NUTRIENT REMOVAL AND PLANT GROWTH IN A SUBSURFACE FLOW CONSTRUCTED WETLAND IN BRISBANE, AUSTRALIA

By

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ABSTRACT

One of the major water quality issues affecting waterways is eutrophication. Controlling the input of nutrients from municipal wastewater treatment plants (WTP’s) is a significant step in reducing eutrophication. Tertiary wastewater treatment for water quality improvement in particular Biological Nutrient Removal (BNR) is often expensive to construct with high maintenance costs. Constructed wetlands (CWs) offer an alternative wastewater treatment and have been used successfully worldwide to treat various types of wastewater.

This study investigated the effectiveness of the Oxley Creek horizontal subsurface flow (SSF) CW for tertiary municipal wastewater treatment and the suitability of four native macrophyte species, *Baumea articulata*, *Carex fascicularis*, *Philydrum lanuginosum* and *Schoenoplectus mucronatus*. The investigation consisted of four main components: 1) Plants: monitoring plant establishment, growth, impact of cropping, gravel size, nutrient content and storage for the four macrophyte species trialed; 2) Water quality - effluent treatment: monitoring water quality and quantity entering and leaving the wetland to determine wastewater treatment; 3) Organic matter: accumulation of organic carbon within the wetland cells for the different gravel sizes (5mm and 20mm) and 4) Mass balance: combining nutrient storage by macrophytes with wastewater nutrient removal to determine proportion of nutrient removal by plant uptake.

The Oxley horizontal SSF CW is situated at the Oxley Creek WTP in Brisbane (South-East), Queensland, Australia which has a sub-tropical climate. The experimental design involved four different substrate treatments: Cell A new 5mm gravel, Cells B and C old 20mm gravel and Cell D old 5mm gravel. Cells B, C and D had been operational since 1995 whereas Cell A had been in use since 2000. The wetland received secondary treated effluent direct from the Oxley Creek WTP at an average flow rate of 8L/min with a median hydraulic loading rate (HLR) of 0.12m/day and a hydraulic retention time (HRT) of 2 to 3 days. Each cell consisted of three gravel sections (Section 1 to 3) separated by 1m wide open water sections. Gravel Sections 2 and 3 were planted out with the four macrophyte species in October 2000, Section 1 remained unplanted.

Plant health and leaf height was monitored to assess plant establishment and growth. Investigations into plant establishment and growth demonstrated that *Carex* was most
suitable. *Carex* achieved the highest maximum leaf height and was not affected by pests and disease unlike *Schoenoplectus* and *Philydrum*.

Above ground biomass was cropped in May and August 2001, with biomass of cropped material measured on both occasions. Plant health and re-growth following cropping of above ground biomass in May and August 2001 demonstrated that cropping retarded re-growth of *Schoenoplectus* and *Philydrum*. *Carex* and *Baumea* recovered quickest following cropping, with *Carex* achieving leaf height prior to cropping within 6 months.

Proportion of biomass contained above and below ground was measured by collecting biomass samples three times over 9 months and dividing into plant components (roots, rhizomes, leaves, flowers and stems). Investigations into the proportion of above and below ground components indicated that >80% of biomass is contained above ground. Therefore cropping above ground biomass would potentially remove a significant proportion of nutrient storage from the CW. The results indicated that the ideal time for cropping was in spring/summer when plants are flowering particularly for *Philydrum*, whose flowering stems comprised 40% of total plant biomass. Flowering stems of *Philydrum* could potentially have a commercial use as a cut flower.

Nutrient content of the four species in each cell was measured for individual plant components when first planted and after three (summer) and six (autumn) months growth. This was combined with biomass data to quantify nutrient bioaccumulation (nitrogen and phosphorus) by the four species in each cell. In terms of ability to bioaccumulate nitrogen and phosphorus, measurements of nutrient content and storage indicated that all four species were suitable. Nutrient storage was highest for *Baumea* and *Carex*. However high nutrient content may make the macrophytes more susceptible to pest and disease attack as found in this study for *Philydrum* and *Schoenoplectus*. Nutrient storage was highest in Cell A (new 5mm gravel) as a result of higher biomass achieved in this cell. The cropping and nutrient storage experiments indicated that *Carex* was the most suitable species for use in SSF CWs. *Carex* achieved the highest nutrient storage and had the fastest regrowth following cropping.

Organic carbon accumulation between gravel particles measured as the proportion of material lost at 500°C was determined for gravel samples collected from each section for all four cells at 10cm depth increments (0-10cm, 10-20cm and 20-30cm).
Investigations into organic carbon accumulation within the gravel substrate showed that organic accumulation was higher in the planted sections particularly for cells that had previously been planted with *Phragmites australis*. Organic accumulation was highest in the top 20cm of the gravel, which can be attributed to litter fall and root material.

The effect of gravel size on plant growth, biomass, root depth and organic accumulation was assessed throughout the study. Investigations indicated that gravel size did not appear to affect biomass, maximum root penetration, re-growth following cropping and organic accumulation.

Water quality from the inlet and outlet of each cell was measured fortnightly over 12 months (May 2001 to May 2002). Water quantity (HLR) was measured weekly using tipping buckets located at the inlet and outlet of each cell. Water quality and quantity were combined to investigate the nutrient removal efficiency of the wetland. The Oxley wetland was highly effective in reduction of TSS (<2mg/L) and COD (<30mg/L). Principal TSS and COD removal mechanism was physical with the first gravel section acting as a filter removing the majority of particulate material. Average loading rates to the wetland were 7.1 kg/ha/d PO₄-P, 14 kg/ha/d NH₄-N and 5.4 kg/ha/d NOx-N. Average daily mass removal rates ranged from 7.3 kg/ha NH₄-N in Cell D to 4.6 kg/ha in Cell C (i.e. 37%-22% removal efficiency respectively); 5.2 kg/ha NOx-N in Cell C to 1.3 kg/ha in Cell A (i.e. 75%-22% removal efficiency) and 0.8 kg/ha PO₄-P in Cell A to 0.1 kg/ha in Cell C (i.e. 10%-1% removal efficiency). Removal efficiency was calculated on a loads basis. Insufficient retention times (2-3 days based on tracer study) and anaerobic conditions (<1mg/L) limited further nitrogen removal. Negligible phosphorus removal for all cells was attributed to short retention time and likelihood of phosphorus adsorption being close to capacity. Investigation into the proportion of nutrient removal attributed to plant uptake demonstrated that nutrient uptake and storage in plant biomass accounted for <12% TN and <5% TP.

This research project has provided several useful outcomes that can assist in future guidelines for designing effective SSF CWs in the subtropics/tropics. Outcomes include the importance of maintaining an adequate water level during the initial establishment phase. Maximising effluent treatment by pre-treatment of wastewater prior to entering SSF CWs to enable ammonia to be converted to nitrate and ensuring adequate hydraulic retention time. *Carex fascicularis* was the most suitable species particularly where
harvesting regimes are employed. *Philydrum* flowering stems could be used as a cut flower in the florist trade.
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This thesis presents the original work of the author. This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously unpublished or written by another person except where due reference is made in the thesis itself.

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Catharine Marie Browning  Date
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CHAPTER 1: CONSTRUCTED WETLANDS FOR WATER POLLUTION CONTROL

1.1 Wetlands

A wetland can be defined as an area that is periodically or permanently waterlogged by surface water or groundwater, and can support the growth of aquatic vegetation (Mitsch and Gosselink 2000). Components of a wetland ecosystem are biotic (plants, animals and microorganisms) and abiotic (water, substrate and air) (Kadlec and Knight 1996). Wetlands are some of the most biologically productive ecosystems in the world (Kadlec and Knight 1996). The major ecological functions of wetlands include water purification, hydrological modification, protection against erosion and deposition, provide habitat for flora and fauna, aesthetic appeal and provide recreational opportunities (IWA 2000).

1.1.1 Water purification

There is a range of biogeochemical (physical, chemical and biological) processes within a wetland that enable water purification. Physical processes include filtration, sedimentation and adsorption of pollutants including suspended solids and phosphorus. Chemical processes include precipitation, adsorption, decomposition and volatilisation reactions whereas biological processes include direct biological uptake of nutrients and processes mediated by microbial activity (IWA 2000; Kadlec and Knight 1996). Descriptions of the major pathways for the removal of phosphorus and nitrogen are detailed below.

1.1.1.1 Phosphorus

Removal mechanisms for phosphorus within a wetland are primarily by 1) direct uptake by plants and microorganisms, 2) sedimentation and burial 3) mineralisation and 4) adsorption and precipitation (Figure 1) (Reddy and D'Angelo 1997). Studies have shown that the primary removal mechanism is phosphorus adsorption onto substrate with plant uptake being negligible (Faulkner and Richardson 1989; Mann and Bavor 1993). Whereas Greenway and Woolley (2001) found that plant uptake accounted for 65% of the influent phosphorus. Phosphorus can bind to substrate from adsorption and precipitation reactions with calcium (Ca), iron (Fe) and aluminium (Al). These reactions are influenced by the pH and redox potential of the substrate and temperature.
The rate of phosphorus adsorption is controlled by the adsorptive surface area, availability of reactive Fe, Al and Ca, substrate pH and redox potential and temperature (Arias et al. 2001; Reddy and D'Angelo 1997).

\[ \text{Figure 1: Wetland phosphorus cycling} \]

1.1.1.2 Nitrogen

Nitrogen removal within a wetland is through a series of biogeochemical processes including 1) direct uptake by plants and microorganisms, 2) mineralisation, nitrification and denitrification 3) ammonia volatilization and 4) sedimentation and burial (Figure 2) (Reddy and D'Angelo 1997; Zhu and Sikora 1995). Of these processes denitrification, ammonia volatilization and plant harvesting result in the net loss of nitrogen from a wetland, while the other processes transform nitrogen from one form to another (Queensland Department of Natural Resources 2000). The step by step processes of ammonification, nitrification and denitrification dominate nitrogen cycling and removal within a wetland (Faulkner and Richardson 1989).

Nitrogen enters a wetland in particulate and dissolved inorganic (nitrate, nitrite and ammonia) and organic (plants, microbial biomass and soil organic matter) forms (Reddy and D'Angelo 1997). Particulate forms of nitrogen are removed by filtration and sedimentation, with dissolved forms removed through a series of processes including...
ammonification, nitrification, denitrification and ammonia volatilization (Reddy and D'Angelo 1997).

Ammonification is the process by which organic carbon is converted to ammonia (NH$_4^+$) (Vymazal et al. 1998). Ammonification rates are dependent on pH, temperature, available nutrients in the system and soil conditions (Reddy and Patrick Jr 1984). The optimum pH range for ammonification is between 6.5 to 8.5, therefore pH is not usually a limiting factor in wetlands where pH values are commonly within this range (Vymazal et al. 1998). Ammonia (NH$_4^+$) can then be assimilated by microorganisms and plants or further converted by ammonia volatilisation or nitrification-denitrification processes (Myrold 1999).

Ammonia volatilisation converts ammonia in solution (NH$_4^+$) to ammonia gas (NH$_3$) which is then released into the atmosphere (Figure 2). This process is usually insignificant as it requires pH values greater than nine, which is generally uncommon in wetlands (Reddy and Patrick Jr 1984).

Nitrification is the oxidation of ammonia in solution (NH$_4^+$) to nitrate by nitrifying bacteria with nitrite produced as an intermediate (Figure 2). Several types of bacteria are involved in the nitrification process, the first stage of the process where ammonium is converted to nitrite primarily involves bacteria from the genus *Nitrosomonas* and the second stage where nitrite is converted to nitrate involves the genus *Nitrobacter* (Kadlec and Knight 1996). The rate of nitrification is controlled by several factors including temperature, pH, alkalinity and supply of oxygen, ammonia (NH$_4^+$) and nitrifying bacteria (Queensland Department of Natural Resources 2000). From studies of nitrification in pure bacterial cultures the optimal temperature for this process is 25°C to 35°C (Kadlec and Knight 1996). Although nitrification rates have been found to decrease markedly at water temperatures above 30°C (Kadlec and Knight 1996). It has also been reported that growth of nitrifying bacteria and thus nitrification rates decrease significantly below 15°C (Reddy and Patrick Jr 1984). Ideal pH range for nitrification has been found to be between 7.2 and 9.0, since treatment wetlands commonly operate under close to neutral conditions pH is generally not a limiting factor (Kadlec and Knight 1996; Metcalf and Eddy 1991). Nitrification requires aerobic conditions so is limited to environments that have sufficient dissolved oxygen concentrations. Studies of a number of different constructed wetlands have found that conversion of ammonium
to nitrate is very low where dissolved oxygen concentrations are below 0.5mg/L (Kadlec and Knight 1996). Although the presence of anaerobic ammonium oxidiser (anamox) pathways in nature has recently been established but further research is needed to determine their role in nitrogen processing within wetlands (Jetten et al. 1999; Tanner and Kadlec 2002; van Loosdrecht and Jetten 1998). In a wetland nitrification primarily occurs in the water column, aerobic layer of substrate and within the oxidised rhizosphere of plant roots. Studies have shown that plants may transport and release oxygen from roots to the surrounding rhizosphere providing aerobic conditions for nitrification to occur (Reddy et al. 1989; Reddy and Patrick Jr 1986). Whereas research by Bedford et al. (1991) indicated the limited ability of macrophytes to oxygenate sediments. Nitrification is often recognised as the limiting stage in the nitrification-denitrification process as wetland environments can often be low in dissolved oxygen (Kadlec and Knight 1996).

Nitrate can be assimilated by plants and microorganisms or converted and removed from the wetland through denitrification. Denitrification is an energy requiring reduction process where nitrate is transformed to atmospheric nitrogen by denitrifying bacteria (Figure 2) (Kadlec and Knight 1996). Commonly found bacterial groups involved in the denitrification process are Bacillus, Enterobacter, Micrococcus, Pseudomonas and Spirillum (Kadlec and Knight 1996). Denitrification is the dominant mechanism for the long term removal of nitrogen from a wetland (Brix 1993; Faulkner and Richardson 1989). Factors known to influence denitrification rates include: temperature, pH, anaerobic conditions, organic carbon, redox potential, denitrifying bacteria and soil type (Vymazal 1995). Denitrification occurs primarily under anaerobic conditions where there is sufficient supply of dissolved organic carbon. Hammer and Knight (1994) reported optimal temperatures for denitrification as 25 to 65°C and marked decrease in rates below 5°C. Though a study by Broderick et al. (1988) on laboratory incubations found that there were significant rates of denitrification at temperatures below 5°C. Ideal pH range for denitrification lies between 7.0 and 8.0, since treatment wetlands commonly operate under close to neutral conditions pH is generally not a limiting factor (Kadlec and Knight 1996; Vymazal et al. 1998).
Figure 2: Nitrogen transformations in a wetland system.

1.1.2 Ecological functions

Other ecological functions of wetlands include: hydrological modification, physical effects, provide habitat, aesthetic appeal and recreation. Wetlands play an important role in floodwater control by storing rainfall and runoff thus dramatically reducing peak, frequency and duration of flood events (Hammer 1997; Mitsch and Gosselink 2000). The physical effects of the wetland itself provide protection against erosion by stabilising substrate and deposition of suspended material by reducing flow and turbulence (Hammer 1997). They can also improve aesthetic appeal and provide opportunities for passive recreation (e.g., walking, jogging and cycling) (Knight 1997).

The favourable ecological functions of wetlands listed above particularly for water purification, has raised interest from environmental engineers to consider wetlands as possible alternatives for conventional wastewater treatment.

1.2 Constructed Wetlands

Constructed Wetlands (CWs) utilise the physical, biological and chemical processes and functions of a natural wetland to improve water quality (Department of Land and Water Conservation New South Wales 1998). Research on the use of CWs for wastewater treatment began in the 1950’s but this has increased substantially in the last 20 years.
largely due to public interest in “green” technology waste treatment (Kadlec and Knight 1996; Moshiri 1993). In Australia at least forty CWs were built between 1990-2000 to treat wastewater (Queensland Department of Natural Resources 2000).

CWs have several advantages over conventional wastewater processes including: 1) relatively low construction and maintenance costs; 2) ability to tolerate variable loading rates; 3) provide habitat for flora and fauna; 4) increase aesthetic appeal 5) provide recreational opportunities and 6) potentially provide an economic benefit (eg. Tea tree oil) (Bastion and Hammer 1992; Bolton and Greenway 1994).

For a CW to be successful at treating wastewater they need to be designed, constructed and operated correctly to provide conditions suitable for biological, chemical and physical processes needed for water purification. CWs have certain limitations in the effective treatment of wastewater, including chemical and biological process rates, hydrological limitations (loading rate and detention time) and environmental limitations (excessive organic matter, nutrients or lack of oxygen) (Department of Land and Water Conservation New South Wales 1998).

Chemical and biological processes are controlled by environmental factors including the presence of oxygen, pH and temperature. Metabolic activity generally decreases with temperature, thus reducing the effectiveness of treatment processes relying on biological activity (Department of Land and Water Conservation New South Wales 1998). Several wetland studies in temperate climates have shown that nitrogen removal rates decrease over winter due to the lower temperatures (Gersberg et al. 1983; Kadlec 1987). While environmental conditions are unfavourable for chemical and biological processes the effectiveness of the wetland to remove pollutants is limited (Department of Land and Water Conservation New South Wales 1998).

Hydrological limitations can also affect the capacity of a wetland to treat wastewater in terms of the amount of water and pollutants entering the wetland. The volume of water entering a wetland is called the hydraulic loading rate and the volume of pollutants entering a wetland is termed pollutant loading rate (Department of Land and Water Conservation New South Wales 1998). The average length of time water stays within the wetland is referred to as the residence or retention time (Queensland Department of Natural Resources 2000). Studies have shown that pollutant removal generally
increases with longer retention times, due to increased wastewater contact time for biogeochemical processes (Kadlec and Knight 1996). Effective wastewater treatment involves balancing pollutant loading rates and detention time. Data collected from wetlands treating effluent wastewater found that optimal wetland performance was linked to relatively low loading rates, with wetland performance decreasing once loading rates were increased (Department of Land and Water Conservation New South Wales 1998). Typical retention times for nutrient removal can range from 7 to 14 days (Crites and Tchobanoglous 1998). Research by Greenway and Woolley (1999) on several CWs in Queensland found that a retention time of 7 days ensured effective removal of nutrients and suspended solids. Detention times will vary depending on the pollutant and the level of treatment required (Queensland Department of Natural Resources 2000). For further discussions on limitations of CWs for effective treatment of wastewater see Chapter 7: Effluent Treatment – Performance Efficiency.

There are two main types of CW: surface flow (SF) or free water surface wetlands and subsurface flow wetlands (SSF) (Queensland Department of Natural Resources 2000). SF wetlands generally consist of shallow channels or basins with flow predominantly above the wetland floor or substrate through planted macrophytes (Figure 3) (Crites 1994; Department of Land and Water Conservation New South Wales 1998; Queensland Department of Natural Resources 2000). SF systems have been used in the Netherlands for almost 30 years for wastewater treatment (Brix 1993).
In a SSF wetland water moves through a substrate (usually soil, sand or gravel), flow is either horizontal or vertical with macrophytes planted into the substrate (Figure 4)
(Department of Land and Water Conservation New South Wales 1998; Moshiri 1993). The choice of media for the wetland substrate is extremely important and can determine wetland performance. The chemical and physical composition of the media can largely determine pollutant removal (Gumbricht 1993). Substrate adsorption is a significant mechanism for phosphorus removal in CWs so ideally media should be selected that has a high phosphorus adsorption capacity (Mann 1990; Wood 1990). Studies by Arias et al. (2001) on the use of sand in SSF systems found that the most important media characteristic determining phosphorus removal was the calcium content, which enhances precipitation reactions.

Important physical characteristics of media include hydraulic conductivity and surface area. US EPA (1988) recommended the use of sands or gravelly sands as substrate in SSF CWs rather than the use of soil which can have hydraulic constraints. Geller (1997) recommends that if soil is to be used it should predominantly consist of sand with a small amount of clay. An extensive surface area is important in pollutant removal providing surfaces for the removal of particulate pollutants, phosphorus adsorption and microbial activity (eg. nitrification and denitrification) (Wood 1994). Fine grained porous media such as sand has a large surface area but can often have low hydraulic conductivity (Zhu et al. 1997).

An advantage of SSF CW over SF CW is that they have a greater ability to treat high concentrations of organic material, suspended solids, nitrate, pathogens and other pollutants (Queensland Department of Natural Resources 2000). This is due to longer retention times and an extensive surface area that increases wastewater contact times for biogeochemical processes to occur including assimilation, adsorption and nitrification (Kloosterman and Griggs 1989; Wood 1994). SSF CWs have greater treatment per unit area of land compared to SF CWs, which generally have higher land requirements (Queensland Department of Natural Resources 2000).

Subsurface flow (SSF) CWs are most suitable for treating secondary effluent or in a treatment train after a settling pond or septic tank in which suspended solids have largely been removed. SSF wetlands have been widely used throughout Europe to provide secondary treatment after screening and primary settlement, for small communities and single households where conventional wastewater treatment can often be too expensive to construct and maintain (IWA 2000).
As there is no direct contact between the water column and atmosphere in a SSF CW they are unlikely to be a public safety problem and there is less risk of mosquitoes or odour compared to SF CW (Crites and Tchobanoglous 1998; Department of Land and Water Conservation New South Wales 1998; Reed and Brown 1992). SSF CWs generally have a higher tolerance to colder weather due to the insulation ability of the media (Queensland Department of Natural Resources 2000).

The disadvantages of SSF over SF CWs is that they tend to have higher construction costs due to the cost of supplying media (gravel, sand or soil) (Queensland Department of Natural Resources 2000). Horizontal SSF wetlands can often be oxygen limited affecting nitrification rates although studies have shown oxygen transfer by macrophytes into the rhizosphere (Brix 1993; Cooper et al. 1996). A common problem of SSF CWs is that pore spaces tend to clog with suspended material decreasing hydraulic conductivity but this can often be overcome with design alterations (Crites 1994). Although clogging will decrease conductivity it can also be a valuable source of organic carbon for de-nitrification (Ingersoll and Baker 1998). See Chapter 6: Organic Carbon Accumulation for more information.

1.3 Macrophytes

The three main types of macrophytes are emergent, free-floating and submerged (Greenway 2003; Sainty and Jacobs 1994). SF CWs often have all three types whereas SSF CWs will only have emergent species, except where they have open water sections (Figures 3 and 4). Macrophytes play a major role in CWs, influencing biological, chemical and physical treatment processes. The most important functions of macrophytes in CWs have been categorised by Brix (1997) as physical and metabolic. Physical effects include: filtration of suspended material, protection against erosion by reducing turbulence and flow velocities, stabilisation of sediments and providing surface area for microorganisms (Brix 1997; Dowling and Stephens 1995; Greenway and Woolley 2000). Metabolic functions of macrophytes include nutrient uptake and oxygen release from roots into the rhizosphere (Brix 1997). Macrophytes have adapted to anaerobic conditions by developing internal air spaces (aerenchyma) which transport oxygen to the root zone (Reddy and D'Angelo 1997). These air spaces form an extensive system throughout the plant and can occupy 60% of the total tissue volume (Studer and Brandle 1984). Research differs on the potential for macrophytes to release oxygen from roots to the surrounding rhizosphere thus providing aerobic conditions for
nitrification to occur (Reddy et al. 1989 and Reddy and Patrick Jr 1986). A study by Armstrong et al. (1991) concluded that internal oxygen movement not only supplied buried plant tissues but also leaked oxygen into the rhizosphere. A study by Brix and Schierup (1990) reported that the respiratory oxygen consumption of *Phragmites* roots and rhizomes was almost equal to that being released leaving only 0.02g O$_2$ m$^{-2}$ day$^{-1}$ released into the surrounding soil. The same study found that for the four species studied (*P. australis, Glyceria maxima, Typha latifolia* and *Iris pseudacorus*) only subapical regions of young roots released oxygen with no release detected from old roots and rhizomes. Macrophytes can also provide habitat for flora and fauna and increase aesthetic appeal (Greenway and Simpson 1996).

Research differs on the significance of plant uptake in nutrient removal with nutrient loading an important part in the proportion of nutrient removed by plant uptake (Brix 1997; Gersberg et al. 1983; Greenway and Woolley 2000; IWA 2000; Mann and Bavor 1993; Tanner et al. 1995). Nutrient content of macrophytes, nutrient storage and harvesting is discussed in Chapter 4: Plant Biomass and the Effects of Cropping on Regrowth and Chapter 5: Nutrient Storage in Plant Biomass. The significance of plant uptake in nutrient removal is further discussed in Chapter 7: Effluent Treatment – Performance Efficiency.
1.4 Study Aims and Approach

In Queensland, Australia limited trials have been conducted on native macrophyte species for use in SSF CWs. Thus the opportunity arose to investigate the suitability of native species for use in SSF CWs. In 1998 Brisbane City Council and Griffith University were successful in obtaining funding through the Natural Heritage Trust under the Clean Seas Program to study a pilot constructed wetland for nutrient removal.

The issues to be addressed under this funding application included: 1) Suitability of native macrophytes for use in SSF CWs; 2) Research into alternative wastewater treatment facilities in a major metropolitan region and 3) Potential of harvested biomass for commercial use (ie. cut flowers). These issues were incorporated into the primary aims of the current study.

The primary aims of the study were: 1) To determine the effectiveness of the wetland for wastewater treatment and 2) Suitability of the four native macrophyte species for use in horizontal SSF CWs receiving secondary treated effluent in South-East Queensland.

The study was comprised of four components:

1) Plants
Study of plant establishment, growth, impact of cropping and gravel size, nutrient content and storage for the four macrophyte species within the Oxley horizontal SSF CW (Chapters 3,4 and 5);

2) Water quality – effluent treatment
Monitoring water quality and quantity entering and leaving the wetland to determine wastewater treatment (Chapter 7);

3) Organic carbon
Investigation of organic carbon accumulation within the wetland cells for the different gravel sizes (5mm and 20mm) (Chapter 6); and

4) Mass balance
Combine nutrient storage by macrophytes with wastewater nutrient removal to determine proportion of nutrient removal by plant uptake (Chapter 7).
Chapter 3: Plant establishment and growth

The aims of the research presented in this chapter were: 1) To investigate plant establishment of the four species in a horizontal SSF CW in Brisbane; 2) Investigate effect of gravel size on plant growth and 3) Investigate differences in plant establishment and growth between the four different species.

The wetland was planted out with the four macrophyte species (*Baumea articulata*, *Carex fascicularis*, *Philydrum lanuginosum* and *Schoenoplectus mucronatus*) to be trialed in October 2000. Plant health and leaf height was monitored to assess plant establishment and growth. Biomass samples were collected and divided into plant components (roots, rhizomes, leaves, flowers and stems) to determine proportion of biomass contained above and below ground.

Chapter 4: Plant biomass and the effects of cropping on regrowth

The aims of the research presented in this chapter were: 1) To investigate the effect of cropping on plant re-growth and biomass; 2) Investigate effect of gravel size on plant re-growth and 3) Investigate differences in plant re-growth after cropping between the four different species.

Above ground biomass was cropped in May and August 2001, with biomass of cropped material measured on both occasions. Samples were also collected to determine any changes in proportion of above ground and below ground components. Following cropping plant health and leaf height was measured to determine effect of cropping on plant growth and biomass for the four species trialed. Maximum root length was measured for all four species in each cell to determine any differences with species, gravel size and wetland age.

Chapter 5: Nutrient storage in plant biomass

The aims of the research presented in this chapter were: 1) Investigate differences in nutrient content between plant components, age/season and species and 2) Investigate nutrient storage of nitrogen and phosphorus in plant biomass for the four species.

Nutrient content of the four species in each cell was measured for individual plant components when first planted and after three (summer) and six (autumn) months.
growth. This was combined with biomass data to quantify nutrient bioaccumulation (nitrogen and phosphorus) by the four species in each cell.

Chapter 6: Organic carbon accumulation
The aims of the research presented in this chapter were: 1) To investigate organic carbon accumulation and 2) Investigate relationship between organic carbon and gravel size, age of wetland and depth.

Gravel samples were collected from each section for all four cells at 10cm depth increments (0-10cm, 10-20cm and 20-30cm). Organic carbon accumulation between gravel particles for the gravel samples collected were measured as the proportion of material lost at 500°C.

Chapter 7: Effluent treatment – performance efficiency
The aims of the research presented in this chapter were: 1) Determine the effectiveness of the wetland for wastewater treatment; 2) Effect of gravel size and age on water treatment and 3) Quantify nutrient removal rates and proportion of nutrient removal contained in plant biomass.

The wetland receives secondary treated effluent direct from the Oxley WTP. Effluent enters pond 1 of each cell and then flows horizontally through the first gravel section where it continues through the wetland

Parameters used to investigate effluent treatment were: water depth, pH, temperature, dissolved oxygen, total suspended solids, total volatile solids, nitrogen, phosphorus and chemical oxygen demand. Water quantity entering and leaving the wetland was measured using tipping buckets located at the inlet and outlet of each cell. Water quality and quantity were combined to investigate the nutrient removal efficiency of the wetland. Nutrient content and biomass for each of the four macrophyte species trialed was combined with wetland nutrient removal to calculate proportion of nutrient removal contained within plant biomass.
CHAPTER 2: SITE DESCRIPTION

2.1 Oxley Pilot Subsurface Flow Constructed Wetland

2.1.1 Climate
The constructed wetland under investigation is located in South-East Queensland, Australia which has a sub-tropical climate, with the majority of rainfall between December and March. The city of Brisbane has an average annual rainfall of 1090mm, with humidity levels around 50 per cent year round. Air temperatures range from a minimum of 10°C in winter to a maximum of 30°C in summer (Bureau of Meteorology 2000). These climatic conditions are extremely favourable for high plant growth rates, and would therefore indicate the potential of this climatic region for CWs for wastewater treatment (Greenway and Simpson 1996).

2.1.2 Site Description
The wetland is situated within the grounds of the Oxley WTP, which supplies the wetland with a constant source of secondary treated effluent and secure surroundings (Figure 5). The Oxley WTP treats approximately 56ML/day of sewage (dry weather flow) with up to 250 ML following rain (Brisbane City Council 1999). The plant treats domestic and industrial waste from the suburbs bounded by Algester to the south, Chapel Hill to the north and Jindalee to the west (Brisbane City Council 1999). Within the Oxley WTP primary treatment of raw sewage involves screening, grit removal and primary sedimentation to settle organic solids with lighter materials which float to the surface skimmed off to sludge pits (Brisbane City Council 1999). Primary treated wastewater is then secondary treated using the “activated sludge process” with wastewater pumped into aeration tanks that provide aerobic conditions for organic breakdown by microbes. Wastewater is then pumped into final settling tanks. Secondary treated effluent is disinfected using chlorine after which it is chlorinated and released into the Brisbane River (Brisbane City Council 1999).

The pilot wetland is a horizontal subsurface flow (SSF) wetland, consisting of four equally sized cells, which are 26m long, 4m wide and 0.5m deep, the total surface area of the wetland is approximately 400m² (Figure 6). The wetland was originally designed
and constructed to treat primary effluent produced by up to 80 people with an average flow of 20m$^3$/day, based on various literature which recommend a wetland size of 5m$^2$ per person (Findlater et al. 1990; King 2002). The base of the wetland cells slope downward from the inlet to the outlet and have an average base slope of 1% and the cell walls slope outwards at approximately 45 degrees from the base as per (King 2002).

Each cell is lined with a single sheet of high-density polyethylene, and has three gravel sections (Section 1 to 3) separated by 1m wide, open water sections. The gravel sections are separated by a wall of masonry blocks, with the large cavities in the blocks covered with a polyethylene mesh to prevent gravel entering the open water sections (King 2002). The wetland receives secondary treated effluent at an average flow rate of 8L/min (11,520L/day) with a median hydraulic loading rate (HLR) of 0.12m/day, hydraulic retention time (HRT) is approximately 2 to 3 days based on tracer studies of wetland cells. Bernhard Zeiringer from the University of Agricultural Sciences Vienna, Austria conducted tracer studies of each cell using Lithium Chloride in 2003, as part of his Engineering Dissertation. Effluent enters each cell separately with the ability to control the flow rate. Flow was set and measured by tipping buckets located at the inlet and outlet of each cell (Figure 6). The tipping buckets were constructed by the Griffith University workshop and had an average volume of 3.5 litres per tip with an error of 6% for flows up to 8 tips per minute. To protect the tipping buckets from the weather and to ensure flow was directed to the wetland the buckets were situated inside 200 litre plastic buckets and covered in plastic as per King (2002). The effluent enters the wetland in the first open water section which has a cover of duckweed and then through the first gravel section where it continues through the wetland. All other open water sections are covered with corrugated metal to prevent algal growth. Following treatment through the wetland the effluent from each cell is piped away and re-enters the WTP.

The pilot wetland was built in 1995 by Queensland University to study the flow hydraulics of a SSF constructed wetland. Part of the study involved studying the effect of emergent macrophytes ($Phragmites australis$) and organic accumulation on flow hydraulics. $Phragmites australis$ were planted in three of the four cells, the last cell left unplanted as a control for water treatment with gravel only. The 0.5m depth for the wetland was chosen by King (2002) to ensure $Phragmites$ roots reached the base of the wetland cell to maximise plant root and water column contact. Two cells had 20mm
size gravel, one 5mm and the other 40mm gravel (Figure 7). Crushed basalt was used for the 40mm, 5mm and one of the cells filled with 20mm gravel, for the other cell with 20mm river gravel was used to compare treatment performance (King and Mitchell 1995).

On the commencement of the study one bed containing 40mm gravel was removed and replaced with 5mm gravel, leaving two cells having 20mm size gravel and two with 5mm gravel. The above ground *Phragmites australis* were cropped and the tips treated chemically. Four different Australian native macrophyte species were planted late October 2000: *Baumea articulata* (Jointed Twigrush), *Carex fascicularis* (Tassel sedge), *Philydrum lanuginosum* (Frogsmouth) and *Schoenoplectus mucronatus*. Gravel Sections 2 and 3 were planted out and Section 1 remained unvegetated with filtration being the primary role of this section. Each section was divided into 4 compartments each 7m$^2$ and planted with one of the four macrophyte species at a density of 9 per m$^2$. Within every cell each species covered a total of 14m$^2$, 7m$^2$ per gravel section. Planting layout was the same for all cells to allow comparison between cells (Figure 6). Due to the decline of plant health and high plant mortality in Cell D following initial planting in October 2000, this cell was replanted in February 2001.
Figure 5: Location of Oxley constructed wetland. (Source: Gregory's, 2000)
### Section 1
- Gravel: 5mm only

### Section 2
- Gravel: New Gravel
  - 5mm
- Gravel: Old Gravel
  - 20mm

### Section 3
- Gravel: Gravel & Plants

#### Effluent
- Cell A
  - Gravel & Plants
  - Ba
  - Cf
  - Pl
  - Sm

#### Gravel Section 2
- Gravel & Plants
  - Ba
  - Cf
  - Pl
  - Sm

#### Gravel Section 3
- Gravel & Plants
  - Ba
  - Cf
  - Pl
  - Sm

#### Influent
- Water

### Macrophytes
- **Ba** – *Baumea articulata* “Jointed Twigrush”
- **Cf** – *Carex fascicularis* “Tassel Sedge”
- **Pl** – *Philydrum lanuginosum* “Frogsmouth”
- **Sm** – *Schoenoplectus mucronatus*

**Planting density:** 9 per m²

**Figure 6:** Current Oxley wetland layout
Figure 7: Previous wetland layout
2.2 Description of Macrophytes

2.2.1 *Baumea articulata* “Jointed Twigrush”

Is an attractive rhizomatous perennial and can reach a maximum height of 2.5m. Stems are cylindrical usually 1cm in diameter and rigid with large air spaces. Flowers are brown open drooping panicles that can reach 50cm in length, usually flower from summer to early autumn (Sainty and Jacobs 1994) (Figure 8). They can form large dense clumps that provide ideal habitat for nesting waterbirds. They are usually found in coastal lagoons and swamps where they tend to be in standing or slow flowing water less than 1m deep, but can sometimes be found in deep mud. They have a wide distribution being found in most states of Australia (Sainty and Jacobs 1994).

![Figure 8: Baumea articulata](image)

Source: Kate Browning

2.2.2 *Carex fascicularis* “Tassel Sedge”

Is a native perennial that generally stands less than 1m in height. Leaves are flat; usually 0.7cm wide and 40cm long (Figure 9). Inflorescence is an arrangement of spikes with a leafy bract. Commonly found in swamps and creek banks, but can tolerate periodic flooding. They are widely distributed and are found in most Australian states (Sainty and Jacobs, 1981).

![Figure 9: Carex fascicularis](image)

Source: Kate Browning
2.2.3 *Philydrum lanuginosum* “Frogsmouth”

Is an attractive native rhizomatous perennial and can reach a maximum height of 2m. Leaves generally reach a maximum height of 0.60m with an erect spike reaching heights up to 2m. Inflorescences on the spike have solitary yellow flowers (Figure 10). Leaf colour can change from green to yellow and pink. They can form dense clumps providing habitat and food for birds and animals (Sainty and Jacobs, 1981, 1994). Commonly found on the margins of swamps, farm dams and ponds where they grow in still or slow flowing water up to 20cm deep. Although usually growing in water they often flower and fruit growing in wet and drying mud. They grow in temperate and tropical climates and are found in most Australian states (Sainty and Jacobs, 1994).

![Figure 10: Philydrum lanuginosum](Image)

*Source: Kate Browning*

2.2.4 *Schoenoplectus mucronatus*

Is an attractive native perennial with triangular stems and a maximum height of 1m. Flowers most of the year, inflorescence is a cluster of up to 12 egg shaped spikelets. Spikelets can be 1.5cm long and 0.15cm wide with many flowers (Figure 11). They can form dense clumps and are often used by waterbirds for nesting and shelter, nuts produced can also be a food source (Sainty and Jacobs, 1994). Commonly found on creek and river banks, floodplains periodically inundated and in billabongs. They are widely distributed and are found in most Australian states (Sainty and Jacobs, 1994).

![Figure 11: Schoenoplectus mucronatus](Image)

*Source: Kate Browning*
CHAPTER 3: PLANT ESTABLISHMENT AND GROWTH

3.1 Introduction

The characteristic feature of a wetland natural or constructed is the presence of aquatic vegetation (macrophytes). The three main types of macrophytes are emergent, free-floating and submerged (Sainty and Jacobs 1994). Surface flow (SF) CWs support a wide range of macrophyte types – emergent, floating, floating leaved attached and submerged whereas the gravel and/or soil filled SSF wetland systems only support emergent species (Greenway, 1997; Greenway 2003; IWA 2000).

Macrophytes play a major role in CWs, influencing biological, chemical and physical treatment processes. The most important functions of macrophytes in CWs have been categorised by Brix (1997) as physical and metabolic. Physical effects include: filtration of suspended material, protection against erosion by reducing turbulence and flow velocities, stabilisation of sediments and providing surface area for microorganisms (Brix 1997; Dowling and Stephens 1995; Greenway and Woolley 2001). Metabolic functions of macrophytes include nutrient uptake and oxygen release from roots into the rhizosphere (Brix 1997). Macrophytes can also provide habitat for flora and fauna and increase aesthetic appeal (Greenway, 2003; Greenway and Simpson 1996).

Desirable plant characteristics for macrophyte species to maximise nutrient uptake in a constructed wetland treating secondary effluent include rapid growth, high plant tissue content and the ability to attain a high standing crop (Reddy and DeBusk 1987). Characteristics listed by Husak (1992) for temperate CWs included: tolerance of high organic matter, nutrient loadings and fluctuating water levels, having a long season of growth (ie. unlike Phragmites), high productivity and the ability to accumulate large amounts of nutrients. The above ground biomass of macrophytes is sometimes cropped to permanently remove nutrients from the CW which have been stored in plant biomass and to invigorate new growth (Greenway, 2000; Kim and Geary 2000). Therefore additional to those characteristics listed above is the ability to recover following cropping and an attractive species is also desirable to increase wetland aesthetics.
Many macrophyte species have been trialed for use in SSF CWs around the world. The most commonly used emergent species in SSF CWs is the reed *Phragmites australis* (IWA 2000; Kvet *et al*. 1999). Species used in Europe include *Phalaris arundinaceae* (reed canary grass), *Glyceria maxima* (sweet manna grass) and *Typha spp* (cattails) and in the USA *Scripus spp* (IWA 2000; Vymazal 1995). Commonly used species in Australia and New Zealand include *Schoenoplectus* and *Juncus*. *Phragmites spp* are probably the most widely used species in SSF CWs particularly in temperate climates. In Europe SSF CWs are sometimes referred to as “Reed Bed Treatment Systems” due to the popularity of using the common reed (*Phragmites australis*) in these wetlands (IWA 2000).

The tropical/sub-tropical climate in Queensland, Australia provides ideal growth conditions for macrophytes. Prior to the 1990s there were no CW systems for the treatment of municipal sewage effluent. Since this time several wetlands have been constructed the majority of which have been free water surface (FWS) wetlands (Greenway 1997; Queensland Department of Natural Resources, 2000). As a result numerous species of aquatic macrophytes have been successfully used in SF CWs but limited trials have been conducted on species suitable for SSF CWs (Greenway 1997; Greenway 2003; Greenway and Woolley 1999; Mitchell and McNevin 2001). Therefore there was a need to investigate the suitability of other Australian native species for use in SSF systems in the tropical/sub-tropical climate in Queensland.

### 3.1.1 Aims

The overall aim of this project was to investigate the suitability of the four native macrophyte species for use in SSF wetlands in Queensland, Australia. This chapter will examine plant establishment and growth for the four species. The aims of the research presented in this chapter are: 1) To investigate plant establishment of the four species in a horizontal SSF CW in Brisbane; 2) Investigate effect of gravel size on plant growth and 3) Investigate differences in plant establishment and growth between the four different species.
3.2 Materials and Methods

3.2.1 Macrophytes
All four species are Australian native emergents and are listed by the Queensland Herbarium as suitable for use in artificial wetlands in Queensland (Dowling and Stephens 1995).

3.2.1.1 Nursery plant stock
Plants were ordered from a nursery due to the large number required and to ensure plants were all generally the same age and height. Ideally plant tube stock should be grown in similar substrate to what they will be planted into, in this case gravel. We had requested that seedlings be grown in gravel however when they arrived it was clear that they had been grown in potting mix. The idea was that they would initially be planted in potting mix with gravel introduced to the pot so the roots would start spreading into the gravel. Several of the plants were ‘pot bound’, and many were in the process of flowering.

3.2.2 Planting layout
In late October 2000 the four macrophyte species were planted in the Oxley SSF CW, details on the wetland setup are presented in Chapter 2: Site Description. Each compartment is approximately 7m², within every cell each species covered a total of 14m², 7m² per gravel section (Figure 6). Each compartment was planted with one of the four macrophyte species at a density of 9 per m² (Figure 12). Planting layout was the same for all cells to allow comparison between cells (Figure 12).

Due to the decline of plant health and high plant mortality (approximately 70%) in Cell D following initial planting in October 2000, this cell was replanted in February 2001 (Figure 13). To increase plant survival Cell D was flooded for two weeks, resulting in considerable improvements in plant health. Outflow height for Cells B and C were raised for two weeks resulting in some flooding. The increased water level enabled some plants to be transplanted from healthy quadrats to quadrats that had a large number of mortalities.
### Figure 12: Oxley wetland macrophyte layout and compartments.

<table>
<thead>
<tr>
<th>Cell A</th>
<th>Cell B</th>
<th>Cell C</th>
<th>Cell D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effluent</strong></td>
<td><strong>Effluent</strong></td>
<td><strong>Effluent</strong></td>
<td><strong>Effluent</strong></td>
</tr>
<tr>
<td>Gravel &amp; Plants</td>
<td>Gravel &amp; Plants</td>
<td>Gravel &amp; Plants</td>
<td>Gravel &amp; Plants</td>
</tr>
<tr>
<td><em>Ba</em></td>
<td><em>Cf</em></td>
<td><em>Ba</em></td>
<td><em>Cf</em></td>
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<tr>
<td>C7</td>
<td>C8</td>
<td>C15</td>
<td>C16</td>
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<td>C5</td>
<td>C6</td>
<td>C13</td>
<td>C14</td>
</tr>
<tr>
<td><em>Pl</em></td>
<td><em>Sm</em></td>
<td><em>Pl</em></td>
<td><em>Sm</em></td>
</tr>
<tr>
<td>C3</td>
<td>C4</td>
<td>C11</td>
<td>C12</td>
</tr>
<tr>
<td>C1</td>
<td>C2</td>
<td>C9</td>
<td>C10</td>
</tr>
<tr>
<td><strong>New Gravel 5mm</strong></td>
<td><strong>Old Gravel 20mm</strong></td>
<td><strong>Old Gravel 20mm</strong></td>
<td><strong>Old Gravel 5mm</strong></td>
</tr>
<tr>
<td><strong>Influent</strong></td>
<td><strong>Influent</strong></td>
<td><strong>Influent</strong></td>
<td><strong>Influent</strong></td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Macrophytes**

- *Ba* – *Baumea articulata* “Jointed Twigrush”
- *Cf* – *Carex fascicularis* “Tassel Sedge”
- *Pl* – *Philydrum lanuginosum* “Frogsmouth”
- *Sm* – *Schoenoplectus mucronatus*
3.2.3 Maintenance

3.2.3.1 Weed removal

Two of the cells (Cell B and D) had previously grown *Phragmites australis* for over five years, which resulted in an extensive root and rhizome system. The above ground material was cropped and remaining shoots were sprayed with herbicide. Prior to planting the roots and rhizomes were physically removed (Figure 14). Figure 14 shows the large volume of root and rhizome material that was removed from Cell D, although volunteers were thorough some roots and rhizomes were not removed. During the initial establishment phase *Phragmites* continued to re-shoot competing with the new plants, shoots were physically removed every week, Cell D required more weeding than Cell B. There were two groups of volunteers from different organisations who separately prepared and planted Cell B and D. The more experienced group removed roots and rhizomes from Cell B which may partially explain why there was less *Phragmites* re-growth in Cell B compared to Cell D. *Phragmites* shoots were continually being removed through the length of this project.
3.2.3.2 General maintenance

There was a considerable amount of maintenance required in the initial establishment phase, the majority of which was weeding and trying to maintain a high water level. Prior to plant establishment there was considerable weed growth (not only *Phragmites*), where plants have high plant coverage the weeds have been forced out but several compartments have under 60% cover so weeds continued to be a problem throughout the length of the study (Figure 6).

3.2.4 Plant establishment and Growth

Parameters used to measure plant establishment and growth are plant health, leaf height and proportion of above and below ground plant components.

3.2.4.1 Plant health

Plant health assessments were made fortnightly for the entire compartment and individual plants. Parameters included: presence of flowering, number of flowers, general health (colour of leaves), and any signs of fungal disease or insect pest.

3.2.4.2 Leaf height

Leaf height was estimated fortnightly by measuring the maximum leaf height of ten randomly chosen healthy plants from each compartment. The maximum leaf height was measured from the surface of the gravel to the tip of the highest leaf, only green plant
material was measured. For *Philydram*, only leaves growing from the base of the plant were measured and number of flowering stems was recorded, but height was not measured. For each visit ten maximum leaf heights from each compartment were averaged to provide a mean maximum height.

### 3.2.4.3 Plant components

Macrophytes are comprised of several different plant components which can be divided into above ground (leaves, stems and flowers) and below ground (roots and rhizomes). The proportion of each plant component (root, leaf, stem, flower and rhizome) for each of the four species was estimated by collecting plant biomass using a 0.04 m$^2$ quadrat and for each compartment there were three replicates. All plant components were carefully removed from the quadrats. Roots and rhizomes were washed on site to remove any adhering gravel. Plants were oven dried at 75°C for 48 hours and weighed to determine plant dry weight (Greenway and Woolley 2001; Kalra 1998). Plants were then sorted into individual plant components and then weighed to calculate the proportion of each plant component to the total dry weight of the biomass sample. Once planted, proportion of plant components was measured three times over 9 months in: February, May and August 2001 over a 3 month interval to determine change in proportion of each plant component for the four species. In February 2001 the biomass samples were not collected for *Carex* and *Schoenoplectus* in Cell B, *Philydram* in Cell C and *Baumea* in Cell C (compartment 17) (Figure 12) due to poor establishment.

### 3.3 Results and Discussion

#### 3.3.1 Leaf height and plant health

Decline of plant health in Cell D was apparent soon after planting with at least 70% of plants dying (Figure 13). Maintaining a high water level in a subsurface flow wetland is vital for macrophyte planting and during the initial establishment phase to ensure plant survival (Queensland Department of Natural Resources, 2000). Cell D which had old 5mm gravel had problems maintaining even a moderate water level for plant establishment; which in turn resulted in the new plants lacking moisture. Cell D was replanted in February 2001.
Mean maximum leaf heights for all four species in Cells A, B, C and D for gravel sections 2 and 3 are presented in Figure 15 to Figure 22. In general leaf height was highest in Cell A for all four species (Figure 15 to Figure 22). This is likely due to the ability to maintain a constant high water level in Cell A during the initial establishment phase as seen in Figure 23 where Cell A has the highest water depth of all four cells over the first seven months (October 2000 to April 2001).

**Figure 15:** Mean maximum leaf height for *Baumea articulata* in Section 2 of Cells A, B, C and D. (Cell D replanted in February 2001)

**Figure 16:** Mean maximum leaf height for *Baumea articulata* in Section 3 of Cells A, B, C and D. (Cell D replanted in February 2001).
The highest mean maximum leaf height for all four species was recorded for Carex at 174cm in Cell A, gravel section 3 (Figure 18). Schoenoplectus suffered from rust in Cell A as can be seen from the gradual decrease in leaf height in May 2001 (Figure 21 and Figure 22). Philydrum was healthy in most cells and produced large numbers of
flowers, following flowering plant health particularly in Cell A declined possibly as a result of insect damage. Studies have shown that herbivory is often increased in constructed wetlands due to the high nutrient conditions (Bolton and Greenway, 1994; Jordan et al., 1990; Newell et al., 1989).

![Figure 19: Mean maximum leaf height for Philydrum lanuginosum in Section 2 of Cells A, B, C and D. (Cell D replanted in February 2001).](image)

![Figure 20: Mean maximum leaf height for Philydrum lanuginosum in Section 3 of Cells A, B, C and D. (Cell D replanted in February 2001).](image)
Figure 21: Mean maximum leaf height for *Schoenoplectus mucronatus* in Section 2 of Cells A, B, C and D. (Cell D replanted in February 2001).

Figure 22: Mean maximum leaf height for *Schoenoplectus mucronatus* in Section 3 of Cells A, B, C and D. (Cell D was replanted in February 2001).
Figure 23: Median water depth for each pond over the initial establishment period (October 2000 – April 2001).
3.3.2 Plant components

Proportion of each plant component for each of the four species in February 2001 (3 months), May 2001 (6 months) and August 2001 (9 months) is presented in Figure 23 to Figure 27. There was little difference in proportion of plant components of total biomass between February, May and August 2001 for all species with *Philydrum* being the only exception (Figure 23 to Figure 27). When *Philydrum* plants were flowering in February 2001, flowering stems dominated the above ground component although the proportion of above ground to below remained relatively the same (Figure 26).

![Figure 24: Percentage composition of each plant component (leaf, root and rhizome) of total plant biomass for *Baumea articulata* over the first 9 months (February, May and August 2001). NB mean biomass values were used to calculate % composition. Flowers comprised less than 1% of total plant biomass.](image-url)
Figure 25: Percentage composition of each plant component (leaf, root and rhizome) of total plant biomass for *Carex fascicularis* over the first 9 months (February, May and August 2001). NB mean biomass values were used to calculate % composition.
Figure 26: Percentage composition of each plant component (leaf, root and rhizome) of total plant biomass for *Philydrum lanuginosum* over the first 9 months (February, May and August 2001). NB mean biomass values were used to calculate % composition.
Figure 27: Percentage composition of each plant component (leaf, root and rhizome) of total plant biomass for *Schoenoplectus mucronatus* over the first 9 months (February, May and August 2001). NB mean biomass values were used to calculate % composition.
The average proportion of plant components of the total biomass over the 9 months was calculated and is presented in Figure 28. Average plant component proportions over the nine months demonstrate that above ground components for all four species comprises on average greater than 80% of the total plant biomass (Figure 28).

**Figure 28:** Percentage composition of each plant component (leaf, stem, flower, root and rhizome) of total plant biomass for the four macrophyte species. NB mean biomass values were used to calculate % composition.

Only 64% of the below ground material for *Baumea* was rhizomatous, which is below that found by Adcock and Ganf (1994) for a CW in temperate Australia which reported 90% rhizomatous for *Baumea* (Figure 29). A similar finding was reported by Greenway (1997) for *Baumea* in a CW in Queensland which reported that rhizomes are less likely to act as a storage organ in Queensland as the sub-tropical\tropical climate is conducive to year round growth. Thus nutrient concentrations and biomass of rhizomes for macrophytes where climate promotes year round growth are likely to be lower than those growing in temperate climates.
Figure 29: Below ground biomass distribution (over 9 months) for *Baumea articulata*.
3.4 Conclusion

Of the four species trialed, *Carex* was the most suitable for use in SSF CWs in sub-tropical / tropical climates in regards to plant establishment as it achieved the highest maximum leaf height and was not affected by pests and disease unlike *Schoenoplectus* and *Philydrum*.

The importance in maintaining an adequate water level during the initial establishment phase particularly in sub-tropical/tropical climates was highlighted with leaf height being highest for the cell that had the highest water depth (Cell A).
CHAPTER 4: PLANT BIOMASS AND THE EFFECTS OF CROPPING ON REGROWTH

4.1 Introduction

One of the many roles macrophytes play in CWs for wastewater treatment include their capacity for nutrient bioaccumulation that is the direct uptake and storage of nutrients (Brix 1997). Nutrients stored within macrophytes can be removed from the wetland by harvesting above ground plant material. Above ground plant material can be cropped approximately 5-10cm above the substrate or water level and removed from the wetland (Greenway and Woolley 2001). Whereas if plants are not cropped there is the possibility that in time plant material will decompose and nutrients will be returned to the wetland (Brix 1997). Wetzel (1993) also reported the potential release of nutrients into the wetland system when plants are senescent. Although it is more probable that senescent plants will translocate nutrients to below ground biomass in autumn and winter, such a growth pattern has been well documented for Phragmites australis (Fiala 1978; Hocking 1989). Research differs into the effectiveness of wetland cropping to enhance nutrient removal in CWs. Reed et al. (1995) reported that routine cropping of above ground material can increase phosphorus uptake by macrophytes. Kim and Geary (2000) reported that although cropping enhanced phosphorus uptake by Schoenoplectus mucronatus overall removal decreased as a result of slow regrowth following cropping with no significant difference found in phosphorus removal between cropped and Uncropped systems. Greenway and Woolley (2001) found that in a Queensland SF CW the effect of wetland cropping on plant re-growth varied between macrophytes species Typha, Schoenoplectus and Eleocharis. Studies of plant growth and biomass after cropping have shown that for some species (eg. Schoenoplectus) cropping retards plant growth whereas other species have shown invigorated growth (eg. Eleocharis) (Greenway and Woolley 2001; Kim and Geary 2000). Greenway and Woolley (2001) reported that cropping appeared to invigorate plant growth in Eleocharis, which took only 2 months to achieve the initial biomass standing stock (biomass prior to cropping), whereas cropping Schoenoplectus appeared to retard growth taking 10 months to achieve the initial biomass standing stock.
Cropping of *Phragmites australis* is not recommended during periods of peak biomass as it can cause considerable damage to plants (Vymazal et al. 1999). To ensure minimal damage to plant *Phragmites* can be cropped in winter but this is when nutrient concentrations are expected to be lowest. Whereas IWA (2000) suggests ideal timing for cropping *Phragmites* is at the start of spring, although cropping in either spring or winter will not be maximising removal of nutrient storage (Greenway 2002). Greenway (2002) reported rapid increases in nutrient storage for *Phragmites* in spring as a result of new shoot growth with nutrient storage doubling by summer.

Where loading rates are low plant uptake can account for a large proportion of nutrient removal. Greenway and Woolley (2001) reported up to 65% and 47% phosphorus and nitrogen removal respectively by plant tissue for a low loaded SF system. Where loading rates are high, the amount of phosphorus and nitrogen taken up by plants accounts for only a small proportion of nutrient removal (Brix 1994; Geary and Moore 1999; Mann and Bavor 1993; Tanner 2000; Tanner and Kadlec 2002; Tanner et al. 1999). This is due to plants only being able to remove a finite amount of nutrients needed to sustain growth (refer to Chapter 6). As nutrient uptake by plants is often not considered a significant removal mechanism biomass in highly loaded system is often not cropped (Vymazal et al. 1998). Kim and Geary (2000) reported biomass removed by cropping only accounted for less than 5% of total phosphorus removal from the system. Whereas Mann (1990) reported 92% phosphorus removal for a small-scale (bucket size) cropping experiment. Proportion of nutrient removal attributed to nutrient uptake by macrophytes is investigated in Chapter 7: Effluent Treatment – Performance Efficiency. A large proportion of nutrients is often contained in the below ground biomass depending on macrophyte species which is inaccessible for removal through cropping (Adcock and Ganf 1994).

Wetland cropping can also disturb the wetland system potentially affecting wetland performance. Cropping has the potential to disturb wetland substrate releasing nutrients and/or sediment from the system (Department of Land and Water Conservation New South Wales 1998). Greenway and Woolley (2001) reported an increase in nutrient removal post harvest compared to removal in an unharvested channel. Timing of cropping is also important and it is often most desirable to crop in spring and/or summer when nutrient content and above ground biomass is generally highest as nutrients are commonly translocated to below ground biomass in winter (Breen 1990).
Various studies have looked at ways to use harvested plant material. Possible commercial uses have included: generation of biogas, organic fertilizers, mushroom cultivation, fibre products, chemical production and livestock feed (DeBusk and Ryther 1987; Gujral et al. 1987; Joglekar and Sonar 1987; Lakshman 1987). Macrophytes in CWs generally have high annual productivity and are generally tolerant to environmental variations making them attractive for commercial uses (Lakshman 1987). The use of Melaleuca trees in constructed wetland can potentially provide an economic benefit from tea tree oil production (Greenway 1996).

If a wetland is to be cropped a desirable plant characteristic to maximise nutrient uptake in a CW treating wastewater is the ability to recover following cropping. Therefore the effect of cropping on plant growth and biomass was investigated to further ascertain the suitability of the four species for use in tropical/subtropical SSF CWs.

4.1.1 Aims

This chapter will investigate the effect of cropping on growth and biomass on the four species. The aims of the research presented in this chapter are: 1) To investigate the effect of cropping on plant re-growth and biomass; 2) Investigate effect of gravel size on plant re-growth and 3) Investigate differences in plant re-growth after cropping between the four different species.

4.2 Materials and Methods

Parameters used to measure effect of cropping on plant growth and biomass are plant health, leaf height, root depth and biomass. Refer to Chapter 3 for plant health, leaf height and plant component methodologies.

4.2.1 Cropping

Plants in each compartment were cropped in August 2001 (9 months growth: Cells A, B and C and 6 months for Cell D due to initial problems with plant establishment) and re-cropped 6 months later (February 2002) to determine regrowth and nutrient removal capacity for each species following cropping. Plants were cropped approximately 10cm from the gravel surface to minimise disturbance. Plants were cropped while flowering (February 2002) to determine if flowers significantly increase above ground nutrient content as suggested by Breen (1990).
4.2.2 Root length

Maximum root length for each species was measured for each compartment with three replicates where there was sufficient number of plants. For *Philydrum* there was often insufficient number of plants to enable three replicates per compartment. Maximum root length was measured once plants were removed from the gravel. Root length was measured in February 2002 following 15 months growth in Cells A, B, and C and 12 months for those in Cell D, this was also 6 months after plants were first cropped (August 2001).

4.2.3 Biomass

Biomass was calculated for actual harvested biomass and total biomass was estimated using the measured harvested biomass and proportion of above to below ground plant components.

4.2.3.1 Harvested biomass

All fresh harvested plant material was immediately weighed on site on cropped using calibrated hanging scales. Representative samples of harvested plant material were collected for each species and oven dried at 75°C to determine moisture content. The mean moisture content for each species was then used to calculate the total harvested biomass per species and cell.

4.2.3.2 Estimated total biomass

Whole plant biomass (leaf, stem, flower, root and rhizome) for 9 months plant growth (6 months growth for plants in Cell D) (August 2001) and 6 months regrowth following cropping (February 2002) was calculated using harvested above ground biomass from 14m² and percentage plant component. August 2001 (9 month) and February 2001 (3 month to approximate likely proportion in February 2002) percentage plant components were used to estimate August 2001 and February 2002 biomass respectively (Refer to Chapter 3: Figure 24 to Figure 27). Estimated total biomass was calculated using above ground biomass minus the bottom 10cm (cropped biomass), estimated biomass of the bottom 10cm plus estimated below ground biomass using mean proportion of below ground components.
4.2.4 Statistical analysis

Root depth results were analysed using one-way ANOVA to identify significant interactions between species and cell. Results from gravel sections 2 and 3 were pooled to increase data size, this assumes that plants grew equally for both gravel sections. Statistical analysis of *Philydrum* data should be viewed with caution due to low replication as there was often not sufficient number of plants to enable three replicates per compartment.

4.3 Results and Discussion

4.3.1 Plant health and leaf height

Plant growth was similar in most cells following cropping in August 2001 and 6 months later in February 2002 compared to leaf height results prior to cropping (Figure 30 to Figure 37).

*Carex* and *Baumea* recovered quickly following cropping in late August 2001 with leaf heights after three months re-growth close to height prior to cropping. Slower regrowth for *Schoenoplectus* and *Philydrum* is likely due to the decline in plant health observed in several cells prior to cropping. Following cropping in February 2002 regrowth was similar for all cells with leaf heights lower than regrowth following initial cropping in August 2001. This suggests that leaf heights for regrowth from further cropping may continue to decrease. As found by Greenway and Woolley (2001) who reported that cropping retarded growth in *Schoenoplectus* but appeared to invigorate growth in *Eleocharis*. *Schoenoplectus* consistently had slowest regrowth similar to results from a study by Kim and Geary (2000) that found regrowth of *Baumea* and *Schoenoplectus* was slow following harvesting. Whereas Greenway (2002) reported re-growth of *Phragmites australis* in a sub-tropical SSF CW (SSF CW also used in this study) following cropping attained plant height of uncropped plants within 17 weeks.
**Figure 30:** Mean maximum leaf height for *Baumea articulata* in Section 2 of Cells A, B, C and D. (Cell D replanted in February 2001).

**Figure 31:** Mean maximum leaf height for *Baumea articulata* in Section 3 of Cells A, B, C and D. (Cell D replanted in February 2001).
Figure 32: Mean maximum leaf height for *Carex fascicularis* in Section 2 of Cells A, B, C and D. (Cell D replanted in February 2001)

Figure 33: Mean maximum leaf height for *Carex fascicularis* in Section 3 of Cells A, B, C and D. (Cell D replanted in February 2001).
Figure 34: Mean maximum leaf height for *Philydrum lanuginosum* in Section 2 of Cells A, B, C and D. (Cell D replanted in February 2001).

Figure 35: Mean maximum leaf height for *Philydrum lanuginosum* in Section 3 of Cells A, B, C and D. (Cell D replanted in February 2001).
**Figure 36:** Mean maximum leaf height for *Schoenoplectus mucronatus* in Section 2 of Cells A, B, C and D. (Cell D replanted in February 2001).

**Figure 37:** Mean maximum leaf height for *Schoenoplectus mucronatus* in Section 3 of Cells A, B, C and D. (Cell D was replanted in February 2001).
4.3.2 Root length

Maximum root depths have been reported by Gersberg et al. (1986) as 30cm, 60cm and 76cm for Typha, Phragmites and Scirpus species respectively growing in CWs. However several studies of macrophyte growth in gravel-bed systems using a variety of species report that maximum root depth rarely exceeds 30cm (Parr 1990; Reed and Brown 1992; Tanner 1994a).

The depth of Oxley pilot wetland (50cm) was designed to accommodate Phragmites root system, which is known to be extensive in a gravel wetland with root depths reported at over 60cm (Gersberg et al. 1986). Mean maximum root length for each species in Cells A, B, C and D are presented in Figure 38. Mean maximum root length obtained for all four species in all cells was below 25cm (Figure 38). Carex consistently had the deepest root penetration of the four species will little variation between cells.

![Figure 38: Root length for all four species in Cells A, B, C and D after 15 months growth in Cells A, B, and C and 12 months for those in Cell D.](image)

Maximum root penetration for Baumea was lower than Adcock and Ganf (1994) where maximum root and rhizome penetration was 30-40cm in a gravel SSF CW. Results are within that found by Greenway (2002) studying Phragmites growth in the Oxley SSF CW prior to the commencement of the current study which reported that greater than 80% of rhizomes occurred in the top 0-20cm of Cells B and D. A study by Tanner
(1996) investigating growth of eight emergent species in gravel-bed wetlands receiving dairy farm wastewater reported that although maximum root depth was recorded at 35-45cm for many of the species, root density generally decreased considerably below 25-30cm.

Mean maximum root depths for each cell were not significantly different between the four species (p≥0.05). Therefore gravel size does not appear to have greatly affected depth of root penetration. A significant difference was observed for root depth for *Baumea* between cells (p<0.05), with root depth significantly different between Cell B (old 20mm gravel) and the other three cells (p<0.05). Mean maximum root depth for *Baumea* in Cell B (18.8cm) was significantly higher (deeper penetration) compared to the other three cells, where mean maximum root depth ranged between 10.3 – 13.8cm. This may be due to the low water depth in Cell B for pond 3 (45cm), *Baumea* was located at the start of gravel section 2 and it is possible that it was necessary for *Baumea* to grow down towards the water thus having obtaining greater root penetration (Figure 39). Refer to Appendix 6 for the complete statistical analysis results.

**Figure 39:** Mean water depth in each pond for all four cells over the length of the study (Oct 2000 to May 2002).
4.3.3 Plant components

Proportion of each plant component for each of the four species in November 2001, 6 months after wetland was first harvested is presented in Figure 40. There was little difference in proportion of plant components of total biomass between February, May and August 2001 compared to November 2001 (Figure 40, Chapter 3: Figure 24 and Figures 26 to Figure 28). November 2001 composition is identical to the February and May 2001 composition for Carex (Figure 40 and Chapter 3: Figure 25). Philydrum composition in November 2001 was similar to that for February 2001 with flowering stems comprising close to 40% of the above ground component although the proportion of above ground to below remained relatively the same (Figure 40 and Chapter 3: Figure 27). This illustrates the potential for Philydrum flowering stems to be cropped thus removing a large proportion of the above ground biomass with the potential for minimal disturbance to the wetland. Additionally, the harvesting of Philydrum flowering stems could provide a product for the florist trade.

![Figure 40: Percentage composition of each plant component (leaf, root, flower and rhizome) of total plant biomass for all four species in November 2001 (12 months growth). NB mean biomass values were used to calculate % composition. Flowers comprised less than 1% of total plant biomass for Carex and Baumea.](image-url)
The average proportion of plant components of the total biomass over the 12 months was calculated and is presented in Figure 41. There is very little difference between average proportion of plant components over 9 months (Chapter 3: Figure 28) compared to 12 months (Figure 41) which is as expected due to the similarities between 12 month composition and 3, 6 and 9 month composition. Adcock and Ganf (1994) reported an even distribution between above and below ground biomass for *Baumea* and therefore a large proportion of nutrients was contained in the below ground biomass and inaccessible for removal through cropping. In this study greater than 80% of biomass is above ground thus cropping above ground material will remove a significant proportion of biomass (Figure 41).

**Figure 41:** Average percentage composition of each plant component (leaf, root, flower and rhizome) of total plant biomass. NB mean biomass values over 12 months were used to calculate % composition. Flowers comprised less than 1% of total plant biomass for *Carex* and *Baumea*. 
4.3.4 Cropped above ground biomass and estimated total biomass

Harvested above ground biomass showed considerable variability between species and cells (Figure 42 and Table 1). The highest shoot biomass cropped in August 2001 after 9 months growth was 25.7kg (from 14m²) for *Baumea*, followed by *Carex* (24.8 kg) (Figure 42). *Carex* consistently had the highest harvested above ground biomass with high regrowth following cropping. Biomass following cropping was generally always lower than biomass attained prior to cropping (Table 1 and Figure 42). Greenway (2002) studying *Phragmites* in the same CW reported that biomass overall was lower for cropped sections compared to uncropped biomass.

Total plant biomass for *Baumea* was lower than found in previous studies by Adcock and Ganf (1994) and Tanner (1996) in SSF wetlands where mean plant biomass was in the range of 8kg/m². As indicated in Figure 42 *Carex* is the only species that consistently had high regrowth following cropping and could achieve a harvestable above ground biomass in 6 months. Kim and Geary (2000) similarly found that regrowth of *B. articulata* and *S. mucronatus* was slow following harvesting. Greenway and Woolley (2001) showed that cropping invigorated growth in *Eleocharis sphacelata* but limited regrowth in *Schoenoplectus validus*. Gravel size does not appear to have affected biomass and recovery following cropping. This same conclusion was also reported by Greenway (2002) studying re-growth of *Phragmites* following cropping in the same CW.

**Table 1**: Estimated total plant biomass (g DW/m²) in each cell by the four macrophyte species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total biomass (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell A Aug-01</td>
</tr>
<tr>
<td></td>
<td>Feb-02</td>
</tr>
<tr>
<td><em>Ba</em></td>
<td>2160</td>
</tr>
<tr>
<td><em>Cf</em></td>
<td>1992</td>
</tr>
<tr>
<td><em>Pl</em></td>
<td>347</td>
</tr>
<tr>
<td><em>Sm</em></td>
<td>241</td>
</tr>
</tbody>
</table>

NB. Wetland was harvested in August 2001 after 9 months plant growth (6 months for Cell D), and harvested again in February 2002 after 6 months re-growth.
Figure 42: Total above ground biomass harvested from 14m² in August 2001 and February 2002 for all four macrophyte species.
4.4 Conclusion

A large proportion of biomass is contained within above ground plant components therefore cropping above ground biomass would potentially remove a significant proportion of nutrient storage from the CW. Ideal time for cropping would be in spring/summer when plants are flowering. Removal of *Philydrum* flowering stems would remove a considerable proportion of total plant biomass. Flowering stems could also potentially have a commercial use as a cut flower. Mean maximum root depth for all four species trialed were less than 25cm indicating that any potential oxygen release into the rhizosphere would occur within the top 25cm.

Cropping above ground biomass was found to retard re-growth of *Schoenoplectus* and *Philydrum*. Of those species trialed *Carex* and *Baumea* recovered quickest following cropping, with *Carex* attaining leaf height prior to cropping within 6 months. This indicates that of the four species trialed *Carex* is the most suitable for use in a SSF CW if the wetland is to be cropped due to rapid re-growth. Gravel size did not appear to affect maximum root penetration or re-growth following cropping.
CHAPTER 5: NUTRIENT STORAGE IN PLANT BIOMASS

5.1 Introduction

Uptake of nutrients by macrophytes is essential for their growth and reproduction. The high productivity of macrophytes enables substantial amounts of nutrients to be stored in plant biomass (IWA 2000). Net productivity (growth rate) and the concentration of nutrient within plant tissues limits the rate of nutrient uptake by macrophytes with nutrient storage dependent on plant tissue nutrient concentrations and potential for biomass accumulation (maximum standing crop) (IWA 2000; Reddy and DeBusk 1987). At low to medium levels of nutrients plant growth is proportional to nutrient supply (Figure 43). Increases in nutrients above this may result in the luxury uptake of nutrients by plants, but does not increase plant growth (IWA 2000; Reddy and DeBusk 1987). Vymazal et al. (1999) studying Phragmites reported a limited range in nutrient concentration with CW concentrations comparable to natural wetlands. Nutrient concentrations for Phragmites growing in CWs treating nutrient rich wastewater did not exceed concentrations for Phragmites growing in eutrophic or polytrophic natural stands (Vymazal et al. 1999). Whereas similar studies using Phalaris reported that plant nutrient concentrations were higher for CWs treating nutrient rich wastewater compared to nutrient rich natural stands (Vymazal et al. 1999).

Nutrient accumulation will eventually plateau although nutrient supply continues to increase, beyond this point increases in nutrient concentration can cause nutrient toxicity (IWA 2000; Reddy and DeBusk 1987). This is further supported by Vymazal et al. (1999) who found little difference in Phragmites nutrient concentrations between inflow and outflow zones of CWs, where there was considerable differences in inflow and outflow water quality. A study by Greenway and Woolley (1999) found no differences in nutrient concentrations of macrophytes between inlet and outlet sections of a CW and suggested nutrient sufficiency. Vymazal et al. (1999) concluded that increase in biomass and nutrient concentration is proportional to a certain level of nutrients in the water and above this level can have negative effects on plant health.
Figure 43: Relationship between nutrient supply and nutrient accumulation and biomass yield.

Nutrient content also changes with plant age, young plants often have a high nutrient content but as the plant reaches maturity nutrient content decreases with biomass and thus total nutrient storage increases (Boyd 1970). Vymazal et al. (1999) studying Phragmites in a temperate climate observed variability in plant nutrient concentration during the growing season, concentrations were generally highest early in the growing season and decreased during winter. Haslam (1968) found that in Phragmites following seed set, nutrients were translocated down into the below ground material with above ground material dying back for the winter season. Greenway (2002) investigating Phragmites growth and nutrient storage in a subtropical/tropical climate reported senescence and translocation of nutrients to below ground components during winter. In this study nitrogen and phosphorus content of leaves and stems was highest in early summer from new growth. Greenway (2002) also found evidence of mobilisation and translocation of nutrients from stems to reproductive structures (flowers) for mature Phragmites plants at varying stages of senescence.

Although biomass has the largest influence over plant storage due to limited variation in plant nutrient concentrations between sites a high plant tissue content is still a desirable characteristic (Reddy and DeBusk 1987; Vymazal et al. 1999). Nutrient concentrations have been found to be higher in leaves compared to stems for both natural and constructed wetlands (Vymazal et al. 1999). Greenway (1997) studying macrophytes in a subtropical/tropical climate reported only slight variability between nutrient content and different plant components (roots, rhizomes, stem and leaves), with nitrogen generally highest in the leaves and phosphorus highest in the roots. Similarly a study by
Mars et al. (2003) that phosphorus storage was highest in below ground components with lowest amount of phosphorus storage in leaves.

In order to assess the suitability of the four species trialed in this project for use in SSF CWs for nutrient removal, nutrient bioaccumulation for the four species needs to be investigated.

5.1.1 Aims
This chapter will investigate nutrient storage of nitrogen and phosphorus in plant biomass for the four species. The aims of the research presented in this chapter are: 1) Investigate differences in nutrient content between plant components, age/season and species and 2) To investigate nutrient storage of nitrogen and phosphorus in plant biomass for the four species.

5.2 Materials and Methods
Parameters used to investigate nutrient bioaccumulation (nitrogen and phosphorus) are biomass and nutrient content of individual plant components for all four species.

5.2.1 Plant biomass and nutrient content
Biomass samples used for determination of nitrogen and phosphorus content were those collected when the macrophytes were first planted (October 2000) for initial nutrient content, summer after 3 months growth (February 2001) and autumn after 6 months growth (May 2001). Plant biomass was determined as per Chapter 4: Effect of Cropping on Growth and Biomass. Once biomass samples were split into plant components as per Chapter 3: Plant Establishment and Growth, individual plant components (shoots, flowers, roots and rhizomes) were analysed for total nitrogen and phosphorus using representative samples for all four species from each cell. This enabled bioaccumulation and nutrient composition for various plant components for each of the four species to be determined.

Prior to analysis all samples were finely ground in a Rocklab ring grinder (Greenway and Woolley, 1999). Total nitrogen was determined using the automated combustion method (Kalra 1998). Two methods were used to determine total phosphorus the first was a sodium sulphate, selenous and sulphuric acid digestion mixture as per methods
for plant analysis outlined in Appendix 1 (Johnson et al., 1985). Digested samples were analysed using the automated ascorbic acid reduction method for colourmetric determination (total phosphorus in kjeldahl digests method 13-115-01-1-B (refer to Appendix 2) (APHA 1997). The second method, which had better colour differentiation, was a mixed acid digestion made of nitric, sulphuric and perchloric acids using a modified method of that by Allen (1989) (Appendix 3). Digested samples were analysed using the ascorbic acid reduction method for manual colourmetric determination (Method 4500-P E.) outlined in Appendix 4 (APHA 1997; Kalra 1998).

For comparison purposes plant samples were also collected for three of the species (Baumea, Carex and Philydrum) growing in their natural environment. Carex was growing in soil within the riparian zone of a tributary of Native Dog Creek, Brisbane. Baumea and Philydrum were growing in an oligotrophic surface flow wetland in 20cm of water. Plant components and nutrient content was determined as described above.

5.2.2 Nutrient storage

Nutrient storage by plants was calculated for each species and each cell by multiplying the mean nitrogen and phosphorus content (mgP/g and mgN/g) of whole plant by the estimated total plant biomass as calculated in Chapter 4: Effect of Cropping on Plant Growth and Biomass (Table 1).

5.2.3 Statistical analysis

All statistical analyses were undertaken using SAS Version 8.0. Nutrient content results were analysed using one-way ANOVA and two-way factorial to identify significant interactions in nitrogen and phosphorus between age/season (summer after 3 months growth (February 2001) and autumn, 6 months growth (May 2001)), species, cell and plant component. Interaction between phosphorus and age/season was not assessed, as there was insufficient number of results to conduct a factorial analysis.

Results from gravel sections 2 and 3 were pooled to increase data size, this assumes that plants received similar nutrient concentrations for both gravel sections. Although nutrient concentrations decreased between gravel sections 2 and 3 (refer to Chapter 7: Effluent Treatment – Performance Efficiency) nitrogen and phosphorus concentrations
for both sections are likely to be sufficient for the macrophytes suggesting little variation in nutrient content between the two gravel sections (Reddy and DeBusk 1987).

Results were all transformed to represent a normal distribution as required for one-way ANOVA and factorials. Samples of rhizomes, stems and flowers were not available for all four species. Insufficient number of *Philydrum* plants for sample collection in several cells reduced replication for statistical analysis. As a result rhizomes, flowers and stems were removed to ensure balanced analysis. Nutrient data for macrophytes growing in their natural environment could not be statistically compared to nutrient data for Oxley SSF CW due to lack of replication. For the three species growing in natural stands three replicates were collected. Statistical analysis of *Philydrum* data should be viewed with caution due to low replication as there was often not a sufficient number of plants to enable three replicates per compartment.

5.3 Results and Discussion

5.3.1 Nutrient content

Mean nitrogen and phosphorus content of the different above ground (leaf, stem and flower) and below ground (root and rhizome) plant components for the four macrophyte species when first planted (initial), February 2001 (3 months growth) and May 2001 (6 months growth) is presented in Table 2.

There was only a slight difference in nitrogen and phosphorus content of plant components when first planted compared to content after 3 and 6 months (Table 2). This can be attributed to plants having been raised in potting mix that contained fertiliser to improve plant survival. Although initial nutrient content of roots and rhizomes and phosphorus content of leaves was often slightly lower than content at 3 and 6 months growth.
Table 2: A comparison of mean nutrient content (mgP/g and mgN/g) in plant components (leaf, stem, flower, root and rhizome) for the four macrophyte species when first planted (initial), February 2001 (3 months growth) and May 2001 (6 months growth) from all four cells.

<table>
<thead>
<tr>
<th>Plant Component and Growth</th>
<th>Baumea (N)</th>
<th>P</th>
<th>Carex (N)</th>
<th>P</th>
<th>Philydrum (N)</th>
<th>P</th>
<th>Schoenoplectus (N)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaves</strong></td>
<td>13.1 ± 2.1</td>
<td>1.8 ± 0.2</td>
<td>16.1 ± 0.6</td>
<td>1.7 ± 0.2</td>
<td>19.6 ± 3.6</td>
<td>1.6 ± 0.3</td>
<td>19.2 ± 2.7</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>Initial</td>
<td>16.6 ± 5.1</td>
<td>2.2 ± 0.3</td>
<td>16.1 ± 1.7</td>
<td>2.8 ± 0.2</td>
<td>16.4 ± 2.4</td>
<td>2.0 ± 0.3</td>
<td>17.1 ± 1.4</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>3 months</td>
<td>16.2 ± 3.5</td>
<td>1.8 ± 0.6</td>
<td>17.0 ± 3.8</td>
<td>2.7 ± 0.5</td>
<td>18.3 ± 3.0</td>
<td>2.5 ± 1.1</td>
<td>18.4 ± 4.5</td>
<td>4.0 ± 1.4</td>
</tr>
<tr>
<td>6 months</td>
<td>13.4 ± 3.5</td>
<td>3.1 ± 1.3</td>
<td>9.4 ± 3.1</td>
<td>2.6 ± 0.7</td>
<td>14.1 ± 5.1</td>
<td>2.6 ± 1.8</td>
<td>12.6 ± 6.9</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td><strong>Stems</strong></td>
<td>22.3 ± 3.6</td>
<td>2.5 ± 0.2</td>
<td>18.7 ± 4.3</td>
<td>2.3 ± 0.2</td>
<td>21.2 ± 4.3</td>
<td>2.4 ± 0.2</td>
<td>17.1 ± 5.7</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Initial</td>
<td>15.4 ± 3.9</td>
<td>1.5 ± 0.6</td>
<td>11.0 ± 2.6</td>
<td>1.1 ± 0.6</td>
<td>13.4 ± 2.1</td>
<td>2.3 ± 0.7</td>
<td>16.0 ± 6.7</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td>3 months</td>
<td>15.6 ± 4.8</td>
<td>2.3 ± 0.2</td>
<td>19.1 ± 4.2</td>
<td>2.1 ± 0.3</td>
<td>21.2 ± 5.1</td>
<td>2.4 ± 0.3</td>
<td>17.1 ± 6.7</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>6 months</td>
<td>10.3 ± 2.7</td>
<td>2.6 ± 0.5</td>
<td>12.9 ± 2.8</td>
<td>1.8 ± 0.7</td>
<td>15.6 ± 2.6</td>
<td>3.3 ± 2.0</td>
<td>13.1 ± 4.3</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td><strong>Flowers</strong></td>
<td>10.3 ± 2.7</td>
<td>2.6 ± 0.5</td>
<td>12.9 ± 2.8</td>
<td>1.8 ± 0.7</td>
<td>15.6 ± 2.6</td>
<td>3.3 ± 2.0</td>
<td>13.1 ± 4.3</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>Initial</td>
<td>18.2 ± 2.3</td>
<td>3.2 ± 0.3</td>
<td>18.4 ± 3.5</td>
<td>2.6 ± 0.5</td>
<td>21.2 ± 5.1</td>
<td>2.4 ± 0.3</td>
<td>17.1 ± 6.7</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>3 months</td>
<td>20.3 ± 4.4</td>
<td>3.6 ± 1.1</td>
<td>19.1 ± 4.2</td>
<td>2.1 ± 0.3</td>
<td>21.2 ± 5.1</td>
<td>2.4 ± 0.3</td>
<td>17.1 ± 6.7</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>6 months</td>
<td>10.3 ± 2.7</td>
<td>2.6 ± 0.5</td>
<td>12.9 ± 2.8</td>
<td>1.8 ± 0.7</td>
<td>15.6 ± 2.6</td>
<td>3.3 ± 2.0</td>
<td>13.1 ± 4.3</td>
<td>3.4 ± 0.9</td>
</tr>
</tbody>
</table>
Nutrient content for above and below ground plant components (3 and 6 month growth) ranged between 1.8 – 4.0 mgP/g and 9.8 – 20.3 mgN/g (Table 2). Nutrient content is within the range of other macrophytes growing in treatment wetlands (Adcock and Ganf, 1994; Greenway, 1997; Greenway 2002; Tanner, 1996). Phosphorus content of rhizomes was lower than that reported from a temperate wetland by Adcock and Ganf (1994). Results are consistent with work carried out in tropical climates where year round growth is promoted, thus reducing the necessity for the rhizome to act as a storage organ for phosphorus (Greenway 1997).

Significant differences in TP and TN plant content between plant components, plant species, and age/season (summer after 3 months growth (February 2001) and autumn, 6 months growth (May 2001) – nitrogen only) are presented in Table 3. Differences were generally not consistent over the four cells. Refer to Appendix 6 for further results of statistical analysis of nutrient data.

Nitrogen content of leaves in summer (3 months growth) was significantly higher (p<0.05) than content in autumn may be due to flowering and new plant growth increasing the above ground nutrient content (Breen 1990). Nutrient content is generally highest for young plants early in the growing season, with nutrient content decreasing once plants reach maturity (Boyd 1970; Greenway, 2002; IWA 2000; Vymazal et al. 1999). Greenway (2002) reported that re-growth of cropped shoots in summer had higher nitrogen and phosphorus content compared to uncropped shoots.

In many instances nitrogen content of leaves was significantly higher than roots (p<0.05). This is in keeping with studies by several authors who have also reported nitrogen content of leaves to be higher than roots (Vymazal et al. 1999; Mars et al. 2003).

Results from the current study support cropping above ground biomass in summer when plants are flowering and nutrient content of leaves is higher compared to autumn. Although cropping in summer may cause more damage to macrophytes compared to cropping in autumn or spring, which is particularly the case when cropping Phragmites (IWA 2000).
Table 3: Significant differences in TN and TP content of different plant components for the four species (p<0.05 indicates significant difference).

<table>
<thead>
<tr>
<th>Cell</th>
<th>Variable</th>
<th>Significance Level (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Total Nitrogen Content of Macrophytes</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>February leaves vs May leaves</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>February roots vs February leaves</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>May roots vs February leaves</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>May leaves vs May roots</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B</td>
<td>No significant differences observed</td>
<td>≥0.05</td>
</tr>
<tr>
<td>C</td>
<td>Carex vs Schoenoplectus</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Philydrum roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Baumea leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Philydrum leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Carex leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Baumea leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Philydrum leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Carex leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>D</td>
<td><strong>Schoenoplectus roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Schoenoplectus roots vs Philydrum leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Schoenoplectus roots vs Carex leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Philydrum roots vs Philydrum leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Philydrum roots vs Carex leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Total Phosphorus Content of Macrophytes</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td><strong>Baumea leaves vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Schoenoplectus leaves vs Philydrum leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Philydrum roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Schoenoplectus roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Schoenoplectus roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Philydrum roots vs Carex leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Philydrum roots vs Philydrum leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea leaves vs Schoenoplectus roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Schoenoplectus roots vs Philydrum leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B</td>
<td>No significant differences observed</td>
<td>≥0.05</td>
</tr>
<tr>
<td>C</td>
<td><strong>Baumea leaves vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Schoenoplectus leaves vs Carex leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Carex roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Schoenoplectus roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Philydrum leaves vs Carex roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea leaves vs Schoenoplectus roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Schoenoplectus roots vs Carex leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>D</td>
<td><strong>Baumea roots vs Philydrum roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Philydrum roots</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Carex roots vs Schoenoplectus leaves</strong></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td><strong>Baumea leaves vs Philydrum roots</strong></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Comparisons between mean nutrient content in plant components for three of the macrophyte species, at Oxley SSF CW and in their natural environment generally show marked differences (Table 2 and Table 4). A study by Greenway (1997) found that nutrient concentrations were generally higher for plants growing in CWs compared to natural stands. Nutrient content is much lower for *Baumea* and *Philydrum* growing in the natural stands of an oligotrophic SF wetland which is nutrient-limiting compared to secondary treated effluent high in nutrients (refer to Chapter 7 for nutrients levels at Oxley SSF CW). Whereas nitrogen and phosphorus content of *Carex* leaves from a natural stand growing in soil is comparable to that in the Oxley SSF CW (Table 2 and Table 4). This may be due to nutrient cycling and accumulation of organic matter in the soil particularly within the surface layer providing sufficient nutrients for plant growth. Vymazal *et al.* (1999) reported that nutrient content of *Phragmites* in eutrophic natural stands was similar to those growing in CWs treating nutrient rich wastewater concentrations.

**Table 4:** A comparison of mean nutrient content (mgP/g and mgN/g) in plant components (leaf, stem, flower, root and rhizome) for the four macrophyte species in their natural habitat. NB. *Baumea* and *Philydrum* samples were collected from Cornubia Nature Reserve and *Carex* samples from Native Dog Reserve.

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf (mg/g)</th>
<th>Stem (mg/g)</th>
<th>Flowers (mg/g)</th>
<th>Root (mg/g)</th>
<th>Rhizome (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Baumea</em></td>
<td>P 0.5 ± 0.04</td>
<td>P 1.2 ± 1.02</td>
<td>P 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N 5.6 ± 0.12</td>
<td>N 6.1 ± 0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Carex</em></td>
<td>P 2.0 ± 0.25</td>
<td>P 0.8 ± 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N 14.9 ± 1.56</td>
<td>N 7.9 ± 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Philydrum</em></td>
<td>P 0.3 ± 0.01</td>
<td>P 0.5 ± 0.31</td>
<td>P 0.7</td>
<td>P 0.5 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N 6.7</td>
<td>N 7.2 ± 4.26</td>
<td>N 10.0</td>
<td>N 6.2 ± 1.41</td>
<td></td>
</tr>
</tbody>
</table>
5.3.2 Nutrient storage

Nutrient storage as plant biomass for the four species in August 2001 and February 2002 is presented in Table 5.

Table 5: Plant biomass (g DW/m²) and nutrient storage (gP/m² and gN/m²) in each cell by the four macrophyte species in August 2001 and February 2002. NB nutrient storage was calculated using mean nitrogen and phosphorus content of whole plant for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cell A</th>
<th>Cell B</th>
<th>Cell C</th>
<th>Cell D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aug-01</td>
<td>Feb-02</td>
<td>Aug-01</td>
<td>Feb-02</td>
</tr>
<tr>
<td>Ba</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total biomass (g/m²)</td>
<td>2160</td>
<td>1307</td>
<td>1195</td>
<td>401</td>
</tr>
<tr>
<td>P storage (g/m²)</td>
<td>5.49</td>
<td>3.32</td>
<td>3.03</td>
<td>1.02</td>
</tr>
<tr>
<td>N storage (g/m²)</td>
<td>33.91</td>
<td>20.51</td>
<td>18.76</td>
<td>6.29</td>
</tr>
<tr>
<td>Cf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total biomass (g/m²)</td>
<td>1992</td>
<td>1928</td>
<td>515</td>
<td>659</td>
</tr>
<tr>
<td>P storage (g/m²)</td>
<td>4.62</td>
<td>4.47</td>
<td>1.20</td>
<td>1.53</td>
</tr>
<tr>
<td>N storage (g/m²)</td>
<td>30.32</td>
<td>29.35</td>
<td>7.84</td>
<td>10.03</td>
</tr>
<tr>
<td>Pl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total biomass (g/m²)</td>
<td>347</td>
<td>263</td>
<td>371</td>
<td>23</td>
</tr>
<tr>
<td>P storage (g/m²)</td>
<td>0.89</td>
<td>0.68</td>
<td>0.96</td>
<td>0.06</td>
</tr>
<tr>
<td>N storage (g/m²)</td>
<td>5.31</td>
<td>4.03</td>
<td>5.68</td>
<td>0.36</td>
</tr>
<tr>
<td>Sm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total biomass (g/m²)</td>
<td>241</td>
<td>114</td>
<td>15</td>
<td>183</td>
</tr>
<tr>
<td>P storage (g/m²)</td>
<td>0.80</td>
<td>0.38</td>
<td>0.05</td>
<td>0.61</td>
</tr>
<tr>
<td>N storage (g/m²)</td>
<td>3.59</td>
<td>1.70</td>
<td>0.22</td>
<td>2.72</td>
</tr>
</tbody>
</table>

Wetland was harvested in August 2001 after 9 months plant growth (6 months for Cell D), and harvested again in February 2002 after 6 months re-growth.

Nutrient storage was generally highest in Cell A due to the high biomass achieved in this cell. Of the four species nutrient storage was highest for *Baumea* in August 2001. Once the wetland was cropped *Baumea* was slow to recover with *Carex* having the highest nutrient storage following cropping as a result of rapid regrowth (Chapter 4: Plant Biomass and the Effects of Cropping on Regrowth, Table 5). Nutrient storage is much lower than a study by Reddy and DeBusk (1987) which reported 140-1560 mgN/m² and 14-375 mgP/m² for *Typha, Juncus, Scirpus* and *Phragmites*. 
Biomass and nutrient storage for *Baumea* and *Carex* in Cell A, was similar to that recorded by Greenway (2002) for *Phragmites australis* also growing in the same CW at Oxley, Brisbane. Biomass and nutrient storage for *Baumea* and *Carex* in Cell A was higher than reported by Greenway and Woolley (2001) for a dense stand of *Typha* growing in a Queensland CW. Comparable nutrient storage for the two species *Baumea* and *Carex* to other nutrient storage for other species used successfully in Queensland CWs indicates the potential of these two species for nutrient removal (Greenway 2002; Greenway and Woolley; 2001).

The proportion of nutrient removal by the Oxley SSF CW contained in plant biomass is investigated in Chapter 7: Effluent Treatment – Performance Efficiency.
5.4 Conclusion

The four species trialed are all suitable for use in CWs, in terms of ability to bioaccumulate nitrogen and phosphorus but high nutrient content may make them more susceptible to pest and disease attack. *Baumea* and *Carex* have the highest nutrient storage with *Carex* having rapid regrowth following cropping. This indicates that *Carex* is the most suitable of all the four species trialed as it not only has comparable nutrient content to other macrophytes used successfully in CWs but has the highest nutrient storage and regrowth following cropping.

Nutrient storage was generally highest in Cell A (new 5mm gravel) as a result of higher biomass achieved in this cell. Higher biomass achieved in Cell A is most likely due to this cell having the highest water depth during the plant establishment phase.
CHAPTER 6: ORGANIC CARBON ACCUMULATION

6.1 Introduction

Wetland ecosystems are comprised of both heterotrophic and autotrophic organisms. Autotrophs are primary producers such as plants whereas heterotrophs are consumers and include animals and microbes. Wetlands are largely autotrophic with primary carbon fixation from the atmosphere by plants through direct or indirect actions largely responsible for organic matter accumulation within many wetlands (IWA 2000; Mitsch and Gosselink 1993). Additional supplies of carbon also enter CWs within the wastewater although the amounts of carbon cycled within the wetland far exceed any wastewater additions (Kadlec and Knight 1996; Tanner et al. 1998).

Organic matter can be biologically converted to ammonia nitrogen through the actions of aerobic and anaerobic microflora, this process is termed “mineralisation or ammonification” (Hamersley and Howes 2002; IWA 2000). Although within oxygen deficient wetlands organic matter accumulates due to decreased microbial activity and organic decomposition (IWA 2000). Accumulation of organic matter in this case generally exceeds any accumulation from sedimentation of incoming suspended material. Such a process is termed “peat accumulation” where accumulated material has originated from plant detritus (IWA 2000).

Within a wetland plant tissues are removed from the wetland (cropping), consumed, or eventually recycled to the substrate as plant litter (Kadlec and Knight 1996). Litterfall and the subsequent decomposition of organic plant material play an important role in wetland functioning (Kadlec and Knight 1996; Reddy and D'Angelo 1994). Decomposing detritus and algal cells provide organic carbon, nitrogen and phosphorus to the wetland as cellulose, lignin, proteins, hemicellulose and phospholipids (Reddy and D'Angelo 1994). Vertebrates, invertebrates and microbes are largely responsible for decomposition of plant detritus within a wetland, with decomposition rates varying between plant species and tissues (Kadlec and Knight 1996). In turn organic carbon produced by plants supports heterotrophic microbes important in nutrient transformations (ie. denitrification) (Reddy and D'Angelo 1994).
Within a CW denitrification is the dominant mechanism for the long term removal of nitrogen from a wetland (Brix 1993; Faulkner and Richardson 1989). Denitrification occurs primarily under anaerobic conditions where there is sufficient supply of dissolved organic carbon.

Accumulated plant derived organic matter can also provide additional adsorption sites for phosphorus removal (Bostrom et al. 1982). Tanner et al. (1999) investigating phosphorus accumulation in gravel CWs suggested that a substantial proportion of TP accumulation was in organic forms or related to organic material and that continued TP removal was likely determined by the wetlands ability to accumulate organic material thus providing additional adsorption sites. Although continued organic accumulation within a gravel CW may lead to pore spaces clogging decreasing hydraulic conductivity creating short-circuiting and ultimately reducing the removal efficiency of the wetland (Crites 1994; Tanner et al. 1999).

Tanner and Sukias (1995) reported increased organic matter accumulation attributed to above and below ground plant production in planted gravel CWs compared to unplanted. Organic matter accumulation in planted SSF CWs is generally greatest on the surface and in the top 100mm of substrate primarily a result of litter fall (Tanner and Sukias 1995). Tanner et al. (1998) and Vymazal (1999) suggested that within a SSF CW most of the organic carbon from plant litter on the substrate surface is unlikely to contribute to organic accumulation within the substrate. This is due to potential losses of plant litter from in situ aerobic decomposition and herbivory which is often increased due to the high nutrient conditions of CWs (Jordan et al. 1990; Mann and Wetzel 1996; Newell et al. 1989). Tanner (2000) studying senescing aerial shoots of Schoenoplectus, Typha and Phragmites suggested that by the time dead shoots fall to the ground as litter the majority of available carbon will have been consumed while dead shoots are still standing. Greenway (2002) reported considerable litter accumulation for a gravel SSF CW with litter at varying stages of decomposition.

Tanner et al (1998) suggests that below ground plant material (roots and rhizomes) shed directly into the substrate is likely to provide the organic carbon source within the substrate for denitrification rather than above ground plant litter. Further study is needed to determine if macrophytes can provide organic carbon for denitrification, and changes in organic matter accumulation with depth.
6.1.1 Aims

This chapter will examine organic carbon accumulation for the four cells, studying differences in accumulation between cells and depth (0 to 10 cm, 10 to 20 cm and 20 to 30 cm). The aims of the research presented in this chapter are: 1) To investigate organic carbon accumulation and 2) Investigate relationship between organic carbon and gravel size, age of wetland and depth.

6.2 Materials and methods

Organic carbon accumulation was estimated as amount of organic carbon trapped between gravel particles.

6.2.1 Organic accumulation

In order to measure organic carbon (OC) accumulation gravel samples were collected from each gravel section in all four cells between August and October 2002. Gravel samples were collected using a plastic corer serrated at the base, with 10 cm depth increments marked within the corer. The corer was gently knocked and twisted into the gravel until it was 30 cm into the gravel bed. Gravel was then removed from the corer with gloved hands in 10 cm depth increments (0-10 cm, 10-20 cm and 20-30 cm) and placed into a labelled plastic bag. Three replicates were taken from each gravel section with cores taken from approximately the front, middle and back of each section to capture any changes in organic accumulation with distance through the gravel section. Samples were then refrigerated (4°C) prior to processing (generally within 48 hours) (Tanner et al. 1998).

Gravel was placed into a 100 mL plastic measuring container to represent an approximate volume of gravel. The gravel was then thoroughly washed and shaken by hand for approximately 5 minutes with 1 L of distilled deionised water to remove any organic matter trapped between gravel particles. The supernatant was collected to determine the total volatile solids (TVS). TVS can be used as an estimate of organic carbon and in the current study was used as a measure of organic carbon (APHA 1997). TVS was analysed according to total suspended solids dried at 103-105°C and volatile solids ignited at 500°C methods (Methods 2540 D and 2540 E) (APHA 1997). Tanner and Sukias (1995) estimated organic accumulation of gravel samples as loss on ignition (LOI; 550°C for 4 hours). LOI levels showed a close linear correlation to total carbon
concentrations, with approximately 0.5% of LOI attributable to non-organic losses (Tanner and Sukias 1995).

6.2.2 Statistical analysis

All statistical analyses were undertaken using SAS Version 8.0. Organic accumulation results were analysed using one-way ANOVA and two-way factorial to identify significant interactions and differences in organic accumulation with cell and depth. Results were all transformed to represent a normal distribution as required for one-way ANOVA and two-way factorial. Only a one-way ANOVA was used for gravel section 1 for all cells due to the limited number of replicates for the three depths, therefore interactions in organic accumulation with depth and cell could not be determined.

6.3 Results and Discussion

6.3.1 Organic accumulation

Mean OC accumulation along each cell for three different depths can be seen in Figure 44 to Figure 47.

Figure 44: Mean organic carbon accumulation for Cell A (new 5mm gravel) at three depths with distance along the cell. NB. 1, 2 and 3 represent gravel sections 1, 2 and 3. N=3. Error bars show standard deviation.
Figure 45: Mean organic carbon accumulation for Cell B (old 20mm gravel) at three depths with distance along the cell. NB. 1, 2 and 3 represent gravel sections 1, 2 and 3. N=3. Error bars show standard deviation.

Figure 46: Mean organic carbon accumulation for Cell C (old 20mm gravel) at three depths with distance along the cell. NB. 1, 2 and 3 represent gravel sections 1, 2 and 3. *Samples not collected from gravel section 1 at 20-30cm. N=3 for sections 2 and 3 and N=2 for section 1. Error bars show standard deviation.
Figure 47: Mean organic carbon accumulation for Cell D (old 5mm gravel) at three depths with distance along the cell. NB. 1, 2 and 3 represent gravel sections 1, 2 and 3. *Samples not collected from gravel section 1 at 20-30cm. N=3 for sections 2 and 3 and N=2 for section 1. Error bars show standard deviation.

Mean OC accumulation was highest in Cell D (old 5mm gravel) with 672 mgOC/100mL of gravel measured in the top 10cm (Figure 47). Organic accumulation measured within the Oxley SSF CW is considerably lower than that measured by Tanner and Sukias (1995). Tanner and Sukias (1995) reported mean organic accumulation (measured as loss on ignition) at three depths for an unplanted and planted wetland between 3-33kg/m$^3$ with majority of organic accumulation within the top 10cm of gravel. Whereas organic accumulation measured in this study once converted to kg/m$^3$ ranged between 0.05–9.47 kg/m$^3$. The wetlands studied by Tanner and Sukias (1995) had been in operation for 22 months whereas in the current study Cells B, C and D had been in operation for close to 7 years and Cell A 2 years. Although TVS loading rates (total TVS loading over 22 months was 1.3 - 4.4 kg/m$^2$ (using 82% of TSS is TVS) for the CW studied by Tanner and Sukias (1995) was considerably higher than for the current study which may explain the large variation in results. It is also possible that the lower amount of organic accumulation within the Oxley wetland is a result of increased plant decomposition in the subtropical/tropical climate of Queensland.

Cells B, C and D all showed an increase in organic accumulation between the unplanted section (gravel section 1) and the planted sections (gravel sections 2 and 3) (Figure 45
to Figure 47). Even though the majority of organic matter contained within wastewater was removed in the unplanted gravel section, illustrated by greatest COD and TSS reduction in the first gravel section (Chapter 7: Effluent Treatment – Performance Efficiency), organic accumulation was still higher in the planted sections. Tanner and Sukias (1995) also reported increased organic accumulation in planted sections compared to unplanted sections. This can largely be attributed to the supply of organic matter from above and below ground plant production (Tanner and Sukias 1995). Comparisons in mean organic accumulation between the four cells show that Cells B and D had the highest mean organic accumulation for the planted sections (Figure 44 to Figure 47). Within the unplanted gravel section (section 1) there were no significant differences in organic accumulation observed between cells and depth. In the planted sections (sections 2 and 3) Cells B and D had significantly higher accumulation compared to Cells A and C (p<0.05). Both Cells B and D prior to the commencement of this study were planted with *Phragmites australis*. Whilst much of the root material was removed prior to commencement of this project trapped particulate organic matter and decomposing below ground material remained, explaining why organic accumulation was highest in these two cells.

OC generally appeared highest in the top 20cm of gravel. In the planted gravel sections for all four cells OC accumulation at 0-10cm and 10-20cm is significantly higher than at 20-30cm (p<0.05). OC accumulation at 0-10cm is most likely attributed to plant litter. The mean maximum root penetration for the four species within the Oxley SSF was also within the top 20cm of gravel (Chapter 5: Figure 38).

OC accumulation in the top 0-10cm was lowest in Cell C compared to the other three cells including Cell A, which similar to Cell C has only been planted for two years. Water depth within Cell C over the length of the study was lowest of all the cells (Chapter 4: Figure 39), which may have provided increased aerobic areas within the gravel for organic decomposition thus reducing accumulation of organic matter.

There were no significant interactions observed in OC accumulation with cell and depth for the two planted sections. Refer to Appendix 6 for the complete statistical analysis results. Gravel size did not seem to have affected OC accumulation, with age of gravel, water depth and previous planting history largely influencing accumulation.
6.4 Conclusion
The presence of macrophytes in the planted sections influenced organic accumulation particularly for cells that had previously been planted with *Phragmites australis*. Organic accumulation was generally highest in the top 20cm of the gravel, which can be attributed to litter fall and mean maximum root penetration recorded for all four species within the Oxley SSF CW wetland. Gravel size did not appear to affect organic accumulation.
CHAPTER 7: EFFLUENT TREATMENT – PERFORMANCE EFFICIENCY

7.1 Introduction

CWs are an approved wastewater treatment device and have been used successfully worldwide to treat various types of wastewater including stormwater, industrial, domestic, agricultural, mine drainage and landfill leachate (Kadlec and Knight 1996). SSF wetlands have been widely used throughout Europe to provide secondary treatment after screening and primary settlement, for small communities and single households where conventional wastewater treatment can often be too expensive to construct and maintain (IWA 2000).

CWs utilise the physical, biological and chemical processes and functions of a natural wetland to improve water quality (Department of Land and Water Conservation New South Wales 1998). Chemical and biological processes are controlled by environmental factors including the presence of oxygen, pH and temperature. Low oxygen concentrations can limit aerobic processes (nitrification) but enhance anaerobic processes (denitrification). Many processes are pH dependent; such as ammonia volitilization, which is only significant when, pH values are above 9 (Reddy and Patrick Jr 1984). Metabolic activity generally decreases with temperature, thus reducing the effectiveness of treatment processes relying on biological activity (Department of Land and Water Conservation New South Wales 1998).

Processes of ammonification, nitrification and denitrification dominate nitrogen cycling and removal (Faulkner and Richardson 1989). A performance evaluation conducted by Reed and Brown (1995) of 14 SSF CWs in America reported low ammonia removal were attributed to insufficient oxygen levels within the gravel bed to support nitrification and short detention time with majority of wetland having <5 day HRT. Horizontal SSF wetlands can often be oxygen limited affecting nitrification rates although studies have shown the potential for oxygen transfer by macrophytes into the rhizosphere (Brix 1993; Cooper et al. 1996). Reed and Brown (1995) also reported net production and export of ammonia for half of the CWs trialed. Addition of ammonia to the systems was likely due to anaerobic decomposition of organic nitrogen trapped within the gravel bed as particulate matter.
Phosphorus generally remains within the wetland absorbed to substrate or in plant material and can be removed from the wetland, when plants are harvested. Phosphorus removal efficiency is generally low in SSF CWs (Vymazal 1999). Long term phosphorus removal is often difficult to achieve as the rate of phosphorus adsorption removal is largely limited by adsorptive surface area as well as availability of reactive Fe, Al and Ca, substrate pH and redox potential and temperature (Arias et al. 2001; Reddy and D'Angelo 1997).

Phosphorus removal rate is generally highest at the commencement of operation when there are numerous sites available for adsorption which efficiency decreasing as sites for adsorption become saturated. In some cases phosphorus output may be higher than input with the release or de-sorption of phosphorus (Greenway and Woolley 1999; Kadlec and Knight 1996; Vymazal et al. 1998).

Research differs on the significance of plant uptake in nutrient removal. Brix (1997) suggests that plant uptake of nutrients is only likely to be of quantitative importance in low loaded systems such as many SF CWs.

Research by Adcock and Ganf (1994) on a Phragmites CW with low nitrogen loadings found that approximately 65% of the nitrogen entering the wetland was found in plant biomass. A study of a low nitrogen loaded SSF CW planted with Schoenoplectus validus reported over 80% of nitrogen removal to be contained in plant biomass (Rogers et al. 1991). Greenway and Woolley (2001) studying a SF constructed wetland in Queensland supporting a mixture emergent macrophytes (Typha, Schoenoplectus, Eleocharis, Marsilea and Nymphaea) and floating duckweed found that over a three year period total mass of nitrogen and phosphorus was reduced by approximately 80% and 20% respectively. Of this plant biomass accounted for 27% to 47% total nitrogen removal and 44 % to 65% reactive phosphorus removal. Retention times varied from 10 to 17 days, with pollutant loading rates ranging from 2.4 to 3.7kg Total N ha$^{-1}$d$^{-1}$ and 2.0 to 3.3kg Total P ha$^{-1}$d$^{-1}$ (Greenway and Woolley 2001).

Other CW studies have suggested that nitrogen removal by emergent macrophytes rarely exceed 10% of the total nitrogen removed from a wetland (IWA 2000). A study by Tanner et al. (1995) of a pilot SSF CW receiving varying wastewater loading rates found that over an annual period plant biomass accounted for only 2-8% and 1.9-5.3%
total nitrogen and phosphorus removal respectively. A study of a SSF CW with high loading rates reported that only a small fraction of nutrient removal could be accounted for by plant uptake (Gersberg et al. 1983). Several studies have shown that phosphorus adsorption onto substrate is the primary removal mechanism with plant uptake being negligible (Faulkner and Richardson 1989; IWA 2000; Vymazal, 1999). Mann and Bavor (1993) reported that in a SSF CW planted with *Typha orientalis* and *Schoenoplectus validus* phosphorus uptake by plants was not significant.

In high nutrient loaded CWs it is likely that direct nutrient uptake by plants can only account for a small fraction of nutrient removal (Tanner 2000). Other roles of the macrophytes including possible oxygen release to the rhizosphere and physical effects of the plants themselves may have a larger influence on nutrient removal as suggested by Tanner (2000).

The main body of work on CWs, their effectiveness in wastewater treatment and significance of plant uptake in nutrient removal has been conducted in temperate climates with considerably less research conducted in sub-tropical/tropical climates such as Queensland, Australia. The majority of research conducted in Queensland on CWs (Greenway and Woolley 1999; Greenway and Simpson 1996) has focused on surface flow CWs. Therefore there is a need for more research on subsurface flow (SSF) CWs, their effectiveness for wastewater treatment and proportion of nutrient removal contained within the plant biomass of the four species trialed.

### 7.1.1 Aims

This chapter will investigate effluent treatment (water quality and quantity) and proportion of nutrient removal contained within plant biomass in each of the four cells. The aims of the research presented in this chapter are: 1) Determine the effectiveness of the wetland for wastewater treatment; 2) Effect of gravel size and age on water treatment and 3) Quantify nutrient removal rates and proportion of nutrient removal contained in plant biomass.

### 7.2 Materials and Methods

The wetland receives secondary treated effluent direct from the Oxley WTP (see Chapter 2 for outline of primary and secondary treatment processes). Effluent enters
pond 1 of each cell and then through the first gravel section where it continues through
the wetland (Chapter 2: Figure 6).

Parameters used to investigate water quality were: water depth, pH, temperature,
-dissolved oxygen, total suspended solids (TSS), total volatile solids (TVS), nitrogen,
-phosphorus and chemical oxygen demand (COD). Water quantity entering and leaving
the wetland was measured using tipping buckets located at the inlet and outlet of each
cell. Water quality and quantity were combined to investigate the nutrient removal
efficiency of the wetland. Nutrient content and biomass for each of the four macrophyte
species trialed was combined with wetland nutrient removal to calculate proportion of
nutrient removal contained within plant biomass.

7.2.1 Water quality

Water samples were collected fortnightly over 12 months from May 2001 to May 2002
from the inlet and outlet of each cell (Chapter 2: Figure 6). Samples were not collected
in December 2001 as water to the wetland was temporarily offline due to maintenance
to the wastewater treatment plant (WTP). Field parameters: pH, temperature, and
dissolved oxygen were measured in situ using a calibrated multiprobe. The water depth
of each open water section was measured using a 1m ruler when water samples were
 collected for analysis. Total phosphorus (TP) analysis was conducted for water samples
but results were consistently below FRP. TP concentration for unfiltered samples was
determined using the persulphate acid digestion method, the TP concentration range for
this method is 0.002 – 1.00 mgP/L for undiluted samples (APHA 1997; Hosomi and
Sudo 1986; Sadler and Wruck 2001). As FRP concentration of water samples was >6
mgP/L samples were diluted (1:10).

Parameters measured in the laboratory included:

- total suspended solids (TSS)
- total volatile solids (TVS)
- chemical oxygen demand (COD)
- total phosphorus (TP)
- filterable reactive phosphorus (FRP)
- total nitrogen (TN)
- oxidised nitrogen (NOx)
- ammonia (NH₄)
Samples were collected, preserved and analysed in accordance with standard methods (APHA 1997). TSS and TVS was analysed according to total suspended solids dried at 103-105°C and fixed and volatile solids ignited at 500°C methods (Methods 2540 D and 2540 E.) (APHA 1997). COD was determined using HACH pre-prepared digestion tubes 0-150 ppm range in accordance with HACH methods for COD determination (Jirka and Carter 1975). Digestion solution contained: mercuric sulfate, demineralised water, chromic acid, silver sulfate and sulfuric acid. COD of digested samples was measured using a HACH spectrophotometer.

Samples for nitrite, nitrate, ammonia and FRP analysis were filtered through 0.45 μm membrane filters and analysed using Flow Injection Analysis (FIA) on a QuickChem 8000 Automated Ion Analyser (Lachat). Standard methods from the operational manual were followed. QuickChem methods used were 10-107-04-1-H for nitrite and nitrate (NOx), 10-107-06-4-D for ammonia and 31-115-01-3-A for orthophosphate, refer to Appendix 2 for reagents used in each method.

Total nitrogen (unfiltered samples) was determined using the persulphate acid digestion method outlined in Appendix 5 (APHA 1997; Hosomi and Sudo 1986). Digested samples were analysed by FIA using the Nitrite and Nitrate (NOx) method for TN and orthophosphate method for TP refer to Appendix 2.

7.2.2 Hydraulic loading rates
The volume of water entering and leaving the wetland was measured using tipping buckets located at the inlet and outlet of each cell (Chapter 2: Figure 6). The volume of water required for the tipping bucket to drop was known (3.3L) and a magnetic counter measured the number of times the bucket tipped. Each tipping bucket counter was read weekly to determine volume of water and to calculate hydraulic loading rates.

7.2.3 Nutrient removal efficiency of the wetland
Nutrient removal efficiency of the wetland was determined by calculating the nitrogen and phosphorus loads in (pond 1) and out (pond 4) using median concentrations multiplied by the volume of water entering and leaving the wetland. The difference between loads in and out of each cell was then calculated and presented as percentage
removal. To enable comparisons in nutrient removal to those reported in published
literature nutrient removal rates were reported as kg/ha/day.

7.2.4 Quantify nutrient removal contained in plant biomass
Using total nitrogen and phosphorus removed within planted sections (ponds 2 to 4)
from each cell and nutrient content and biomass for each species and cell an indication
of nitrogen and phosphorus removal contained in plant biomass can be estimated as per
Greenway and Woolley (2001). Estimates assumed a 6 month turnover of plant
biomass although as previously discussed in Chapter 4 this is only likely for Carex.

7.2.5 Statistical analysis
Serial correlation between open water sections (ponds) within cells limited statistical
analysis as each preceding open water section influences the next open water section.
The outlet water quality for each treatment (cell) could not be compared statistically, as
experimental design did not meet assumptions required in order to conduct ANOVA.
7.3 Results and Discussion

7.3.1 General water quality

The median inlet and outlet water quality and loading rates for each of the four cells are presented in Table 6. Hydraulic retention time from tracer studies is approximately 2 to 3 days for the four cells.

Table 6. Median water quality concentrations and loading rates for Oxley Wetland over 12 months (May 2001 – May 2002).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cell A</th>
<th>Cell B</th>
<th>Cell C</th>
<th>Cell D</th>
</tr>
</thead>
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<tr>
<td>Water temperature (°C)</td>
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<td>21.3</td>
<td>21.6</td>
<td>21.5</td>
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<td>7.1</td>
<td>7.2</td>
<td>7.1</td>
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<tr>
<td>DO (mg/L) IN</td>
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<td>2.4</td>
<td>2.0</td>
<td>1.6</td>
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<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
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<tr>
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<td>44.0</td>
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<td>COD (mg/L) OUT</td>
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<td>24.0</td>
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<tr>
<td>TSS (mg/L) IN</td>
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<td>9.6</td>
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<td>Oxidised N (mgN/L) IN</td>
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</tbody>
</table>

All cells were effective in reducing suspended solids from 9.4mg/L (mean of pond 1 for all four cells) to <2mg/L, and COD concentration from 45mg/L (mean of pond 1 for all four cells) to <30mg/L. Greatest TSS and COD removal occurred within the first gravel section (Figure 48 and Figure 49). The first gravel section acts as a filter removing particulate material majority of, which was organic with little reduction in TSS and COD after this gravel section indicating that concentrations are at background levels.
(Figure 48). Larger biogeochemical cycles in CWs compared to natural oligotrophic wetlands due to additions of organic carbon and nutrients produce higher background concentrations (IWA 2000).

Figure 48. Median total suspended solids (inorganic and organic component) in each pond for all four cells.

Figure 49. Median chemical oxygen demand (COD) concentration each pond for all four cells.
pH was consistently close to neutral for all cells (Table 6). Dissolved oxygen concentrations decreased once water entered the first gravel section as a result of the COD of inlet wastewater quickly creating anaerobic conditions (Figure 50). SSF CWs are often oxygen limited as a result of excessive water depth and oxygen-demanding material contained within the wastewater and substrate (IWA 2000).

Horizontal SSF CWs can often be oxygen limited affecting nitrification rates (Cooper et al. 1996). However some studies have shown that macrophytes can potentially transport and release oxygen from roots to the surrounding rhizosphere providing aerobic conditions for nitrification to occur (Reddy and Patrick Jr 1986; Reddy et al. 1989). Since root penetration for the four species is less than the depth of the wetland any potential oxygen release from macrophytes into the rhizosphere is only likely to occur within the top 30cm of gravel. Although studies of horizontal SSF CWs where oxygen levels are apparently low have shown low numbers of nitrifying bacteria in wastewater but high numbers on root and gravel surfaces (May et al. 1990; Ottova et al. 1996).

![Figure 50. Median dissolved oxygen (DO) concentration in each pond for all four cells.](image-url)
7.3.2 Phosphorus

TP concentrations were consistently below FRP results as seen in Table 6. This is partly due to there being very little particulate phosphorus with TSS results often <2 mg/L, which indicates low suspended material with TP, comprised almost wholly of FRP (Table 6). The large dilution factor (1:10) required to use the persulphate acid digestion method (analysis range 0.002 – 1.00 mgP/L for undiluted samples) when concentration of particulate P is likely to be low may also further explain discrepancies in TP results.

FRP concentrations were similar for all cells with median outflow concentrations higher than inflow (Table 6). Removal of FRP in all cells was low. Daily mass removal rates for FRP over the total 12 month period were 0.8kg (10%), 0.4 kg (5%), 0.1 kg (1%) and 0.4 (5%) kg/ha/d in Cells A, B, C and D respectively (Table 7). Cell A (5mm new gravel) had the highest daily mass removal, whereas Cell C (20 mm old gravel) had the lowest FRP removal with export for 9 of the 12 months (Table 7). As Cell A has been in operation for less than 2 years it is likely that the phosphorus adsorption capacity of substrate had not yet reached saturation unlike the older cells (Greenway and Woolley 1999). It is commonly accepted that phosphorus adsorption and precipitation in CWs is finite and generally only effective short-term (Vymazal 1999). Low phosphorus removal can also be attributed to the low retention time (2-3 days based on tracer study) limiting treatment time.

Low phosphorus removal for all cells may also be attributed to the limited number of adsorption sites for gravel compared to soil-based systems. Brix (1987) hypothesised that soil-based systems have increased number of binding sites for phosphorus adsorption compared to gravel-based systems and thus higher phosphorus removal could be expected for soil-based systems. A study reviewing wetland treatment for CWs in North America reported average TP removals of 22% for SSF CWs at similar loading rates (5.1 kgP/ha/d) to the current study (IWA, 2000; Kadlec and Knight 1996). Studies of SSF CWs in Poland and U.S. (phosphorus loading rates ranging between 54.8 and 179 g/m²/y and 52.6 and 274 g/m²/y for Poland and U.S. respectively) had average phosphorus removal of 50.4 (±27.4)% (Poland) and 37.9 (±37.3)% (U.S.) (Kowalik and Obarska-Pempkowiak 1998; Reed 1993).

From December 2001 to February 2002 all cells exported FRP. For much of this period water supply to the wetland was disrupted with only 49 days of flow. Disruption to
water supply may have facilitated the remobilisation of inorganic phosphate as found in a previous study by Greenway and Woolley (2001). Long term phosphorus removal is often difficult to achieve. Once phosphorus storage capacity is saturated removal rates decline with potential for output to be higher than input with the release or de-sorption of phosphorus (Kadlec and Knight 1996; Vymazal et al. 1998). Such a process has sometimes been referred to as the “aging phenomena” (Kadlec 1985). Many studies of CWs have reported periods of phosphorus export from aged constructed wetlands (Greenway and Woolley 1999; Mann 1990; Tanner et al. 1995). Mann and Bavor (1993) investigating phosphorus removal in three gravel SSF CWs reported considerable variability over the two years of operation with removal varying between – 40% (export) to 40%. In the current study gravel size did not appear to influence FRP removal.

Table 7. FRP loading (kg), efficiency of removal (%) and removal rate (kg/ha/d) for each cell at Oxley Wetland over 12 months.

<table>
<thead>
<tr>
<th>Period</th>
<th>Days Flow</th>
<th>Cell</th>
<th>FRP In (kg)</th>
<th>FRP Out (kg)</th>
<th>Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>Kg/ha/d</td>
<td></td>
</tr>
<tr>
<td>Jun – Aug 2001</td>
<td>90</td>
<td>A</td>
<td>8.96</td>
<td>7.72</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>9.48</td>
<td>9.09</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>9.00</td>
<td>7.97</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>9.81</td>
<td>9.74</td>
<td>1</td>
</tr>
<tr>
<td>Sept to Nov 2001</td>
<td>88</td>
<td>A</td>
<td>6.95</td>
<td>5.81</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>7.70</td>
<td>6.76</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>7.93</td>
<td>8.73</td>
<td>Export</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>7.35</td>
<td>5.27</td>
<td>Export</td>
</tr>
<tr>
<td>Dec 2001 to Feb 2002</td>
<td>49*</td>
<td>A</td>
<td>2.68</td>
<td>4.11</td>
<td>Export</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>2.47</td>
<td>2.66</td>
<td>Export</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>3.30</td>
<td>3.32</td>
<td>Export</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>3.14</td>
<td>4.81</td>
<td>Export</td>
</tr>
<tr>
<td>Mar – May 2002</td>
<td>92</td>
<td>A</td>
<td>3.08</td>
<td>1.87</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>2.46</td>
<td>2.51</td>
<td>Export</td>
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<tr>
<td></td>
<td></td>
<td>C</td>
<td>2.49</td>
<td>2.55</td>
<td>Export</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>2.03</td>
<td>1.44</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>270</td>
<td>A</td>
<td>21.68</td>
<td>19.51</td>
<td>10</td>
</tr>
<tr>
<td>June 01 – May 02</td>
<td></td>
<td>B</td>
<td>22.11</td>
<td>21.01</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>22.72</td>
<td>22.57</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>22.32</td>
<td>21.27</td>
<td>5</td>
</tr>
</tbody>
</table>

*Water supply to the wetland was disrupted for most of December 2001 and much of January 2002. Outflow rate and volume was estimated for Cell A (June to August 2001) and Cell B (December 2001 to February 2002) due to problems with tipping buckets.
7.3.3 Nitrogen

Median outflow concentrations of TN and NH$_4$ over the 12 months were lower than inflow effluent concentrations for all cells particularly Cell D (Table 6). Median outflow concentrations of NOx were also lower except in Cell A where the outflow concentration was higher (Table 6). Concentration of NOx decreased considerably after the first gravel section for Cells B, C and D most likely a result of denitrification (Figure 51 and Figure 52). There was little reduction in TN concentration between inlet and outlet water quality for Cell A (Table 6).

![Figure 51: Median oxidised nitrogen (NOx) concentration in each pond for all four cells.](image)

Through the remainder of the cells there is a steady decline in NH$_4$, although oxygen levels are anaerobic after the first gravel section limiting nitrification. A proportion of ammonia would be removed by plant uptake in the planted gravel sections (pond 3 and 4) (Figure 52). A recent study by Tanner and Kadlec (2002) of SSF CWs suggested that oxygen transfer across wetland surface and via macrophytes is insufficient to support nitrification-denitrification levels generally reported in SSF CWs. Tanner and Kadlec (2002) suggested researchers look to alternative explanations, which may include
denitrifying from nitrite, rather than nitrate, which would reduce oxygen demand and anaerobic ammonium oxidisers (anamox).

Figure 52: Median ammonia (NH₄) concentration in each pond for all four cells.

Mass loads of TN, NH₄-N and NOx-N over four 3 month periods is given in Table 8. Daily mass removal rates and removal efficiency for TN over 12 months were 8.30kg (28%), 12.22kg (40%), 12.49kg (38%) and 12.76kg/ha-1d-1 (43%) in Cells A, B, C and D respectively (Table 8). A study reviewing wetland treatment for North American treatment wetlands reported average mass removal rates of 15.6kg/ha/d with average mass reductions of 53.8%, loading rates were similar to that in this study (Knight et al. 1985). A study reviewing wetland treatment for 12 SSF CWs in North America reported average TN removals of 44% and 42% mean TN removal was recorded for 24 SSF CWs in Czech (IWA, 2000). TN removal efficiency is at the low end of TN removal results reported for several studies of horizontal SSF CWs treating wastewater at comparable loading rates where TN removal ranged between 47-74% (Bavor et al. 1987; Fisher 1990; Gersberg et al. 1986; Tanner 1994b). Tanner and Kadlec (2002) reported 82% and 93% TN load removal in SSF CWs at comparable TN loading rates to the current study.
Largest proportion of TN was in the form of ammonium generally comprising 50 to 90%. Low nitrogen removal can also be attributed to the low retention time, with a study by Reed and Brown (1995) suggesting that 6 to 8 days residence time in SSF CWs is required to achieve low concentrations of NH$_4$ with longer time required for temperature climates in winter.

Table 8: Nitrogen loading (kg), efficiency of removal (%) and removal rate (kg/ha/d) in each cell at Oxley Wetland over 12 months.

<table>
<thead>
<tr>
<th>Period</th>
<th>Days Flow</th>
<th>Cell</th>
<th>NH$_4$ – N</th>
<th>NOx – N</th>
<th>TN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In (kg)</td>
<td>Out (kg)</td>
<td>%</td>
<td>Kg/ha/d</td>
<td>In (kg)</td>
</tr>
<tr>
<td>Jun – Aug 01</td>
<td>90</td>
<td>A</td>
<td>17.69</td>
<td>14.14</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>18.68</td>
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<td></td>
<td>C</td>
<td>18.26</td>
<td>16.55</td>
<td>9</td>
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<tr>
<td></td>
<td></td>
<td>D</td>
<td>18.85</td>
<td>13.28</td>
<td>30</td>
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<td></td>
<td></td>
<td></td>
<td>88</td>
<td>12.52</td>
<td>11.01</td>
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<td>13.59</td>
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<td>13.62</td>
<td>13.72</td>
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<td></td>
<td></td>
<td></td>
<td>49*</td>
<td>15.65</td>
<td>8.09</td>
</tr>
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<td></td>
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<td></td>
<td>14.99</td>
<td>6.25</td>
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<td></td>
<td></td>
<td>20.97</td>
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</tr>
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<td></td>
<td></td>
<td>17.40</td>
<td>11.00</td>
</tr>
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<td>92</td>
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<td>9.43</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>7.36</td>
<td>3.38</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>6.98</td>
<td>4.54</td>
<td>35</td>
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<td></td>
<td></td>
<td>D</td>
<td>6.43</td>
<td>1.76</td>
<td>73</td>
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<tr>
<td>Mar – May 02</td>
<td>270</td>
<td>A</td>
<td>55.29</td>
<td>37.52</td>
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<tr>
<td></td>
<td></td>
<td>B</td>
<td>54.62</td>
<td>39.00</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>59.82</td>
<td>46.92</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>55.06</td>
<td>34.59</td>
<td>37</td>
</tr>
</tbody>
</table>

* Water supply to the wetland was disrupted for most of December 2001 and much of January 2002. Outflow rate and volume was estimated for Cell A (June to August 2001) and Cell B (December 2001 to February 2002) due to problems with tipping buckets. NB. ex = export

Average daily mass removal rates and removal efficiency for NH$_4$-N over 12 months were 6.3 kg (32%), 5.6 kg (29%), 4.6 kg (22%) and 7.3 kg/ha$^{-1}$d$^{-1}$ (37%) in Cells A, B, C and D respectively (Table 8). NH$_4$-N removal efficiency is considerably lower than that reported for several studies of horizontal SSF CWs treating wastewater at comparable loading rates where NH$_4$-N removal ranged between 57-89% (Bavor et al. 1987; Fisher 1990; Gersberg et al. 1986). Tanner (1994b) reported 40-90% TN removal for four
horizontal SSF CWs treating dairy farm wastewater which had a mean ammonia composition of 57%. Removal of NH₄ and NOx was variable for all cells, occasionally including export (Table 8). Cell D consistently performed better than the other cells in the removal of NH₄. Low removal efficiencies for NH₄-N suggest that nitrification is limiting due to the lack of aerobic conditions.

Daily mass removal rates and removal efficiency for NOx-N over the 12 months were 1.3 kg (22%); 3.6 kg (62%); 5.2 kg (75%) and 3.3 kg ha⁻¹ d⁻¹ (55%) in Cells A, B, C and D respectively (Table 8). The lowest removal in Cell A suggests a more limited capacity for denitrification with organic carbon accumulation lower in Cells A and Cell C compared to the other two cells. Denitrification rates can be limited by organic carbon, as approximately 2.5 g of organic carbon are required to denitrify 1 g of nitrate (Kadlec and Knight, 1996). The decay of litter and microdetritus can provide an internal carbon supply for denitrification. A study by Gersberg et al. (1983) found that in a vegetated SSF wetland without additional organic carbon, nitrogen removal was relatively low. For more details on OC accumulation refer to Chapter 6: Organic Carbon Accumulation.

TN removal rates for Cells A, B and C were highest for the three month period December 2001 to February 2002, NH₄ removal in all cells was also highest for this time period with performance almost 50% higher (Table 8). Over this period all four cells experienced elevated NH₄ inflow concentrations (Figure 53).

Figure 53: Inflow nitrogen concentration for each cell at Oxley Wetland over 12 months.
Several factors may be responsible for this improved performance including elevated nitrification rates and a possible increased nutrient demand by plants for flowering and rapid growth in February post cropping. During this period dissolved oxygen concentrations and water temperature within all four cells were slightly elevated, median temperatures for all cells were close to 25°C which is the optimal temperature for nitrification and denitrification (Hammer and Knight 1994; Vymazal 1995). All four cells experienced NOx export as a result of low inflow concentrations and effective conversion of NH4 to NOx through wetland nitrogen processes (Figure 53 and Table 8).

### 7.3.4 Proportion nutrient removal contained in plant biomass

There was considerable variability between cells with biomass in August 2001 accounting for 11%, 3%, 2% and 1% nitrogen removal in Cells A, B, C and D respectively; and 3%, 3%, 1%, 1% of phosphorus removal (Table 9). These removal percentages are lower than those found in a previous study of a SSF system by Gersberg et al. (1986) but within the range found by Tanner et al. (1995), where over an annual period plant biomass accounted for 2-8% TN removal and 1.9-5.3% TP removal. In a tropical surface flow wetland in Cairns where plants (includes duckweed and submerged species) accounted for up to 47% for the nitrogen removal and 65% of the phosphorus removal (Greenway and Woolley, 2001). However, nutrient loading in the Cairns wetlands was low with 3.6 kg TN and 3.3 kg TP/ha/d compared to Oxley wetland with an average loading of 25 kg TN and 7.1 kg FRP/ha/d (Table 6). Tanner and Kadlec (2002) concluded that net uptake and storage of nutrients by macrophytes once established generally comprises less than 10-15% of total nutrient removal.

<table>
<thead>
<tr>
<th>% nutrient in plant biomass</th>
<th>Cell A</th>
<th>Cell B</th>
<th>Cell C</th>
<th>Cell D</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>P</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 9. Proportion of nutrient removal contained in plant biomass estimated for a 6 month period. NB. Nutrient removal between ponds 2 to 4 (planted sections)

Results from the present study are in agreement with the majority of literature suggesting direct nutrient uptake by the four species in the Oxley SSF system can only
account for a small fraction of nutrient removal in a highly loaded system. Other roles of the macrophytes including possible oxygen release to the rhizosphere and physical effects of the plants themselves may have a larger influence on nutrient removal as suggested by Tanner (2000).
7.4 Conclusion

The Oxley Wetland was highly effective in reducing concentrations of TSS and COD to background concentrations. Nitrogen removal rates were within reported removal rates for SSF CWs treating wastewater of similar loading rates. Insufficient retention time and low dissolved oxygen concentrations limited nitrogen removal efficiency. Negligible phosphorus removal for all cells was attributed to short retention time and likelihood of phosphorus adsorption being close to capacity.

Nutrient uptake by the four macrophytes species trialed accounted for less than 12% TN and less than 5% TP removal for the period studied. Proportion of nutrient removal contained in plant biomass was highest in Cell A due to biomass being highest in this cell.
CHAPTER 8: RECOMMENDATIONS

This research project has provided several useful outcomes that can assist in future guidelines for designing effective SSF constructed wetlands in the subtropics/tropics.

To improve wastewater treatment within SSF CWs the following recommendations should be considered in the wetland design:

1. Pre-treatment of wastewater to enable ammonia to be converted to nitrate prior to entering SSF CWs could potentially improve nitrogen removal, as the nitrification is limited most likely due to low oxygen concentrations.

2. Increasing hydraulic retention time to provide greater treatment time, current retention time of approximately 2 days is insufficient for efficient nitrogen and phosphorus removal.

3. Although proportion of nutrient removal contained in plant biomass was low, results indicate that wetland cropping spring/summer when plants are flowering would be most suitable.

4. Cropping of Philydrum flowering stems could remove a large proportion of plant biomass and could potentially have a commercial use such as cut flowers.

To increase the success of plant establishment in subtropical/tropical regions it is advisable for planting to occur in July/August prior to high summer temperatures. During the initial plant establishment phase it is vital that a high water level is maintained in a SSF CW to increase plant survival. Weed maintenance is also important during the initial establishment phase. Plant cover is generally low during the establishment period this combined with nutrient enriched water provides ideal growth conditions for weeds. Therefore it is important to maintain weeds in order to reduce competition with newly planted macrophytes.

Trials indicated that of the four macrophyte species, Carex fascicularis was the most suitable for use in SSF CWs in Queensland particularly where harvesting regimes are employed. Investigate the potential of Philydrum flowering stems for commercial use as
a cut flower in the florist trade, by determining if there is a market and suitability of the *Philydrum* flowering stem.
REFERENCES


Brisbane City Council (1999). Oxley Creek Wastewater Treatment Plant. *Brisbane City Council Pamphlet*.


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Queensland Department of Natural Resources (2000). *Guidelines for Using Free Water Surface Constructed Wetlands to Treat Municipal Sewage*. Brisbane, Department of Natural Resources.


Appendix 1: Digestion of plant material – sodium sulphate, selenous and sulphuric acid digestion

Reagents were all of analytical reagent quality and distilled deionised water was used as the water source.

Digestion matrix
Digestion matrix was made to the following recipe outlined in CSIRO Standard Methods (Johnson et al., 1985).

In a 1L Schott bottle 100.0g anhydrous sodium sulphate, 1.633g selenous acid and approximately 800mL concentrated sulphuric acid were added. The bottle was covered with parafilm and stirred overnight using a magnetic stirrer. This was then made up to the mark with concentrated sulphuric acid.

Digestion procedure
Into 75mL digestion tubes approximately 0.4g of ground plant samples was weighed to 4 decimal places. 6mL digestion matrix was then added to each tube and mixed with a vortex stirrer to wet all plant material. Tubes were then placed into an AIM 450, 50 chambered block digestor controlled by an AIM 500 microprocessor. For every 50 samples 3 blanks (digestion matrix only) and 3 reference samples (plant samples of known phosphorus content) were digested. The block digestor was set to the following program:

1. Ramp to 230°C at 2°C per minute
2. Hold for 120 minutes
3. Step to 320°C
4. Hold for 180 minutes
5. End program

Once digested samples were allowed to cool slowly to room temperature water was added almost to the 75mL the tube was then stoppered and inverted several times to mix. Since the addition of water and acid produces heat the tube was not diluted to the 75mL mark until the tubes had reached room temperature again. In a FIA tube digested
samples were diluted (1:5) with water and analysed as per QuickChem Method 13-115-01-1-B (Appendix 2)
Appendix 2: QuickChem methods and reagents

Samples were analysed using Flow Injection Analysis (FIA) on a QuickChem 8000 Automated Ion Analyser (Lachat). Standard methods from the operational manual listed below were followed. Standard curves were created using standard concentrations of nitrate, nitrite, ammonia and orthophosphate.

Plant analysis

*Total phosphorus in kjeldahl digests method 13-115-01-1-B*

Reagents:
- stock ammonium molybdate solution
- stock antimony potassium tartrate solution
- molybdate colour reagent
- ascorbic acid reducing solution
- matrix blank

TP was calculated according to the following equation:

\[
TP \text{ (mgP /g DW) = } \frac{TP \text{ (mg/L) } \times V \times D}{M}
\]

\[
V = \text{diluted volume of digested samples (L) which was 0.075 L}
D = \text{dilution factor}
M = \text{mass of digested plant sample (g)}
\]

Water analysis

*Nitrite and nitrate method 10-107-04-1-H.*

Reagents:
- ammonium chloride buffer – acceptor solution
- ammonium chloride buffer – donor solution
- sulfanilamide colour reagent solution
Ammonia method 10-107-06-4-D.

Reagents:
- sodium citrate donor solution
- salicylate / citrate acceptor solution
- sodium dichloroisocyanurate solution

Orthophosphate method 12-115-01-1-A.

Reagents:
- stock ammonium molybdate solution
- stock antimony potassium tartrate solution
- molybdate colour reagent solution
- ascorbic acid reducing solution
Appendix 3: Digestion of plant material – tri-acid digestion

Reagents were all of analytical reagent quality and distilled deionised water was used as the water source.

Digestion procedure outlined below has been adapted from Allen (1989).

1. 0.1g ground plant material was weighed into 75ml digestion tubes.
2. 5ml 70% nitric acid and 0.5ml 98% sulphuric acid was added to the tubes, swirled gently and left to digest slowly overnight.
3. Following day 1ml 72% perchloric acid was added to each tube and swirled gently.
4. Tubes were then placed into a AIM 450, 50 chambered block digestor controlled by an AIM 500 microprocessor. For every 50 samples 3 blanks (digestion matrix only) and 3 reference samples (plant samples of known phosphorus content) were digested. The block digestor was set to the following program:

   1. Step to 100°C; Hold for 30 minutes
   2. Step to 125°C; Hold for 15 minutes
   3. Step to 150°C; Hold for 60 minutes
   4. Step to 200°C; Hold for 15 minutes
   5. Step to 275°C; Hold for 30 minutes
   6. Step to 350°C; Hold for 15 minutes

5. Once digested samples were allowed to cool slowly to room temperature water was added almost to the 75mL the tube was then stoppered and inverted several times to mix. Since the addition of water and acid produces heat the tube was not diluted to the 75mL mark until the tubes had reached room temperature again.

6. 10ml of sample was required for analysis, digested samples were diluted with water 1:20.

7. Standard curves were created using standard concentrations of orthophosphate. To each working standard add 0.335mL of diluted sulphuric acid (0.67mL 98% sulphuric acid diluted to 100ml) this is equivalent to the concentration of acid remaining in diluted digested samples.
8. Samples were then analysed as per the ascorbic acid reduction method for manual colourmetric determination outlined in Appendix 4 (APHA 1997).
Appendix 4: Manual colourmetric determination of TP for plant samples

Total phosphorus was measured according to ascorbic acid method (Method 4500-P E.) (APHA 1997). Samples were analysed using a spectrophotometer, standard curves were created as per Appendix 5. Reagents were all of analytical reagent quality and distilled deionised water was used as the water source.

The following reagents were prepared:

**Sulphuric acid**
In a 500mL volumetric flask 70mL concentrated sulphuric acid was added to approximately 300mL water. Since the addition of water and acid produces heat the solution was not made to the mark until it had reached room temperature.

**Potassium antimonyl tartrate solution**
In a 500mL volumetric flask 1.3715g antimony potassium tartrate was added to approximately 400mL water. This was diluted to the mark and mixed with a magnetic stirrer until dissolved. This solution was stored in a dark bottle and refrigerated.

**Ammonium molybdate solution**
In a 500mL volumetric flask 20.0g ammonium molybdate tetrahydrate was added to approximately 400mL water. This was diluted to the mark and mixed with a magnetic stirrer until dissolved.

**Ascorbic acid solution**
In a 100mL volumetric flask 1.76g ascorbic acid was added to approximately 50mL water. This was diluted to the mark and mixed with a magnetic stirrer until dissolved.

**Combined reagent**
The above solutions in the following proportions to a 100mL Schott bottle; 50mL sulphuric acid, 5mL potassium antimonyl tartrate solution, 15mL ammonium molybdate solution and 30mL ascorbic acid solution.
Procedure

10ml of diluted digested sample (Appendix 3) was poured into a test tube. 2mL of combined reagent was added and mixed thoroughly using a vortex stirrer. All standards were treated in the same manner as for normal samples. After 11 minutes absorbance of each sample was measured using a spectrophotometer at a wavelength of 880nm following creation of a standard curve.

TP was calculated according to the following equation:

$$TP (\text{mgP/g DW}) = \frac{TP (\text{mg/L}) \times V \times D}{M}$$

$V =$ diluted volume of digested samples (L) which was 0.075 L
$D =$ dilution factor
$M =$ mass of digested plant sample (g)
Appendix 5: Digestion of water samples for TP and TN analysis

Reagents were all of analytical reagent quality and distilled deionised water was used as the water source.

Digestion matrix
Digestion matrix was made to the following recipe outlined in Hosomi and Sudo (1986).

In a 1L volumetric flask 30.0g potassium persulphate and 4.5g sodium hydroxide were added to approx 750mL water. This was diluted to the mark and mixed with a magnetic stirrer.

Borate buffer solution
Borate buffer solution was made to the following recipe according to persulphate method (4500-N C) (APHA 1997). Borate buffer was only added for TN analysis.

In a 1L volumetric flask 61.8g boric acid and 8.0g sodium hydroxide were added to approx 750mL water. This was diluted to the mark and mixed with a magnetic stirrer.

Digestion procedure
10mL of sample (previously diluted 1:5) and 5mL digestion matrix were added to a 30mL polycarbonate tube, capped tightly and inverted to mix. Samples were then heated in an autoclave for 1 hour at 100 – 110°C. Samples were slowly cooled to room temperature. For TN analyses only, 1.0mL borate buffer was added and mixed by inverting at least twice (APHA 1997).

A standard curve was created using standard concentrations of 0, 0.2, 0.5, 1.0, 2.0, 5.0, 8.0, 10.0, 20.0 and 40.0 mg NO₃-N/L (sourced from potassium nitrate) and mgPO₄-P/L (sourced from potassium dihydrogen phosphate). For TN analysis a glutamic acid digestion check standard of 2.9 mgN/L was used to assess digestion efficiency. All standards were treated in the same manner as for normal samples.
Appendix 6: Statistical analysis results

Appendix 6.1: ANOVA analysis for significant differences in root depth between species and cells. P values in bold indicate significant difference (p<0.05).

<table>
<thead>
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<td><em>Baumea</em> vs Cell</td>
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Appendix 6.2: ANOVA analysis for significant difference in root depth between cells for *Baumea*. P values in bold indicate significant difference (p<0.05).

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<tr>
<td>C 0.0349</td>
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<td>E</td>
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<table>
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<th>B</th>
<th>C</th>
<th>D</th>
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Appendix 6.3: ANOVA analysis for significant differences and interactions in organic matter accumulation between cells and depth for the three gravel sections (S1-S3).

P values in bold indicate significant difference (p<0.05).

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Appendix 6.4: ANOVA analysis for significant difference in organic matter accumulation between cells and depth for gravel section 2. P values in bold indicate significant difference (p<0.05).

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Appendix 6.5: ANOVA analysis for significant differences in organic matter accumulation for Gravel Section 3 between cells and depth for gravel section 2. P values in bold indicate significant interaction (p<0.05).

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<td>&lt;0.0001</td>
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Appendix 6.6: ANOVA analysis for significant differences and interactions in nitrogen content of macrophytes between species, plant component and season (February and May 2001). P values in bold indicate significant difference (p<0.05).

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<th>Significance Level (P value)</th>
</tr>
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Appendix 6.7: ANOVA analysis for significant differences and interactions in phosphorus content of macrophytes between species, plant component and season (February and May 2001). P values in bold indicate significant difference (p<0.05).

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