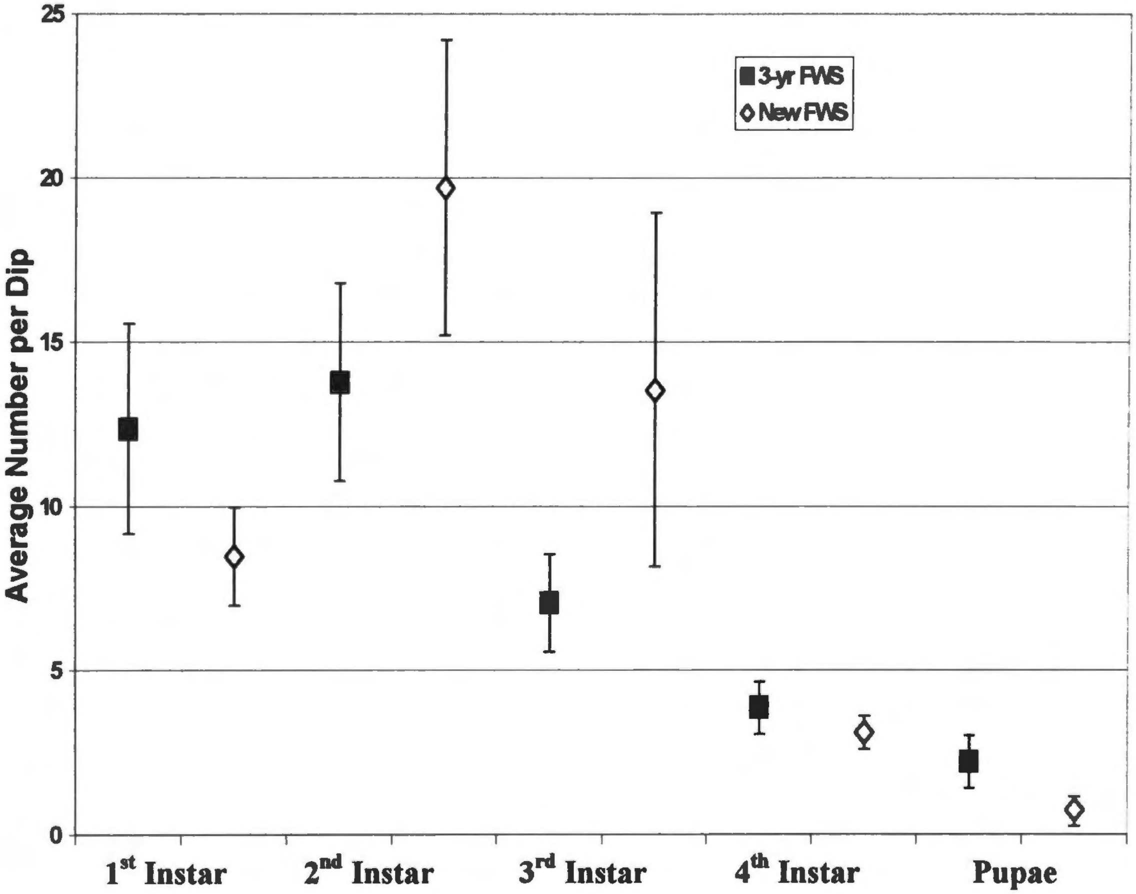


Figure 12. *Average composition of mosquito larvae (1st – 4th instar) and pupae in dip samples immediately preceding drain cycles plotted with one standard error.*

contrasts with contrast estimates, significance, and 95% confidence intervals on the estimate for COD, TN, NH_3-N, and TP are reported on pages 47, 48, 50 and 52.

COD

Mean COD removal rates and standard error (kg ha^{-1} d^{-1}) for each CW type are illustrated in Figure 13. With a 7-d HRT Group 1 removed, on average, 235 kg ha^{-1} d^{-1} COD. Group 2 removed 222 kg ha^{-1} d^{-1} COD. Group 3 removed 230 kg ha^{-1} d^{-1} COD. Group 1, 2, and 3 removed an additional 119, 85, and –31 kg ha^{-1} d^{-1} respectively with an additional 7-d HRT.

After the first 7 days of treatment, no statistically significant differences among the three Groups were observed (Table 2). This trend changes with an additional 7 days of treatment, with Group 1 and Group 2 having significantly greater COD removal (150 and 117 kg ha^{-1} d^{-1} respectively) than Group 3. Groups 1 and 2 were not significantly different from one another at an additional 7 days of treatment.

Total Nitrogen

Mean TN removal rates and standard error (kg ha^{-1} d^{-1}) for each CW type are illustrated in Figure 14. With a 7-d HRT Group 1 removed, on average, 57 kg ha^{-1} d^{-1} TN. Group 2 removed 48 kg ha^{-1} d^{-1} TN. Group 3 removed 42 kg ha^{-1} d^{-1} TN. Group 1, 2, and 3 removed an additional 17, 15, and –9 kg ha^{-1} d^{-1} respectively with an additional 7-d HRT.

Custom contrasts indicate no significant differences among Group 1 and Group 2 at either the first 7-d HRT or with an additional 7 days of treatment (Figure 14, Table 3). Group 1 cells outperformed Group 3 cells, removing 16 kg ha^{-1} d^{-1} more TN at a 7-d HRT and an additional 26 kg ha^{-1} d^{-1} more TN with the additional 7 days of treatment. Group 2

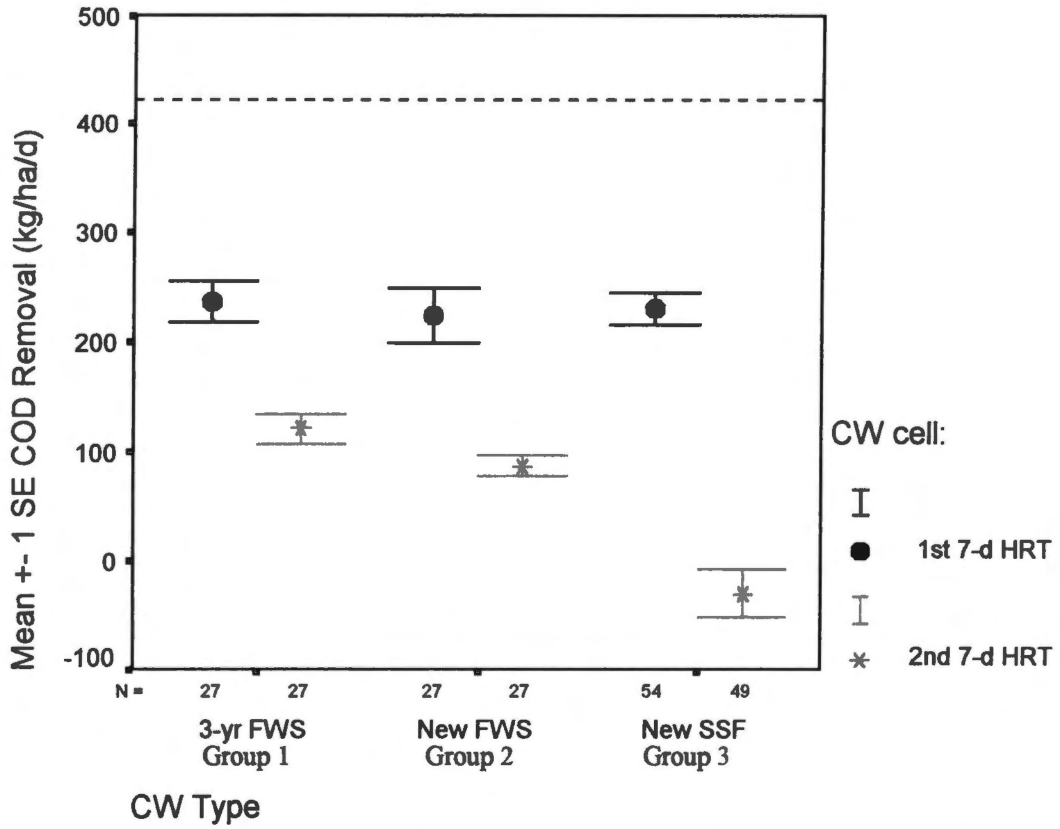


Figure 13. *Mean COD mass removal (kg ha^{-1} d^{-1}) plotted with standard error (SE) for each type of CW and CW cell in series. The horizontal dashed line represents mean COD mass influent (kg ha^{-1} d^{-1}) of influent wastewater.*

Table 2. *Contrast results indicating contrast estimates for COD mass removal (kg ha^{-1} d^{-1}) with F-values, significance levels, and 95% confidence intervals for the difference.*

Custom Contrast	Contrast Estimated COD (kg ha^{-1} d^{-1})	F value	Sig.	95% Confidence Interval for Difference	
				Lower Bound	Upper Bound
3-yr FWS vs. New FWS @ 7-d HRT	14	0.201	0.655	-48	76
3-yr FWS vs. New SSF @ 7-d HRT	6	0.048	0.827	-47	59
New FWS vs. New SSF @ 7-d HRT	-8	0.090	0.765	-61	45
3-yr FWS vs. New FWS @ an additional 7-d HRT	33	1.130	0.289	-28	95
3-yr FWS vs. New SSF @ an additional 7-d HRT	150	29.62	0.000*	96	204
New FWS vs. New SSF @ an additional 7-d HRT	117	17.94	0.000*	62	171

*. The difference is significant at the 0.05 level.

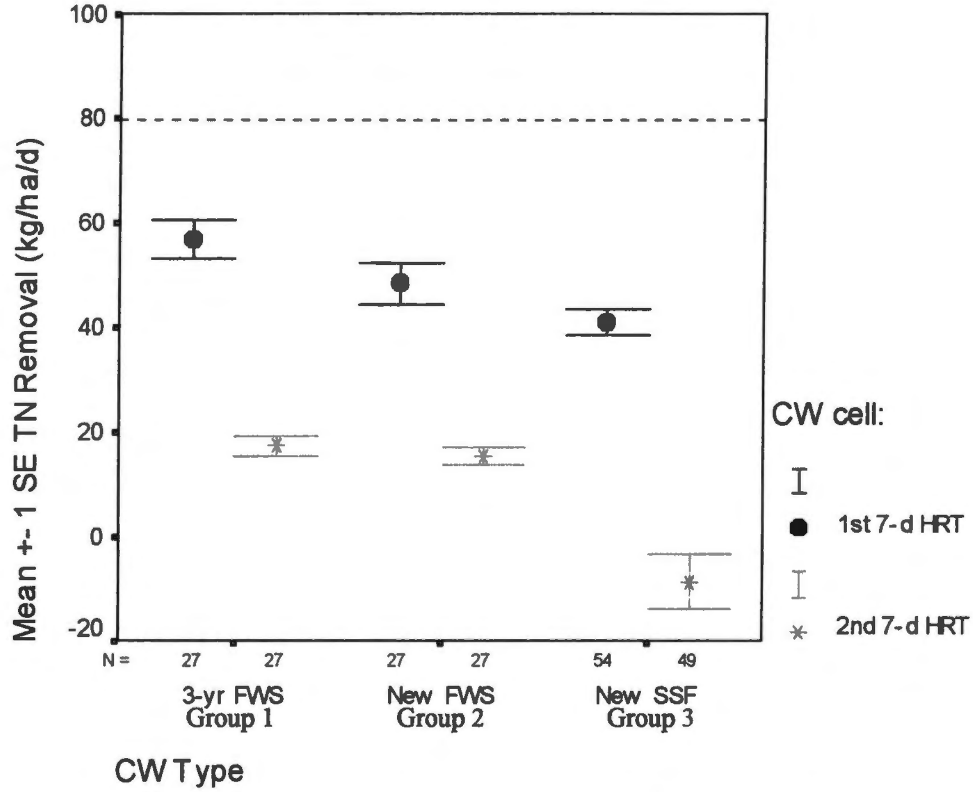


Figure 14. *Mean Total Nitrogen mass removal (kg ha^{-1} d^{-1}) plotted with standard error (SE) for each type of CW and each HRT. The horizontal dashed line represents mean TN mass influent (kg ha^{-1} d^{-1}) of influent wastewater.*

Table 3. *Contrast results indicating contrast estimates for TN mass removal (kg ha^{-1} d^{-1}) with F-values, significance levels, and 95% confidence intervals for the difference.*

Custom Contrast	Contrast Estimated TN (kg ha^{-1} d^{-1})	F value	Sig.	95% Confidence Interval for Difference	
				Lower Bound	Upper Bound
3-yr FWS vs. New FWS @ 7-d HRT	9	1.693	0.195	-4	21
3-yr FWS vs. New SSF @ 7-d HRT	16	7.818	0.006*	5	27
New FWS vs. New SSF @ 7-d HRT	7	1.665	0.198	-4	18
3-yr FWS vs. New FWS @ an additional 7-d HRT	2	0.091	0.763	-11	15
3-yr FWS vs. New SSF @ an additional 7-d HRT	26	20.49	0.000*	15	37
New FWS vs. New SSF @ an additional 7-d HRT	24	17.5	0.000*	13	35

*. The difference is significant at the 0.05 level.

cells did not outperform the Group 3 cells during the initial 7 day treatment; however, there was a significant difference with the additional 7-d HRT (Refer to Table 3 for details). The poor performance of Group 3 may be explained by lower ET associated with a newly planted SSF CW coupled with an excess of rainfall. A net efflux of TN occurred frequently from the secondary SSF CWs, perhaps due to the effect of periods of heavy rainfall and possible biomass washout. Groups 1 and 2 rarely demonstrated this phenomenon since net rainfall typically did not exceed net ET in these groups.

NH_3-N

Mean NH_3-N removal rates and standard error (kg ha^{-1} d^{-1}) for each CW type are illustrated in Figure 15. With a 7-d HRT Group 1 removed, on average, 49 kg ha^{-1} d^{-1} NH_3-N. Group 2 removed 42 kg ha^{-1} d^{-1} NH_3-N. Group 3 removed 31 kg ha^{-1} d^{-1} NH_3-N. Group 1, 2, and 3 removed an additional 14, 14, and -6 kg ha^{-1} d^{-1} respectively with an additional 7-d HRT.

The trends observed are identical to those seen in the TN data with the addition of a statistical difference between Group 2 and Group 3 in the secondary 7-d HRT cells. Mean removal rates for each treatment level, as well as statistical results are provided in Table 4. The superior performance of the Group 1 and Group 2 in NH_3-N removal suggest that ammonia volatilization or nitrification/denitrification and subsequently diffusion into the soil played a critical role in N removal. The virtually identical removal rates between Group 1 and Group 2 suggest plant density (i.e. assimilation) had little effect on N removal. A net efflux of NH_3-N was observed from the secondary SSF CWs. This result may have been due to washout of biomass in sediment during high rainfall events.

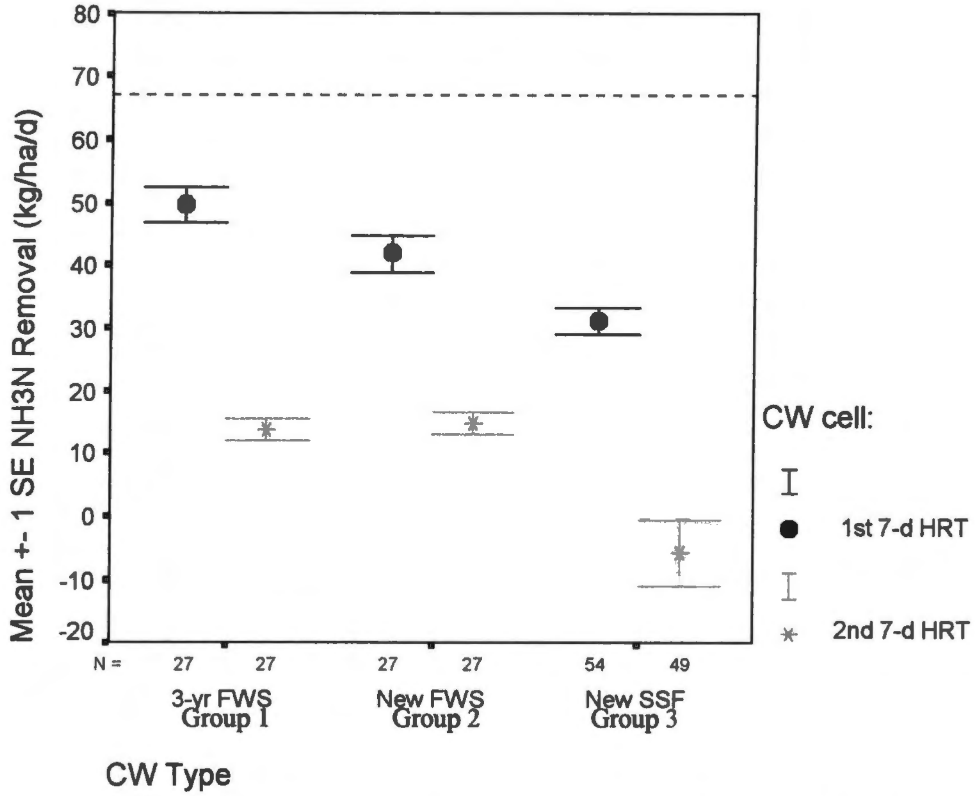


***Figure 15.** Mean NH_3-N mass removal (kg ha^{-1} d^{-1}) plotted with standard error (SE) for each type of CW and each HRT. The horizontal dashed line represents mean NH_3-N mass influent (kg ha^{-1} d^{-1}) of influent wastewater.*

***Table 4.** Contrast results indicating contrast estimates for NH_3-N mass removal (kg ha^{-1} d^{-1}) with F-values, significance levels, and 95% confidence intervals for the difference.*

Custom Contrast	Contrast Estimated NH_3-N (kg ha^{-1} d^{-1})	F value	Sig.	95% Confidence Interval for Difference	
				Lower Bound	Upper Bound
3-yr FWS vs. New FWS @ 7-d HRT	8	1.790	0.182	-4	19
3-yr FWS vs. New SSF @ 7-d HRT	18	13.10	0.000*	8	28
New FWS vs. New SSF @ 7-d HRT	10	4.291	0.040*	0.5	20
3-yr FWS vs. New FWS @ an additional 7-d HRT	-1	0.046	0.830	-12	10
3-yr FWS vs. New SSF @ an additional 7-d HRT	19	14.32	0.000*	9	29
New FWS vs. New SSF @ an additional 7-d HRT	20	16.23	0.000*	11	31

*. The difference is significant at the 0.05 level.

Phosphorous

Mean TP removal rates and standard error (kg ha^{-1} d^{-1}) for each CW type are illustrated in Figure 16. With a 7-d HRT Group 1 removed, on average, 6 kg ha^{-1} d^{-1} TP. Group 2 removed 5 kg ha^{-1} d^{-1} TP. Group 3 removed 6 kg ha^{-1} d^{-1} TP. Group 1, 2, and 3 removed an additional 3, 3, and 0 kg ha^{-1} d^{-1} respectively with an additional 7-d HRT.

Custom contrasts indicate no significant differences between the three Groups for removal of TP after the initial 7 days of treatment (Refer to Table 5, Figure 16). An additional 7 days of treatment distinguished Group 1 and Group 2 to be significantly different from Group 3—each removing an additional 3 kg ha^{-1} d^{-1} TP compared to Group 3.

pH

Custom contrasts indicated significant statistical differences of pH among the three groups of CW mesocosms, both at the initial 7-day treatment and at the additional 7-day treatment cell. However, mean pH values among the twelve mesocosms were relatively neutral, ranging between the values of 7.2 and 8.2.

Dissolved Oxygen

Mean DO concentrations (mg/L) were not statistically different for the three Groups. Oxygen inputs from surface and plant diffusion were not typically able to raise the mean DO concentrations above 2.5 mg/L in any of the CW types. This is significant because even the lowest-oxygen-tolerant fish species require DO concentrations above 2.0 mg/L to survive--making control of mosquitoes by fish predators difficult to implement and manage in FWS CWs.

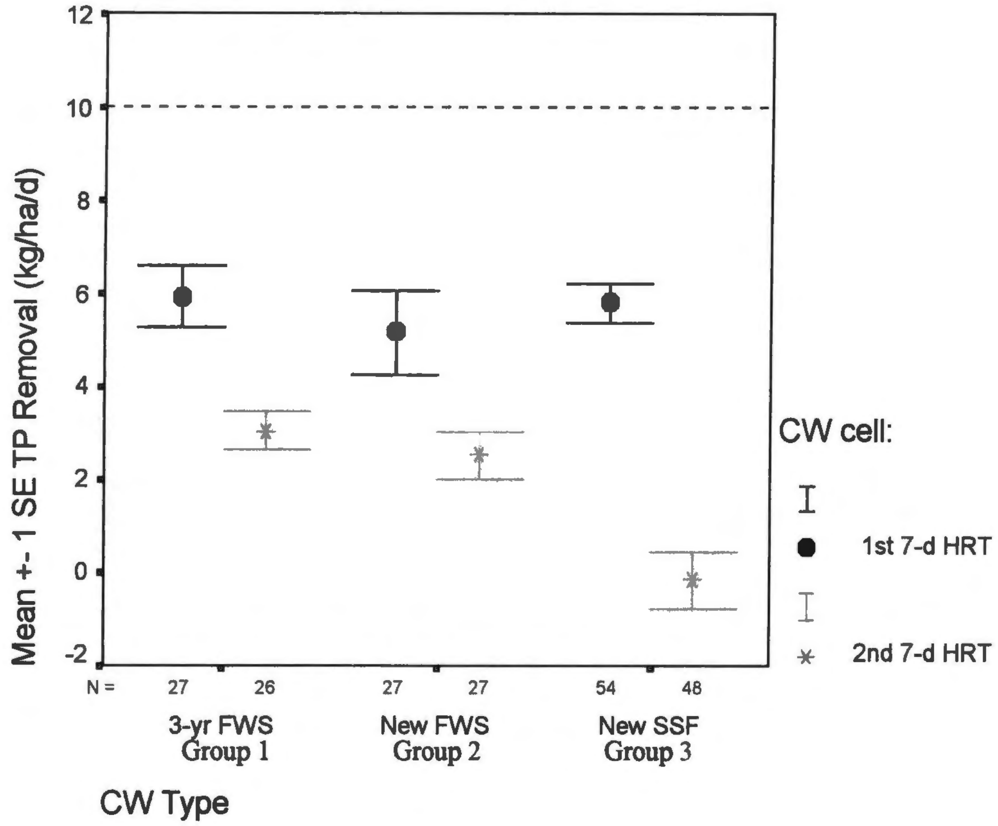


Figure 16. *Mean TP mass removal (kg ha^{-1} d^{-1}) plotted with standard error (SE) for each type of CW and each HRT. The horizontal dashed line represents mean TP mass influent (kg ha^{-1} d^{-1}) of influent wastewater.*

Table 5. *Contrast results indicating contrast estimates for TP mass removal (kg ha^{-1} d^{-1}) with F-values, significance levels, and 95% confidence intervals for the difference.*

Custom Contrast	Contrast Estimated TP (kg ha^{-1} d^{-1})	F value	Sig.	95% Confidence Interval for Difference	
				Lower Bound	Upper Bound
3-yr FWS vs. New FWS @ 7-d HRT	0.7	0.622	0.431	-1	3
3-yr FWS vs. New SSF @ 7-d HRT	0	0.010	0.922	-2	2
New FWS vs. New SSF @ 7-d HRT	-0.6	0.664	0.416	-2	1
3-yr FWS vs. New FWS @ an additional 7-d HRT	0.5	0.298	0.586	-1	2
3-yr FWS vs. New SSF @ an additional 7-d HRT	3	13.13	0.000*	1	5
New FWS vs. New SSF @ an additional 7-d HRT	3	9.338	0.003*	1	4

*. The difference is significant at the 0.05 level.

Time-averaged Pollutant Removal

When periodic draining is implemented the removal rates in those cells must be adjusted to account for the 50% duty cycle on which they operate. This effectively halves the instantaneous removal rates previously reported for FWS CWs. Thus, with a 7-d HRT, Group 1 and 2 removed 117 and 111 kg ha^{-1} d^{-1} COD, and an additional 60 and 43 kg ha^{-1} d^{-1} with an additional 7-d HRT. Group 1 and 2 TN removal rates were 29 and 24 kg ha^{-1} d^{-1} after the first 7 days of treatment, and were 8.5 and 7.5 kg ha^{-1} d^{-1} in the subsequent 7-d HRT. Ammonia nitrogen removal rates become 25 and 21 kg ha^{-1} d^{-1} for the initial 7-d HRT, and an additional 7 and 7 kg ha^{-1} d^{-1} for the second HRT. Finally, TP removal rates were 3 and 2.5 kg ha^{-1} d^{-1} for the first 7-d HRT, and an additional 1.5 and 1.5 kg ha^{-1} d^{-1} after an additional 7 days. Therefore, FWS CWs operated for mosquito control achieved roughly half the areal removal rates of continuously fed SSF CWs (230, 42, 31, and 6 kg ha^{-1} d^{-1} for COD, TN, NH_3-N, and TP, respectively for the first 7-d HRT). However, based on economic calculations reported by Tchobanoglous (1997) it would still be more economical to install FWS CWs.

Chapter 5

CONCLUSIONS AND FUTURE WORK

Data from this study indicated that periodic drying of free water surface constructed wetland cells (FWS CW) can control adult mosquito populations. However, the first one to two days of the drying phase is critical, and rainfall during this time can negate the effect of the drying period. It may be possible to overcome this limitation by activating drain pumps when rain occurs during a dry-phase. Also, floss filters were used to prevent transfer of mosquito larvae or pupae between CWs during drain periods. This type of barrier may be difficult to implement in a full size system. Future studies should assess the effect of large centrifugal pumps on mosquito larvae and pupae viability. The internal forces of large pumps may be enough to kill larvae or pupae during a transfer.

Water quality data indicated that FWS CW cells with 3-yr established plants do not achieve significantly greater pollutant removal rates than newly established FWS cells for COD, TN, NH_3-N, or TP at a 7-d HRT. It has been reported that subsurface flow constructed wetland cells (SSF CW) achieve areal removal rates four times greater than FWS CW cells. This result was not confirmed by this experiment. Rather, results show both newly established FWS and 3-yr old FWS CWs achieving within 50% removal rates as their SSF counterparts. Long-term observation is needed to determine if this trend will continue beyond the six-month experimental period.

During the seasonal timeframe of this study (March-October), FWS CWs operating at a 7-d HRT achieved average TN mass removal rates of roughly 25 times that of a corn crop planted to yield 150 bu ac^{-1} yr^{-1} (Midwest Plan Service, 1993).

Additionally, average TP mass removal rates were approximately 15 times that of corn planted to yield 150 bu ac^{-1} yr^{-1} (Midwest Plan Service, 1993). While average winter mass removal rates in CWs are likely to decrease, it is reasonable to expect that with an additional six months of removal by CWs, the removal differences between CWs and corn would widen to some degree. This demonstrates a significant savings in the land requirement for waste disposal at land-limited farms.

Finally, this study demonstrated the possible synergism of high-rate anaerobic digestion and constructed wetlands, as treatment systems for dairy wastewater. The DFAF had average pollutant reduction rates of 23% (COD), 5% (TN), 4% (NH_3-N), and 24% (TP). Pollutant reductions of 75% (COD), 85% (TN), 82% (NH_3-N), and 86% (TP) were observed for the Group 1 CW system, operating at a total hydraulic retention time of just under 1 month. (True HRT is estimated as 28.6 d, based on doubling the 14-d HRT of the wet-dry cells to account for their inactivity 50% of the time.) Furthermore, the plants are thriving in the DFAF effluent, with greatest plant growth occurring in the cells directly receiving DFAF effluent, rather than the 2nd stage cells. Such a system may have practical application, if issues of mosquito control, maintenance requirements, and capital costs can be successfully addressed.

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