Plant Harvesting from a Constructed Wetland: Nutrient Removal and Plant Attributes

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DECLARATION

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I consent to this thesis being made available for copying and loan if accepted for the award of the degree.

Michelle Bald

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ABSTRACT

The capacity for wetlands to function as sinks for nutrients has been widely exploited to treat a variety of wastewaters. Much debate has centred on the contribution to nutrient storage via acquisition and assimilation, by aquatic macrophytes to the overall sink capacity of constructed wetlands. This study investigated the nutrient storage and removal potential of four aquatic macrophyte species (*Bolboschoenus medianus, Phragmites australis, Triglochin procerum* and *Typha domingensis*) at the Willunga constructed wetland system, South Australia.

The macrophytes at Willunga had large above ground biomass and high tissue nutrient concentrations compared with maximum values in other studies. The storage capacity for nitrogen and phosphorus of two species not commonly used in constructed wetlands (*Triglochin procerum* and *Bolboschoenus medianus*) were 70.7 g N m^{-2} 6.8 g P m^{-2} respectively. These were significantly higher than the values recorded for species commonly used in constructed wetlands (*Typha domingensis* and *Phragmites australis*). In addition, *Triglochin* maintained a high tissue nutrient concentration throughout the year (29.1 - 36.6 mg N g^{-1} dwt; 3.6 - 4.8 mg P g^{-1} dwt), whereas the tissue nutrient concentrations for *Bolboschoenus* and *Phragmites* were low during winter senescence.

A comparative study was undertaken to determine the influence of single versus multiple harvest on the removal of nutrients. Generally, potential nutrient removal was higher under multiple regimes than single regimes for *Bolboschoenus, Triglochin* and *Typha.* Multiple harvests of *Phragmites* did not remove greater quantities of nitrogen and phosphorus due of poor regrowth after harvesting.

The range of parameters measured at the Willunga system was used to construct a simple predictive computer model which calculated nutrient removal via harvesting as a percentage of total annual nutrient load. Under the current plant densities, nutrient loading and wetland area, a single harvest of all above ground plant material during late summer (February) would only remove 6.1% of annual N load and 2.2% of annual P load. A multiple harvest regime (harvest in November followed by a harvest in February) would increase the removal to 8.7% N and 3.3% P. The model was used to make nutrient removal predictions under a variety of hypothetical wetland configurations.

Other ecological aspects were investigated for the four species (*Typha* and *Triglochin* in particular) in both field and controlled conditions. This included investigations into the implications of the contrasting rhizome extension strategies observed at the Willunga system for *Triglochin* and *Typha*. Also a controlled pond based experiment demonstrated that location of nutrient supply (sediment versus water nutrient addition) had little influence on total above ground biomass, however differences in storage capacity resulted from variation in nutrient concentration of that above ground biomass. Another pond based experiment investigated the resulting biomass and growth performance for *Triglochin* and *Typha* when grown in a replacement series design.

Overall, it was concluded that wetland design and management were crucial to nutrient storage and removal by aquatic plants. Appreciation of plant responses to environmental factors such as nutrient addition regime and water depth, was integral to an understanding of the processes which optimise nutrient removal from wetlands via aquatic plants.

CHAPTER 1: INTRODUCTION:

1.1. Wetlands:

1.1.1. Definition:

Wetlands are zones of transition between terrestrial and aquatic systems (Hammer and Bastian 1989) where the duration and frequency of flooding alter the chemical and physical properties of the soil (Hammer and Bastian 1989; Kadlec and Knight 1996; Paijmans *et al.* 1985). These interactions between the water and soil become the driving factor determining the biota within the systems. The biota have adapted to take advantage of the abundant water while overcoming the periodic shortage of resources such as oxygen, which would exclude most terrestrial biota. Consequently, most wetlands support a floristic community consisting predominantly of macrophytes within an area of water logged, anaerobic soils (Hammer and Bastian 1989).

1.1.2. Wetlands as sinks, sources and transformers:

Wetlands consist of a series of interactive components; the water, substratum, microbiota, flora and fauna (Breen 1990). The interaction of these components give wetlands the capacity to function as sinks, sources and transformers of chemicals and nutrients (Mitsch and Gosselink 1993). A wetland is considered a sink for a particular nutrient if the input exceeds the output, i.e. there is a net retention of that nutrient. A wetland is considered a nutrient source if the system's export of a nutrient exceeds the input. If the level of nutrients leaving the system is similar to that entering, but in a different form, the wetland is defined as a transformer of nutrients. The functioning of a wetland as a sink, source and transformer of nutrients can occur simultaneously. For example, a wetland can act as a sink for an inorganic nutrient and as a source for an organic form of the same nutrient (Mitsch and Gosselink 1993).

1.2. Wastewater:

1.2.1. Sources and characteristics of wastewater:

Wastewater is water that has been polluted or altered chemically or physically by human activity, either through point source (localised origin) pollution or non-point source (diffuse or widespread) pollution. The pollutants present in wastewater and their concentrations depend on the origin and nature of the pollution source.

There are three main origins of wastewater: agricultural, industrial and municipal; each of which has typical characteristics and therefore different treatment requirements.

Agricultural wastewaters are generated from a wide variety of agricultural endeavours including dairies, feedlots and aquaculture (Kadlec and Knight 1996) and can be of point source or more diffuse origins. These waters are generally characterised by high turbidity, high pesticide concentrations, high nutrient concentrations (80-200 g/m^3 N and 10-25 g/m^3 P, van Oostrom and Cooper 1990) and the potential for biochemical oxygen demand (BOD, measure of oxygen consumption) levels exceeding those commonly reported in domestic sewage (for example, 3000 mg/L, Gray *et al.* 1990) (see Table 2.1. for parameters typical of domestic sewage).

Industrial wastewaters are generated from operations such as food processing, paper production and various chemical manufacturing (Kadlec and Knight 1996) and are generally point source origins. The major pollutants from these industrial wastewaters include nutrients (particularly nitrogen as NO_3^- or NH_4^+), BOD, various salts, stains, organics, heavy metals and oils (Kadlec and Knight 1996).

Municipal wastewater can be subdivided into two main categories; stormwater and domestic sewage. Stormwater is usually generated after a rain event as a result of runoff from urbanised areas such as roads, drains, car parks and roofs, and is usually of non-point source origin. Therefore, unlike the other forms of wastewater, the production of stormwater tends to be somewhat unpredictable and intermittent (Raisin *et al.* 1997).

Domestic sewage originates from households and public buildings, and is therefore a point source form of wastewater. Generally, sewage wastewaters have high nutrient loads and high BOD. The Willunga wetland system, the study site for this thesis, receives domestic wastewater from a small community south of Adelaide, South Australia. Characteristics typical of domestic sewage are described in section 2.2.2 and parameters specific to the Willunga system outlined in Table 2.3. Further references to wastewater throughout this study apply to domestic sewage unless otherwise specified.

1.2.2. Traditional methods of wastewater treatment:

Primary wastewater treatment removes gross pollutants and suspended solids through sedimentation and formation of sludge in relatively deep (3 to 5m) ponds. Floating pollutants such as grease, oils and plastics form the scum and are physically removed from the surface (Kadlec and Knight 1996).

Secondary wastewater treatment removes solids and dissolved organic matter through microbial activity (Kadlec and Knight 1996). The aerobic conditions required for the microbial communities (bacteria and fungi) are provided through oxidation ponds, facultative ponds, aeration basins and trickling filters (Kadlec and Knight 1996). Secondary treatment is the minimum level required before discharge into receiving water bodies, and the target BOD is 30 mg/L.

The processes of secondary treatment focus on the reduction of BOD and undesirable bacteria such as *Escherichia coli.* The concentration of nutrients in secondary effluent may still be above desirable levels. Advanced, or tertiary wastewater treatment reduces nutrients such as nitrogen and phosphorus through a variety of chemical, physical and biological processes (Kadlec and Knight 1996).

Effluents from secondary or tertiary treatment may be discharged into fresh or marine water bodies for disposal. High concentrations of pollutants (particularly nutrients such as nitrogen and phosphorus) remaining in the effluent may cause detrimental changes to these receiving ecosystems, such as eutrophication and subsequent algal blooms (Gersberg *et al.* 1983). Ensuring that ecosystems receive treated effluent of loadings no greater than that of natural systems (approximately 22 g N m^{-2} yr^{-1} in North American systems, Mitsch and Gosselink 1993) will reduce detrimental impacts. Alternatively, the treated water can be recycled for irrigational use in parks and gardens or natural aquifer recharge (Tomlinson *et al.* 1993).

1.2.3. Alternative approach to wastewater treatment:

Due to an abundance of resources such as light, water and nutrients and the adaptations of aquatic flora to take advantage of these conditions (Westlake 1963), wetland systems and the associated aquatic flora are considered to be among the most productive ecosystems in the world (Brix and Schierup 1989b; Klopatek 1978; Westlake 1963). Moreover, many of the processes important in the treatment of wastewater occur naturally within the sink/source/transformer properties of wetland systems.

The combination of these factors has led to the widespread use of wetlands to treat wastewaters (Cooper and Findlater 1990; Hammer 1989), particularly in small communities (Gersberg *et al.* 1984). These systems can operate at approximately half the corresponding cost of conventional secondary treatment and provide other benefits such as the creation of wildlife habitat (Gersberg *et al.* 1984).

1.3. Wetlands for Wastewater Treatment:

Wetlands have been reported as receiving wastewaters of varying degrees of pre-treatment (from no pre-treatment to tertiary treatment). The level of pre-treatment required depends on the nature of the wastewater but wastewater has usually received at least primary treatment before entering a wetland system. Wastewaters of industrial origin may have high concentrations of toxic substances and therefore a high degree of pre-treatment may be required, and in extreme cases, treatment within a wetland system will not be appropriate. The higher the degree of pre-treatment, the greater the potential for effective treatment within the wetland system.

Many pollutants from many sources of wastewater have been treated in wetlands; nutrients, heavy metals, BOD, suspended particles. Most attention in the treatment of domestic wastewater has focused on major nutrients (nitrogen and phosphorus) and these two nutrients will form the foundation for this review and thesis. Most attention has been given to storage of nitrogen and phosphorus and to some micronutrients, with only limited attention given to the storage of carbon. However, quantifying the storage of carbon may gain increased focus with the implementation of carbon credits in the regulation of greenhouse gas emissions. Micronutrients are dealt with in more detail in section 3.2.2.1. and 3.2.3.1. Nitrogen and phosphorus retention varies enormously between wetland systems due to variations in climate, system design and management. For example, (Mitsch *et al.* 2000) reported ranges of nitrogen and phosphorus retention of 3 to 1022 g NO_3-N m^{-2} yr^{-1} and 0.4 to 38.5 g P m^{-2} yr^{-1}.

1.3.1. Natural wetlands:

Natural wetlands have been used successfully for the treatment of wastewaters. However, questions have been raised regarding potential detrimental effects on these natural systems. Alterations to community structure and floristic composition can result from the changes in nutrient status from increased pollutant loads (Kadlec 1994).

Osborne and Totome (1994) investigated the impact on a tropical wetland in Papua New Guinea after 25 years of receiving primary treated sewage effluent. The system was reducing the load of suspended solids and nutrients, however, over 25 years the system suffered a complete loss of submerged and floating-leaved plants, and a significant alteration to the composition of the emergent vegetation. These floristic changes were attributed to a combination of low light conditions, changes in water regime and direct effects of nutrient enrichment. Significant floristic changes (Vaithiyanathan and Richardson 1999) and an acceleration in the rate of primary succession (Portielje and Roijackers 1995) have also been recorded in response to nutrient enrichment of natural wetlands.

The recognition of detrimental effects to natural wetlands, combined with increased focus on the conservation value of wetland systems has discouraged their use of natural wetland systems for the treatment of wastewater (Brix 1993; Gersberg *et al.* 1983; Guntenspergen *et al.* 1989; Howard-Williams 1985; Osborne and Totome 1994). The inability to control and manipulate the hydraulics of natural wetland systems, plus other factors contributing to extreme variability and unpredictability, add to the impracticality of using natural wetlands for the treatment of wastewater (Brix 1993; Kadlec 1994). Consequently, this has led to the widespread use of constructed wetlands, purpose built for the treatment of wastewaters. These systems provide the benefits of wastewater treatment with minimal impact on the extent and function of natural ecosystems (Gersberg *et al.* 1983).

1.3.2. Constructed wetlands:

Constructed wetlands are wetland systems designed and built for the treatment of wastewater (Platzer and Netter 1994) or for direct benefit to humans (Hammer and Bastian 1989) and are located at sites where natural wetlands did not exist at the time of construction (Platzer and Netter 1994). Life span (i.e. period where the system continues to operate as a sink) of these constructed systems is highly variable and largely dependent on the quality of the influent water and the general system maintenance. Kadlec and Knight (1996) suggested that systems receiving low pollutant loading and undergoing regular maintenance controls would experience minimal ecological change over time and therefore operate indefinitely. These systems vary markedly in their substrate type, flow regime, and biota and generally it is these factors that form the basis for the classification of constructed wetlands.

Constructed wetlands can be broadly classified by flow regime as surface flow or subsurface flow designs (Brown and Reed 1994; Kadlec and Knight 1996; Steiner and Freeman 1989). Both flow types can be incorporated into a single system (Steiner and Freeman 1989), and see section 2.2.3.

1.3.2.1. *Surface Flow Designs*

Water is delivered to surface flow wetlands in a sheet flow across the sediment surface, resulting in standing water overlying the substrate. Surface flow systems have been recommended for the treatment of municipal, agricultural and industrial wastewaters of secondary or greater treatment (Kadlec and Knight 1996). These systems can be classified by the vegetation type present in the system. The three major groups under this criteria are: 1) emergent plants with root systems in the substrate and a large proportion of their photosynthetic material above the water surface, 2) submergent plants which also have root systems in the substrate but their photosynthetic material below the water surface, and 3) free floating forms which have roots in the water column and floating leaves (Brix 1993; Brix and Schierup 1989b). Multistage systems consist of a combination of two or more of these basic vegetation types (Brix 1993).

1.3.2.2. *Subsurface Flow Designs*

Delivery and exit of water to subsurface flow wetlands is through the substrate and therefore, subsurface flow systems generally have little or no standing water, and consequently, emergent macrophytes are the only suitable vegetation type (Brix and Schierup 1989b). These systems are recommended for the treatment of municipal, agricultural and industrial wastewaters of primary or greater treatment. Subsurface flow systems are inappropriate for the treatment of pulse derived inflows such as stormwater (Kadlec and Knight 1996). Subsurface flow systems are often lined with an impermeable membrane to prevent seepage (Brix and Schierup 1989b; Mackney 1989). The classification of subsurface flow wetlands is based on the hydraulic flow through the system. The most common forms are horizontal subsurface flow and vertical subsurface flow systems (Brix 1993; Brix and Schierup 1989b).

A horizontal flow system forces water through the wetland primarily in a horizontal direction to the outflow, theoretically making contact with the plant root zone as it travels (Brix and Schierup 1989b). However, in some cases the water moves across the bottom of the system, following the path of least resistance, avoiding contact with plant roots, therefore decreasing the efficiency of the system (Brett 1989). The vertical flow system is designed to force water from the bottom of the system, vertically to or near the substrate surface before reaching the outflow, thus increasing potential contact with plant roots (Brett 1989). Other systems such as the hydroponic nutrient film technique described by Jewell (1994) use basic principles of wetland ecology in a more highly modified design.

1.3.2.3. *Substrate considerations*

The substrate type can influence nutrient retention via differences in binding capacity and flow regime. Gravel substrates are commonly used in subsurface flow systems (Cullen 1990; Mackney 1989), as the large particle size allows for unimpeded root growth, increased hydraulic conductivity and reduces potential clogging problems (Sanford *et al.* 1995). However, due to the size and nature of the particles, the binding capacity of gravel (for nutrient pollutants) is less than that of other substrates. This may influence the efficiency of the system, especially for pollutants such as phosphorus for which sediment storage is a major storage process (see section 1.5.1.).

1.4. Components of Wetland Systems:

Efficient wastewater treatment wetlands tend to mimic the processes of natural wetlands (McComb *et al.* 1989). Therefore, in order to maximise treatment efficiency, it is important to understand the natural wetland processes and design and manage constructed wetlands to enhance the processes which maximise the sink potential of the system.

The wetland sink can be divided into a series of interactive components, both abiotic and biotic (Breen 1990). Biotic components include aquatic macrophytes (vascular plants), algae (periphyton, phytoplankton, epiphytes) fauna and microorganisms (Howard-Williams 1985). The major abiotic component is the sediment (Howard-Williams 1985). The water is the primary means of nutrient supply into the wetland system. A variety of processes facilitate the movement of nutrients from the source water into the wetland sink.

These processes result in either the storage of nutrients, where the nutrients are no longer dissolved in the water, but still remain within the system, or the complete removal of nutrients from the system. Examples of storage include nutrient uptake and assimilation by plants and algae, whereas an example of removal would be bacterial transformations resulting in gaseous emissions (Gersberg *et al.* 1986).

Interactions exist between these processes and components, such that plants not only comprise a storage component, but also provide energy source and sites for attachment for microbial communities (Kadlec 1994). Asaeda *et al.* (2000) suggested that the major nutrient pathway in wetlands was from the sediment to water via the macrophytes.

The processes leading to nutrient storage are reversible and therefore nutrients held within the storage components can, under certain conditions (such as plant decomposition, changes in chemical condition or physical disturbance), become soluble and re-enter the water (Klopatek 1978), potentially turning the system into a nutrient source. Plants may act as a transformer of nutrients, converting inorganic nitrogen (ammonium or nitrate) to organic forms of nitrogen. Soluble organic nitrogen has been recognised as an important nitrogen source for microbes. Recently, the importance of soluble organic nitrogen as a nitrogen source for higher plants has been highlighted (Aerts and Chapin 2000).

Nutrients may be transferred from one storage component to another. For example, plant litter may enter the long-term abiotic storage as peat upon decomposition (Richardson and Craft 1993), or nutrients released from the abiotic component may become available for plant uptake.

1.5. Storage Components:

1.5.1. Abiotic storage:

Nutrients can enter the abiotic storage component of the sediment through a number of processes including; precipitation, adsorption, sedimentation and complexation (Brix and Schierup 1989b as cited by Reddy *et al.* 1999; Mitsch *et al.* 2000; Richardson and Craft 1993; Watson *et al.* 1989; Tchobanoglous, 1987 as cited by Gearheart 1992). These processes occur for a variety of nutrients entering the system, however, they are perhaps most important for phosphorus and cations such as iron, aluminium, magnesium, manganese and calcium. Of the four processes, adsorption has received the most attention in terms of nutrient storage within constructed wetlands.

The adsorption capacity of a wetland varies with the substrate type. Krom and Berner (1980) demonstrated that coatings of organic matter on sediment particles were largely responsible for P adsorption. Suzuki *et al.* (1989a) also highlighted the importance of organic matter, with a positive correlation between organic matter and metal content in the soil. Brix (1987, as cited by Mann 1990) hypothesised that gravel bases, used in many systems for hydraulic permeability, would provide limited binding sites for nutrients and therefore offer a lower phosphorus removal capacity than soil based systems. Therefore, the design of wetland systems needs to strike a compromise between the hydraulic permeability advantage of large particle sizes and the nutrient removal capacity of smaller sized particles (Mann 1990).

The active sites for adsorption of nutrients such as phosphorus will become saturated over time. The ability of a system to continue as a sink would therefore be expected to decrease over time (Mann 1990). For example, White *et al.* (2000) investigated P storage across 1246 ha in a northern prairie wetland in Canada. It was found that the sediments at the inflow had a lower capacity for further P-sorption than sediments closer to the outflow.

Adsorption can only be considered a short-term storage mechanism, as desorption back to the water column can easily take place (Richardson and Craft 1993). Nutrients which undergo chemical precipitation and form insoluble oxides and hydrous oxides (Ann *et al.* 2000a; Reddy and D'Angelo 1994) can become more permanently stored as part of the peat (Richardson and Craft 1993). However, under times of high wave action or disturbance these oxides can be resuspended (Sanvill *et al.* 1976, as cited by Ann *et al.* 2000a). The incidence and severity of resuspension may be controlled by wetland vegetation (Cooke 1992). Nguyen (2000) suggested that sorption-precipitation of phosphorus followed by subsequent binding with iron and aluminium oxides may be the most important process of phosphorus retention in constructed wetlands treating wastewater.

The regulation of these oxide complexes is dependent on the interaction between parameters such as pH, redox, temperature and concentration (Gale *et al.* 1994; Watson *et al.* 1989; Richardson and Davis 1987 as cited by Mann 1990). For example, phosphorus forms insoluble complexes with aluminium and iron under conditions of low pH, and forms complexes with calcium and magnesium under high pH (Reddy and D'Angelo 1994). However, iron complexes are sensitive to changes in redox (Ann *et al.* 2000b; Dunbabin and Bowmer 1992). Under anaerobic conditions, ferric phosphate compounds dissolve (due to reduction of Fe^{3+} to Fe^{2+}) potentially releasing iron and phosphorus back into the water column (Ann *et al.* 2000b). Organic acids (intermediate products of anaerobic decomposition processes) promoted the formation and stability of $Al\text{-}PO_4$ complexes (Huang and Violnate 1986, as cited by Gale *et al.* 1994).

1.5.2. Biotic storage:

Living organisms (biota) require nutrients for their growth and survival, and biomass accumulation by the biota results in the incorporation of nutrients into biotic tissue. Most attention on biotic storage has been directed towards the vascular plants, however all organisms can represent a biological store of nutrients as highlighted by the increased attention on the role of algae and floccular bacteria in nutrient storage (Cromar *et al.* 1992; Richardson and Craft 1993).

1.5.2.1. *Nutrient Storage in Plants*

There has been much debate in the literature as to the relative importance of the biological storage, particularly that represented by plants, in the sink capacity of wetlands. There is a general consensus that plants play an important role in the processes of nutrient storage and removal within wetland systems. However, it has been suggested that this is limited to potential enhancement to the process of nitrification (see section 1.6.2.) (Gersberg *et al.* 1986) or as a potential source of carbon fuel for the process of denitrification (Gersberg *et al.* 1983). This line of thought suggests that the storage component represented by the plants is minimal compared to other storage (eg abiotic storage, section 1.5.1.) and removal processes (bacterial transformations section 1.6.2.) within wetland systems.

Estimates for the storage capacity of the plant component range from less than 10% (Herskowitz 1986, as cited by Watson *et al.* 1989) up to 75% (Reddy and DeBusk, 1987 as cited by Reddy and D'Angelo 1994) of annual incoming nutrient load. Comparisons of this sort are confounded by species variation in seasonal growth patterns, productivity and nutrient uptake ability, as well as differences in the annual nutrient load to the wetland. The most effective storage as expressed as percentage of annual inflow occurs at low loading, for example the 25 g N m^{-2} yr^{-1} suggested by Kadlec (1994).

However, it has also been suggested that the highly productive nature of aquatic plants under optimum conditions leads to significant storage, even if not necessarily the single greatest storage component within the system. For example, Asaeda *et al.* (2000) found that the storage capacity of macrophytes was second only to the sediment, and was still considered to constitute a significant component of nutrient storage.

A mass balance approach was taken by Breen (1990) in order to quantify the nutrient storage capacity of the plant (*Typha orientalis*) component compared to other components. Planted and unplanted experimental systems (in 10 L buckets) received a moderate nutrient loading of 160 g N m^{-2} yr^{-1} and 58 g P m^{-2} yr^{-1} (see section 2.2.2. for typical nutrient loadings). Nutrient storage and removal was greater in planted than in unplanted systems. Overall, the plants proved to be the major nutrient storage compartment in the planted systems with an accumulation of 50.7% and 57.3% of the influent nitrogen and phosphorus, respectively (Breen 1990).

1.5.2.1. *Factors Determining Storage in Plants*

Storage, in this context, is the total mass (g) of nutrients contained within the plant biomass (dry weight) of the wetland. Increased nutrient storage in plants is a function of, and controlled by, increases in tissue nutrient concentration, and increases in biomass. Interactions may exist between the two processes, for example plants under high nutrient load may have a greater relative growth rate and therefore an increased ability for nutrient uptake (Guntenspergen *et al.* 1989). Many factors control nutrient storage in plants, including nutrient uptake, incorporation of nutrients into plant tissue and retention and cycling of nutrients within the plant.

The uptake of nutrients by plants is regulated by complex interactions between a number of factors. For example, many wetland systems experience low pH conditions and anaerobic soils. Under these anaerobic conditions, nitrogen will be available to plants predominantly in the NH_4^+ form. The uptake of NH_4^+ across the plasma membrane of plant roots is decreased at low pH, and at pH<4 a net loss may occur (Marschner 1995).

As discussed in section 1.5.1., the solubility of phosphorus and associated cations is dependent on a range of parameters such as oxygen and pH. It is only when nutrients are in a soluble form that they will be available for plant uptake. Macrophytes with a floating habit access soluble nutrients directly from the water column. Emergent and submerged macrophytes can access nutrients from the sediment via roots, and therefore require the nutrients within the substrate pore water to be soluble to be available for uptake. Many submerged plants have demonstrated ability to access nutrients directly from the water column, and this is explored in more detail in chapter 4.

Increased nutrient supply in the source water may lead to increased storage potential by plants (Guntenspergen *et al.* 1989) through rapid nutrient capture and high growth rates

(Aerts and Chapin 2000). Breen (1990) found that even though biomass production increased over time, the system performance (determined primarily by the nutrient storage capacity of the plants) remained relatively constant due to reductions in tissue nutrient concentration over the same period.

The degree of nutrient uptake will be, in part, dependent on the external concentration of available nutrients (a function of nutrient loading). The accumulation of nutrients in plant tissue beyond that necessary for plant growth, a process known as luxury uptake, has been widely reported (Brett 1989; Cullen 1990; Osborne and Totome 1994). Cary and Weerts (1984) investigated the response of tissue nutrient concentrations in *Typha orientalis* to a range of external nutrient treatments. Tissue nitrogen concentration ranged from 0.87 to 2.58 % of dry weight under nitrogen treatments ranging from 0.02 to 20 mg N L^{-1} and tissue phosphorus concentration ranged from 0.22 to 0.498 % of dry weight under phosphorus treatments ranging from 0.01 to 40 mg P L^{-1}; i.e. the tissue nutrient concentration increased with increased loading. In contrast, Hocking (1989) reported that *Phragmites australis* responded to nutrient enriched conditions (inland Australia swamp) by increasing dry matter production, while maintaining nutrient concentrations within a narrow range. Reddy *et al.* (1989a) reported greater total biomass of *Eichhornia crassipes* in response to increased external nitrogen supply, up to a concentration of 5.5mg N L^{-1}. Increases in nitrogen supply beyond that concentration resulted in no further increase in biomass, however, total storage potential continued to increase due to increases in tissue nutrient concentration. A similar response by *Eichhornia crassipes* was reported by Reddy *et al.* (1990) for external phosphorus supply.

Reddy *et al.* (1990) and Reddy *et al.* (1989a) found that the uptake and storage of nitrogen and phosphorus by *Eichhornia crassipes* was dependent not only the external concentration of the respective nutrient, but also the N:P ratio. Ulrich and Burton (1985) suggested that the N:P ratio of wastewater should be manipulated to encourage optimum plant growth. In contrast, Cary and Weerts (1984) found that there was no significant interaction between the levels of supply of nitrogen and phosphorus. It was found that effects of nitrogen and phosphorus supply on plant tissue concentrations were much greater in the leaves than in roots and rhizomes. The concentrations of N, K, Ca and Mg were higher in the leaves than in the below ground organs (Cary and Weerts 1984).

1.5.2.3. *Nutrient Retention and Plant Decomposition*

Nutrient uptake by plants occurs mainly during the growing season and can be significantly reduced or completely suspended during periods of dormancy. Nutrients stored within plant tissue can potentially be returned to the water or enter other components of the wetland upon senescence. Many aquatic plants undergo complete senescence prior to winter dormancy, potentially releasing soluble nutrients to the water. Therefore, understanding the mechanisms of decomposition and nutrient retention is important for maximising the longer term storage potential of macrophytes.

Many rooted macrophytes translocate nutrients from the above ground tissue to below ground storage organs prior to senescence. The extent of this translocation appears to vary with species and environment. For example, nutrient use efficiency, which is a function of "nutrient retention strategy", decreases with increasing soil fertility (Aerts and Chapin 2000; Davis 1991) and it may therefore be expected that plants from constructed wetlands may have lower nutrient use efficiencies than plants from low nutrient environments.

Large quantities of labile nutrients are lost from leaves via translocation of nutrients prior to shoot death and leaching from the senesced leaves (Davis and van der Valk 1978). However, it can be difficult to determine the relative contribution of each of these processes. Decomposing tissue from macrophytes in high nutrient environments may still contain high concentrations of nutrients. These nutrients may become available for further plant growth, move into other components of the wetland, or act as a potential nutrient source or transformer, especially during seasonal senescence. Mechanisms of decomposition may determine the fate of these nutrients. For example, Lenssen *et al.* (1999) observed that phytotoxic organic acids released under anaerobic decomposition of macrophyte litter had a detrimental impact on plant growth. Such impacts on growth may ultimately restrict nutrient movements into the plant component.

Decomposition of plant material results from a combination of leaching, microbial respiration, fragmentation and shredding of material caused by biological and physical activities (Rodgers *et al.* 1983). Rates of decomposition depend on the original fibre, hemi-cellulose, cellulose and lignin content of the plant (Asaeda *et al.* 2000). Secondary thickening and lignification required to support aerial shoots in emergent macrophytes results in slower rates of decomposition than for submerged plants (Rodgers *et al.* 1983). However, decomposition rates for emergent macrophytes are still greater than those observed for terrestrial plants (Rodgers *et al.* 1983), therefore the nutrient source potential of these plants is still relatively high.

Environmental factors, such as nutrient load and moistures also regulate the rates of decomposition for aquatic plants. High productivity and tissue turnover rates for plants from nutrient rich environments can result in large quantities of litter with high nutrient concentrations and small degree of secondary compounds (Aerts and Chapin 2000).

Davis and van der Valk (1978) compared the rates of decomposition in standing and fallen litter. The most important factors regulating rates of decomposition for submerged material were microbial decay of tissue, leaching, microbial uptake and adsorption. In standing litter, it was found that leaching and fragmentation were the most important processes regulating decomposition and microbial activity was seemingly of minor importance, except at the base of stalks (Davis and van der Valk 1978). The use of standing litter in this study rather than the more conventional litter bags suspended above the water surface was seen as significant because the suspended bag method virtually eliminates the process of fragmentation, which in fact proved to be important for non-submerged litter (Davis and van der Valk 1978).

The importance of temperature in the regulation of decomposition rates is unclear. Puriveth (1980) found that losses of soluble compounds by leaching to be greater at 18°C than at 10°C, however Kaushik and Hynes (1971) found that temperature had little effect on early leaching rate.

1.6. Natural Processes of Nutrient Removal from the wetland system:

1.6.1. Abiotic removal:

Nitrogen can be permanently removed from wetlands via volatilisation of NH_3. However, this process is considered to be insignificant under wetland conditions where pH is lower than 8 (Koottatep and Polprasert 1997).

Many nutrients, such as phosphorus and micronutrients, stored in the abiotic component of the wetland (the sediment) have limited capacity for removal. Nutrients in the sediment can remain stored in the system indefinitely, i.e. permanent storage, with many compounds being buried as peat deep within the system. However there is potential for nutrients to return to the water column from both the long and short term abiotic storage component under certain chemical conditions or after a physical disturbance (see section 1.5.1.).

1.6.2. Biotic Removal:

Biological removal of nitrogen from wetlands occurs predominately via bacterial transformations. Denitrification is a bacteria mediated process that removes nitrogen permanently from wetlands (Gersberg *et al.* 1989). Depending on the form of nitrogen entering the system, nitrification may need to occur before nitrogen is released to the atmosphere by denitrification. Nitrogen entering a wetland as nitrate will be removed from the system via denitrification in anoxic zones. Nitrogen entering the wetland in the form of ammonia will need to go through sequential nitrification-denitrification, where the ammonia is first oxidised (aerobically) to nitrate via nitrification and then lost as nitrogen gas via anaerobic denitrification (Gersberg *et al.* 1989).

Nitrification is also a two part process that must occur under aerobic conditions (May *et al.* 1990; Watson *et al.* 1989). In the first step, ammonia is oxidised by nitrifying bacteria (genus *Nitrosomonas*) to nitrite ions (Davies and Hart 1990) and in the second step, nitrite ions are oxidised to nitrate by *Nitrobacter* bacteria (Davies and Hart 1990). The process of nitrification requires approximately 7 mg of alkalinity to oxidise 1 mg of ammonia (Watson *et al.* 1989). However, in many wetlands, it is the supply of oxygen that ultimately limits the rate of nitrification (Gersberg *et al.* 1989).

Denitrification occurs under anoxic conditions as the denitrifying bacteria degrade organic matter by using nitrate as an electron acceptor in place of oxygen (Gersberg *et al.* 1989; May *et al.* 1990). The nitrate entering the system or made availably by nitrification is converted by denitrification to nitrogen gas or nitrous oxide by denitrifying bacteria such as *Pseudomonas denitrificans* (Davies and Hart 1990; May *et al.* 1990; Watson *et al.* 1989).

The processes of nitrification and denitrification act independently under environments of contrasting oxygen status. However, the presence of aerobic and anaerobic micro-sites (May *et al.* 1990) allows for the coexistence of these two processes within a single system (Hsieh and Coultas 1989). Rates of denitrification are generally an order of magnitude greater than nitrification (Davies and Hart 1990). Therefore it is often the location of aerobic micro-sites within the predominately anaerobic conditions of wetlands, the bacteria which inhabit these sites (Davies and Hart 1990; May *et al.* 1990), and the resultant rate of nitrification (Brix and Schierup 1990; Davies and Hart 1990; Willadsen *et al.* 1990) that ultimately limits biotic nitrogen removal.

Many species of aquatic macrophyte have adapted to meet the high oxygen demand the below ground tissues experience living in highly anaerobic environments. It has been widely suggested that the oxygen supplied via these adaptations can lead to the oxygenation of the macrophyte's rhizosphere (the area of soil influenced by the root system) and may therefore facilitate the process of nitrification in an otherwise oxygen deprived environment (Davies and Hart 1990 as cited by McBride and Tanner 2000; Gersberg *et al.* 1989; Mitsch *et al.* 2000). Reddy *et al.* (1989b) detected gaseous nitrogen above the macrophyte root zone but not above unvegetated soil in controlled experimental conditions. This supported the hypothesis that both nitrification and denitrification occur within the root zone. However, others have expressed doubt that rate of oxygen supply to the rhizosphere would be sufficient to significantly influence nitrification, particularly at very high nitrogen loadings (Brix and Schierup 1990; van Oostrom and Russell 1994). For example, Brix and Schierup (1990) found that the plant initiated flux of oxygen from the atmosphere perfectly balanced the respiratory demand of the below ground tissue, suggesting that rhizosphere oxygenation was poor, and the resulting microbial nitrification would be minimal.

The microbial reactions involved in nitrification and denitrification are largely temperature dependent, and rates of nitrogen removal tend to be greater in warm climates than in seasonal climates (Mitsch *et al.* 2000). However, nitrate elimination via denitrification can occur throughout the year, even in low temperatures, under conditions of low oxygen and organic carbon (Stengel and Schultz-Hock 1989).

Carbon can be removed from the wetland system in the form of CO_2 and CH_4 as a product from a variety of biotic processes, including respiration and methanogenesis.

1.7. Human induced mechanisms of nutrient removal:

The incorporation of human induced methods of nutrient removal within the management regime of constructed wetlands may enhance the efficiency and longevity of the system.

Long-term storage of nutrients such as phosphorus and heavy metals can be enhanced by artificially promoting precipitation of insoluble oxides and hydrous oxides (as discussed in section 1.5.1.). Ann *et al.* (2000a) investigated the use of various soil amendments to bind phosphorus. The order of effectiveness for these amendments in immobilizing phosphorus was found to be; $FeCl_3$>alum>$Ca(OH)_2$>calcite>dolomite. However, it was concluded that high levels of amendments would be required to overcome the competition with organic matter for phosphorus binding cations (Al, Fe and Ca). The addition of such soil amendments as a mechanism for encouraging long term storage needs to be given special attention. For example the $FeCl_3$ complex is very sensitive to changes in redox, and the formation of stable Ca-P compounds at pH levels of approximately 8, can actually lead to increased phosphorus levels due to the solubilization of organic matter (Ann *et al.* 2000a).

Such manipulations only lead to enhancements of long term storage and do not result in the permanent removal of nutrients from the system. Processes which permanently remove nutrients from the system are therefore desirable in long term management of constructed wetlands.

Much work has focused on ways to enhance nitrogen removal through nitrification/denitrification. As discussed in section 1.6.2., designing wetlands which encourage alternate aerobic/anaerobic microsites can improve removal (eg, planting aquatic macrophytes with the ability to initiate high rates of convective flow). Other manipulations, such as the addition of limestone to encourage nitrification (7 mg of alkalinity required to oxidise 1 mg of ammonia by nitrification) (Watson *et al.* 1989), or the return of plant material to the system to provide a carbon source for denitrification (Gersberg *et al.* 1983), have also been suggested.

The permanent removal of phosphorus and cations such as iron from wetland systems relies on active management protocols (Swindell and Jackson 1990). Processes such as dredging and harvesting of biotic material can be effective in the removal of many nutrients, including carbon, nitrogen, phosphorus and various cations (Gearheart 1992).

Dredging involves the partial or complete removal (and replacement) of wetland substrate as a means of permanently removing nutrients held within both long and short term abiotic storage components. Even though this renewal of substrate may provide a large number of new binding sites for adsorption and the re-establishment of plant communities may encourage rapid growth and nutrient uptake, the disadvantages associated with expense and labour probably limit it to use as an infrequent management tool.

Harvesting plant biomass may be a viable method for permanently removing stored nutrients, (particularly for phosphorus), which may ultimately increase the longevity of wetland systems (Breen 1990). Encouraging movement of nutrients from the abiotic storage component (sediment) to the harvestable biotic component may decrease the frequency of destructive management protocols such as dredging and further increase the longevity of the system. The re-use (as compost, animal fodder or an energy source) of the resources recovered form harvesting may provide additional benefit (Brix and Schierup 1989b).

Many factors determine the success of harvesting as a mechanism for nutrient removal and much research and debate has focused on its potential as a management tool.

1.8. Harvesting as a tool for removing nutrients from constructed wetland systems:

Factors such as species differences, external nutrient load and season influence storage of nutrients by aquatic macrophytes (see section 1.5.2.). Therefore, species selection and timing of harvest can greatly influence the success of harvesting as a tool for nutrient removal. Other factors such as regeneration after harvesting and resource allocation responses to nutrients and flooding will also influence the total nutrient removal.

The harvestable fraction depends primarily on the growth form of the plant. Harvesting floating plants involves the removal of the entire plant (shoot and root material) from the water. For submerged and emergent plants only the above ground portion (i.e. the shoot material) is easily accessible for harvesting.

1.8.1. Response to harvesting:

Plants respond to removal of above ground material through the production of new material. This regrowth can be achieved by an allocation of resources within the plant or relying on external resources. Sundblad and Robertson (1988) found that rhizome growth was suppressed by the internal re-allocation of carbohydrate stores in response to harvesting in *Glyceria maxima*. Lower nitrogen content in the above ground tissue was recorded in the spring following the harvest treatment, indicating a nutrient limitation relative to the previous year. The study by Sundblad and Robertson (1988) was conducted within a natural system, and it was suggest that under the high nutrient conditions within constructed wetlands, the reliance on external nutrient supply would increase and largely overcome this effect.

Long term detrimental effects to plant growth need to be considered before establishing harvesting protocols within constructed wetlands. Regrowth of *Typha angustifolia* (Husak 1978) and *Glyceria maxima* (Chat 1984, as cited by Sundblad and Robertson 1988) may be severely affected the year after imposing a cutting regime. Furthermore, Sale and Wetzel (1983) found that cutting shoot material below the water surface induced anaerobic respiration within the below ground organs. This resulted in almost complete death of the below ground material if the cutting protocol was repeated several times in a season. Biomass allocation within plants responds to the relative limitations of resources. Plants will allocate resources to above ground components (leaves and stems) under conditions of carbon deficiency relative to nutrient deficiency (Chapin *et al.* 1987) and also see Grace (1989). The nutrient enriched conditions experienced in constructed wetlands may encourage allocation of biomass to the above ground component, which will enhance the potential nutrient removal via harvesting. Additional allocation to the above ground component may be achieved under conditions which further induce relative carbon deficiency, such as flooding (Grace 1989).

1.8.2. Timing of harvests:

The timing and frequency of harvests must account for the short term nature of biological storage and therefore harvests should take place before nutrients are returned to a soluble form upon plant decomposition or translocated to below ground storage organs (Gearheart 1992). Asaeda *et al.* (2000) suggested that the high growth rate of *Potamogeton pectinatus* meant it may be useful in nutrient removal, however with the rapid rate of decomposition, harvesting was essential in order to prevent those nutrients returning to the system. Harvesting will be most successful if conducted not only at times of maximum nutrient storage, but also at times of maximum allocation to the harvestable portion.

As discussed in section 1.5.2., storage capacity of plants is determined by the total biomass and the tissue nutrient concentration of that biomass. The interaction between these parameters determines the timing for optimum harvest potential (Haberl and Perfler 1990; Suzuki *et al.* 1989b). For example Haberl and Perfler (1990) found that a harvest in summer would result in three to five times greater removal of nitrogen and phosphorus than a harvest during autumn and winter after the commencement of senescence. Due to the large investment in the production of new leaf material (Hopkinson and Schubauer 1984) tissue nitrogen and phosphorus concentrations are usually high in younger tissue and decrease with maturity (Dykyjova 1978; Suzuki *et al.* 1989b). Hopkinson and Schubauer (1984) further suggested that the decline in nitrogen concentration in older tissue was due to dilution of the nitrogen relative to carbon as the rate of carbon fixation increased over time. Tissue nutrient concentration of emergent macrophytes may vary four to five-fold over the growing season (Klopatek 1978), and flowering can also significantly increase the phosphorus content of the above ground fraction (Breen 1990).

Above ground material may by replaced up to one to two times each year (McComb *et al.* 1989), therefore the frequency of harvests within a season may also be important for overall nutrient removal. Suzuki *et al.* (1989b) conducted harvest regimes which removed above ground material multiple times throughout a single growing season, and therefore maintained tissue in a younger, more nutrient-concentrated state. The maximum nutrient removal from the stand of *Phragmites australis* on which a double harvest strategy was imposed was 22% and 10% greater for nitrogen and phosphorus respectively than for a single harvest during the growing season. Nitrogen and phosphorus removal were 90% and 175% greater respectively from a double harvest than for a single end of growing season harvest (Suzuki *et al.* 1989b).

The plant's recovery from such an intensive harvest regime relies on nutrient translocation from the below ground stores to the above ground tissue, or rapid nutrient uptake strategies. Encouraging rooted plants to move below ground stores above ground where they are easily accessible via harvesting will increase the removal from the system (Wetzel 1993).

1.8.3. Translocation of nutrients in rooted macrophytes:

Much work has focused on translocation of nutrients within plants, in particular in response to season. However, the mechanisms which regulate translocation are complex and to what extent plants rely on it for survival is largely unknown. Simply measuring the loss of nutrients from senescing tissue does not discriminate between processes of internal nutrient translocation and those of decomposition (such as leaching and microbial attack). Therefore, most studies have not been able to fully quantify the translocation of nutrients to below ground organs at the end of the growing season.

It has been suggested that up to half the nutrients contained within leaves can be transported during senescence to the rhizome where they would be available for next season's growth (Congdon and McComb 1980; Hocking 1989). Haberl and Perfler (1990) showed nitrogen and phosphorus content of the below ground organs to be 2-4 times greater during winter dormancy than in summer, suggesting a significant degree of translocation. A nitrogen budget by Hopkinson and Schubauer (1984) found that 54% of the above ground nitrogen requirements for *Spartina alterniflora* were met through internal cycling. Therefore the reliance on nutrient cycling within the plant may influence the ultimate removal from the wetland system. Harvesting above ground material on a frequent basis may be able to break such cycles of internal supply and therefore shift the nutrient reliance from one of internal supply to one of external supply, thus enhancing overall removal from the system.

1.9. Plant Selection and Propagation:

Water regime, defined as the depth and duration of inundation, is an important factor controlling plant growth and survival in southern hemisphere wetland systems (Mitchell and Rogers 1985; Rea and Ganf 1994a). Therefore, selection of plant species for use in constructed wetlands must account for water regime preferences as well as the nutrient processing attributes discussed above (Wong *et al.* 1999). Selection of native species is most desirable as those plants are more likely to thrive under local conditions and any impacts on neighbouring aquatic environments will be minimised.

Macrophytes of all three major growth form categories, i.e. floating, emergent and submerged, have been used in constructed wetlands to treat wastewater. The choice of growth form depends on the wetland type (i.e. subsurface vs. surface flow) and treatment requirements (e.g. Brix and Schierup 1989a).

Wetland plants can be established via a number of methods including planting of nursery stock, transplantation of natural material, direct seeding (Kadlec and Knight 1996; Wong *et al.* 1999) or transplantation of seed banks (Wong *et al.* 1999). The most appropriate methods of propagation vary between species. For example, Chambers and McComb (1994) advised against the use of rhizome fragments for establishment of *Typha domingensis* and *T. orientalis* via due to high disturbance by birds and insufficient anchorage for heavy leaf material. In contrast, rhizome transplantation was the preferred method of propagation for *Leptocarpus diffusus* due to the difficulty in obtaining germination.

The propagule density required within a wetland depends on the desired floristic composition, the rate of plant establishment and the cost of plant material. Kadlec and Knight (1996) suggested an average planting density of 10,000 plants per hectare (1m spacing), with a higher density to achieve faster vegetative cover, or a lower density in large-scale systems where cost may be an issue. In order to reduce weed invasion and/or achieve a specific floristic composition, a very high (e.g. 80% cover) initial planting density may be required (Wong *et al.* 1999).

Competitive interactions between species and the ability of some species to preempt space under various planting protocols will influence floristic composition and ultimately system performance. Work by Mitsch (Anon. 2000) found that a wetland (1 hectare) where macrophytes were allowed to self-establish was less diverse, but 20 percent more productive after six years than a similar wetland originally planted with approximately 12 macrophyte species.

1.10. Constructed Wetland Research within Australia:

Research within Australia into the use of constructed wetlands to treat wastewater began in the late 1970s (Mitchell *et al* 1995). However, work which quantified overall system performance and the rigorous assessment of aquatic plants within constructed wetland systems did not begin until the mid 1980s. Much of this early work was conducted in small scale (microcosm) subsurface flow systems and focussed on the treatment of agricultural wastes such as that from piggeries (Bowmer 1985) and chicken abattoirs (Finlayson and Chick 1983; Breen 1990; Mitchell *et al* 1990). These studies were somewhat specialised (in sources of wastewater and system design), hence limiting the scope of conclusions drawn from the work. However, a number of important conclusions on design, species selection and scaling were drawn from these studies.

Mitchell *et al* (1995) concluded that research within Australia had confirmed the potential of constructed wetlands for treatment of wastewater, even though their performance and operation was not fully quantified. The authors identified a number of reasons for this uncertainty. These included problems of scaling up from small to large scale systems, the dilemma as to whether systems should mimic natural wetlands or exist as highly engineered structures, and the uncertainty about species selection and the role of aquatic plants in constructed wetland systems.

The original small scale systems generally recorded high removal efficiencies, for example, 95% removal of nitrogen and 99% removal of phosphorus recorded by Breen (1990). However, the high performance observed in small-scale systems is not necessarily translated into larger-scale systems. In addition, reported performace of these large-scale systems has been highly variable. For example Soukup et al (1994) recorded 99.8% nitrogen removal and 99% phosphorus removal for a wetland receiving sewage effluent, whereas Raisin et al (1997) recorded only 11% nitrogen and 17% phosphorus removal in a system receiving stormwater.

Similarly to scaling issues observed with system performance, nutrient storage recorded within the plant component also varied between small and large-scale systems. Breen (1990) observed that plants respresented the major storage component within a microcosm system, accounting for 50.7% of nitrogen and 67.3% of phosphorus. Davies and Cottingham (1993), however, concluded that harvesting of above ground portion of emergent plants from a constructed wetland would remove only 6% of the phosphorus.

Constructed wetlands, both in Australia and overseas, have traditionally been planted with only a select number of aquatic macrophyte. *Typha* spp. and *Phragmites* sp. have received the most attention (Lantzke et al 1999; Raisin et al 1997; Adcock *et al* 1995; Davies and Cottingham 1993; Breen 1990; Mann 1990; Mitchell *et al* 1990; Bowmer 1985; Finlayson and Chick 1983).

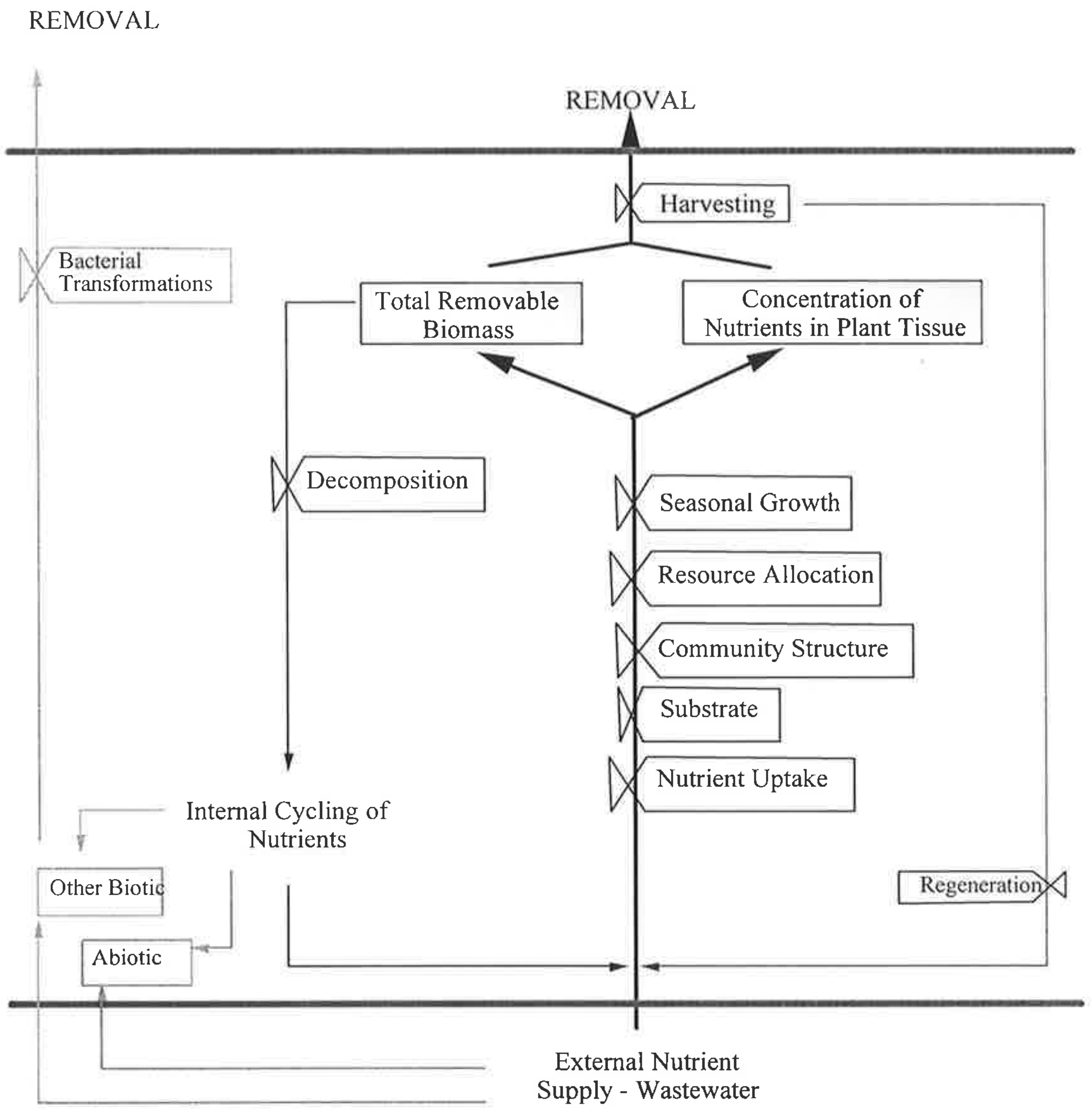
One of the major disadvantages associated with these commonly used species (*Typha* and *Phragmites*) is the seasonal growth pattern and winter die-back (Hocking 1985). In contrast, many other Australian species, such as *Cyperus involucratus* (Hocking 1985) and *Triglochin procerum* (Adcock and Ganf 1994), will maintain active growth throughout the year thus continuing to function as a sink for nutrients over the winter period. Consequently, recent attention has been given to the selection of alternative genera for the use in constructed wetlands, for example; *Scirpus* sp. (Finlayson and Chick 1983), *Schoenoplectus* sp. (Lantzke et al 1999; Raisin et al 1997) and *Triglochin* spp. (Mars *et al* 1999; Adcock and Ganf 1994).

In one of the most comprehensive investigations to date, Greenway and Woolley (1999) assessed the nutrient bioaccumulation (nutrient storage) of over 15 aquatic macrophyte species (both traditional such as *Typha orientalis* and non-traditional such as *Bolboschoenus caldwellii*) within nine pilot constructed wetlands throughout Queensland. The storage potential of plants and the nutrient concentrations of the inflow of one particular wetland indicated that almost 100% of phosphorus and 52% of the nitrogen could potentially be incorporated into plant biomass. This demonstrated that plants within a full-scale system could represent a high nutrient storage potential. However, as this study was conducted within the tropical climates of Australia, it still remains unclear whether the same potential can be achieved in the Mediterranean climates of Southern Australia.

1.11. Objective of This Study:

The movement of nutrients into and through the various wetland components determines the storage and ultimate nutrient removal achieved within wetlands. Nutrient removal from the perspective of the plant component was highlighted in a schematic model (Figure 1.1.). The potential nutrient removal (via the process of harvesting) is a function of the total removable biomass (above ground) and the nutrient concentration of that biomass. These two parameters are determined by a variety of processes inherent in plant growth and performance such as nutrient uptake, substrate conditions, community structure (due to species variations), resource allocation and patterns of seasonal growth. Plant regeneration after the harvesting and the influence of the growth parameters on that regenerated material will determine the long term nutrient removal potential.

Figure 1.1: Schematic model of nutrient movements through a wetland system via the plant component.



Despite the vast number of studies into numerous aspects of constructed wetlands, the relative importance of nutrient storage and removal capacity of aquatic plants remains disputed. As identified in section 1.10., there is still uncertainty regarding nutrient storage within the aquatic vegetation of large scale systems (especially in southern Mediterrean climates) and also in the selection of appropriate species. The primary aim of this study was to quantify the potential nutrient removal via harvesting. In conjunction with this primary aim, investigations into some of the processes governing removal and investigations into regeneration after harvesting were conducted.

These aims were addressed via field based observations and controlled experiments. Patterns of distribution and observations of seasonal variation in dry weight (Chapter 2) and nutrient storage potential (Chapter 3) were recorded for four aquatic macrophytes indigenous to South Australia in a constructed wetland receiving domestic wastewater (The Willunga wetland system). In addition, a number of harvesting protocols were investigated in order to determine optimum potential nutrient removal (Chapter 3).

Secondary aims arose from observations made during the main investigation. Growth and performance comparisons between two species across two environmental zones were conducted at the Willunga wetland system (Chapter 2) to obtain basic plant trait data. Plant growth responses to nutrient addition regime were investigated under controlled supply of nutrients to the water and/or the substrate (Chapter 4). Species interactions were investigated through biomass and growth responses of two species under particular planting combinations (Chapter 5).

Information from all of the above aims was combined to develop a model of nutrient storage and removal via the plant component at the Willunga wetland system. The model could also be used to predict nutrient removal under various protocols of management and wetland design.

CHAPTER 2: THE WILLUNGA WETLAND SYSTEM: PHYSICAL PARAMETERS AND THE FLORA:

2.1. Introduction:

Plant performance within constructed wetlands is related to parameters of the wastewater inflow, such as nutrient load, BOD and pH. These parameters vary with the origin of the wastewater and degree of pre-treatment (see section 1.2). In order to address the primary aim of this study, i.e. to quantify nutrient storage and removal by the plant component, it was necessary to know the nature of the wastewater entering the Willunga wetland system and place the parameters within the context of other constructed wetlands.

Variations between and within species, plus responses to local conditions, determine storage and removal potential of particular species. Therefore, the floristic composition of the wetland can influence the total nutrient storage represented by the plant component. Biomass, one of the major determinants of biotic nutrient storage (see section 1.5.2.2.), varies seasonally and between species. Species which maintain active growth throughout the year and achieve large above ground biomass may represent a greater store and removal of nutrients than species with a high degree of seasonal variation.

Depending on the competitive interactions (both intra- and inter-specific), as well as other biological and physical interactions, the floristic composition of wetlands may change over time. Maintenance of existing distributions and colonisation of new regions by macrophytes is achieved via sexual reproduction (flowering and seed establishment) or asexual reproduction (clonal growth). Both methods of reproduction are important for macrophytes (Grace 1993; Rea and Ganf 1994b), even though the degree of reliance on each method may vary between species. Lovett Doust (1981) defined two extreme strategies of rhizome extension in clonal growth; the opportunistic "guerrilla" strategy resulting in long distances between shoots, and the more conservative "phalanx" strategy resulting in a slow radial spread with a tightly packed front of ramets. These contrasting strategies may lead to different patterns of colonisation.

Plants adjust the allocation of biomass to the above ground portion in response to biological and environmental parameters. For example, inundation, or increased water depth, has increased shoot height in *Typha, Triglochin* and *Bolboschoenus* (Grace 1993; Lieffers and Shay 1981; Rea and Ganf 1994c; Siebentritt and Ganf 2000) and above ground biomass (Grace 1993) in *Typha*. These responses to inundation were correlated with increased emergent (exposed) leaf area and therefore attributed to limitations in light (Grace 1989) and/or gaseous CO_2 (Blanch *et al.* 1999). Understanding the mechanisms of such responses may suggest management strategies to enhance overall nutrient storage.

Knowing the floristic composition and the biomass represented by that composition will permit estimates of total plant nutrient storage. Changes in floristic composition, due to species interactions and reproductive capacities, will influence estimates of nutrient storage. Understanding basic plant traits and responses of plant storage to environmental parameters will provide the foundation for quantifying plant nutrient storage and removal.

2.1.1. Aims:

This chapter aimed to describe the features of the Willunga wetland system, introduce the macrophyte species planted within the system and provide background data for later chapters. The biomass (dry weight) per shoot and per area is a major determinate of the nutrient storage capacity represented by the plants. Nutrient storage capacity of a whole system will be influenced by the distribution and floristic composition of the system, changes to that distribution over time and plant responses to different environments within the system.

The aims of this chapter were:

1) Describe the history and function of the Willunga wetland system and place the specific parameters within the context of other constructed wetlands.

2) Determine seasonal variation in standing biomass of the four species of macrophyte at Willunga.
3) Quantify the distribution of the four species within the system.
4) Identify changes in distribution and determine mechanisms of successful colonisation.
5) Determine plant growth responses in the environments where colonisation was successful, and describe the mechanisms of those responses.
6) Quantify the implication to above ground nutrient storage (biomass and nutrients) of those plant responses.

Hypotheses for this chapter are stated in section 2.2.5.5.

2.2. Descriptions:

2.2.1. History of the Willunga Wastewater Treatment System:

The municipal wastewater treatment system located at Willunga (35°16′S 138°33′E, approximately 50 km south of Adelaide, South Australia) receives household waste (both from septic tanks and direct sewer lines) from the townships of McLaren Vale, McLaren Flat and Willunga. In 1993, this represented a total population of approximately 5000 people, and grew to approximately 7000 people in the year 2000. The oxidation pond consists of four sections with an average depth of 1.5 m, a capacity of 21 ML, an annual flow of 255 ML, and therefore an average retention time of approximately 30 days. The system relies on oxygenation to initiate the biological processes of treatment and was intended to provide secondary treatment (see section 1.2.2.) of the wastewater.

The retention time of the oxidation pond was insufficient to ensure complete secondary treatment. Prior to 1994, effluent from the oxidation pond was discharged directly into the adjacent Willunga Creek. State legislation implemented in 1990 required the council to complete secondary treatment of wastewater before discharge into natural water bodies. Therefore, in 1992, the council commissioned the construction of a wetland system to complete secondary level treatment and provide effluent of a sufficient quality for discharge or re-use.

Construction of the system was completed by late January 1993. The three emergent macrophyte species (*Typha, Phragmites* and *Bolboschoenus*) were planted in March 1993, and the semi-emergent species (*Triglochin*) was planted in September 1993. Effluent from the oxidation pond began entering the wetland system in January 1994, and the water level was raised gradually to reach maximum depth by March 1994. By November 1994 the discharge from the wetland system was diverted from the creek to the 120 ML storage lagoon where it was ponded for intended reuse in irrigation of the local golf course.

2.2.2. Oxidation Ponds:

Municipal oxidation ponds receive domestic wastewater from households' grey water and sewage. These waters are high in nutrients such as nitrogen (20 to 85 mg/L Eckenfelder and Yerachmiel 1991) and phosphorus and may also have a very high BOD. The nutrient input to the oxidation pond depends on the population and domestic use within the service area. Estimates of phosphorus contributions to wastewater per capita for the U.S.A. are for human waste 0.6 kg/capita/yr; laundry detergents 0.3 kg/capita/yr; and other household detergents and cleaners 0.1 kg/capita/yr i.e. a potential total of 1.0 kg/capita/yr (Jenkins and Hermanowicz 1991). The typical composition of raw municipal wastewater and secondary effluent plus the percentage removal expected at the primary and secondary stages of treatment are summarised in Table 2.1.

The nutrient loading (g m^{-2} yr^{-1}) of a wetland is a function of nutrient concentration of the wastewater, and the area of the wetland. Consequently, nutrient loadings vary widely between constructed wetland systems (Table 2.2). Nitrogen and phosphorus loadings of constructed wetlands are generally significantly higher (over 10 fold) than loadings considered typical for natural wetlands (approximately 22 g N m^{-2} yr^{-1} Mitsch and Gosselink 1993). Leaf tissue N:P mass ratio of 10:1 is considered optimal for growth

(Aerts and Chapin 2000). The N:P ratios of wastewater tend to be less than 10, therefore wastewaters have proportionately more phosphorus than is required for balanced plant growth.

Table 2.1: Typical composition (mg L^{-1}) of municipal wastewater, from Table 18-2 (Kadlec and Knight 1996): Biochemical oxygen demand (BOD), ammonia (NH4), nitrate-nitrite (NO3+NO2), organic nitrogen (org N), inorganic phosphorus (inOrg P), and total phosphorus (Total P).

Parameter	Raw Wastewater (mg/L)	% Removal Primary	% Removal Secondary	Secondary Effluent (mg/L)
BOD	110-400	0-45	65-95	10-45
NH4	12-50	0-20	8-15	<1-20
NO3+ NO2	-	-	-	<1-20
Org N	8-35	0-20	15-50	2-6
TKN	20-85	0-20	20-60	10-20
Total N	20-85	5-10	10-20	10-30
InOrg P	4-15	-	-	2-8
Org P	2-5	-	-	0-4
Total P	6-20	0-30	10-20	4-8

Table 2.2: Nitrogen and phosphorus loadings (g m^{-2} yr^{-1}) and N:P ratio of wastewaters from a variety of published sources.

Reference	g N m^{-2} yr^{-1}	g P m^{-2} yr^{-1}	N:P
Finlayson *et al.* (1986)	1643	292	5.6:1
Burgoon *et al.* (1991)	219	43.8	5:1
Burgoon *et al.* (1991)	803	164.3	4.9:1
Burgoon *et al.* (1991)	1606	346.8	4.6:1
Mitchell *et al.* (1990)	160.6	58.04	2.8:1
Girts and Knight (1989)	87.6	13.5	6.5:1

2.2.3. Description of the Willunga Wetland System:

The volume (21 ML), retention time (30 days) and annual flow (255 ML) of the Willunga constructed wetland system are all similar to that of the oxidation pond. The wetland system consists of three separate basins in series, and the entire system is gravity fed from the oxidation pond to the storage lagoon (Figure 2.1). Each wetland basin consists of a perimeter zone, a 3 m deep open water section ending in a 60 cm deep shelf and two 60 cm deep surface flow sections (Figure 2.3). A gravel barrage area (4 m wide by 44-58 m across) was constructed at the front edge of each 60 cm section, with the direction of flow perpendicular to the long side of the barrage. The barrages were designed to encourage subsurface flow of the water through the gravel to encourage contact with plant roots as well as aeration of the water.

2.2.4. Input/Output:

The only study of water quality parameters at the Willunga wetland system was conducted by Piranti (1995). The major parameters from this study are summarised in Table 2.3.

Table 2.3: Water quality parameters of the Willunga wetland system, from Piranti (1995): Influent flow rate, total phosphorus (TP), total Kjeldahl nitrogen (TKN), 5 day biochemical oxygen demand (BOD_5), phosphorus loading (P loading), nitrogen loading (N loading), pH.

	Mean Inflow	Mean Outflow	% Removal
Flow rate (L/sec)	4.3	2.9	
Concentration			
TP (mg/L)	13.9 (±1.27)	13.6 (±0.41)	
TKN (mg/L)	36.8 (±2.25)	6.8 (±1.61)	
Total nutrients			
P (mg/sec)	59.8	31.3	33.7%
TKN (mg/sec)	158.2	19.7	87.5%
BOD_5	36.3 (±1.22)	20.37 (±6.49)	
P loading (g m^{-2} yr^{-1})	126.2 (±20.5)		
N loading (g m^{-2} yr^{-1})	334.9 (±57.6)		
pH	7.86	7.52	

Figure 2.1: Aerial view of the oxidation pond, wetlands 1, 2 and 3 and storage pond of the Willunga wetland system. Photograph taken March 1995 by Nina Johansen.

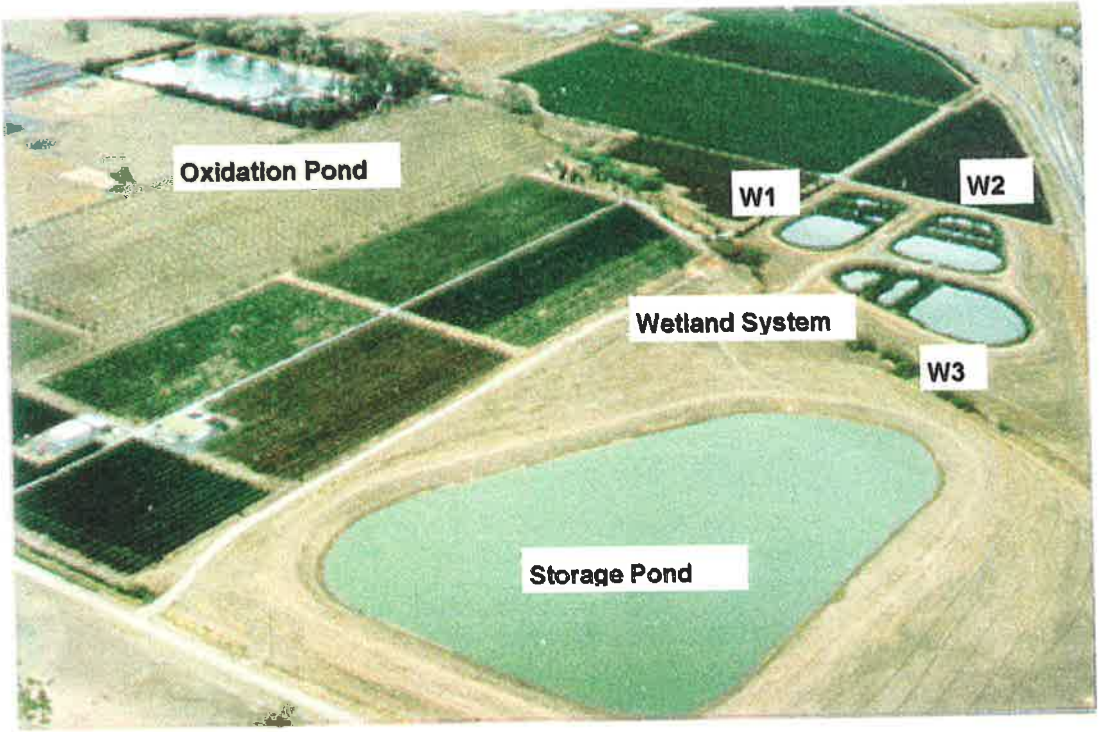
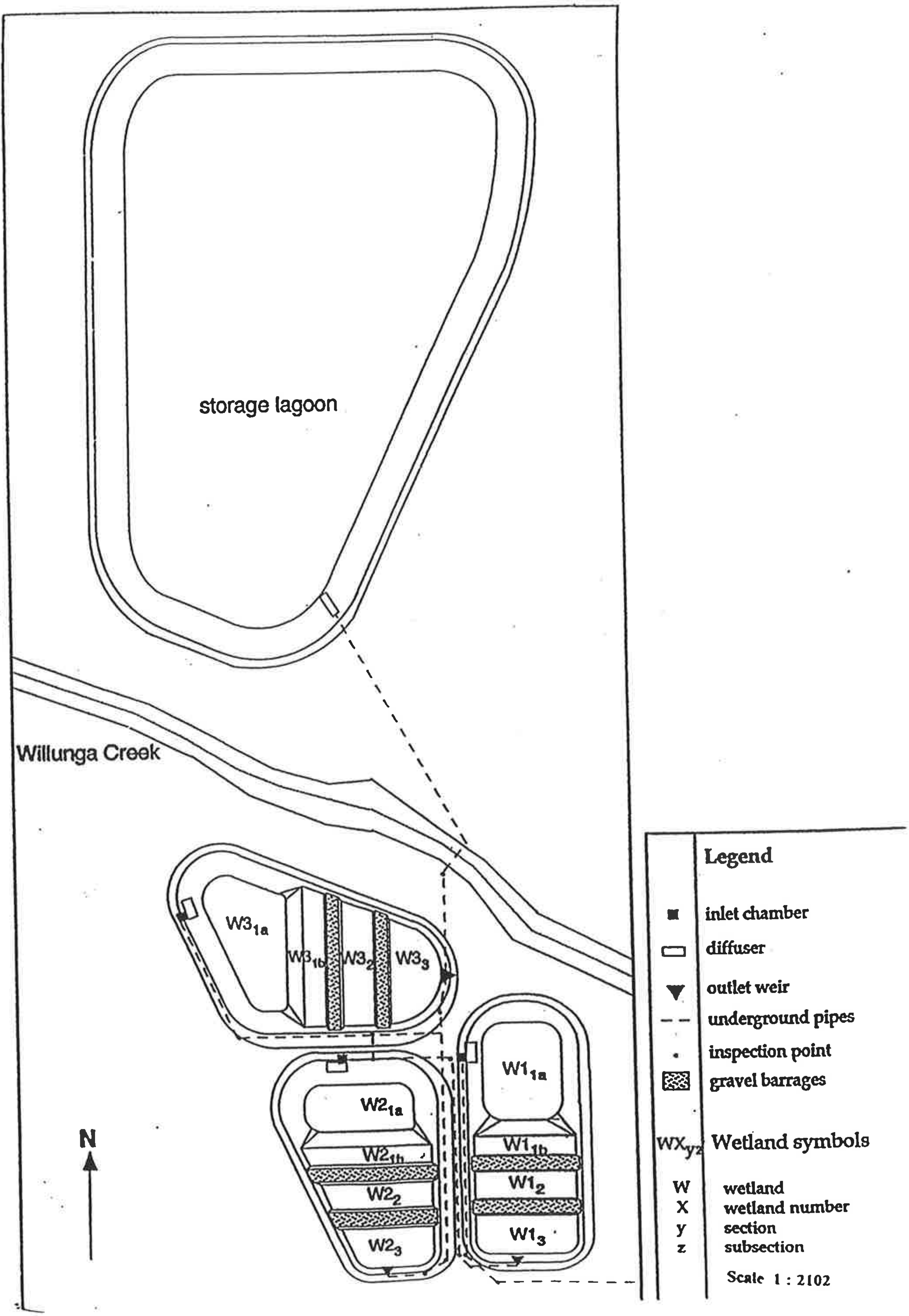


Figure 2.2: Perimeter, open water, barrage and surface flow zones of the Willunga wetland system. Photograph taken March 1995 by Nina Johansen.

Figure 2.3: Features of the Willunga wetland system.



Even though the total phosphorus concentration did not differ significantly between the inflow and outflow, a 33.6% reduction was observed for total phosphorus due to the change in flow rate between the inflow and outflow. The lower flow rate seen at the outflow was due to water loss from either seepage into the surrounding substrate/ground water or loss to the atmosphere by evaporation and/or evapotranspiration.

Piranti (1995) estimated the inflow of phosphorus to be 5.13 kg per day over the five week study period (January to February 1995). Assuming a constant rate over 12 months, 1872 kg of phosphorus would enter the system in one year. This estimate is only 37% of the 5000 kg per year that would be estimated for a population of 5000 people using the per capita values of Jenkins and Hermanowicz (1991). Even if cleaners and detergents were removed (assuming low phosphorus detergents were used), the figure reported by Piranti (1995) was only 62% of the estimate of human waste per capita for a population of 5000 people. This may be due to the fact that at the time of the study by Piranti, many of the residences in the municipal area were still on septic tank systems and therefore the input into the oxidation pond may have been less than the total population of the district in 1994. Additionally, phosphorus concentration may have been lowered through losses within the oxidation pond and pipe networks. The estimates of nutrient loading by Piranti (1995) were constructed from a five week study and therefore may not be representative of the annual loading to the system. It is therefore possible that the nutrient loadings given by Piranti (1995) are in fact an underestimate of the true loading in the Willunga constructed wetland system, however, as it has been the only study into the loading, these values will be used for the purpose of this study.

2.2.5. Description of Wetland Flora:

Desirable characteristics of plants to be used in constructed wetlands include rapid and constant growth, ease of propagation, reasonable capacity for absorbing nutrients, tolerance of hyper-eutrophic conditions, ease of harvesting (Mitchell 1978 as cited by Hocking 1985), robust habit, high biomass throughout the year and be readily available in the local area (Chambers and McComb 1994). Many emergent macrophytes fit these criteria and constructed wetland systems have been planted with numerous species. *Typha* spp. and *Phragmites* spp. are the two most commonly used plants (see Cooper and Findlater 1990; Hammer 1989), but others including *Baumea* sp. (Adcock and Ganf 1994; Tanner 1996; Wong *et al.* 1999), *Bolboschoenus* sp (Greenway and Woolley 1999), *Carex* sp. (Wong *et al.* 1999), *Eleocharis* sp, (Greenway and Woolley 1999; Wong *et al.* 1999), *Juncus* sp. (Tanner 1996; Wong *et al.* 1999), *Schoenoplectus* sp (Greenway and Woolley 1999; Tanner 1996), and *Triglochin* sp (Adcock and Ganf 1994; Mars *et al.* 1999), have also been suggested. Four species, two traditional and two more non-traditional, were selected for the Willunga wetland system.

2.2.5.1. *Bolboschoenus medianus* (V. Cook) Sojak (Cyperaceae)

Bolboschoenus medianus has an emergent habit with a creeping woody rhizome bearing hard globose tubers. The stems grow up to 200 cm and the leaves up to 50 cm in a grass-like form. The inflorescence is terminal and umbel-like, and the plant usually flowers from February to May (Jessop and Toelken 1986). *Bolboschoenus medianus* was reported (Brix *et al.* 1992) to produce insignificant convective through-flow of oxygen, and may account for the limited attention the plant has received for use in constructed wetlands (see section 1.6.2. for advantages of aeration).

Tissue nutrient concentrations recorded for the genus *Bolboschoenus* (synonym *Scirpus)* receiving domestic sewage are high (23.0 mg N g^{-1} dwt, 4.3 mg P g^{-1} dwt, Oki 1992). Even though some studies have reported low biomass (232 g dwt m^{-2}, under field conditions, Ennabili *et al.* 1998), Morris (1998) recorded similar RGR (42 mg g^{-1} d^{-1}) for *Bolboschoenus medianus* and *Typha domingensis* under nutrient rich conditions. This potential for high productivity and nutrient concentrations led to the selection of *Bolboschoenus medianus* for the Willunga system.

2.2.5.2. *Phragmites australis* (Cav.) Trin. Ex Steud (Gramineae)

The bamboo-like culm of *Phragmites australis* arises from a large rhizome system. The hollow stems, which may reach 3.5 m in height, have many nodes and flat leaves alternately arranged (Aston 1973). The terminal branched inflorescence produces many small wind-dispersed seeds. The high annual biomass production, plus ability to initiate convective flow, (Armstrong and Armstrong 1988; Armstrong and Armstrong 1991; Brix *et al.* 1992), which potentially enhances oxygen supply and nitrogen transformations (see section 1.6.2), has led to the extensive use of this species in constructed wetlands (see Cooper and Findlater 1990; Hammer 1989).

2.2.5.3. *Triglochin procerum* R.Br (Juncaginaceae)

Triglochin procerum has a thick fibrous rhizome, 4.5-18 cm long, and fleshy roots which end in an elongated tuber. The leaves are flat, erect to semi-erect and up to 2 m long, arising from central base. The erect inflorescence has many flowers on a spike-like raceme at the terminal end of a long cylindrical scape (Aston 1973). *Triglochin procerum* produces a large number of short-lived, water-dispersed seeds which do not contribute significantly to the long term seed bank (Rea and Ganf 1994b). Adult plants grow in water up to 1.5m deep, and deep conditions stimulate flowering and allow dispersal of seed via hydrochory (Rea and Ganf 1994b). Germination and establishment occur under drawdown conditions. *Triglochin* relies on sexual reproduction for colonisation of new areas, with slow and short ranged (phalanx) clonal growth (Rea and Ganf 1994b). There are no reports on the ability for *Triglochin* to initiate convective flow.

This genus has not been widely used in constructed wetlands, with only investigations by Adcock and Ganf (1994) into *Triglochin procerum*, and Mars *et al.* (1999) into *Triglochin huegelii*. The investigations by Adcock and Ganf (1994) found that *Triglochin procerum* maintained vigorous growth throughout the year with high nutrient concentrations (22.63 mg N g^{-1} dwt, 5.19 mg P g^{-1} dwt) and biomass (15000 g m^{-2}). The potential displayed in this initial investigation promoted the use of this species on a larger scale at the Willunga wetland system.

2.2.5.4 *Typha domingensis* Pers (Typhaceae)

Typha domingensis is a robust perennial emergent macrophyte with a branched rhizome which terminates in a leaf-bearing stem. The leaves are concentrated at the base of the stem and can grow to 3 m high. The inflorescences are densely packed cylindrical spikes, the upper portion male, and the lower portion female. The seeds are very small and easily dispersed by either wind or water. This species forms dense monospecific stands in water up to 1.5m deep (Aston 1973). *Typha* spp can achieve high biomass production under Australian conditions (Roberts and Ganf 1986), and like *Phragmites*, members of this genus have demonstrated an ability for convective flow (Brix *et al.* 1992). These attributes have resulted in wide spread use of *Typha* spp. in constructed wetlands (see Cooper and Findlater 1990; Hammer 1989) and the selection of *Typha domingensis* for the Willunga system.

Throughout the remainder of this text, reference to the four species at the Willunga system will be by genus name only, and species names will be given elsewhere as required.

2.2.5.5 *Species Comparison and Hypotheses*

Variations in growth form between the four species will be reflected in parameters such as biomass production, nutrient concentration and growth performance. The ability for *Phragmites* and *Typha* to facilitate convective flow has been linked to competitive advantage in deep water environments (Brix *et al.* 1992). However, *Triglochin*, which presumably relies on diffusive gas supply to below ground organs, flowers under flooded conditions and has been reported to grow at depths of 1.5 m. Even though *Bolboschoenus medianus* was reported to be more flood tolerant than the co-occurring *Bolboschoenus caldwellii* (Siebentritt and Ganf 2000), Blanch *et al.* (1999) reported a reduction in RGR with increasing water depth. Therefore, it may be expected that *Bolboschoenus* at Willunga would achieve optimum performance in the perimeter and shallow zones, whereas the other three species would additionally be able to colonise the surface flow sections (60cm).

Hypotheses of species-specific responses to conditions in constructed wetlands can be suggested. The characteristics of the non-traditional species suggest that they may perform equally with, or even out-perform, the traditional species in terms of seasonal standing biomass per area. The seasonal dynamics of *Typha, Phragmites* and *Bolboschoenus* would suggest that the peak biomass would be achieved in the summer season and biomass lows in winter. However, *Triglochin* may be expected to maintain high biomass throughout the year due to lack of winter senescence. Changes in distribution and floristic composition may influence nutrient storage capacity of the plant component. Generally, plants in inundated zones would be expected to have greater above ground nutrient storage potential as a result of greater biomass allocation to the above ground portion (but minimal change in nutrient concentration).

2.3. Floristic Measurements:

2.3.1. Methods:

2.3.1.1. Standing Above Ground Biomass

Monthly measurements were conducted over a 12 month period (February 1996 to February 1997) on five to ten randomly selected replicate shoots of *Phragmites, Typha* and *Bolboschoenus*. Shoots were collected from stands within the perimeter and gravel barrage regions of wetlands 1 and 3 for a direct calculation of plant biomass. All standing above ground material was collected, i.e. live plus senescent tissue, and shoots were cut level with the substrate. This material was dried at 80°C for 96 hours for dry weight determination. The shoot density of each species was recorded in a 1 m^2 quadrat around the shoot collected for dry weight determination (n=5-10) and used to convert information collected on per shoot basis to a per square metre basis. This method of biomass determination was chosen over more destructive methods because of the high frequency of measurements and the need to minimise disturbance to the operation of the council-operated wetland system.

Recruitment of new shoots of the three species occurred almost exclusively in early spring (September) and as a result, the observations made throughout the year followed the progress of a single cohort. Shoot density was also relatively uniform throughout the wetland, and therefore calculating biomass per unit area was considered appropriate.

Due to the low number of *Triglochin* shoots within the wetland, it was not viable to determine monthly shoot dry weight through direct collection as for the other three species. Instead, *Triglochin* biomass in the surface flow sections (60cm) of wetlands 1 and 3 was estimated indirectly by measuring the length and width of all leaves on 10 replicate shoots in each wetland. Individual leaves were collected every season (late summer (February), late autumn, late winter, late spring, late summer) from a subsample of 10 replicates per wetland in order to derive a relationship between leaf length, leaf width and dry weight.

Data were analysed using a model 1, two-tailed three-way analysis of variance with wetland, species and time as factors. Post-hoc analyses were performed using a Tukey-Kramer test. Data were natural log transformed to achieve a normal distribution. All statistical analysis was performed using JMP software (SAS Institute Inc. 1989). All standing above ground material of wetland 3 was harvested by the wetland managers in February 1997. Therefore, to avoid artificial interactions, data from this month are presented (in Tables 2.4 and 2.5), but excluded from the analysis. P values stated in the text were from the highest level interaction where a significant result was recorded. For example, if the three-way interaction was not stated then that level of interaction was not significant, and similarly, only those two-way interactions with significant interactions were stated in the text. Tables of ANOVA results can be obtained from the author upon request.

2.3.1.2. *Distribution and Subsequent Colonisation*

The four species were originally planted in monospecific stands and therefore the distribution of each species was determined by measuring the area of each stand. Distribution of all four species within wetlands 1 and 3 were determined in early 1995 by directly measuring the area of monospecific stands. These measurements were repeated in early 1997 for wetland 1 after changes in distribution were observed.

The incidence of flowering and fruiting was recorded in order to gain insight into the plant's investment in sexual reproduction. The percentage of individuals with inflorescence (flowering or fruiting) was calculated for each month over the 12 months using shoots from the standing above ground biomass measurements.

The number of *Triglochin* clumps per wetland section was identified by the presence of emergent leaves. These were counted five times over the 12 month period (February 1996, May 1996, August 1996, November 1996 and February 1997) and multiplied by the average number of shoots per clump (from the 10 replicates per wetland for the standing above ground biomass) to estimate the total number of shoots per section.

2.3.1.3. *Implications of changes in distribution on plant growth and nutrient storage*

Plant growth responses and potential nutrient storage of *Typha* and *Triglochin* were compared between the areas of original distribution and the colonised zones. These comparisons were conducted in February 1997 with shoots from two environmental zones within wetland 1; zone 1 the gravel barrage, 0-5 cm deep water; zone 2 the clay-based centre of the surface flow wetland basin, 60 cm deep water.

The following parameters were determined for six randomly selected replicate shoots for both species from each zone : Length of the longest leaf (to quantify the maximum height response), leaf area, above ground dry weight, number of leaves per shoot and the density of shoots within a 1 m^2 area around each replicate shoot. Shoots in the deep zone were divided into emerged and submerged above ground material and dried at 80°C for 96 hr. Four leaves (of material above the water surface) from three of the six replicate shoots were measured directly for leaf surface area using a Delta T meter and the relationship between area and dry weight determined. This relationship was used to estimate the total leaf area (one side of leaf) above the water surface from the total dry weight above the water surface for the six replicate shoots for each species at the two environmental zones. Insufficient data were available to determine leaf area ratio (LAR equation 5.4), therefore the ratio of emergent leaf area to above ground biomass was calculated to further compare leaf structure and form.

Nutrient concentration was measured for the two species by sampling three replicates each of emerged and submerged leaf portions in the deep zone and above ground material from the shallow zone. Above ground tissue of each replicate was ground using a grinding mill. The ground material was thoroughly mixed and a 500 – 700 mg subsample analysed for carbon and nitrogen concentration (by oxidative combustion using a CN200 carbon nitrogen analyser at Roseworthy campus of the University of Adelaide) and phosphorus concentration (by ICP at Waite Analytical Services).

Prior to drying, the structural density (mass per stem volume; g per cm^3) of plant material was determined for both species across the two environmental zones. Longitudinal sections, 15 cm long, were cut from the aerial portion of 15 leaves and the fresh weight of these sections was determined. The cross-sectional area was determined from a thin slice of each shoot section, placed on a transparency with a grid of known area under a dissecting microscope. The volume of these sections was estimated by multiplying the cross sectional area by the length of the section. The structural density ($g\ cm^{-3}$) was calculated by dividing the fresh weight (g) of the shoot section by the volume of that section (cm^3).

Data were analysed using a model 1 two-tailed two-way analysis of variance, with species (*Triglochin* and *Typha*) and environmental zone (shallow gravel zone, and deep clay zone) the two factors. Post-hoc analyses were performed using Tukey-Kramer tests. All proportion and percentage data were arcsine square root transformed and all other data were natural log transformed (Zar 1987). All statistical analysis was performed using JMP software (SAS Institute Inc. 1989). P values stated in the text were from the highest level interaction where a significant result was recorded. For example, if the two-way interaction was not stated then that level of interaction was not significant, and therefore the P values of the individual factors was stated. Tables of ANOVA results can be obtained from the author upon request.

2.3.2. Results:

2.3.2.1. *Standing Above Ground Biomass*

Bolboschoenus, Phragmites and *Typha* all recorded seasonal variation in dry weight per shoot with maxima in February. Minima occurred in August and also in September (for *Bolboschoenus* and *Phragmites*) with the onset of new shoots (Figure 2.4, wetland 1; Figure 2.5 wetland 3; data in Table A.1). *Triglochin* displayed a less pronounced seasonal variation than the other species, but generally minimum shoot dry weight was recorded in autumn and maximum in summer (Figure 2.4, 2.5). The analysis demonstrated that these responses could only be explained by an interaction between the three factors (species, wetland and month) ($P = 0.0001$). *Triglochin* was the only species to record a wetland response with greater weight per shoot in wetland 1 compared to wetland 3 (see Table A.1). *Typha* shoots were heavier than *Triglochin* at the beginning of the observational period, however, by the end of that time, *Triglochin* shoots were greater.

Peak shoot density (shoots per square metre) for *Bolboschoenus* and *Phragmites* was recorded in summer (February 96 greater than February 97) and minimum density recorded in winter (August) (Table 2.4). Density of *Typha* shoots remained relatively constant over the year, with only a slight decline in autumn and winter (and into February 1997 in wetland 3). Shoot density in *Triglochin* increased over the observational period (with a decline in February 1997 in wetland 3) (Table 2.4). Shoot densities were higher in the species which recorded the lower dry weight per shoot (i.e. *Bolboschoenus* and *Phragmites*). These responses were supported by a species x wetland x month interaction ($P < 0.0001$).

Triglochin, which recorded high biomass per shoot, compared very poorly to the other three species when expressed on a biomass per square metre basis (due to the low density of shoots in the surface flow sections of the wetland) (Table A.1). This species was distributed as large aggregates of shoots originating from a single rhizome. These aggregates, or clumps, were made up of approximately 20 shoots within a 1 m^2 area and each clump was isolated from the next clump by up to several metres. Therefore, in order to compare more directly the dry weight of all species per area, the total dry weight (g) per clump (thus per area of approximately 1 m^2) was calculated.

Figure 2.4: Above ground dry weight per shoot (g) for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 1 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

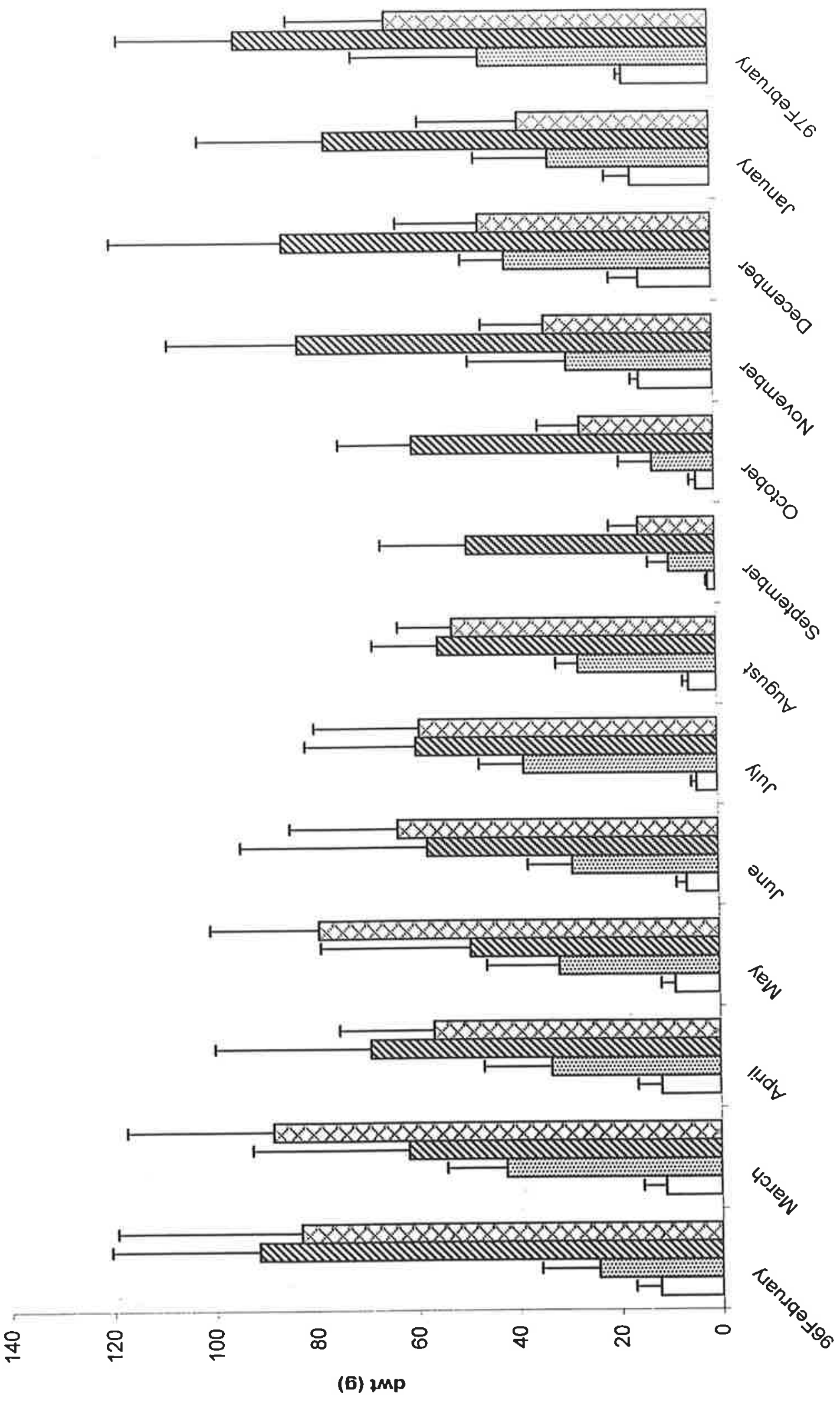
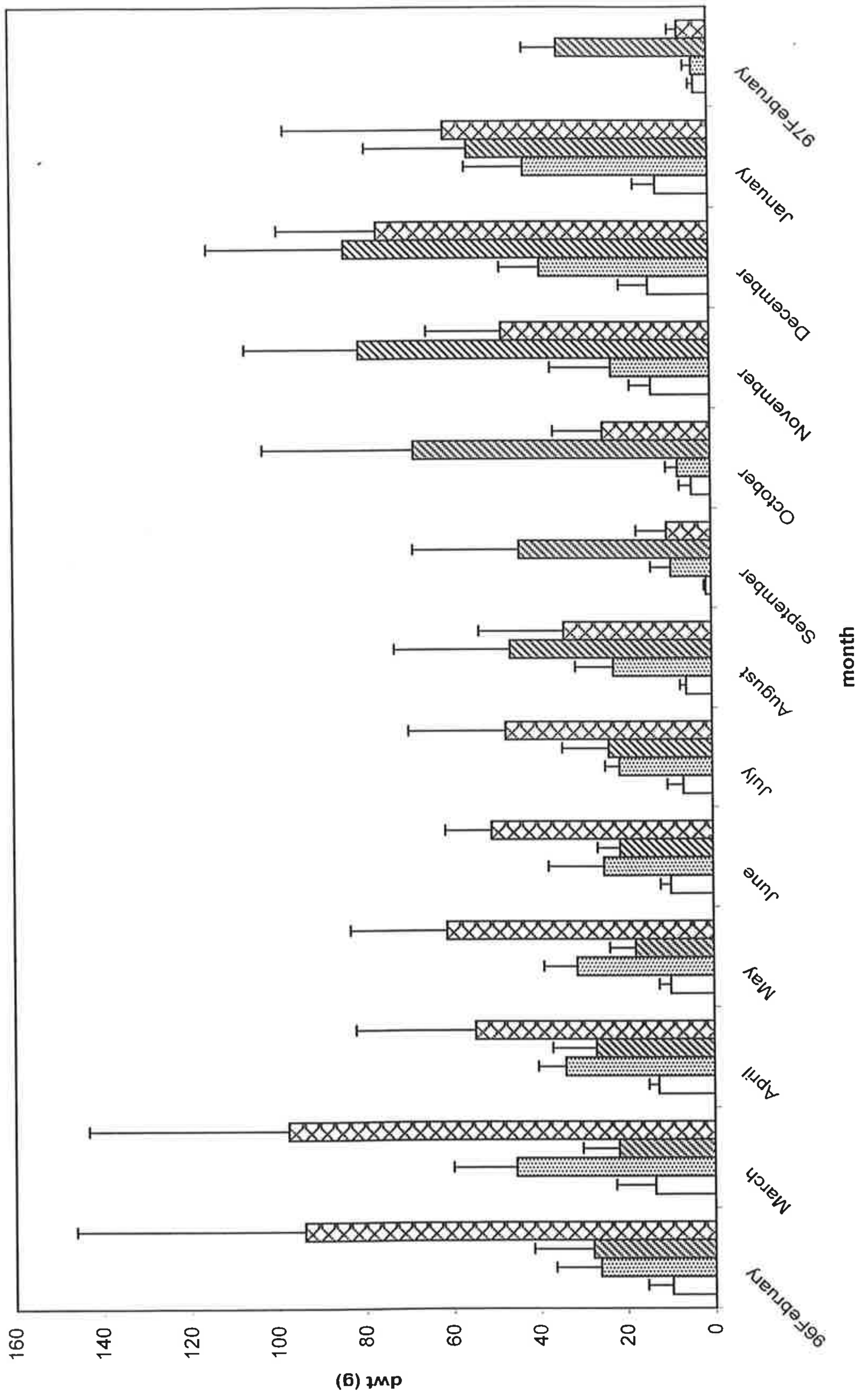


Figure 2.5: Above ground dry weight per shoot (g) for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 3 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).



Dry weight per square metre for *Bolboschoenus*, *Phragmites* and *Typha* showed a similar pattern to that observed per shoot, with minima observed in August and September, and maxima in February (Figure 2.6, wetland 1; Figure 2.7, wetland 3; data presented in Table A.2). The maximum dry weights recorded were similar between the three species, while the minima were noticeably lower in *Bolboschoenus.* The dry weight per square metre for *Triglochin* (per clump data) was lower than the other species at the beginning of the observational period (Figure 2.6, Figure 2.7). The dry weight of *Triglochin* increased over the winter period and reached values comparable with the other species by November. These differences resulted in a significant species x time interaction ($P < 0.0001$). *Triglochin* was the only species to demonstrate a dry weight per square metre response between wetlands, with greater dry weight recorded for wetland 1 compared to wetland 3. This was demonstrated by the significant wetland x species interaction ($P < 0.0001$).

Table 2.4: Density of shoots per square metre for four species for four species of aquatic macrophyte in wetland 1 (W1) and wetland 3 (W3) at Willunga over 12 month period (n= 5-10, ± standard deviations).

	Bolboschoenus		*Phragmites*		*Triglochin* Total area		*Triglochin* Within clump		*Typha*	
	W1	**W3**	**W1**	**W3**	**W1**	**W3**	**W1**	**W3**	**W1**	**W3**
February 96										
	335	208	151	102	0.13	0.13	9.6	6.1	43	40
	(±75.8)	(±35.4)	(±73.7)	(±18)	(±0.005)	(±0.028)	(±3.9)	(±3.3)	(±6.6)	(±7.5)
May										
	94	82	74	72	0.13	0.12	10	6.4	36	29
	(±15.9)	(±20.5)	(±22.4)	(±13.3)	(±0.008)	(±0.038)	(±6.1)	(±3.0)	(±4.0)	(±7.7)
August										
	56	56	53	53	0.14	0.15	12	5.9	37	30
	(±7.5)	(±8.9)	(±13.1)	(±6.6)	(±0.003)	(±0.045)	(±5.9)	(±5.4)	(±5.2)	(±4.6)
September										
	159	126	119	108	0.14	0.15	15	9.2	45	47
	(±26.2)	(±18.7)	(±19.1)	(±30.1)	(±0.001)	(±0.046)	(±9.1)	(±6.2)	(±6.6)	(±5.9)
November										
	159	126	119	108	0.14	0.15	20	14	45	47
	(±26.1)	(±18.6)	(±19.0)	(±30.1)	(±0.003)	(±0.045)	(±8.8)	(±6.9)	(±6.6)	(±5.9)
February 97										
	158	37	109	86.4	0.14	0.18	21	7.3	46	40
	(±36.3)	(±38.5)	(±10.8)	(±39.0)	(±0.005)	-	(±11.5)	(±3.8)	(±5.4)	(±10.2)

Figure 2.6: Above ground dry weight per square metre (g m^{-2}) for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 1 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

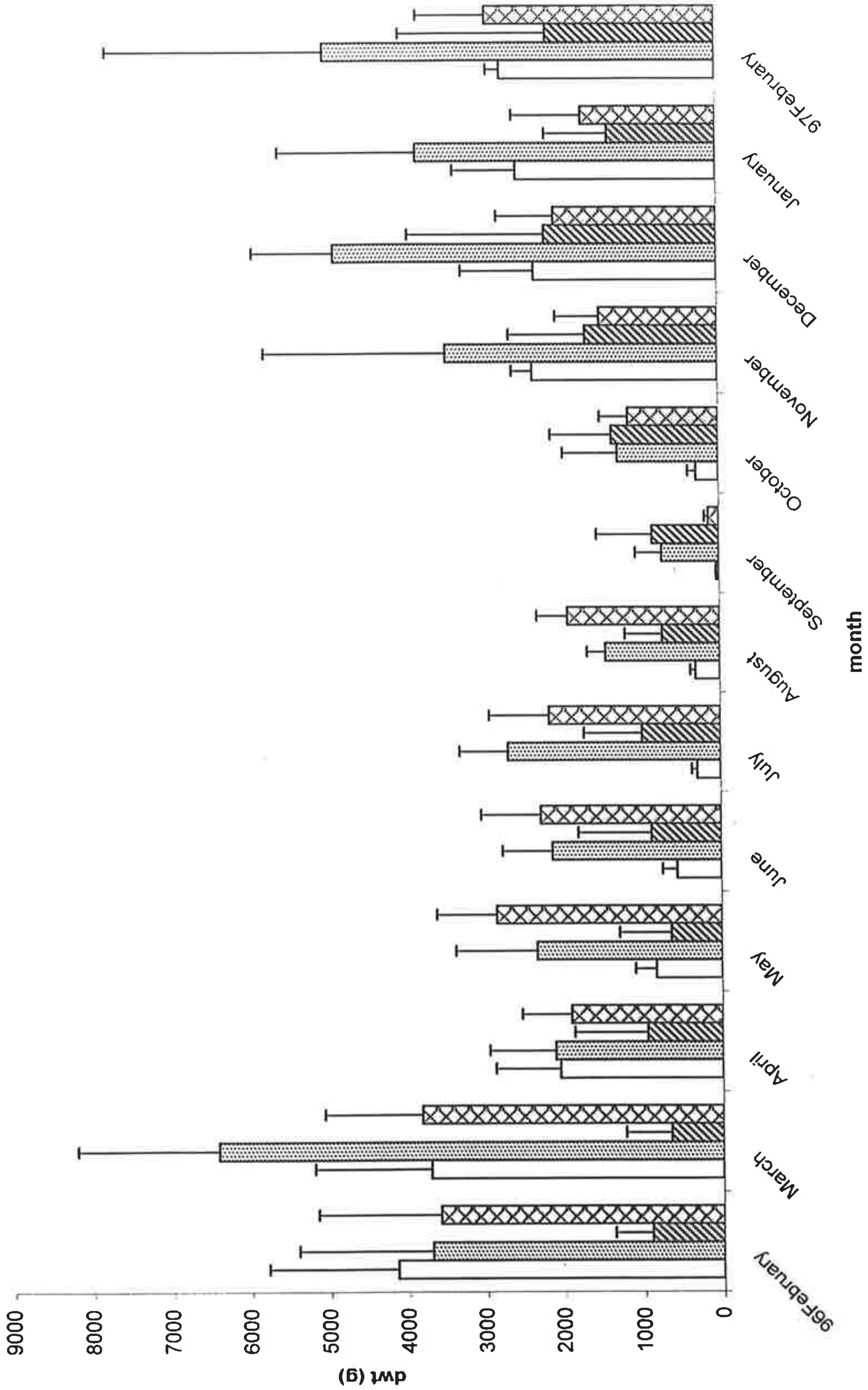
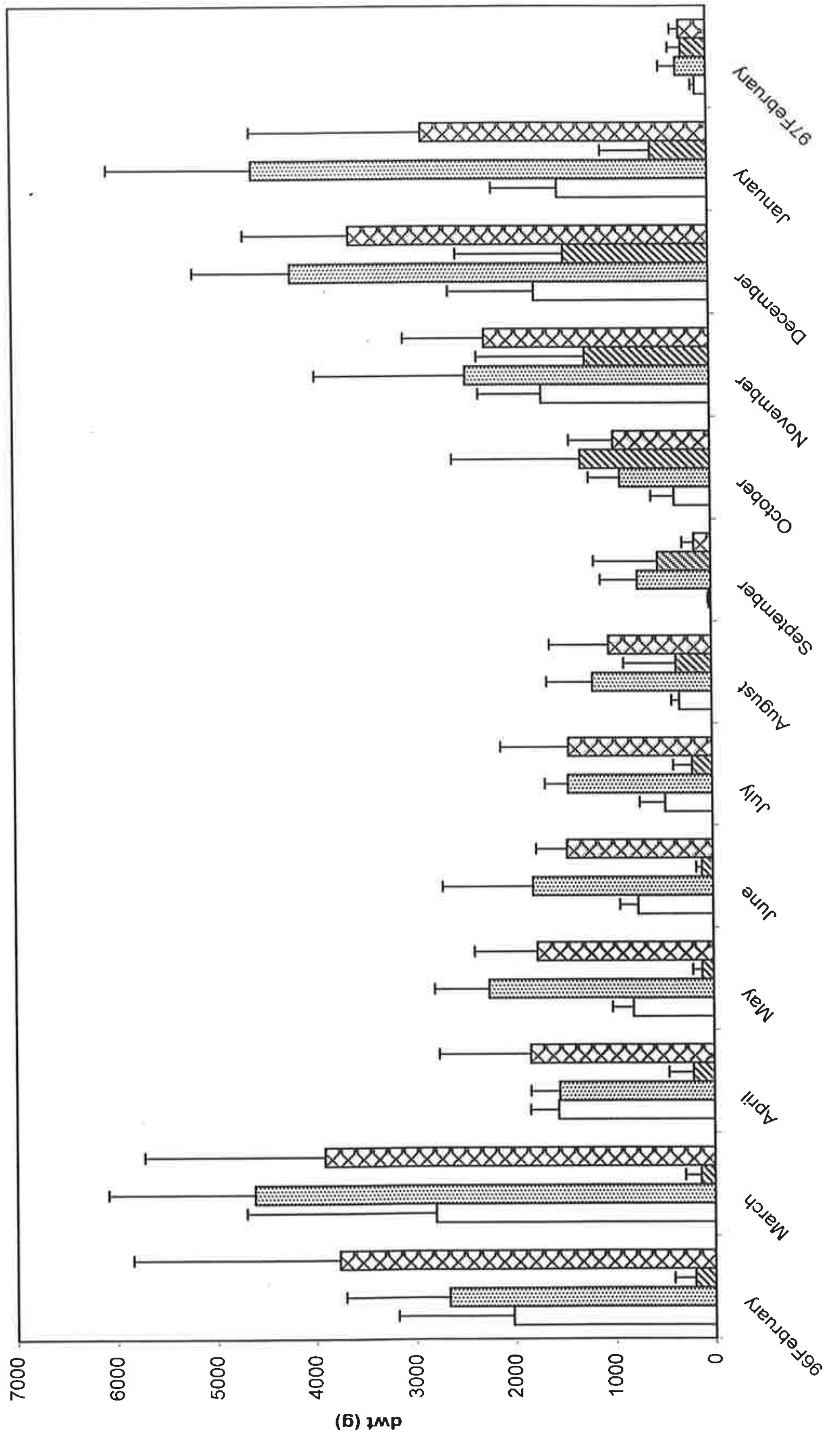


Figure 2.7: Above ground dry weight per square metre (g m^{-2}) for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 3 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).



2.3.2.2. *Distribution and Subsequent Colonisation*

The distribution of *Bolboschoenus* in 1995 was restricted to the barrage areas in both wetlands (i.e., where original planting had occurred). By 1997, small areas of this distribution in wetland 1 had been displaced by *Triglochin* (7 m^2 on each barrage). This displacement occurred after the council removed vegetation and scraped the gravel areas in an attempt to correct short-circuiting of flow within the wetland. *Triglochin* seeds were water dispersed in the surface flow sections (original distribution) and germinated in these unvegetated areas of the barrage.

Typha was originally planted on the barrages and perimeter of wetlands 1 and 3 and subsequently recorded the greatest wetland 1 distribution change of all species. A displacement of *Typha* by *Triglochin* (total of 3 m^2 on each barrage) similar to the displacement of *Bolboschoenus*, was observed on the barrages of wetland 1 between 1995 and 1997. *Typha* successfully colonised the surface flow sections via clonal growth (20% of section 2; 26% of section 3) over the observation period. The colonisation of 10 m^2 of the barrages was the only change in *Triglochin* distribution between 1995 and 1997.

Phragmites occupied 17.4% and 16.8% of vegetated areas of wetland 1 and 3 respectively, in early 1995 (Table 2.5). There was no noticeable change in this distribution in wetland 1 between 1995 and 1997.

The number of clumps and the total number of *Triglochin* shoots per wetland section were counted over the 12 month period of study (Table 2.6). Data previous to that period of observation was obtained from Johansson (1997). *Triglochin* was originally planted by the council in 1993 as single shooted rhizomes. The number of shoots increased but the number of clumps decreased over the period from 1993 to 1997. The most dramatic decrease in the number of clumps was observed in the first year (i.e., between September 1993 and October 1994) and a more gradual decrease was observed through to February 1997. Within an annual cycle, the minimum number of clumps was observed late summer or late autumn 1996 (except for W3, which appeared to be adversely affected by the harvest of that wetland), followed by a sustained increase through late winter.

Table 2.5: Original areas (m²) of distribution (1995) of four species in the zones of the Willunga constructed wetland system, and alterations to distribution in wetland 1 (1997).

	Bolboschoenus	*Phragmites*	*Triglochin*	*Typha*	Total Area
Wetland 1					
Barrage 1-2					
1995	132	-	-	88	220
1997	125	-	10	85	
Barrage 2-3					
1995	132	-	-	88	220
1997	125	-	10	85	
Perimeter					
1995	-	461	-	461	922
1997	-	461	-	461	
Surface flow Section 2					
1995	-	-	520	-	520
1997	-	-	416	104	
Surface flow Section 3					
1995	-	-	766	0	766
1997	-	-	567	199	
Total area					
1995	264	461	1286	637	2648
1997	250	461	1003	934	
Wetland 3					
Barrage 1-2					
1995	150	-	-	100	250
Barrage 2-3					
1995	174	-	-	116	290
Perimeter					
1995	-	480	-	480	960
Surface flow section 2					
1995	-	-	663	-	663
Surface flow section 3					
1995	-	-	693	-	693
Total area					
1995	324	480	1356	696	2856

Table 2.6: Number of *Triglochin* clumps, number of *Triglochin* shoots and water depth (cm) of the first surface flow section of wetland 1 and wetland 3 from September 1993 to February 1997.

	Wetland 1			Wetland 3		
	Clumps	Shoots	water depth (cm)	Clumps	Shoots	water depth (cm)
September 1993	192	192		224	224	
October 1994	98	576		117	454	
March 1995	68	243		101	348	
December 1995	83	730	53	97	795	60
February 1996	70	532	55	65	403	63
May 1996	65	520	53			
August 1996	76	760	58	77	262	67
November 1996	76	1110	56	77	801	62
February 1997	70	1218	55			

The investment in sexual reproduction by the four species was quantified by recording the percentage of flowering and fruiting shoots over a 12 month period from February 1996 to February 1997 (Table 2.7). *Bolboschoenus* shoots flowered and set seed between late spring (November 1996) and late summer (February) with a maximum of 60% of plants flowering or fruiting recorded several times over that period. *Phragmites* shoots began to flower in summer and flowering and fruiting was prolific with 100% incidence recorded at several times from early autumn to late winter. Flowering in *Typha* shoots was recorded over many seasons, and overall, was more frequent in wetland 1 than wetland 3. Flowering and/or fruiting was generally less than 40%, but the highest (60%) and most consistent flowering was observed in early through late summer. *Triglochin* shoots commenced flowering in late winter (August 1996) and incidence of flowering and/or fruiting reached a peak of 80% in late spring before diminishing through late summer.

Table 2.7: Percentage of individuals flowering and/or fruiting for four species of macrophyte in wetland 1 (W1) and wetland 2 (W2) at the Willunga constructed wetland system over a 12 month period (n = 5-10).

	Bolboschoenus		*Phragmites*		*Triglochin*		*Typha*	
	W1	W3	W1	W3	W1	W3	W1	W3
March	0	40	100	100	0	0	0	0
April	0	0	80	100	0	0	20	0
May	0	0	100	60	0	0	20	0
June	0	0	80	80	0	0	0	0
July	0	0	100	100	0	0	0	0
August	0	0	80	80	40	30	0	0
September	0	0	0	0	80	40	20	20
October	0	0	0	0	80	60	0	0
November	40	60	0	0	70	80	0	0
December	60	60	0	0	70	70	60	0
January	40	20	60	60	50	60	40	20
February 97	60	0	80	0	30	20	40	0

2.3.2.3. *Implications of changes in distribution on plant growth and nutrient storage*

Total shoot height, as demonstrated by the length of the longest leaf, was greater for *Typha* shoots than *Triglochin* shoots ($P < 0.0001$). Both species demonstrated a response to water depth, with longer leaves ($P = 0.0001$) in the deep zone compared to the shallow zone. *Typha* shoots in the shallow zones had a greater number of leaves than shoots in the deep zones. In contrast, *Triglochin* maintained a consistent number of leaves per shoot across environmental zone ($P = 0.0043$ species x environmental zone interaction).

Even though both species recorded a greater shoot density in the shallow zone compared to the deep zone, shoot density for *Typha* was slightly greater than shoot density for *Triglochin* in the deep zone, whereas shoot density for *Triglochin* was dramatically greater than that for *Typha* in the shallow zone ($P < 0.0001$, species x environmental zone interaction).

Typha exhibited greater leaf area per shoot in the deep zone compared to the shallow zone, whereas the opposite trend was observed for *Triglochin*, with maximum leaf area observed in the shallow zone ($P = 0.0122$, species x environmental zone interaction) (Table 2.8). The fleshy leaf of *Triglochin* responded to environmental zone with a change in leaf structure and form (Table 2.8). *Triglochin* leaves had a greater structural density (the ratio of fresh weight to volume) and lower ratio of leaf area to above ground biomass in the deep zone compared to the shallow zone. As a consequence, *Triglochin* leaves in the shallow zone were flat and blade-like, whereas leaves from the deep zone were thick and rigid. In contrast, no alteration in leaf form was observed across environmental zones for *Typha* leaves, with a constant structural density ($P = 0.0062$ species x environmental zone interaction) and ratio of leaf area to above ground biomass ($P < 0.0001$ species x environmental zone interaction).

Total above ground biomass was greater ($P < 0.0001$) for *Typha* shoots than *Triglochin* shoots, and for both species, shoots in the deep zone had significantly ($P = 0.0001$) greater biomass than shoots in the shallow zone (Table 2.8). *Typha* recorded significantly ($P < 0.0001$) greater emergent biomass (above water surface for deep shoots, and all above ground biomass for shallow shoots) than *Triglochin*, and the aerial biomass was significantly ($P = 0.0343$) greater for deep shoots than shallow shoots for both species. *Typha* recorded significantly ($P = 0.0016$) greater below water surface shoot material (deep shoots only) compared to *Triglochin* shoots.

Table 2.8: Length of longest leaf per shoot (cm), number of leaves per shoot, density of shoots (m^2), structural density of shoots (ratio of fresh weight to volume), emergent leaf area per shoot (cm^2), ratio of leaf area to above ground biomass (LA:AG) and above ground biomass per shoot (g) measured for *Triglochin* and *Typha* at the Willunga constructed wetland system (n=6 ± standard deviations). Measurements were made across two environmental zones; in zones of original distribution and zones of colonisation (Deep surface flow sections; Shallow subsurface flow sections). Above ground material from the deep zone was divided into above and below the water surface and (where applicable) given as a total of the two.

	Triglochin				***Typha***			
	Deep			**Shallow**	**Deep**			**Shallow**
	Above water	**Below water**	**Total**		**Above water**	**Below water**	**Total**	
Longest Leaf (cm)			134.3 (±17.12)	91.5 (±9.79)			235.7 (±30.79)	197.3 (±20.33)
Number of Leaves			7.17 (±1.47)	8.33 (±2.16)			14.4 (±2.07)	10.3 (±1.97)
Density of Shoots (m^2)			21.8 (±10.71)	96.7 (±15.88)			30.0 (±9.03)	44.7 (±5.32)
Structural Density	0.38 (±0.034)			0.31 (±0.047)	0.44 (±0.052)			0.44 (±0.041)
Leaf Area (cm^2)	877.3 (±381.8)			1211.8 (±410.4)	2526.2 (±898.4)			1431.7 (±679.6)
LA:AG	27.6 (±3.63)			52.0 (±1.23)	23.0 (±0.66)			23.2 (±1.44)
AG Biomass per shoot (g)	33.7 (±18.34)	20.2 (±9.16)	53.9 (±26.10)	23.5 (±8.40)	110.9 (±42.32)	50.1 (±8.93)	161.0 (±47.30)	63.3 (±32.40)

Carbon concentration (mg g^{-1}) of above ground tissue was greater ($P = 0.0005$) for *Typha* than for *Triglochin* (Table 2.9), reflecting the higher structural integrity of *Typha* shoots. Carbon concentration did not respond to environmental zone in either species ($P = 0.2167$), suggesting water depth did not influence the overall production of carbon based compounds.

Table 2.9: Carbon, nitrogen and phosphorus concentration (mg g^{-1}) and storage (g m^{-2}) measured for *Triglochin* and *Typha* at the Willunga constructed wetland system (n=6 ± standard deviations) in February 1997. Measurements were made across two environmental zones; in zones of original distribution and zones of colonisation (Deep surface flow sections; Shallow subsurface flow sections). Above ground material from the deep zone was divided into above and below the water surface and (where applicable) given as a total of the two.

	Triglochin				***Typha***			
	Deep			**Shallow**	**Deep**			**Shallow**
	above water	**Below water**	**total**		**above water**	**Below water**	**total**	
Carbon mg g^{-1}	402.5 (±3.34)	395.9 (±10.65)		394.2 (±16.99)	430.1 (±12.93)	411.7 (±17.07)		434.4 (±7.40)
Nitrogen mg g^{-1}	30.2 (±5.77)	31.4 (±0.89)		32.5 (±13.77)	14.5 (±1.55)	6.53 (±0.35)		11.47 (±1.89)
Phosphorus mg g^{-1}	3.62 (±0.59)	5.74 (±0.45)		3.49 (±0.54)	1.63 (±0.18)	1.78 (±0.24)		2.17 (±0.49)
Nitrogen g $shoot^{-1}$	1.02 (±0.554)	0.64 (±0.288)	1.65 (±0.798)	0.76 (±0.273)	1.61 (±0.614	0.33 (±0.058)	1.94 (±0.644)	0.73 (±0.372)
Phosphorus g $shoot^{-1}$	0.12 (±0.066)	0.12 (±0.053)	0.24 (±0.112)	0.082 (±0.029)	0.18 (±0.069)	0.11 (±0.019)	0.29 (±0.080)	0.14 (±0.070)
Nitrogen g m^{-2}			33.3 (±20.5)	73.5 (±28.7			57.4 (±24.6)	32.8 (±18.4)
Phosphorus g m^{-2}			4.82 (±2.99)	7.89 (±3.08)			8.63 (±3.60	6.21 (±3.49)

Nitrogen concentration of above ground tissues was lower ($P < 0.0001$) in *Typha* compared to *Triglochin* (Table 2.9). As with carbon, the concentration of nitrogen in above ground tissue did not respond to environmental zone ($P = 0.2249$). Phosphorus concentration of above ground tissue was also lower in *Typha* than *Triglochin* (Table 2.9). The concentration of phosphorus in the below water surface tissue of *Triglochin* was greater than emergent tissue (of both environmental zones). This response was not observed in *Typha* tissue, which recorded a constant concentration across environmental zone. The contrasting responses were reflected in a significant ($P = 0.0021$) species x environmental zone interaction.

Nitrogen and phosphorus storage (i.e. the grams of nutrient in above ground tissue) per shoot and per area were compared across the environmental zones for the two species (Table 2.9). Nitrogen and phosphorus storage per shoot (g) was similar between species ($P = 0.8198$ and $P = 0.0823$, respectively). Driven primarily by differences in dry weight per shoot, nitrogen and phosphorus storage per shoot was greater in the deep zone compared to the shallow zone ($P = 0.002$ and $P < 0.0001$ respectively).

The nitrogen and phosphorus stored per square metre for *Triglochin* was greater in the shallow zone whereas the accumulation of nutrients in *Typha* was greatest in the deep zone (nitrogen, $P = 0.0041$; phosphorus $P = 0.0393$ species x depth interaction).

2.3.3. Discussion:

2.3.3.1. *Standing Above Ground Biomass*

Dry weight of above ground material per square metre recorded at the Willunga constructed wetland system was high compared to that recorded in other studies (Table 2.10). *Bolboschoenus* and *Phragmites* both recorded over 4000 g m^{-2} during peak seasons at Willunga. Just under 4000 g m^{-2} was recorded for *Typha* and a maximum of 2184 g m^{-2} was recorded for *Triglochin*. The 3900 g m^{-2} recorded for *Zizania latifolia* by Tanner (1996) was comparable to the emergent species of this study. Adcock and Ganf (1994) recorded 13171 g m^{-2} for *Triglochin,* which was 6 times greater than the values recorded for the same species in this study.

The dry weight of all four species displayed some degree of seasonal variation. The variations observed for *Bolboschoenus, Phragmites* and *Typha* resulted from complete (first two specie) or partial (second two species) seasonal senescence. The fluctuations in dry weight per square metre observed for *Bolboschoenus* and *Phragmites* were a function of seasonal changes in both shoot dry weight and shoot density, whereas the fluctuations in *Typha* were due primarily to changes in dry weight per shoot. The increased *Triglochin* dry weight per square metre over the observational period was driven primarily by an increase in shoot density. As minimum *Triglochin* dry weight per shoot coincided with the end of the flowering season, it may be suggested that energy expenditure by the plant for flower and seed production was the major cause of seasonal biomass fluctuations.

This comparison has given insight into one aspect of the nutrient storage capacity of the four macrophyte species, i.e. seasonal fluctuations in above ground biomass. This information is incorporated with the seasonal fluctuations in nutrient concentration to calculate total nutrient storage in section 3.2.2.2.

Table 2.10: Comparison of standing above ground biomass (g m^{-2}) from this study and published sources.

Source	Species	Notes	dwt g m^{-2}
Willunga			
	Bolboschoenus medianus	Late Summer	4145
	Phragmites australis	Late Summer	4992
	Triglochin procerum	Late Summer	2145
	Typha domingensis	Early Autumn	3906
Adcock and Ganf (1994)			
	Triglochin procerum		13171
	Phragmites australis		788
	Baumea articulata		2139
Ennabili *et al.* (1998)			
	Phragmites australis		2296
	Typha angustifolia		2158
	Scirpus maritimus		232
	Scirpus litoralis		567
Greenway and Woolley (1999)			
	Typha domingensis		1120
Tanner (1996)			
	Bolboschoenus fluviatilis	Late Autumn	1500
	Phragmites australis	Late Autumn	1600
	Juncus effusus	Late Autumn	750
	Zizania latifolia	Late Autumn	3900

2.3.3.2. *Distribution and Subsequent Colonisation*

Expansion of species' ranges within the Willunga wetland system was achieved via two means; 1) asexual reproduction i.e., clonal growth through rhizome extension and 2) sexual reproduction. *Typha* relied on asexual methods of colonisation and was overall the most successful invader. Rhizomes originating from the gravel barrages and perimeter extended into the surface flow sections of the wetland via rafting (rhizomes located above the sediment surface), resulting in the occupation of 20% of the surface flow sections of wetland 1 in 1997. As the populations of *Typha, Phragmites* and *Bolboschoenus* were very dense on the barrage and perimeters zones, the surface flow regions were the only zones available for colonisation (apart from small regions of the barrage disturbed by wetland maintenance).

The original planting of *Triglochin* rhizomes within the surface flow sections was sparse (192 rhizomes in 520 m^2; section W12) and subsequent clonal growth was limited to short rhizome extensions. This led to the clumped distribution of *Triglochin* shoots and associated rhizomes. *Typha* colonisation filled the spaces between the *Triglochin* clumps, resulting in a reduction in the potential distribution of *Triglochin* without affecting the existing biomass. Long term observations would be required to determine whether eventual displacement of *Triglochin* would occur.

Typha colonisation more closely followed the guerrilla strategy described by Lovett Doust (1981) with long distances between shoots. In contrast, the clumped distribution of *Triglochin* was indicative of the conservative radially spreading phalanx-style strategy. The reliance on these alternative clonal growth strategies by the two species may be related to their strategies for satisfying oxygen demand of the below ground organs.

Macrophyte species found commonly in anoxic environments have developed strategies to compensate for the high oxygen demand of below ground organs. One such strategy is the ability for selected macrophytes to initiate convective flow of oxygen via specialised tissue structures (Armstrong and Armstrong 1991; Brix *et al.* 1992). White and Ganf (1998) reported longer rhizome extension for *Typha domingensis* plants able to sustain convective flow than plants unable to sustain convective flow (leaves pierced to prevent flow). This effect was more pronounced at greater depths of inundation. Thus the guerrilla style long rhizome extension responsible for the successful colonisation by *Typha* may have been related to its ability to supply abundant oxygen to the below ground organs. Barclay and Crawford (1982) demonstrated that *Typha angustifolia* rhizomes were able to sustain shoot extension even under anoxic conditions. Other species, including *Phragmites australis,* were able to survive the anoxic treatment, but were unable to sustain any shoot extension while subjected to the anoxic environment (i.e. they resumed shoot extension when returned to an oxic environment). This may explain why *Typha* was highly successful at colonising the surface flow sections of the wetland whereas *Phragmites* showed no change from the original perimeter distribution.

As with *Phragmites*, the distribution of *Bolboschoenus* did not expand between 1995 and 1997. Barclay and Crawford (1982) reported shoot extension under anoxic conditions similar to that of *Typha angustifolia* in *Scirpus maritimus*. Therefore, it may be suggested that factors other than the potentially anoxic conditions excluded *Bolboschoenus* from colonising the surface flow sections. The displacement of small sections of *Bolboschoenus* by *Triglochin* resulted from physical disturbance at a time when *Triglochin* was able to successfully invade through establishment of seed.

Convective flow has not been reported for *Triglochin*, therefore, this species may rely on passive methods, such as diffusion, to satisfy the below ground oxygen demand. The lack of active oxygen transport may be a major factor restricting *Triglochin* to short rhizome extensions, and therefore limiting its vegetative reproduction. *Triglochin* rhizomes in the surface flow sections experienced high mortality (approximately 50%) in the first 12 months after planting, followed by a relatively stable population dynamic with some degree of seasonal fluctuation. As clumps were identified via emergent leaves, the seasonal increases in clump number were most likely from the re-emergence of existing rhizomes rather than production on new rhizomes.

Despite the occurrence of flowering and fruiting in all four species, *Triglochin* was the only species to exhibit successful colonisation through sexual reproduction. Establishment was confined to the favourable environment of the gravel barrages. The limited extent of colonisation (10 m^2 on each barrage) appeared to be due primarily to a lack of space for seed establishment rather than poor recruitment ability.

Rea and Ganf (1994b) highlighted the importance of sexual reproduction in shaping the distribution of *Triglochin* in a natural system (Bool Lagoon). *Triglochin* establishment observed at Willunga appeared similar to that for Little Bool Lagoon, where the static deep water level promoted flowering, but restricted vegetative growth as the steep shoreline offered a very limited zone for establishment (Rea and Ganf 1994b). In contrast, the fluctuating water levels of Bool Lagoon provided favourable conditions for seed establishment of *Triglochin.* The high water levels promoted seed production and dispersal, while the falling water levels at the time of seed germination allowed widespread seedling establishment. As a result, the population in Little Bool Lagoon was small and controlled whereas the population under the fluctuating water regime in Bool Lagoon was rapidly expanding.

In order to support a more densely vegetated population of *Triglochin* at the Willunga wetland system, it would be necessary to either increase the initial planting density (which would require a large number of plants) or provide conditions favourable for widespread colonisation. The above information would suggest that a fluctuating water regime, similar to that described in Bool Lagoon (Rea and Ganf 1994b), may provide conditions favourable for widespread seed establishment. Allowing wet and dry cycles within the system may have additional management benefits such as increased rates of decomposition (Brinson *et al.* 1981 as cited by Mitsch and Gosselink 1993), and may therefore enhance wetland performance and longevity. Wong *et al.* (1999) recommended the use of wet and dry cycles in constructed wetland management as a mechanisms to enhance the vigour of wetland plants and control the invasion of weed species. Such management protocol may, however, result in a pulse of previously stored phosphorus upon reflooding (Kadlec and Knight 1996), and this would need to be controlled via enhanced movement of phosphorus into storage and removal components of the wetland (see section 1.7 for human induced mechanisms for nutrient removal).

2.3.3.3. Implications of changes in distribution on plant growth and nutrient storage

The two environmental zones were distinguished by two factors: substrate type and depth of inundation. Results from section 5.3. indicated no difference in performance between clay or gravel substrates for either species. Therefore, unless an interaction between substrate and depth occurred under field conditions, the major factor driving the observed response was inundation. Inundation, or increased water depth, has two major stresses: 1) a reduction in oxygen availability, i.e. increase in oxygen demand to below ground organs (see White 1999); 2) carbon budget stress (reduced photosynthetic capacity) induced by decreased light availability and/or decreased access to gaseous CO_2 (Blanch *et al.* 1999; Grace 1989).

Numerous responses by macrophytes to inundation have been reported in the literature. The decreased shoot density, increased shoot height, and increased above ground biomass observed for *Typha* and *Triglochin* at Willunga were consistent with observations in other studies (Grace 1989; Lieffers and Shay 1981; Rea and Ganf 1994a; Siebentritt and Ganf 2000).

White and Ganf (1998) suggested that changes in shoot density for species such as *Typha domingensis* may be controlled by rhizome length plasticity. It was also proposed that decreasing shoot density may be a strategy to avoid shoot competition or to retreat to more favourable environments. As *Typha* at Willunga was moving from the shallow zone to the deep zone, the decreased shoot density was more likely an avoidance of shoot competition or perhaps strategy for the preemption of available space (see chapter 5 for more detail on preemption) than a retreat to more favourable environments. The very high density of *Triglochin* shoots in the shallow zone compared to the deep zone was a direct result of seed dispersal and establishment and was possibly independent of the strategies suggested by White and Ganf (1998).

Macrophytes generally respond to the stresses of inundation by reallocating resources to increase the acquisition of atmospheric resources. Grace (1989) concluded that light limitation was of primary importance for *Typha* sp, whereas Blanch *et al.* (1999) suggested that CO_2 limitation forced the response in *Bolboschoenus medianus*. Grace (1989) identified two strategies for carbon adjustment within the genus *Typha*; *Typha domingensis* increased total (above ground plus below ground) biomass per ramet with increasing water depth, but the proportion of biomass allocated to the leaves remained constant, whereas *Typha latifolia* increased the proportion of total biomass to the above ground fraction under greater water depths. As the below ground biomass was not measured in this study, the mechanism for above ground biomass increase in *Typha* and

Triglochin at Willunga, (i.e., via a reallocation of resources within the plant, or an overall increase in total ramet size) could not be determined.

Even though the above ground biomass (and therefore, potential nutrient storage capacity) of both *Triglochin* and *Typha* increased in response to inundation, species-specific responses were observed for emergent (above water level) leaf area. *Typha* shoots increased emergent leaf area with inundation, whereas, Triglochin recorded lower leaf area in the deep zone compared to the shallow zone. Blanch *et al.* (1999) observed a linear relationship ($r^2 = 0.979$) between emergent leaf area and total plant biomass for *Bolboschoenus medianus*, suggesting plant performance was related to an ability to access atmospheric resources. Due to the maintenance of guerrilla style rhizome extension under inundated conditions (see section 2.3.3.2), resource demand may be greater in *Typha* than *Triglochin*. The large emergent leaf area observed for *Typha* may aid in satisfying the high resource demand.

Investigation of leaf structure and form outlined the mechanism controlling the apparently counter intuitive response to inundation observed in *Triglochin* shoots (i.e. increased above ground biomass, but decreased leaf area). Structural density (mass per volume) of *Triglochin* shoots increased, and leaves changed from a flat blade-like form to a thick, more rigid form with inundation. The alteration in leaf structure and form allowed *Triglochin* shoots to maximise leaf area in the shallow zone, and maximise rigidity and structure, at the expense of leaf area, in the deep zone. In contrast, *Typha* shoots appeared to lack either the ability or the need to alter leaf form or structure.

Fractional porosity (% internal gas volume), calculated from the volume of plant tissue and the assumption that specific weight of fresh tissue is 1 g cm^{-3}, has been used to compare oxygen transport potential of macrophytes (Brix *et al.* 1992; White *et al.* 2000). Fractional porosity is inversely proportional to the measure of structural density used in this study. Therefore, *Triglochin* shoots in the shallow zone had a relatively greater fractional porosity (i.e. air spaces made up a greater percentage of total shoot volume) than shoots in the deep zone. *Typha* shoots, which presumably initiate convective flow, had a lower porosity than *Triglochin* shoots. This response appears to contradict the hypothesis that inundated shoots would adapt to satisfy the increased oxygen demand to below ground organs. The alteration in *Triglochin* leaf form may have been for structural support of the fleshy leaf to enable the recorded heights of up to 1.34m, despite the support offered by the water column.

The increased rigidity and structure of inundated *Triglochin* leaves was achieved with no alteration in the carbon: nitrogen ratio of leaf tissue. This suggested that the response was due to changes in physical form rather than alterations in chemical composition (such as increased lignification). The higher carbon concentration observed in leaves of *Typha* compared to *Triglochin* suggested that the rigidity and structure in the former species was achieved via carbon based structural compounds such as lignin and starches.

Epiphytic growth, or other associated biota, may have been responsible for the higher phosphorus concentration in the submerged portion of *Triglochin* leaves compared to emergent tissues. However, as this response was recorded for phosphorus and not nitrogen, it may be suggested that phosphorus was bound or loosely associated with the leaf surface.

Despite differences in biomass and leaf area, the nitrogen and phosphorus storage capacities per shoot were similar between species in equivalent zones. The greater storage capacity of shoots of both species in the deep zone was a direct consequence of the responses to inundation described above (i.e. increased shoot height, and above ground biomass). The interactive response of nitrogen and phosphorus storage per square metre (*Triglochin* maximum storage in shallow zone, and *Typha* maximum storage in deep zone) was a result of the very high *Triglochin* shoot density in the shallow zone.

2.4. Conclusion:

Overall, the biomass accumulations at Willunga were high compared to other published values for similar species in constructed wetland environments. Seasonal fluctuations in above ground biomass were observed in all four species. The fluctuation observed for *Triglochin* appeared to be induced by flowering and fruiting rather than the winter die-back experienced by the other species.

Peak above ground biomass for *Bolboschoenus* was similar to that of the species traditionally used in constructed wetlands (*Typha* and *Phragmites*). Biomass of *Triglochin* per shoot was high compared to the other species, but low shoot densities and poor rhizome extension capacity meant that biomass per area was lower than anticipated.

Providing favourable conditions, such as fluctuating water levels to promote *Triglochin* seedling establishment, may enhance colonisation and potentially increase the longevity of the wetland system. Changes in distribution (through asexual and sexual colonisation) will influence the nutrient storage represented by the plant component.

Typha and *Triglochin* both demonstrated an ability to colonise new regions within the Willunga system. *Typha* colonised the surface flow sections and *Triglochin* the barrage zones. The nutrient storage potential of both species (*Triglochin* per shoot level only) was greater in the inundated zone than the barrage zone. The mechanisms of this response to inundation varied between the two species.

CHAPTER 3: SEASONAL NUTRIENT STORAGE IN ABOVE GROUND TISSUE AND THE ROLE OF HARVESTING IN NUTRIENT REMOVAL:

3.1. Introduction:

Nutrient storage within aquatic macrophytes is a function of above ground biomass and the nutrient concentration of that biomass (see section 1.5.2). Nutrient concentration of plant tissue depends on the nutrient load, the availability of those nutrients to plants (i.e. whether nutrients are added to water column or substrate (see chapter 4) chemistry of the system as governed by redox and pH), season, genetic, phenotypic and physiological variation between plants and distribution within plants (Kadlec and Knight 1996). Biomass also varies between species and fluctuates seasonally (see section 2.3.2.1.).

Storage of nutrients in plant tissue is not permanent, and nutrients can be returned to the system upon senescence (Prentki *et al.* 1978), (also see section 1.5.2.3). Harvesting plant material ensures that nutrients stored within the plant tissue are removed from the system (see section 1.8). This may be of particular importance for nutrients such as phosphorus where naturally occurring permanent methods of removal are limited. The time of optimum nutrient storage and potential removal (via harvesting) may vary between species depending on the relationship between nutrient concentration and biomass.

Nitrogen and phosphorus concentrations are generally higher in younger tissue than old and senescent tissue (Dykyjova 1978; Hocking 1989; Klopatek 1978; Oki 1992; Suzuki *et al.* 1989b). For example; Klopatek (1978) found maximum concentrations of 32.5 mg g^{-1} for nitrogen and 8.1 mg g^{-1} for phosphorus in the young tissue of above ground fraction of emergent macrophytes and this decreased by approximately 80% by the end of the growing season.

Micronutrient (e.g. Fe, Mn, Cu and Zn) concentrations also fluctuate with season. In contrast to macronutrients (nitrogen and phosphorus), higher concentrations of micronutrients are often recorded in older, senescent tissue (Dunbabin and Bowmer 1992; Hocking 1989). The potential use of aquatic macrophytes for micronutrient removal has been widely investigated using both floating and emergent macrophytes (e.g. Boyd 1970; Dunbabin and Bowmer 1992; Finlayson *et al.* 1984; Staves and Knaus 1985). Wetland plants display a high degree of micronutrient tolerance, recording concentrations much higher than terrestrial species and maintaining growth in highly contaminated soils. Mechanisms of micronutrient tolerance include; exclusion, accumulation (Dunbabin and Bowmer 1992), Baker 1981 as cited by Ye *et al.* 1997), or avoidance, detoxification and biochemical tolerance (Berry 1986 as cited by Ye *et al.* 1997).

Only the above ground fraction of emergent plants is readily harvestable. The below ground tissues (roots, rhizomes and other storage organs) play an important role in the internal cycling of nutrients and the regrowth of above ground material in response to harvesting (Congdon and McComb 1980; Haberl and Perfler 1990). Macrophytes which allocate a high proportion of total biomass to the above ground component, while maintaining sufficient below ground resources to promote viable regrowth after harvest, will be most valuable in nutrient removal. Plants preferentially allocate resources above ground under conditions which limit resource acquisition by that fraction (Chapin *et al.* 1987). For example, under conditions of high nutrient availability or inundation, atmospheric resources become limiting relative to nutrient resources. The nutrients contained in the new shoots following a harvest may be acquired from re-allocation from below ground stores or uptake from an external source (Sundblad and Robertson 1988).

Harvesting emergent plants more than once in a growing season may potentially enhance the overall removal by maintaining tissue in an active state thus encouraging further nutrient allocation to above ground tissues (Sundblad and Robertson 1988; Suzuki *et al.* 1989b). Timing and frequency of these multiple harvests will determine the total nutrient removal and regrowth capacity.

Many studies (e.g. Greenway and Woolley 1999; Tanner 1996), have investigated the tissue nutrient concentration and total nutrient storage (N m^{-2} & P m^{-2}) of various aquatic macrophytes in both natural and constructed wetlands. However, very few studies have clearly quantified the seasonal variation, especially in relation to southern Australian conditions with local species. In addition, only a limited number of emergent macrophyte species have been used widely in constructed wetlands (predominantly *Phragmites* and *Typha* see Cooper and Findlater 1990; Hammer 1989), and the potential of alternative species has not been extensively investigated. The Willunga system was planted with the two traditional species *Phragmites* and *Typha*, as well as two non-traditional species, *Bolboschoenus* and *Triglochin*. *Bolboschoenus* was selected as the growth rate (42 mg g^{-1} d^{-1}) was recorded to be similar to that of *Typha domingensis* (42 mg g^{-1} d^{-1}) under nutrient rich conditions (Morris 1998). *Triglochin* was selected because high tissue nutrient concentrations and active growth were reported over the winter period (Adcock and Ganf 1994).

This chapter is divided into two sections with distinct but related aims and hypotheses:

3.1.1. Aims and Hypotheses:

3.1.1.1. *Section 1*

Section 1 focused on the nutrient storage capacity of the four macrophyte species. These aims were investigated in conjunction with the survey of seasonal biomass (see section 2.3.2.1.).

- Determine species differences and seasonal variations in tissue nutrient concentration (major nutrients, carbon, nitrogen and phosphorus; micronutrients, iron, manganese, zinc and copper).
- Determine species differences and seasonal variation in nutrient storage (nitrogen and phosphorus) and potential nutrient removal.
- Determine whether nutrient storage of *Triglochin* is different to that of other species during winter.

The hypothesis for this section was that nitrogen and phosphorus concentration of plant tissue (for *Typha*, *Phragmites* and *Bolboschoenus*) would be higher and micronutrient concentration lower, early in the growing season when young tissue dominated the community. Maximum nutrient (nitrogen and phosphorus) storage would occur in times of high growth and high tissue nutrient concentration in spring or summer. *Triglochin* would maintain active growth in all seasons and therefore record consistent macronutrient concentration and nutrient storage potential throughout the year.

3.1.1.2. Section 2

The focus of this section was on nutrient removal and the specific aims were:

- Determine whether multiple harvest regimes (encompassing variation in timing and frequency of harvest) increase potential nutrient (nitrogen and phosphorus) removal from the Willunga constructed wetland system.
- Determine the most effective multiple harvest regime for each species.

A long term experiment (12 months) run in conjunction with section 1 investigated nutrient removal from a variety of multiple harvest regimes for *Bolboschoenus* and *Typha*. These two species were chosen for this initial investigation as they formed dense monospecific stands, grew in similar regions of the wetland and provided a comparison between a traditional species (*Typha*) and a local species not commonly used in constructed wetlands (*Bolboschoenus*). The optimum multiple harvest regime determined from this investigation was employed in a shorter-term experiment for the other two species (*Triglochin* and *Phragmites*) and *Bolboschoenus* (to serve as a direct comparison to the first experiment).

The hypothesis for both components of section 2 was that multiple harvest regimes would maintain plant tissue in a younger, more nutrient enriched state and ultimately increase nutrient removal from the system.

3.2. Section 1:

Seasonal Nutrient Concentration and Storage of Four Aquatic Macrophyte Species at the Willunga Constructed Wetland System:

3.2.1. Methods:

Nutrient concentration of the plant tissue was determined on a seasonal basis, i.e. summer (February 96 & 97), autumn (May 96), winter (August 96) and spring (November 96). Three replicates for each species were randomly selected from the 10 replicates collected for the seasonal species comparison of dry weight (section 2.3.2.1) at each interval. All above ground tissue of each replicate was ground using a grinding mill. The ground material was thoroughly mixed and a 500 - 700 mg subsample analysed for carbon and nitrogen concentration (by oxidative combustion using a CN200 carbon nitrogen analyser at Roseworthy campus of the University of Adelaide) and phosphorus, iron, manganese, copper and zinc concentration (by ICP at Waite Analytical Services). Material from *Phragmites, Typha* and *Bolboschoenus* was also analysed from early spring (September 96) upon the onset of new shoots after winter senescence. Dry weight data (per square metre) from each replicate of the species comparison (Table 2.5) was multiplied by the mean nutrient concentrations to determine the total grams of nutrient per unit area.

Data were analysed using a model 1, two-tailed three-way analysis of variance, with species, month and wetland the factors. Post-hoc analyses were performed using Tukey-Kramer tests. Nutrient data collected for the new growth tissue (September 1996) of *Bolboschoenus, Phragmites* and *Typha*, was included in the presentation (Figures 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6, Table A.3, Table 3.1 and 3.3), but excluded from analysis in order to maintain full factorial status (as September data was not collected for *Triglochin*). Arcsine transformations were applied to percentage and proportion data. Logarithmic transformations were applied to all other data (due to skewed distribution). Data were found to be normal using a Shapiro-Wilk test. All statistical analysis was performed using JMP software (SAS Institute Inc. 1989). P values stated in the text were from the highest level interaction where a significant result was recorded. For example, if the three-way interaction was not stated then that level of interaction was not significant, and similarly, only those two-way interactions with significant interactions were stated in the text. Tables of ANOVA results can be obtained from the author upon request.

3.2.2. Results:

3.2.2.1. *Nutrient concentration*

Carbon, nitrogen and phosphorus:

Triglochin and *Phragmites* both recorded maximum carbon concentration in November, whereas *Typha* exhibited minimum carbon concentration in that same period and *Bolboschoenus* exhibited minimum concentration in September (Figure 3.1 and 3.2, data in Table A.3). This was reflected in the analysis by a significant ($P = 0.0057$) species x month interaction. The concentration of carbon in the above ground tissue of the four species was significantly greater in wetland 1 than in wetland 3 ($P = 0.0067$).

Nitrogen concentration in above ground tissue was greater in *Triglochin* than the other three species and this high concentration was maintained throughout the year (29.1–36.6 mg g^{-1}) (Figure 3.3 and 3.4, data in Table A.3). The other three species displayed similar trends to each other, with the minimum concentration in August (shoots either fully senescent (*Phragmites* and *Bolboschoenus*) or in an over wintering (*Typha*) state) and maxima after the onset of new shoots in November. These differing responses between species over time were confirmed in the analysis by the species x month interaction ($P < 0.0001$).

Phosphorus concentration in above ground tissue of *Triglochin* was, also, consistently high throughout the year (3.6–4.8 mg g^{-1}) (Figure 3.5 and 3.6, data in Table A.3). The other three species recorded seasonal variations in phosphorus concentrations similar to that observed for nitrogen concentration, again resulting in a significant ($P < 0.0001$) species x month interaction. The highest phosphorus concentration recorded for all species was for *Bolboschoenus* in September (6.8 mg g^{-1} wetland 1) and November (6.3 mg g^{-1} wetland 3). The phosphorus concentration recorded for *Bolboschoenus* in February 1997 was lower than that of the previous summer (February 1996). No response to wetland was detected for either nitrogen or phosphorus concentration for all species.

Figure 3.1: Carbon concentration (mg g^{-1}) in above ground tissue for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 1 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

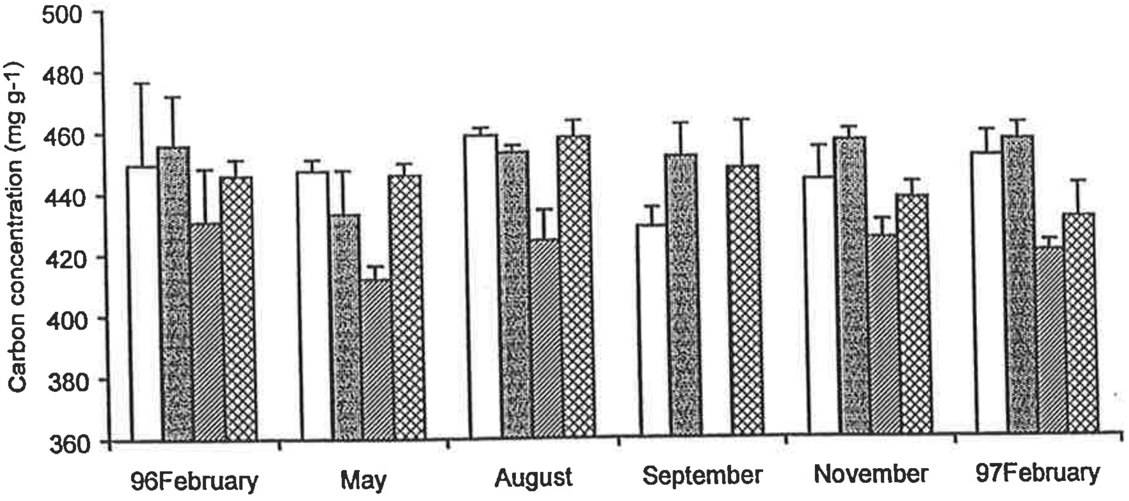


Figure 3.2: Carbon concentration (mg g^{-1}) in above ground tissue for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 3 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

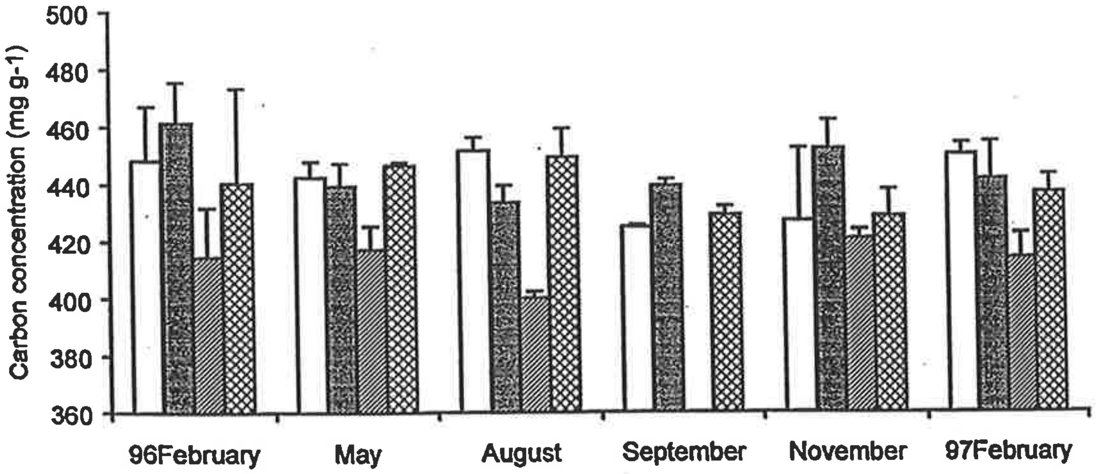


Figure 3.3: Nitrogen concentration (mg g^{-1}) in above ground tissue for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 1 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

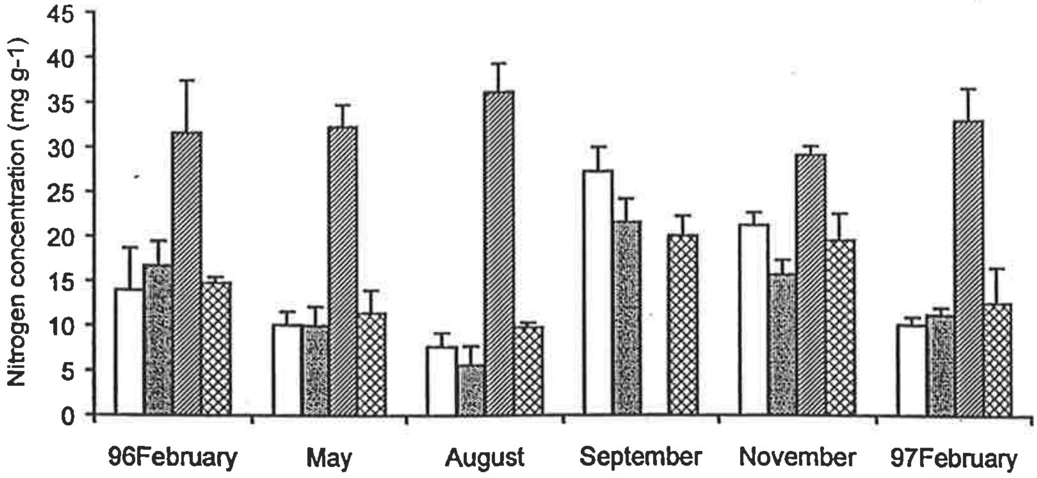


Figure 3.4: Nitrogen concentration (mg g^{-1}) in above ground tissue for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 3 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

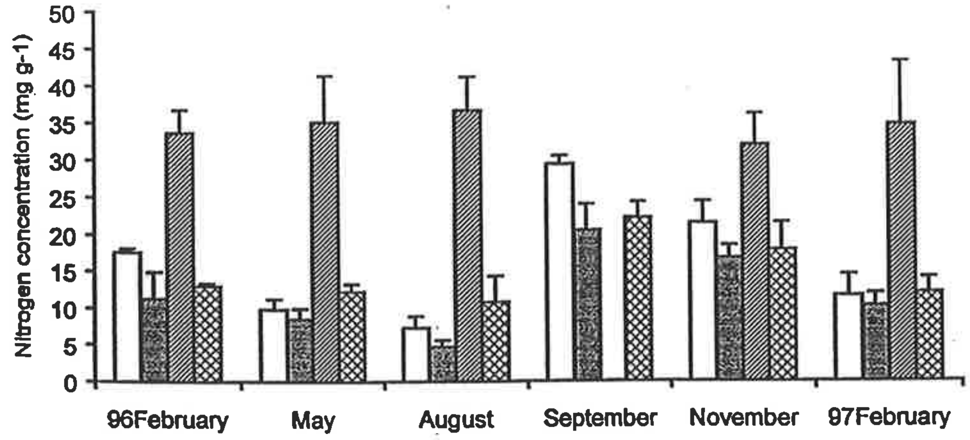


Figure 3.5: Phosphorus concentration (mg g^{-1}) in above ground tissue for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 1 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

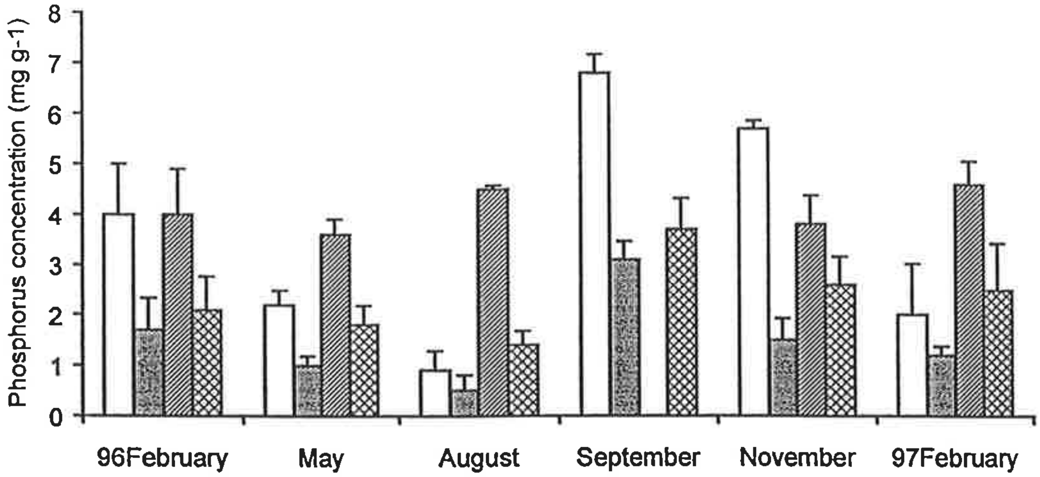
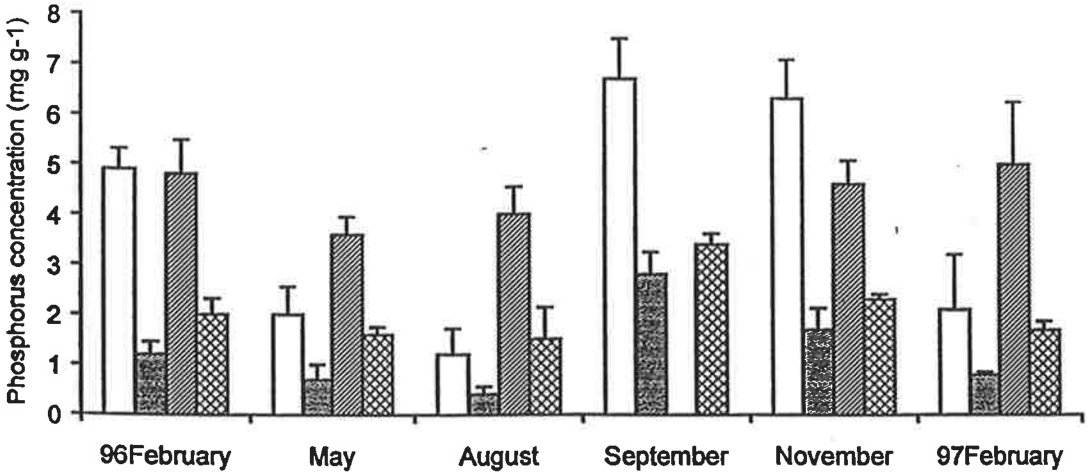


Figure 3.6: Phosphorus concentration (mg g^{-1}) in above ground tissue for *Bolboschoenus* (open), *Phragmites* (stippled), *Triglochin* (hatched), *Typha* (cross hatched) in wetland 3 of the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).



Micronutrients:

The concentration of iron in the above ground tissue of *Triglochin*, *Phragmites* and *Typha* was relatively constant throughout the year (Table 3.1.). Large seasonal variation in iron concentration was observed in *Bolboschoenus* with a maximum (141.3 $\mu g\ g^{-1}$) recorded in senescent tissue in August and a minimum in February 1996 (18.8 $\mu g\ g^{-1}$) and 1997 (17.6 $\mu g\ g^{-1}$). The contrasting response of iron concentration in *Bolboschoenus* compared to the other species was confirmed by a significant ($P = 0.0011$) species x month interaction.

A response of iron concentration to wetland was observed for *Triglochin*, but not the other three species ($P < 0.0001$, wetland x species interaction) (Table 3.1.). For example, a concentration in *Triglochin* of 126.9 $\mu g\ g^{-1}$ was recorded in wetland 1 and 381.4 $\mu g\ g^{-1}$ in wetland 3 during February 1996.

The concentration of manganese in the above ground tissue was greater in wetland 3 than wetland 1 for both *Triglochin* and *Bolboschoenus*. However, the manganese concentration was similar across wetlands for the other two species, as indicated by the significant ($P = 0.0152$) wetland x species interaction.

Seasonal variations in copper concentration were observed in *Phragmites*, *Typha* and *Bolboschoenus* with maxima in November and minima in May to August. In comparison, the copper concentration in *Triglochin* tissue remained relatively constant throughout the year ($P < 0.0001$, species x month interaction) (Table 3.1).

Table 3.1: Micronutrient concentration; iron (Fe), Manganese (Mn), copper (Cu) and zinc (Zn) ($\mu g\ g^{-1}$) for four species of aquatic macrophyte in wetland 1 (W1) and wetland 3 (W3) at Willunga over 12 month period (n= 5-10, ± standard deviations).

		Bolboschoenus		***Phragmites***		***Triglochin***		***Typha***	
		W1	**W3**	**W1**	**W3**	**W1**	**W3**	**W1**	**W3**
February 96	**Fe**	18.8 (±6.11)	22.6 (±5.71)	33.2 (±6.63)	39.7 (±6.92)	126.9 (±41.01)	381.4 (±232.48)	48.4 (±50.03)	48.1 (±15.69)
	Mn	150.0 (±108.5)	237.7 (±145.3)	124.3 (±80.8)	187.3 (±37.5)	205.7 (±48.5)	1010.7 (±647.8)	758.7 (±795.4)	207.3 (±120.2)
	Cu	1.55 (±0.77)	0.83 (±0.56)	3.62 (±1.25)	2.12 (±0.41)	1.36 (±0.85)	1.56 (±0.72)	1.49 (±0.64)	1.31 (±0.35)
	Zn	6.83 (±1.66)	12.74 (±6.15)	11.78 (±3.90)	6.33 (±2.13)	9.48 (±0.29)	15.26 (±0.56)	6.63 (±1.19)	7.71 (±2.78)
May	**Fe**	47.1 (±16.01)	49.7 (±8.23)	58.7 (±3.27)	54.7 (±8.20)	137.2 (±19.74)	269.3 (±105.8)	16.8 (±8.98)	17.2 (±7.56)
	Mn	234.3 (±56.66)	444. 7 (±90.34)	363.7 (±41.04)	194.3 (±51.05)	389.3 (±135.65)	627.0 (±207.30)	209.0 (±156.09)	503.7 (±338.90)
	Cu	0.48 (±0.18)	0.34 (±0.19)	2.23 (±0.91)	3.00 (±2.01)	1.36 (±0.12)	1.02 (±0.35)	1.27 (±0.36)	0.55 (±0.57)
	Zn	5.83 (±0.43)	6.91 (±1.10)	4.91 (±0.15)	3.73 (±0.37)	12.98 (±0.65)	18.55 (±3.16)	5.98 (±1.62)	6.44 (±2.32)
August	**Fe**	119.0 (±34.92)	141.3 (±100.75)	47.5 (±7.00)	46.1 (±13.73)	192.7 (±110.45)	338.3 (±84.20)	35.3 (±9.09)	31.5 (±14.58)
	Mn	598.2 (±673.7)	595.7 (±145.8)	179.0 (±130.5)	196.0 (±24.6)	450.9 (±136.1)	695.4 (±238.5)	414.9 (±543.8)	651.1 (±680.1)
	Cu	5.90 (±9.17)	0.86 (±0.14)	1.29 (±0.51)	1.40 (±0.34)	2.26 (±0.91)	1.30 (±0.57)	0.99 (±0.31)	1.63 (±1.96)
	Zn	5.77 (±1.43)	12.45 (±6.59)	3.29 (±1.58)	3.70 (±0.80)	17.58 (±2.12)	16.40 (±2.92)	6.58 (±1.56)	8.47 (±1.21)
September	**Fe**	30.5 (±13.45)	24.2 (±13.06)	27.8 (±4.43)	31.1 (±7.28)			24.5 (±4.80)	27.7 (±8.97)
	Mn	434.1 (±369.3)	307.3 (±148.1)	96.5 (±59.3)	178.3 (±50.2)			301.1 (±496.9)	155.5 (±145.0)
	Cu	6.64 (±1.61)	3.39 (±0.39)	7.42 (±2.88)	6.03 (±0.90)			2.75 (±0.39)	2.24 (±1.06)
	Zn	20.67 (±6.40)	15.62 (±3.83)	19.76 (±5.52)	18.07 (±1.53)			14.12 (±3.80)	13.88 (±0.95)

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		Bolboschoenus		*Phragmites*		*Triglochin*		*Typha*	
		W1	**W3**	**W1**	**W3**	**W1**	**W3**	**W1**	**W3**
Table 3.1 continued									
November	**Fe**	34.5	22.8	45.5	38.8	108.6	326.3	22.0	22.7
		(±1.98)	(±1.90)	(±6.33)	(±4.01)	(±33.39)	(±74.29)	(±3.95)	(±4.10)
	Mn	119.7	380.0	180.6	155.7	254.8	763.7	314.2	333.0
		(±34.0)	(±201.2)	(±67.5)	(±72.5)	(±10.2)	(±133.2)	(±335.67)	(±316.2)
	Cu	4.62	3.06	3.96	4.48	1.56	1.40	2.70	1.95
		(±1.00)	(±1.87)	(±1.22)	(±1.03)	(±0.11)	(±0.40)	(±0.26)	(±0.82)
	Zn	16.82	22.13	11.08	9.53	10.77	13.67	14.06	11.97
		(±2.99)	(±10.95)	(±2.78)	(±2.89)	(±1.37)	(±0.64)	(±5.09)	(±1.41)
February 97	**Fe**	17.6	17.8	53.7	38.3	134.0	216.9	15.9	31.3
		(±6.39)	(±6.63)	(±5.87)	(±14.01)	(±26.53)	(±100.37)	(±3.69)	(±14.65)
	Mn	220.7	521.7	199.7	185.0	134.3	235.0	162.0	301.0
		(±185.1)	(±324.9)	(±53.0)	(±79.1)	(±15.0)	(±64.5)	(±207.0)	(±97.6)
	Cu	1.80	2.62	2.50	2.89	1.67	0.93	2.45	2.78
		(±1.64)	(±0.97)	(±0.18)	(±0.53)	(±0.58)	(±0.35)	(±0.57)	(±1.43)
	Zn	4.54	8.93	4.94	4.75	10.09	20.07	8.12	10.39
		(±0.27)	(±0.78)	(±0.70)	(±0.66)	(±0.09)	(±14.85)	(±0.66)	(±4.31)

The concentration of zinc in the above ground tissue of *Triglochin* displayed seasonal variation with a maximum concentration recorded in May (18.55 μg g^{-1}) and August (17.58 μg g^{-1}) and a minimum concentration in November (10.77 μg g^{-1}). The other three species recorded the opposite trend in seasonal zinc concentration variation, with maxima in November and minima in May and August ($P < 0.0001$, species x month interaction) (Table 3.1).

Similarly to manganese concentration, a response to wetland was recorded for tissue zinc concentration in both *Triglochin* and *Bolboschoenus* (wetland 3 greater than wetland 1) but not the other two species ($P = 0.0005$, wetland x species interaction).

3.2.2.2. *Nutrient storage per unit area*

Seasonal variations in carbon and nitrogen storage per square metre observed in *Phragmites*, *Typha* and *Bolboschoenus* were driven by the changes in both dry weight and concentration. In contrast, due to the relatively constant nutrient concentration, the seasonal variation in nutrient storage observed for *Triglochin* was driven primarily by changes in dry weight. Minimum carbon and nitrogen storage for *Triglochin* was recorded in May and increased over the period of observation with maxima for each nutrient observed in November. The species specific seasonal responses were represented by a significant species x month interactions (carbon, $P < 0.0001$; nitrogen, $P < 0.0001$). As with dry weight, the carbon per square metre was generally lower in *Triglochin* than the other species, however, due to *Triglochin*'s high tissue nitrogen concentration, storage per square metre was similar between all species (Table 3.2.).

A response to wetland for carbon and nitrogen storage was recorded for *Triglochin* (wetland 1 greater than wetland 3) but not the other three species ($P < 0.0001$, wetland x species interaction). As no response to wetland was observed in carbon and nitrogen tissue concentration, this interaction was driven primarily by the response to wetland observed in *Triglochin* dry weight.

Seasonal patterns of phosphorus storage in all species were similar to those observed for storage of carbon and nitrogen ($P < 0.0001$, species x month interaction). Large seasonal variation in phosphorus storage was recorded for *Bolboschoenus*, due primarily to the variation in phosphorus concentration.

As with carbon and nitrogen storage, *Triglochin* was the only species to record a phosphorus storage response to wetland (wetland 1 greater than wetland 3) ($P < 0.0001$, wetland x species interaction). However, in this case, the response to wetland was month specific ($P = 0.0307$, wetland x month interaction) with no difference in *Triglochin* phosphorus storage between wetlands observed in November, and maximum difference observed in February 1997.

Table 3.2: Carbon (C), nitrogen (N) and phosphorus (P) storage (g m-²) for four species of aquatic macrophyte in wetland 1 (W1) and wetland 3 (W3) at Willunga over 12 month period (n= 5-10, ± standard deviations).

		Bolboschoenus		*Phragmites*		*Triglochin*		*Typha*	
		W1	**W3**	**W1**	**W3**	**W1**	**W3**	**W1**	**W3**
February 96	**C**	1864	897	1686	1231	388	84	1601	1657
		(±735.1)	(±514.4)	(±775.6)	(±476.0)	(±202.9)	(±85.9)	(±694.4)	(±915.1)
	N	58.0	35.6	61.8	30.2	28.5	6.8	53.0	48.8
		(±22.89)	(±20.43)	(±28.42)	(±11.66)	(±14.90)	(±6.97)	(±22.98)	(±26.94)
	P	16.5	9.8	6.2	3.2	3.6	1.0	7.5	7.4
		(±6.50)	(±5.64)	(±2.86)	(±1.23)	(±1.90)	(±0.99)	(±3.24)	(±4.07)
May	**C**	373	356	1012	989	265	50	1272	787
		(±117.9)	(±93.3)	(±454.1)	(±241.6)	(±269.5)	(±37.8)	(±343.9)	(±283.9)
	N	8.4	7.9	23.4	19.1	20.8	4.2	32.7	21.6
		(±2.66)	(±2.07)	(±10.48)	(±4.68)	(±21.14)	(±3.18)	(±8.84)	(±7.78)
	P	1.8	1.6	2.3	1.7	2.3	0.4	5.3	2.9
		(±0.57)	(±0.42)	(±1.02)	(±0.41)	(±2.34)	(±0.32)	(±1.42)	(±1.04)
August	**C**	143	147	657	518	311	142	882	463
		(±30.6)	(±33.7)	(±105.2)	(±198.4)	(±200.6)	(±210.3)	(±180.3)	(±266.6)
	N	2.38	2.38	8.16	5.58	26.5	13.0	18.9	11.1
		(±0.51)	(±0.55)	(±1.31)	(±2.14)	(±17.10)	(±19.22)	(±3.87)	(±6.37)
	P	0.29	0.39	0.68	0.49	3.29	1.42	2.77	1.50
		(±0.06)	(±0.09)	(±0.11)	(±0.19)	(±2.13)	(±2.10)	(±0.57)	(±0.86)
September	**C**	14.6	9.6	331	323			60.4	72.6
		(±3.66)	(±3.74)	(±149.9)	(±163.9)			(±22.70)	(±50.42)
	N	0.93	0.66	15.8	15.0			2.7	3.8
		(±0.23)	(±0.26)	(±7.17)	(±7.60)			(±1.02)	(±2.60)
	P	0.23	0.15	2.23	2.05			0.50	0.57
		(±0.06)	(±0.06)	(±1.01)	(±1.04)			(±0.19)	(±0.40)
November	**C**	1043	718	1582	1108	711	525	655	967
		(±118.2)	(±272.3)	(±1055.8)	(±679.2)	(±414.9)	(±458.2)	(±242.8)	(±349.2)
	N	50.0	35.9	54.5	40.8	48.8	39.8	29.4	40.2
		(±5.67)	(±13.62)	(±36.37)	(±25.04)	(±28.45)	(±34.69)	(±10.88)	(±14.50)
	P	13.5	10.6	5.37	4.11	6.41	5.77	3.83	5.21
		(±1.53)	(±4.01)	(±3.58)	(±2.52)	(±3.74)	(±5.04)	(±1.42)	(±1.88)

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		Bolboschoenus		*Phragmites*		*Triglochin*		*Typha*	
		W1	**W3**	**W1**	**W3**	**W1**	**W3**	**W1**	**W3**
Table 3.2 continued									
February 97	C	1237 (±78.8)	677 (±298.8)	2280 (±1265.9)	2020 (±644.2)	902 (±793.1)	103 (±52.7)	1260 (±381.6)	1253 (±753.3)
	N	27.7 (±1.76)	17.4 (±7.70)	55.7 (±30.95)	46.5 (±14.83)	70.7 (±62.14)	8.7 (±4.42)	36.8 (±11.15)	34.5 (±20.75)
	P	5.49 (±0.35)	3.13 (±1.38)	6.01 (±3.34)	3.83 (±1.22)	9.80 (±8.61)	1.24 (±0.63)	7.16 (±2.17)	4.99 (±3.00)

3.2.3. Discussion:

3.2.3.1. *Nutrient concentration*

Carbon, nitrogen and phosphorus (CNP):

Tissue nutrient concentrations are an important determinant of the functioning of plant species in their habitat (Aerts and Chapin 2000). High concentrations of N, P, K, Ca and Mg are correlated with high capacity for rapid growth in productive conditions and inability to sustain yield under limiting nutrients (Aerts and Chapin 2000). Therefore, species which exhibit high nutrient concentrations would be desirable for use in constructed wetlands.

Macronutrient concentrations in above ground tissue of *Typha, Phragmites* and *Bolboschoenus* adhered to the hypothesis and other reports (Dykyjova 1978; Hocking 1989; Klopatek 1978; Oki 1992; Suzuki *et al.* 1989b) that tissue macronutrient concentrations were greater in the beginning of the growing season compared to late in the season. Carbon, nitrogen and phosphorus tissue concentrations recorded in the four species at Willunga fell within the range considered typical of plants in constructed wetland systems (Table 7-7, (Kadlec and Knight 1996); ranges 290-500 mg g^{-1} C, 14.6-39.5 mg g^{-1} N and 0.8-6.3 mg g^{-1} P).

Triglochin recorded the highest tissue nitrogen and phosphorus concentration of all species at the Willunga system at all sampling intervals (except for *Bolboschoenus* P concentration in September and November; note, concentration not recorded for *Triglochin* in September). The lack of winter dormancy and consequently the continual production of new above ground material meant that these high nutrient concentrations were maintained in *Triglochin* throughout the year. In contrast, a seven-fold increase in phosphorus concentration was recorded for *Bolboschoenus* between the dormant material in winter and the new growth in spring. This seasonal increase in concentration was greater than the four to five fold seasonal increase suggested by Klopatek (1978).

Specific nutrient concentrations for a variety of wetland plants from constructed and natural wetlands are plentiful in the literature. However, accurate comparisons are limited by variations in climate, nutrient supply and season of sampling. Nitrogen and phosphorus concentrations in the above ground fraction of emergent plants from a number of sources were collated for comparison with data from Willunga (Table 3.3.).

The high phosphorus concentration (6.8 mg g^{-1}) recorded for *Bolboschoenus* in early spring was greater than concentrations recorded in the literature for other emergent species (including other species of *Bolboschoenus,* see Table 3.3). The range of N & P concentrations in *Triglochin* (29.1-36.6 mg g^{-1} and 3.6-5.0 mg g^{-1} respectively) were at the high end of concentrations recorded for other species (Table 3.3.) and higher than those recorded by Mars *et al.* (1999) for *Triglochin huegelii.* The range of nitrogen and phosphorus concentrations recorded for *Phragmites* and *Typha* in the Willunga system were similar to values recorded for those species in the literature (Table 3.3.), with nitrogen concentration of *Typha* at Willunga slightly lower than other recorded values. The minimum concentrations recorded at Willunga (during the senescent period for *Phragmites* and *Bolboschoenus* and the over-wintering state of *Typha*) were low in comparison to other studies, and was probably a sampling bias in the literature away from senescent tissue.

Macronutrient concentrations are generally similar between above and below ground organs of emergent macrophytes (see Ennabili *et al.* 1998; Hocking 1989; Nogueira *et al.*

1995). Below ground nutrient concentrations were not measured in this study, but given the high concentrations in the above ground tissue, it could be hypothesised that below ground tissues were also at the high end of the recorded range.

Table 3.3: Nitrogen (N) and phosphorus (P) concentration (mg g^{-1}) and storage (g m^{-2}) for a variety of aquatic macrophytes from this study and published sources.

	N mg g^{-1}	P mg g^{-1}	N:P	N g m^{-2}	P g m^{-2}
Willunga					
Bolboschoenus	7.3-29.3	0.9-6.8	4.5:1-8:1	0.66-58.0	0.15-16.5
Phragmites	4.7-21.6	0.4-3.1	7:1-12:1	5.58-61.8	0.49-6.2
Triglochin	29.1-36.6	3.6-5.0	7.5:1-8:1	4.20-70.7	0.43-9.8
Typha	9.8-22.1	1.4-3.7	6:1-7:1	0.50-53.0	0.50-7.5
Adcock and Ganf (1994)					
Triglochin procerum	25.7	4	6.5:1	338	52.6
Phragmites australis	23	1.8	13:1	18.1	1.4
Baumea articulata	11.8	1.5	8:1	25.2	3.2
Breen (1990)					
Typha orientalis					
Initial (mid summer)	12.2	2.1	6:1		
Final (late summer)	6.1	1.6	4:1		4.34
Ennabili *et al.* (1998)					
Typha angustifolia	19.4	1.7	11.5:1	42	3.7
Phragmites australis	10.4	1.5	7:1	25.4	2.8
Scirpus maritimus	17.2	1.3	13:1	4	0.3
Greenway and Woolley (1999)					
Bolboschoenus caldwellii	14.3	3.0	5:1		
Phragmites australis	20.4	2.0	10:1		
Typha domingensis	15.8	2.0	8:1	12	2.24
Lemna spp.	39.6	10.5	4:1	1.5	0.6
Hocking (1989)					
Phragmites australis					
May	9.0	1.2	7.5:1		
September	29	3.3	9:1		
November	14	2.1	6.5:1		

Continued next page.

	N mg g^{-1}	P mg g^{-1}	N:P	N g m^{-2}	P g m^{-2}
Table 3.3 continued					
Mars *et al.* (1999)					
Triglochin huegelii					
initial	20.8	2.6	8:1		
final	25.2	3.8	6.5:1		
Schoenoplectus validus					
initial	9.7	1.6	6:1		
final	13.5	2.6	5:1		
Oki (1992)					
Scirpus fluviatilis					
late autumn	11.3	1.8	6.5:1		
late spring	23	4.3	5.5:1		
early autumn	21.9	3.0	7.5:1		
Typha angustifolia					
late autumn	6.4	1.2	5.5:1		
late spring	18.7	2.4	8:1		
early autumn	17	2.7	6.5:1		
Phalaris arundinacea					
late autumn	30.7	5.4	5.5:1		
late spring	34.2	3.7	9:1		
early autumn	47.5	4.4	11:1		
Surface *et al.* (1993)					
Phragmites australis					
Constructed wetland	20.9	0.5	42:1		
Adjacent leachate seep	16.8	0.8	21:1		
Tanner (1996)					
Bolboschoenus fluviatilis	15	1.2	12.5:1	20	2
Phragmites australis	32	3.0	10.5:1	55	4.5
Juncus effusus	26	3.5	7.5:1	20	2.5
Zizania latifolia	20	2.5	8:1	75	9.5

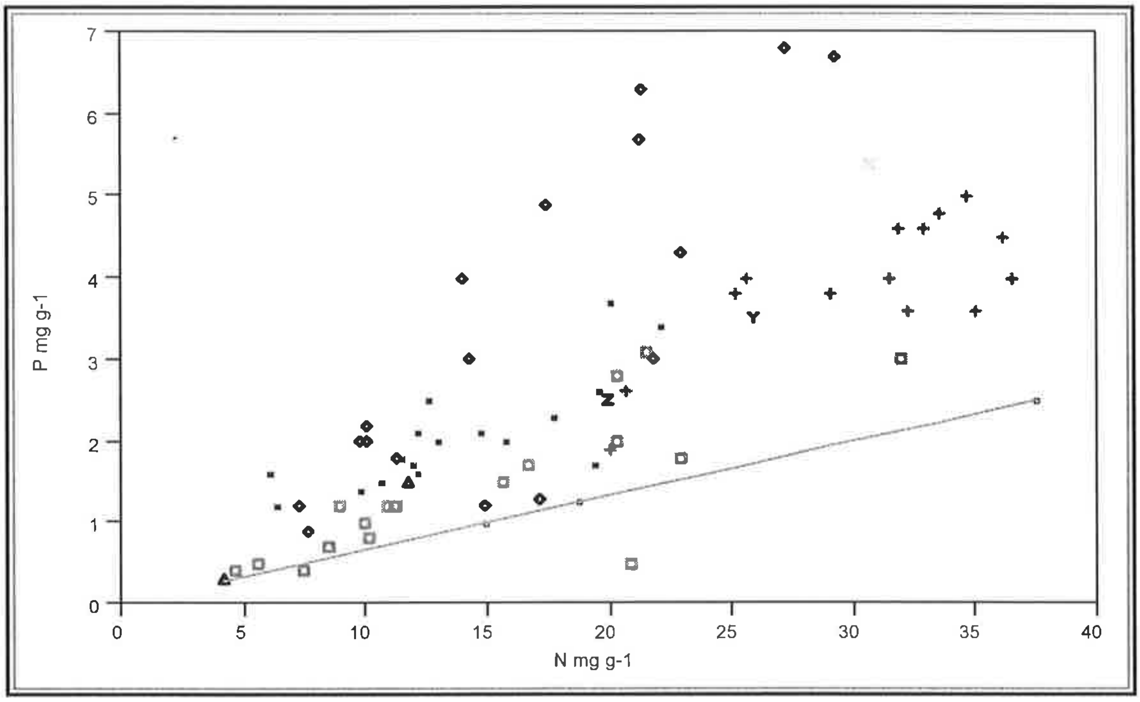
* Denotes from Kadlec and Knight (1996).

In order to compare the nitrogen and phosphorus ratios of above ground tissue for a number of genera simultaneously (Willunga, Table A.3 and published sources, Table 3.3), a scatter plot similar to that of figure 1 from Aerts and Chapin (2000) was constructed (Figure 3.7.). The line depicting the 15:1 N:P ratio on Figure 3.7, represents the relative limitation of nitrogen to phosphorus as modelled by Koerselman and Meuleman (1996). The majority of plants from constructed wetlands had N:P ratios less than 15:1 (i.e. above the 15:1 line of figure 3.7). This was in contrast to the plants from the Aerts and Chapin (2000) which had more widely distributed ratios, with many plants recording N:P ratios less than 15:1 (i.e. below the 15:1 line).

These ratios of tissue nutrients express the content of one nutrient relative to another, and do not necessarily describe an absolute nutrient limitation. Wastewater generally supplies nutrients in excess of requirements for plant growth and the N:P ratio of the wastewater is low (see Table 2.1).

Nutrient content for plants supplied with an of excess nutrients will depend on the assimilation and allocation of nutrients within the plant and the degree of luxury uptake. Two distinct species groups were evident in the N:P scatter plot (Figure 3.7). A low N:P ratio was recorded for *Bolboschoenus,* i.e. high phosphorus storage relative to nitrogen, and a lower N:P was recorded for *Triglochin* (of the constructed wetland species) with high nitrogen storage relative to phosphorus.

Figure 3.7: Comparison of N vs. P concentration (mg g^{-1}) for different genera of aquatic macrophytes from published and experimental sources (Table 3.3). ▪ *Typha*; □ *Phragmites*; ◆ *Bolboschoenus*; + *Triglochin*; Y *Juncus*; ▲ *Baumea*; **Z** *Zizania*, X *Phalaris*. The line represents the N:P ratio of 15:1.



Micronutrients:

As with the major nutrients, abundant information is available on micronutrient concentrations for a variety of wetland plants under of range of conditions. Concentrations of micronutrients in the above ground material of emergent plants was collated from this study and the literature for comparison (Table 3.4.). The micronutrients recorded at the Willunga wetland system were within the range observed in other studies.

Micronutrient concentrations reported in wetland plants can be up to 10 times greater (Kadlec and Knight 1996) than those considered toxic to grass species (Mn 600–1000 µg g^{-1}; Fe > 70 µg g^{-1}, Reuter and Robinson 1986). Even though micronutrient tolerance has been recognised in many wetland plants, the mechanisms (Dunbabin and

Bowmer 1992), and the levels at which toxicity effects may occur, are poorly understood. Plants at Willunga did not display visual symptoms of toxicity, and even the decline in dry weight observed in *Triglochin* over the late summer (see section 2.3.2.1.) was not correlated with high levels of Fe, Mn Cu or Zn (in fact only zinc displayed significant seasonal variation in *Triglochin*).

Storage of micronutrients in the below ground organs has been widely reported (see (Hocking 1989; Peverly 1985; Peverly *et al.* 1995; Suzuki *et al.* 1989a) and is generally considered to be a mechanism of micronutrient tolerance (Dunbabin and Bowmer 1992). Peverly *et al.* (1995) reported that the roots and/or their iron coating, acted as a filter for metal movement to the rhizome and shoots of *Phragmites australis.* As the below ground organs were not analysed in this study, it was not possible to compare the nutrient concentrations of above to below organs.

Table 3.4: Micronutrient concentrations; iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) (µg g-1) from this study and published sources.

	Fe µg g^{-1}	Mn µg g^{-1}	Cu µg g^{-1}	Zn µg g^{-1}
Willunga				
Bolboschoenus	17.6-141	120-598	0.34-6.6	4.5-22.1
Phragmites	27.8-58.7	96.5-364	1.29-7.4	3.29-18.1
Triglochin	109-381	134.3-1011	0.93-2.3	9.5-20.1
Typha	15.9-48.4	156-759	0.55-2.8	6.0-14.1
Boyd (1970)				
Typha latifolia				
Young stands	120	412	37	30
***Haberl & Perfler (1989)**				
Phragmites spp				
Domestic sewage			10-38	18-51
Hocking (1989)				
Phragmites australis				
May	870	374	3.6	37.3
September	350	72.4	8.1	57.3
November	150	210	6.2	29.7

Continued next page.

	Fe μg g^{-1}	Mn μg g^{-1}	Cu μg g^{-1}	Zn μg g^{-1}
Table 3.4 continued				
***Meiorin (1989)**				
Scirpus spp and *Typha* spp				
Urban runoff		100-1200		7-41
***Lan et al (1990)**				
Typha and Phragmites				
Lead-zinc mine effluent			3-13.9	30-341
***Ohlendorf (1986)**				
Emergent species				
Agricultural			4.92	13.7
Peverly (1985)				
Sparganium, Spagittaria and Scirpus	3200-3600		7.9-19.3	74-131
Peverly *et al.* (1995)				
Phragmites australis				
September	94.2	87.5	16.6	2.88
Surface *et al.* (1993)				
Phragmites australis				
Landfill leachate	65.8	68.9		17.4
Adjacent leachate seep	92.0	60.2		21.4
Suzuki *et al.* (1989a)				
Phragmites				
Unpolluted			8	40
Taylor and Crowder (1983)				
Typha latifolia				
Tailings sites	21-905	21-3080	3-24	5-101
***Zhang et al (1990)**				
Typha				
Urban runoff and treated effluent			17-45	38-170

* Denotes from Kadlec and Knight (1996).

Allocation of micronutrients within the plant to prevent toxicity effects in young tissue is also regarded as a tolerance mechanism (Dunbabin and Bowmer 1992; Baker 1981 as cited by Ye *et al.* 1997). Dunbabin (1989 as cited by Dunbabin and Bowmer 1992) reported higher micronutrient concentrations in older senescent tissue than in the younger green tissue. In the study by Hocking (1989), this generalisation held for Mn and Fe concentrations in *Phragmites australis*, but not for other metals such as Zn and Cu.

The present study did not discriminate between senescing or young tissue at each sampling interval, however, young (September and November) and senescent (May and August) tissue dominated at particular seasons (except for *Triglochin*). Generally, very little seasonal variation in micronutrient concentration was observed, however, in the case where seasonal differences were observed, *Phragmites*, *Typha* and *Bolboschoenus* exhibited minimum Cu and Zn concentrations in tissue of the winter season rather than young, early season tissue.

The increase in Fe, Mn and Zn concentrations in the above ground tissue of *Triglochin* and *Bolboschoenus* (Mn and Zn only) in wetland 3 compared to wetland 1 suggests that plants either altered the exclusion and distribution of metals within the plant, or that metals were more available for plant uptake in wetland 3. Generally metals such as Fe become more bioavailable under anaerobic conditions (e.g., ferric (Fe^{3+}) to the more soluble ferrous (Fe^{2+}) iron, (Dunbabin and Bowmer 1992; Kadlec and Knight 1996) and conditions of low pH (increased dissolution of hydrous metal oxides, and increased competition for exchange sites, Dunbabin and Bowmer 1992). The Willunga system had a near neutral pH and high BOD (see section 2.2.4.). Therefore, the higher concentrations of metals in the plant tissue of wetland 3 may have been due to an increased bioavailability of those metals induced by the anaerobic nature of the wetland. Differences in micronutrient concentration between the two wetlands were not observed in *Phragmites* and *Typha*. Potential rhizosphere modifications in these two species, resulting from oxygen supplied via convective flow (Armstrong and Armstrong 1991; Brix *et al.* 1992), may have been sufficient to convert the iron (and other micronutrients) to the less available ferric (Fe^{3+}) form (Ye *et al.* 1997) in microsites of the rhizosphere, therefore, restricting the uptake of the micronutrients despite the anoxic nature of the wetland.

Predicting micronutrient concentrations in above ground tissue of emergent plants for a given season is difficult due to changes in micronutrient availability and uptake determined by wetland chemistry (governed by redox and pH) and plant level tolerance mechanisms such as exclusion and accumulation. Therefore, using harvesting as a tool for micronutrient removal may be limited by the unpredictable storage in plant tissue (Dunbabin and Bowmer 1992).

3.2.3.2. *CNP Storage*

Seasonal variations in nutrient storage (g nutrient per m^2) recorded in the four Willunga species over the 12 month period were due to changes in both nutrient concentration (section 3.2.2.1) and dry weight per square metre (section 2.3.2.1). Generally, the seasonal patterns of nutrient concentration, dry weight and therefore, storage were similar (i.e. concentration, dry weight and storage low in winter and high in summer). The fact that nutrient concentration decreased and this decrease was disproportionate (i.e. decrease in nitrogen and phosphorus concentration was greater than decrease in carbon concentration) suggests that processes other than fragmentation (i.e. translocation, leaching, microbial activity) were contributing to the observed reductions in nutrient storage. In contrast, tissue nutrient concentration of *Triglochin* was relatively constant throughout the year, and therefore, changes in dry weight per m^2 were the major contributor to the variation in nutrient storage.

Nitrogen and phosphorus storage from the literature were assembled for comparison in Table 3.3. The total nutrient storage recorded for the four species at the Willunga constructed wetland system were similar to or higher than other studies for the corresponding season and species. The nitrogen and phosphorus storage recorded for *Triglochin* by Adcock and Ganf (1994) was over four times higher than that recorded for the same species at Willunga. Even though the nitrogen and phosphorus concentration recorded by Adcock and Ganf (1994) was less than that recorded at Willunga (see Table 3.3), the very large biomass (13171 g m^{-2}, see Table 2.11) resulted in a greater nutrient storage than that for *Triglochin* at Willunga.

A measure of efficiency was constructed (Table 3.5) by expressing the maximum nutrient storage for the four species as a percentage of the annual nutrient loading to the wetland (loadings from section 2.2.4; 334.9 g N m^{-2} yr^{-1} and 126.2 g P m^{-2} yr^{-1}). The maximum storage indicates the greatest potential nutrient removal from a single annual harvest (February 1997 for *Triglochin* and February 1996 for the other three species), and the efficiency expresses that removal as percentage of the total annual nutrient loading. This measure of efficiency does not account for any reduction in nutrient load of wetland 3 by prior nutrient removal within wetlands 1 and 2. The current value of efficiency may therefore underestimate efficiency in wetland 3 and using the true loading specific to wetland 3 would yield a more accurate estimate. *Triglochin* recorded the greatest nitrogen efficiency, potentially removing 21% of the annual load and *Bolboschoenus* the greatest phosphorus efficiency potentially removing 13% of the annual load. The high nitrogen efficiency observed for *Triglochin* and high phosphorus efficiency for *Bolboschoenus* was consistent with the observations of high nitrogen and phosphorus accumulation from the N:P ratios (Figure 3.7).

Table 3.5: Nitrogen (N) and phosphorus (P) efficiency (maximum nutrient storage expressed as a percentage of annual nutrient input) for four species of aquatic macrophyte at the Willunga wetland system.

	Bolboschoenus	*Phragmites*	*Triglochin*	*Typha*
N efficiency	17%	18%	21%	16%
P efficiency	13%	5%	8%	6%

This information could be applied to management of systems and to the removal of nutrients through plant harvesting. Wetlands treating a source water high in phosphorus may benefit from plantings of a high phosphorus accumulator such as *Bolboschoenus*, whereas if nitrogen is the nutrient of concern, planting and harvesting *Triglochin* or *Zizania* may be more appropriate. However, in some cases of high phosphorus, the large seasonal variation and potential source of nutrient during winter senescence in *Bolboschoenus* may be undesirable and therefore, *Triglochin* (which maintained constant storage with season) may be more appropriate.

3.3. Section 2:

Manipulations of Harvest Protocol for Maximum Nutrient Removal:

3.3.1. Methods:

3.3.1.1. 12 month harvest experiment

The experiment was conducted in wetland 1 over a 12 month period from February 1996 to February 1997. Ten 0.5 x 0.5 m randomly selected replicate plots were cut for each harvest regime in areas of monospecific stands of emergent species: *Typha domingensis* and *Bolboschoenus medianus* at the time intervals indicated in Table 3.6. All above ground material within each plot was removed to the substrate level at each harvest interval. The single harvest of regime 4 (in February 1997) served as a control for the multiple harvest regimes 1, 2 and 3. These regimes were chosen to encompass both timing and frequency of harvest. In particular, the regimes were designed to encourage new shoot growth more than once within a growing season, and determine the potential nutrient removal from such a rigorous harvest regime.

Table 3.6: Harvest protocol for four harvest regimes imposed at the Willunga wetland system on *Bolboschoenus* and *Typha*. Shaded area (*) represent intervals where harvest occurred.

Harvest Regime	Summer (Feb 96)	Autumn (May 96)	Winter (Aug 96)	Spring (Nov 96)	Summer (Feb 97)
Regime 1	*	*	*	*	*
Regime 2	*		*		*
Regime 3		*		*	*
Regime 4					*

At each harvest interval, a single shoot from the middle of each 0.5 x 0.5 m plot was collected and the density of shoots (in a 0.25 x 0.25 m area around the single shoot) recorded. Plant material was dried at 80° C, and analysed for C, N & P using the methods described in section 3.2.1. The dry weight (dwt), C, N & P removed at each harvest interval was summed to determine the total removal for each regime. The total storage (dwt, C, N & P) per shoot was multiplied by the density for the corresponding plot to present the data per area. In order to minimise the number of nutrient analyses, it was assumed that plots were similar until a harvest had been imposed, therefore, one set of replicate plots (10 for dwt and 3 for nutrient analysis) was used to construct the data for all regimes at the beginning of the experiment (February 1996) and two sets after the imposition of the first harvest (May 1996).

Data were analysed using a model 1, two-tailed three-way analysis of variance, with species, month and harvest regime the factors. Post-hoc analyses were performed using Tukey-Kramer tests. Arcsine transformations were applied to percentage and proportion data. Logarithmic transformations were applied to all other data (to adjust for the skewed distribution). Data were found to be normal using a Shapiro-Wilk test for normality. All statistical analysis was performed using JMP software (SAS Institute Inc. 1989). P values stated in the text were from the highest level interaction where a significant result was recorded. For example, if the three-way interaction was not stated then that level of interaction was not significant, and similarly, only those two-way interactions with significant interactions were stated in the text. Tables of ANOVA results can be obtained from the author upon request.

3.3.1.2. *Three species harvest experiment*

The experiment was conducted in wetland 1 and wetland 2 at Willunga over a three month period from November 1999 to February 2000. Eight randomly selected replicate 0.5 x 0.5 m plots were chosen for each of the harvest regimes (Table 3.7) in monospecific stands on the perimeter regions for *Phragmites* and *Bolboschoenus*. All above ground material within each plot was removed at each harvest interval. Two harvest protocols were applied to *Triglochin* in the surface flow sections: harvest of emergent material (above the water surface) and complete harvest of above ground material (to substrate level, below water surface). Eight 0.5 x 0.5 m plots were cut both above and below the water surface for each regime. Regimes were selected on the basis of result from the 12 month harvest experiment (section 3.3.2.1) that determined the times of maximum nutrient removal were late spring and late summer.

Table 3.7: Harvest protocol for three harvest regimes imposed at the Willunga wetland system on *Bolboschoenus*, *Phragmites* and *Triglochin*. Shaded area (*) represent intervals where harvest occurred.

Harvest Regime	Spring (Nov 99)	Summer (Feb 00)
Regime 1	*	*
Regime 2	*	
Regime 3		*

The same methodology as described in section 3.3.1.1 was employed for plant collection and nutrient determination, except that nitrogen concentration was analysed using ICP techniques (by Waite Analytical Services) and carbon concentration was not determined. Data were analysed and transformed in a similar manner to those in section 3.3.1.1.

3.3.2. Results:

3.3.2.1. *12 month harvest experiment*

Concentration (mg g^{-1}) of carbon in above ground tissue was similar between the two species (*Bolboschoenus* and *Typha*) across harvest regime (three multiple and one single regimes) and time (12 month period, five sampling times) ($P = 0.9905$) (Table 3.8).

The tissue nitrogen concentration in the standing above ground material at each sampling interval responded to a harvest regime x month interaction ($P < 0.0001$) (Table 3.8.). Generally, tissue from plants which had been harvested in the immediately preceding season had a higher nitrogen concentration than tissue which had not been harvested (exception being after the onset of new shoots in spring when all tissue had a high nitrogen concentration). Seasonal variations in tissue nitrogen concentration were similar between *Bolboschoenus* and *Typha*, except in February 1997 when the concentration in *Bolboschoenus* tissue was greater than that of *Typha* tissue ($P = 0.0033$, species x month interaction).

The phosphorus concentration in the above ground tissue at each sampling interval could only be explained by an interaction between species, harvest regime and month ($P = 0.0365$) (Table 3.8). As observed in section (3.2.2.1.), *Bolboschoenus* generally had a higher phosphorus concentration and under went greater seasonal variation than *Typha*. As with nitrogen concentration, tissue from plants harvested in the immediately preceding season had a higher phosphorus concentration than tissue which had not been harvested (exception being after the onset of new shoots in spring when all tissue had a high phosphorus concentration).

Table 3.8: Carbon (C), nitrogen (N) and phosphorus (P) concentration (mg g^{-1}) at various intervals over a 12 month period for two species subjected to four harvest regimes (n=10, ± standard deviation). Zero values indicate that there was no standing biomass at that interval.

		Bolboschoenus				*Typha*			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
February 96	**C**	450	450	450	450	446	446	446	446
		(±27.2)	(±27.2)	(±27.2)	(±27.2)	(±5.6)	(±5.6)	(±5.6)	(±5.6)
	N	14.0	14.0	14.0	14.0	14.8	14.8	14.8	14.8
		(±4.68)	(±4.68)	(±4.68)	(±4.68)	(±0.64)	(±0.64)	(±0.64)	(±0.64)
	P	4.0	4.0	4.0	4.0	2.1	2.1	2.1	2.1
		(±1.00)	(±1.00)	(±1.00)	(±1.00)	(±0.66)	(±0.66)	(±0.66)	(±0.66)
May	**C**	443	443	448	448	444	444	446	446
		(±9.87)	(±9.87)	(±3.60)	(±3.60)	(±9.24)	(±9.24)	(±3.95)	(±3.95)
	N	17.1	17.1	10.1	10.1	19.7	19.7	11.5	11.5
		(±1.63)	(±1.63)	(±1.47)	(±1.47)	(±3.81)	(±3.81)	(±2.49)	(±2.49)
	P	2.4	2.4	2.2	2.2	2.5	2.5	1.8	1.8
		(±0.87)	(±0.87)	(±0.28)	(±0.28)	(±0.29)	(±0.29)	(±0.38)	(±0.38)
August	**C**	0.0	457	0.0	459	449	450	447	458
		(±0.00)	(±9.52)	(±0.00)	(±2.6)	(±3.9)	(±3.0)	(±4.2)	(±5.5)
	N	0.0	9.8	0.0	7.7	18.3	17.0	18.4	9.8
		(±0.00)	(±1.57)	(±0.00)	(±1.53)	(±3.68)	(±1.81)	(±1.23)	(±0.50)
	P	0.0	0.3	0.0	0.9	3.1	3.3	2.8	1.4
		(±0.00)	(±0.03)	(±0.00)	(±0.38)	(±0.89)	(±1.22)	(±0.70)	(±0.28)
November	**C**	443	436	435	444	447	444	443	438
		(±6.6)	(±5.2)	(±8.4)	(±10.8)	(±13.1)	(±3.7)	(±6.3)	(±5.2)
	N	23.0	23.4	23.2	21.3	20.5	21.4	21.2	19.6
		(±1.45)	(±2.15)	(±2.93)	(±1.39)	(±1.80)	(±2.00)	(±3.65)	(±2.97)
	P	5.4	5.6	5.5	5.7	2.5	2.6	2.6	2.6
		(±0.95)	(±0.22)	(±0.08)	(±0.16)	(±0.63)	(±0.25)	(±0.54)	(±0.56)
February 97	**C**	444	447	450	452	440	439	438	431
		(±5.6)	(±5.5)	(±4.6)	(±7.9)	(±2.9)	(±7.9)	(±16.9)	(±11.0)
	N	23.9	19.2	19.3	10.1	16.4	10.9	15.9	12.6
		(±3.57)	(±3.77)	(±0.68)	(±0.87)	(±1.65)	(±1.68)	(±3.96)	(±4.00)
	P	5.0	3.8	4.0	2.0	2.1	1.4	2.3	2.5
		(±0.92)	(±1.04)	(±0.15)	(±1.02)	(±0.49)	(±0.05)	(±0.38)	(±0.94)

The standing above ground biomass present at each sampling interval indicated regrowth potential of the plants after the imposition of the various harvest treatments (Table 3.9.). Harvesting decreased dry weight per square metre at each sampling interval for plants under any harvest regime compared to control plants (i.e., previously unharvested shoots, regime 4). *Bolboschoenus* plants (control and all harvest regimes) recorded greater seasonal variation in dry weight per square metre than *Typha*. This variation was such that the dry weight of *Bolboschoenus* was similar to *Typha* in February (1996 and 1997, significantly less than *Typha* in May, and significantly greater than *Typha* in November. These differences in dry weight could only be explained by a regime x species x month interaction ($P < 0.0001$).

Storage of carbon, nitrogen and phosphorus per square metre at each interval was a function of changes in both dry weight and tissue nutrient concentration (Table 3.9.). The observed trends in nutrient storage per square metre were more similar to the responses of dry weight per square metre than to the responses of nutrient concentration (regime x species x month interactions: carbon, $P < 0.0001$; nitrogen, $P < 0.0001$; and phosphorus, $P < 0.0001$). These variations were most pronounced for nitrogen and phosphorus.

The total dry weight and nutrients accumulated by the end of the experimental period under each harvest regime was calculated by summing the appropriate (from Table 3.6.) dry weight, carbon, nitrogen and phosphorus storage per square metre at each sampling interval. Harvest regime significantly influenced the total dry weight ($P < 0.0001$), carbon ($P < 0.0001$) and nitrogen ($P < 0.0001$) accumulated by the end of the experiment (to February 1997), with greatest accumulation under regime 1 and 2 and the least under regime 4 the least (Table 3.10.). There was no difference between species for the corresponding harvest regime in the total accumulation of dry weight, carbon or nitrogen (dry weight, $P = 0.2685$, carbon $P = 0.3737$ and nitrogen $P = 0.8653$).

Table 3.9: Dwt weight (dwt) carbon (C), nitrogen (N) and phosphorus (P) storage (g m^{-2}) at various intervals over a 12 month period for two species subjected to four harvest regimes (n=10, ± standard deviations). Zero values indicate that there was no standing biomass at that interval.

		Bolboschoenus				***Typha***			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
February 96	**dwt**	4145	4145	4145	4145	3593	3593	3593	3593
		(±1635)	(±1635)	(±1635)	(±1635)	(±1558)	(±1558)	(±1558)	(±1558)
	C	1864	1864	1864	1864	1601	1601	1601	1601
		(±735)	(±735)	(±735)	(±735)	(±694)	(±694)	(±694)	(±694)
	N	58.0	58.0	58.0	58.0	53.0	53.0	53.0	53.0
		(±22.9)	(±22.9)	(±22.9)	(±22.9)	(±23.0)	(±23.0)	(±23.0)	(±23.0)
	P	16.5	16.5	16.5	16.5	7.5	7.5	7.5	7.5
		(±6.5)	(±6.5)	(±6.5)	(±6.5)	(±3.2)	(±3.2)	(±3.2)	(±3.2)
May	**dwt**	104	104	834	834	494	494	2852	2852
		(±74.0)	(±74.0)	(±263)	(±263)	(±271)	(±271)	(±514)	(±771)
	C	46.1	46.1	373.1	373	219	219	1272	1272
		(±32.8)	(±32.8)	(±118)	(±118)	(±121)	(±121)	(±229)	(±344)
	N	1.8	1.8	8.4	8.4	9.7	9.7	32.7	32.7
		(±1.3)	(±1.3)	(±2.7)	(±2.7)	(±5.3)	(±5.3)	(±5.9)	(±8.8)
	P	0.3	0.3	1.8	1.8	1.2	1.2	5.3	5.3
		(±0.2)	(±0.2)	(±0.6)	(±0.6)	(±0.7)	(±0.7)	(±0.9)	(±1.4)
August	**dwt**	0.0	15.7	0.0	311	121	412	135	1925
		(±0.0)	(±12.1)	(±0.0)	(±66.7)	(±138)	(±287)	(±67.5)	(±394)
	C	0.0	7.2	0.0	143	54.5	186	60.5	882
		(±0.0)	(±5.6)	(±0.0)	(±30.6)	(±62.0)	(±129)	(±30.2)	(±180)
	N	0.0	0.2	0.0	2.4	2.2	7.0	2.5	18.9
		(±0.0)	(±0.1)	(±0.0)	(±0.5)	(±2.5)	(±4.9)	(±1.2)	(±3.9)
	P	0.0	0.0	0.0	0.3	0.4	1.4	0.4	2.8
		(±0.0)	(±0.0)	(±0.0)	(±0.1)	(±0.4)	(±1.0)	(±0.2)	(±0.6)
November	**dwt**	1235	1028	1570	2350	153	1164	727	1496
		(±315)	(±244)	(±423)	(±266)	(±174)	(±757.)	(±681)	(±554)
	C	547	449	682	1043	68.7	517	322	655
		(±139)	(±106)	(±184)	(±118)	(±78.1)	(±336)	(±302)	(±243)
	N	28.4	24.1	36.4	50.0	2.8	24.9	15.4	29.4
		(±7.2)	(±5.7)	(±9.8)	(±5.7)	(±3.2)	(±16.2)	(±14.4)	(±10.9)
	P	6.7	5.8	8.7	13.5	0.5	3.1	1.9	3.8
		(±1.7)	(±1.4)	(±2.3)	(±1.5)	(±0.5)	(±2.0)	(±1.8)	(±1.4)

Continued next page

		Bolboschoenus				***Typha***			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Table 3.9 continued									
February 97	**dwt**	745 (±403)	1018 (±490)	943 (±414)	2738 (±175)	1058 (±592)	2075 (±1335)	685 (±578)	2921 (±885)
	C	331 (±179)	455 (±219)	425 (±186)	1237 (±79)	473 (±265)	910 (±585)	300 (±253)	1260 (±382)
	N	17.8 (±9.6)	19.5 (±9.4)	18.2 (±8.0)	27.6 (±1.8)	21.7 (±12.1)	22.6 (±14.5)	10.9 (±9.2)	36.8 (±11.1)
	P	3.7 (±2.0)	3.8 (±1.9)	3.8 (±1.7)	5.5 (±0.4)	2.6 (±1.5)	2.9 (±1.9)	1.5 (±1.3)	7.2 (±2.2)

The total phosphorus accumulated by the end of the experiment was influenced by an interaction between regime x species ($P < 0.0001$) (Table 3.10.). *Bolboschoenus* accumulated more phosphorus per square metre (27.1g, 20.3g and 14.3g respectively) under the multiple harvest regimes (regimes 1, 2 & 3) than *Typha* (12.2g, 11.8g and 8.7g). However, under the single harvest regime (4), phosphorus accumulated by *Typha* (7.2g) exceeded that of *Bolboschoenus* (5.5g). For both species, most phosphorus was harvested under regime 1 (*Typha* regime 1 = regime 2), and the least under regime 4.

Table 3.10: Dry weight (dwt), carbon (C), nitrogen (N) and phosphorus (P) harvested (g m-2) over a 12 month period for two species subjected to four harvest regimes (n=10, ± standard deviations).

	Bolboschoenus				***Typha***			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
dwt	6229 (±1797)	5179 (±1486)	3347 (±613)	2738 (±175)	5418 (±1459)	6080 (±1429)	4264 (±1114)	2921 (±885)
Carbon	2788 (±807)	2326 (±668)	1480 (±272)	1237 (±78.8)	2417 (±650)	2697 (±632)	1894 (±492)	1260 (±382)
Nitrogen	106 (±27.7)	77.7 (±20.6)	63.0 (±12.2)	27.7 (±1.8)	89.4 (±23.3)	82.5 (±18.9)	59.0 (±19.3)	36.8 (±11.1)
Phosphorus	27.1 (±7.5)	20.3 (±5.9)	14.3 (±2.8)	5.5 (±0.4)	12.2 (±3.2)	11.8 (±2.6)	8.7 (±2.6)	7.2 (±2.2)

3.3.2.2. *Three species harvest experiment*

Nitrogen concentration in the above ground tissue of *Triglochin* was significantly ($P < 0.0001$) greater than *Bolboschoenus* and *Phragmites* tissue (Table 3.11.) at each sampling interval. Additionally, the nitrogen concentration in *Triglochin* tissue cut below the water surface was significantly ($P < 0.0001$) greater than that cut above the water surface. In contrast to previous observations (section 3.3.2.1.), neither month ($P = 0.7718$) or harvest regime ($P = 0.2153$) influenced the tissue nitrogen concentration of all three species.

Phosphorus concentration in above ground tissue of *Bolboschoenus* was significantly ($P < 0.0001$) greater than in *Triglochin* tissue cut above the water surface (tissue cut below the water surface was intermediate). *Phragmites* recorded the lowest tissue phosphorus concentration of all species ($P < 0.0001$) (Table 3.11.). Neither month ($P = 0.4919$) or harvest regime ($P = 0.4539$) influenced tissue phosphorus concentration for all three species, which was consistent with nitrogen concentration in this experiment, but contrary to previous observations (section 3.3.2.1.).

Table 3.11: Carbon (C), nitrogen (N) and phosphorus (P) concentration (mg g^{-1}) at various intervals over a three month period for three species subjected to three harvest regimes (n=10, ± standard deviations).

	Bolboschoenus			*Phragmites*			*Triglochin* (cut above water)			*Triglochin* (cut below water)		
	1	2	3	1	2	3	1	2	3	1	2	3
November												
N	21.2	21.2	21.2	21.3	21.3	21.3	31.4	31.4	31.4	35.1	35.1	35.1
	(±1.6)	(±1.6)	(±1.6)	(±2.0)	(±2.0)	(±2.0)	(±7.7)	(±7.7)	(±7.7)	(±0.64)	(±0.64)	(±0.64)
P	4.9	4.9	4.9	2.0	2.0	2.0	4.0	4.0	4.0	4.6	4.6	4.6
	(±1.8)	(±1.8)	(±1.8)	(±0.3)	(±0.3)	(±0.3)	(±0.5)	(±0.5)	(±0.5)	(±0.01)	(±0.01)	(±0.01)
February												
N	20.1	20.1	17.2	21.3	21.3	18.6	32.9	32.9	30.7	43.3	43.3	32.0
	(±3.7)	(±3.7)	(±3.4)	(±5.5)	(±5.5)	(±2.9)	(±4.5)	(±4.5)	(±7.2)	(±9.1)	(±9.1)	(±6.8)
P	5.2	5.2	4.5	2.0	2.0	2.3	4.3	4.3	3.7	5.3	5.3	3.9
	(±1.0)	(±1.0)	(±1.0)	(±0.3)	(±0.3)	(±0.3)	(±0.6)	(±0.6)	(±0.1)	(±1.4)	(±1.4)	(±0.2)

The standing above ground biomass present at each sampling interval was an indicator of regrowth potential of the plants after the imposition of the harvest treatments (Table 3.12.). *Bolboschoenus* and *Triglochin* plants under regimes 1 and 2 regrew after the harvest in November, and by February achieved biomass similar to that at the time of the harvest in November (but less than plants previously unharvested at February). *Phragmites,* however, displayed poor regrowth ability with only 84.9 g dwt m^{-2} achieved by February after a harvest in November. This variation in dry weight responses to harvest regime was explained by a regime x species x month interaction ($P = 0.0435$).

The nitrogen and phosphorus storage per square metre at each interval appeared to be driven primarily by the changes in dry weight, as changes in concentration were only minor. Patterns of variation and the regime x species x month interaction ($P = 0.0204$) for phosphorus storage were similar to that for dry weight, and even though not significant ($P = 0.0529$), the same trend was observed for nitrogen storage.

Maximum accumulation of dry weight was recorded under regime 1 (1930.5g) for *Bolboschoenus*, regime 1 (1893.6g) and regime 2 (1808.7g) for *Phragmites,* and regime 1 (1797.2g) and 3 (1709.2g) for *Triglochin* (Table 3.13). These varying responses were confirmed by a significant regime x species interaction ($P = 0.0317$).

The nitrogen accumulated to the end of experiment was greater in *Triglochin* than for the other species and *Triglochin* plants cut below the water surface accumulated more than plants cut above the water surface (Table 3.13.). Like the response observed for dry weight, the response of total nitrogen storage in the three species could only be explained by a regime x species interaction ($P = 0.0169$) (Table 3.13) i.e., *Bolboschoenus* accumulated maximum nitrogen under regime 1 (40.1g N), *Phragmites* under regime 1 (40.2g N) and 2 (38.4g N), *Triglochin* under regime 1 (67.5g N) and 3 (54.6g N).

Table 3.12: Dry weight (dwt) carbon (C), nitrogen (N) and phosphorus (P) storage (g m^{-2}) at various intervals over a three month period for three species subjected to three harvest regimes (n=10, ± standard deviations).

		Bolboschoenus			*Phragmites*			*Triglochin* (cut above water)			*Triglochin* (cut below water)		
		1	2	3	1	2	3	1	2	3	1	2	3
November													
	dwt	1209	1209	1209	1809	1809	1809	679	679	679	968	968	968
		(±426)	(±426)	(±426)	(±841)	(±841)	(±841)	(±217)	(±217)	(±217)	(±374)	(±374)	(±374
	N	25.6	25.6	25.6	38.4	38.4	38.4	21.3	21.3	21.3	34.0	34.0	34.0
		(±9.0)	(±9.0)	(±9.0)	(±17.9)	(±17.9)	(±17.9)	(±6.8)	(±6.8)	(±6.8)	(±13.1)	(±13.1)	(±13.1
	P	5.9	5.9	5.9	3.7	3.7	3.7	2.7	2.7	2.7	4.4	4.4	4.4
		(±2.1)	(±2.1)	(±2.1)	(±1.7)	(±1.7)	(±1.7)	(±0.9)	(±0.9)	(±0.9)	(±1.7)	(±1.7)	(±1.7)
February													
	dwt	722	722	2107	84.9	84.9	1272	947	9467	1499	541	541	1709
		(±780)	(±780)	(±1584)	(±121)	(±121)	(±922)	(±1078)	(±1078)	(±648)	(±592)	(±592)	(±301
	N	14.5	14.5	36.3	1.8	1.8	23.6	31.1	31.1	46.0	23.4	23.4	54.6
		(±15.7)	(±15.7)	(±27.3)	(±2.6)	(±2.6)	(±17.1)	(±35.4)	(±35.4)	(±19.9)	(±25.6)	(±25.6)	(±9.6)
	P	3.8	3.8	9.5	0.2	0.2	2.9	4.1	4.1	5.5	2.9	2.9	6.7
		(±4.1)	(±4.1)	(±7.1)	(±0.2)	(±0.2)	(±2.1)	(±4.6)	(±4.6)	(±2.4)	(±3.2)	(±3.2)	(±1.2)

Total phosphorus accumulation was greater ($P = 0.0045$) under harvest regime 1 than under regime 2 (regime 3 intermediate) (Table 3.13). *Bolboschoenus* accumulated significantly more phosphorus per square metre ($P < 0.0001$) than *Triglochin* cut above the water surface and *Phragmites* (*Triglochin* cut below the water surface intermediate).

Table 3.13: Dry weight (dwt), carbon (C), nitrogen (N) and phosphorus (P) accumulated (g m^{-2}) over a three month period for three species subjected to three harvest regimes (n=10, ± standard deviations).

	Bolboschoenus			*Phragmites*			*Triglochin* (cut above water)			*Triglochin* (cut below water)		
	1	2	3	1	2	3	1	2	3	1	2	3
dwt	1931	1209	2107	1894	1808.7	1272	1660	679	1499	1797	968	1709
	(±1045)	(±426)	(±1584)	(±951)	(±841)	(±922)	(±1125)	(±217)	(±648)	(±509)	(±374)	(±301)
Nitrogen	40.1	25.6	36.3	40.2	38.4	23.6	53.5	21.3	46.0	67.5	34.0	54.6
	(±21.4)	(±9.0)	(±27.3)	(±20.2)	(±17.9)	(±17.1)	(±36.9)	(±6.8)	(±19.9)	(±22.7)	(±13.1)	(±9.6)
Phosphorus	9.7	5.9	9.5	3.8	3.7	2.9	6.9	2.7	5.5	8.6	4.4	6.7
	(±5.3)	(±2.1)	(±7.1)	(±1.9)	(±1.7)	(±2.1)	(±4.8)	(±0.9)	(±2.4)	(±2.8)	(±1.7)	(±1.2)

3.3.3. Discussion:

3.3.3.1. *12 month harvest experiment*

Bolboschoenus and *Typha* responded favourably to multiple harvest regimes. Harvesting promoted new growth throughout the year (except harvest of *Bolboschoenus* in late autumn, May), and generally, regrowth tissue had a higher nutrient concentration than tissue not previously harvested. In terms of dry weight, carbon and nitrogen removal, there was no advantage in adopting the more intensive regime 1 (five harvests) over regime 2 (three harvests) and no species selection advantage. However, for phosphorus removal choice of species and harvest regime became important.

Efficiencies of nutrient removal were calculated in the same way as in section 3.2.3.2.. The efficiency from the single harvest regime (4) (Table 3.14) yielded less than the optimum storage from the seasonal study (Table 3.5). This suggests that either the harvest regime 4 under-estimated the potential efficiency from a single harvest, or that the seasonal storage study over-estimated efficiency. Regime 3 yielded similar efficiency to that from a single optimum harvest (Table 3.5.) for both species.

Bolboschoenus subjected to regime 1 had an efficiency almost twice that achieved from a single optimum harvest (Table 3.5.), whereas *Typha* subjected to regime 1 had an efficiency slightly less than double that of a single optimum harvest (Table 3.5.). Therefore *Bolboschoenus* responded most favourably to the multiple harvest regime and could achieve a 32% and 22% removal of the annual nitrogen and phosphorus load respectively.

Table 3.14: Nitrogen (N) and phosphorus (P) efficiency (maximum nutrient storage expressed as a percentage of annual nutrient input) for two species of aquatic macrophyte under four harvest regimes (see Table 3.6 for protocol of each regime) at the Willunga wetland system.

	Bolboschoenus				*Typha*			
Regime	1	2	3	4	1	2	3	4
N efficiency	32%	23%	19%	8%	27%	25%	18%	11%
P efficiency	22%	16%	11%	4%	10%	9%	7%	6%

The values of nitrogen and phosphorus storage (g m^{-2} yr^{-1}) from the various sources documented in Table 3.3. represent the potential nutrient removal upon harvesting of the above ground material at that time. The nutrient removal from the multiple harvest regimes was greater (almost double for *Bolboschoenus*) than potential removal from a single harvest at the times data were collected. *Bolboschoenus* under an optimum multiple regime (Table 3.10) achieved increases of 50% dry weight, 50% carbon, 83% nitrogen and 65% phosphorus over a single optimum harvest (Table 3.2.). *Typha* under an optimum multiple regime (Table 3.10.) achieved increases of 55% dry weight, 63% carbon, 69% nitrogen and 63% phosphorus over a single optimum harvest (Table 3.2.).

Suzuki *et al.* (1989b) subjected *Phragmites australis* to a multiple harvest regime; where the first harvest occurred at time of peak nutrient content (storage) and a reharvest in late autumn after growth had ceased. Maximum dry weight, nitrogen and phosphorus recovered from the multiple regime were 3,400 g dwt m^{-2}, 36.5 g N m^{-2}, 3.88 g P m^{-2}, respectively. This represented increases of 14 %, 22 % and 10 % greater than a single optimum harvest, and up to 175% of a single end of season harvest. The potential nutrient recoveries recorded for *Bolboschoenus* and *Typha* in this study were substantially greater than those recorded for *Phragmites australis* by Suzuki *et al.* (1989b) (approximately 2, 3 and 7 times greater for dwt, nitrogen and phosphorus respectively). The higher nutrient removal was due to both greater dry weight and higher nutrient concentrations in plants from the Willunga system. As Suzuki *et al.* (1989b) made no mention of the nutrient load, it is difficult to conclude whether these differences were due to variations in species assimilation capacity, differences in nutrient source, or local growing conditions. The harvest regimes imposed in the current study were more intensive (i.e. more frequent harvesting) than Suzuki *et al.* (1989b) and the long term (2-5 years) sustainability of the wetland system under this type of regime needs be to investigated. As in section 3.2.3.2., *Bolboschoenus* demonstrated a high potential for nutrient removal compared to the more traditional wetland species *Typha* and *Phragmites*.

3.3.3.2. *Three species harvest experiment*

Contrary to the results of the 12 month harvest experiment (3.3.3.1.), harvested plants in this study did not record higher nutrient concentrations compared to previously unharvested plants. However, nutrient concentrations in *Bolboschoenus* and *Phragmites* during the February season were higher than for plants from the 12 month seasonal comparison at an equivalent season (Table A.3). The nutrient storage for *Bolboschoenus* at each month was much lower in this study compared to the previous multiple harvest experiment (section 3.3.3.1.) and the seasonal observations (section 3.2.3.2.). The nutrients accumulated by the end of the experiment for all species, under the multiple regimes, were much less than any of the multiple regimes from the previous experiment (3.3.31.).

The limited regrowth of *Phragmites* after a harvest in November implied an adverse response of that species to harvesting, and therefore, the prolonged use of *Phragmites* for nutrient removal may be limited. However, Suzuki *et al.* (1989b) recorded a positive response in *Phragmites australis* to a multiple harvest regime.

The high nitrogen and phosphorus concentration in *Triglochin* and *Bolboschoenus* respectively was consistent with previous observations (section 3.2.3.1.). The higher nitrogen concentration observed in the *Triglochin* tissue cut below the water surface was similar to the increased phosphorus concentration observed for the submerged portion of *Triglochin* in section 2.3.2.3. Consistent with previous observations (sections 3.3.2.1. and 3.2.2.2.), *Triglochin* recorded the most favourable nitrogen storage and *Bolboschoenus* the most favourable phosphorus storage.

Efficiency was calculated for the three harvest regimes, in the same way as for the 12 month experiment, section 3.3.3.1. *Triglochin* cut below the water surface with a multiple regime recorded the greatest nitrogen efficiency (Table 3.15.). *Bolboschoenus* and *Triglochin* subjected to multiple regimes were similar to each other and recorded the greatest phosphorus efficiency. The maximum efficiency from a multiple harvest was lower than the optimum season efficiencies for each species recorded in Table 3.5. (similar for *Triglochin* cut below the water surface).

Table 3.15: Nitrogen (N) and phosphorus (P) efficiency (maximum nutrient storage expressed as a percentage of annual nutrient input) for two species of aquatic macrophyte under three harvest regimes (see Table 3.7 for protocol of each regime) at the Willunga wetland system. *Triglochin* cut above and below the water level.

	Bolboschoenus			*Phragmites*			*Triglochin* above			*Triglochin* below		
Regime	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
N efficiency	12%	8%	11%	12%	11%	7%	16%	6%	14%	20%	10%	16%
P efficiency	8%	5%	8%	3%	3%	2%	5%	2%	4%	7%	3%	5%

The results from this multiple harvest experiment were not consistent with the results from section 3.3.2.1. and 3.2.2.2. Generally, the nutrient storage (g m^{-2}) was lower for an equivalent time of year, and this was due primarily to differences in dry weight rather than concentration. This study was conducted three years after the multiple harvest experiment and seasonal storage observations. That period of time should have been sufficient to allow removal of the system from any adverse effects of the previous harvesting regimes. Therefore the changes seen may have been due to either annual variations or long term changes to the wetland system. The long term impact of harvesting and the impact of long term wetland changes to nutrient storage within the plant components needs further investigation.

3.4. Conclusion:

Generally, the hypotheses that macronutrient concentrations would be greater early in the growing season and that nutrient storage would be greatest in spring/summer were supported. *Triglochin* also maintained high nutrient concentrations throughout the year, as predicted. However, the hypothesis that micronutrient concentrations would be lower in younger tissue than in older tissue was not generally supported.

Two of the species investigated in this study (*Triglochin* and *Bolboschoenus),* are used infrequently in constructed wetlands, but demonstrated superior nutrient storage ability compared to the more commonly used species; *Typha* and *Phragmites*. *Bolboschoenus* showed the greatest potential for phosphorus storage, whereas *Triglochin* showed greatest potential for nitrogen storage. There may be an advantage to having more than one species present in the wetland, i.e., to have polycultures. For example, *Bolboschoenus* and *Triglochin* have different habitat preferences with the former preferring shallow environments and the latter open water (0.3 m to 1 m). Therefore, a constructed wetland that supports a variety of habitats may be able to achieve high nutrient storage across the entire system. However, in order to maximise removal of plant biomass and associated nutrients, uniformity of habitat may be preferred to allow for ease of harvesting whether manual or mechanical. By employing this information at planning and management stages of constructed wetlands, nutrient storage and removal via the plant sink can be maximised and wetland longevity enhanced.

As anticipated from the hypothesis, nutrient removal by *Bolboschoenus* and *Typha* was increased significantly by imposing multiple harvest regimes. The harvest intervals which contributed the most to the nutrient removal (especially in the case of *Bolboschoenus*) was the harvest in late summer (February) and that in late spring (November). Therefore, high nutrient removal may also be achieved by conducting a multiple harvest regime which imposes the first harvest in late spring and a second in late summer. However, imposing that type of regime on *Triglochin*, *Bolboschoenus* and *Phragmites* failed to achieve the anticipated removal, possibly due to seasonal or long term changes in the wetland rather than flawed harvest protocol.

CHAPTER 4: GROWTH AND PERFORMANCE OF *TRIGLOCHIN* IN RESPONSE TO NUTRIENT DELIVERY IN WATER VERSUS SUBSTRATE:

4.1. Introduction:

Floating macrophytes have aerial and/or floating leaves and access nutrients directly from the water column via their root systems submerged in the water column. Many floating macrophytes, such as *Eichhornia* spp and *Salvinia* spp have been used extensively in surface flow wetlands receiving wastewater due to their very high growth rates, high tissue nutrient concentrations (for example; Oki 1992; Reddy and DeBusk 1985; Tripathi *et al.* 1991) and ability to access nutrients in the water. The highly productive nature of these plants has resulted in many detrimental environmental and social impacts such as clogging of irrigation and navigation channels (Brett 1989; Brix and Schierup 1989a). As a result, floating plants such as *Eichhornia* spp and *Salvinia* spp have been declared noxious weeds in Australia (Sainty and Jacobs 1994), therefore excluding their use in local constructed wetlands.

Many submerged macrophytes have demonstrated ability to access nutrients directly from the water column, via uptake through the stems and leaves (Best and Mantai 1978; Carignan 1982; Huebert and Gorham 1983; Waisel *et al.* 1982). The leaves of submerged macrophytes are thin and/or dissected, giving a large surface area for uptake and the leaf cuticle are thin and relatively permeable for direct ion uptake. However, submergent plants often perform poorly in constructed wetlands (unless being used for a "polishing" of secondarily treated wastewater) as they are not suited to the conditions of high BOD and heavy infestation of epiphytic growth (Brix and Schierup 1989a).

The ability of emergent macrophytes to access nutrients from the water column through leaves and shoots has received limited attention in the literature. Emergent macrophytes may need to maintain some form of cuticle in order to prevent desiccation of the biomass exposed to the atmosphere, therefore reducing the permeability to ions (Warwick and Bailey 1997). Therefore, emergent macrophytes generally rely on their root systems for nutrient uptake. In surface flow systems, emergent macrophytes may require that nutrients move from the water into the substrate before becoming available for uptake. Sub-surface designs may be considered more efficient in the delivery of nutrients to emergent plants as nutrients are supplied at the point of uptake rather than relying on the movement from the water to the substrate.

Triglochin has a "semi-emergent" habit, with leaves able to either float on the water surface, or grow fully emergent. It is unclear whether this species accesses nutrients from the water column similar to submerged species, or relies on uptake via the root system similar to emergent species. Warwick and Bailey (1997) suggested the ability of *Triglochin* to support an emergent habit implies the presence of rudimentary leaf cuticles (to minimize desiccation) and this would restrict movement of solutes through the above ground tissue. Therefore, like fully emergent species, *Triglochin* may rely on the root system for nutrient uptake.

The location of nutrient supply may therefore influence the growth and potential nutrient storage and removal capacity of emergent macrophytes. Emergent plants in surface flow systems may experience nutrient deficiencies relative to other resources such as light and water. The degree of limitation will depend on the nutrient loading and the activity of the other storage (see section 1.5) and removal (see section 1.6) processes. Plants respond to such relative limitations by allocating resources to those organs which acquire the resource in short supply (Chapin *et al.* 1987). Nutrient limitation may induce an allocation of resources to the below ground fraction in order to increase nutrient uptake. Where nutrient availability is high, resources may be allocated to the above ground fraction in order to overcome a relative carbon deficiency.

Triglochin at the Willunga wetland system was established in sections of surface flow as well as sections of subsurface flow (see Table 2.6). It may be expected that the contrasting forms of nutrient delivery may influence the growth and performance *Triglochin*. As depth, sediment type and flow regime were confounded in the design it was difficult to determine the response specifically to nutrient delivery. *Typha* and *Phragmites* have been used in both surface and sub-surface flow constructed wetlands. However, the focus of these studies has generally been flow type, not nutrient supply location (water vs. substrate) therefore, comparisons of plant performance in response to nutrient delivery from published studies are difficult.

A controlled pond-based experiment was conducted to investigate the influence of nutrient supply location and loading on growth and performance of *Triglochin*. Two nutrient loadings were chosen, the lower loading to support maximum productivity (100 g N $m^{-2} yr^{-1}$ 20.8 g P $m^{-2} yr^{-1}$) (when supplied to substrate Morris 1998) and the higher loading (300 g N $m^{-2} yr^{-1}$, 62.5 g P $m^{-2} yr^{-1}$) to investigate responses such as biomass allocation at loads beyond the threshold for maximum productivity.

The aim of this experiment was to:

Investigate the influence of nutrient supply location (i.e. water vs. substrate) at two loadings, on the growth, resource allocation and potential storage and removal capacity of *Triglochin*.

The hypothesis was that *Triglochin* would rely on nutrient uptake from the substrate and therefore total biomass and RGR would be lower when nutrients were supplied to the water column than an equivalent loading added to the substrate.

4.2. Methods:

Single shoot, first season (six month old) *Triglochin* plants, including intact root system, were collected from the barrage zone of the Willunga Constructed Wetland in May 1995. Total fresh weight of all individuals was recorded. An initial harvest of eight representative plants was conducted to establish the fresh to dry weight ratio of the experimental plants. The number of leaves and the length and width of longest leaf were measured and the eight representative plants were separated into various components; shoots, rhizomes, storage roots, fine roots and tubers. This material was dried at 80°C for 72 hr for dry weight determination.

The dry weight for all experimental plants at time zero was estimated using a fresh:dry weight ratio determined from the eight representative plants of the initial harvest (Equation 4.1).

$$dwt = (0.0819768 \times fwt) - 1.373801$$

$$r^2 = 0.98$$

(Equation 4.1)

Nutrients were supplied at three loadings: 0, 100 and 300 g N m^{-2} yr^{-1}. The two nutrient additions (100 and 300 g N m^{-2} yr^{-1}) were chosen to provide sufficient nutrients to support near optimum RGR (Bald, 1994) and were achieved by adding a slow release fertilizer (Osmocote Plus 8-9 month). The weight of product required to achieve the desired loading was calculated using the release rate of Osmocote (Equation 4.2 provided by manufacturer) and the nutrient content (NPK 15+3.3+9.1).

$$\%release = 0.3653 \times days - 0.5219$$

(Equation 4.2)

Nutrient supply location treatments were imposed via nutrient additions to the water only, the substrate only, or by an equal proportion added to the water and substrate (Table 4.1).

Table 4.1: Treatment codes for the nutrient addition regimes and the separate components of each treatment.

Treatment code	Water Loading ($g\ N\ m^{-2}\ yr^{-1}$)	Substrate Loading ($g\ N\ m^{-2}\ yr^{-1}$)
WS0	0	0
W100S0	100	0
W0S100	0	100
W50S50	50	50
W300S0	300	0
W0S300	0	300
W150S150	150	150

Series 2000 Nally bins (product of Nally Ltd.; inside dimensions: 502 x 388 x455 mm; surface area: 0.19 m^2; approximate volume 88 L) were used to create independent environments for individual replicates. Two replicate shoots were planted per bin, with eight replicate plants giving a total of four bins per treatment. With the seven treatments, this gave a total of 56 plants in 28 bins.

Low nutrient sandy loam was added to each bin to a depth of 0.17 m and nutrients for substrate loading treatments were mixed into the substrate before planting. Water depth within the bins was 0.18 m above the surface of the substrate. Nutrient loadings were applied to the water by distributing the Osmocote evenly between two nylon mesh bags (one at each end of the bin) and suspending these bags in the water column using fishing line. Empty bags were suspended in the treatments without water nutrient loads to control for any effect due to the nylon mesh. The sandy loam was capped with a layer of "cricket clay" to act as a buffer for nutrient movement between the substrate and water media. All bins were distributed between two experimental outdoor ponds (4.5m x 3.5 x 1.2m deep) in a semi-random design (i.e. two bins of each treatment in each pond, then randomly arranged within the ponds). Water level within the pond was 0.1 m below the top of the bins, maintaining independent water columns for each replicate bin, but providing even temperature regulation. The plants were given a three week recovery period after planting, and the experiment began in mid July 1995. The experiment ran for 112 days and plants were harvested at the end of October 1995.

At time zero (after the three week recovery), the first measurement of the above ground demographic variables was made. These measurements were repeated at approximately 14 day intervals for the duration of the experiment. The demographic variables measured were; the number of green leaves per shoot, the length and width of the longest leaf per shoot, the number of shoots per plant and the number of new leaves produced since the previous measurement per shoot (determined by marking the youngest recognized leaf at time of each measurement). The area of the longest leaf was calculated by multiplying the length and width of the longest leaf (assuming leaves to be rectangular) and was used to compare the size of leaves between treatments.

At the end of the experiment, plants were removed from the bins and separated into various plant components; above ground components of shoots and flowers; below ground components of rhizomes, storage roots, fine roots and tubers. All material was labelled and dried at 80°C for 72 hours for dry weight determination.

The net relative growth rate was calculated for each treatment using the standing biomass (dry weight) at the beginning and the end of the experiment using Equation 4.3 (from Hunt 1990).

$$RGR = \frac{\ln W_2 - \ln W_1}{(t_2 - t_1)}$$

(mg g^{-1} d^{-1})

(Equation 4.3)

W_1 = Initial dry weight

W_2 = Final dry weight

t_1 = time one

t_2 = time two

Three replicates of whole shoot material were ground, subsampled (500 – 800 mg), and analysed for carbon and nitrogen. Three replicates from treatments W100S0, W0S100 and W300S0 were chosen at random and all above ground tissue of each replicate was ground using a grinding mill. The ground material was thoroughly mixed and a 500 – 700 mg subsample analysed for carbon and nitrogen concentration (by oxidative combustion using a CN200 carbon nitrogen analyser at Roseworthy campus of the University of Adelaide) and phosphorus concentration (by ICP at Waite Analytical Services). As funds did not permit a comprehensive nutrient determination of all treatments, these three treatments were chosen to encompass both variation in loading and location of nutrient supply for treatments which yielded similar total above ground biomass (see Table 4.3). Nutrient storage of the above ground fraction was determined by multiplying the concentration by the total above ground biomass.

Data were analysed using model 1, two-tailed, nested one-way analysis of variance (two way analysis with location and loading as factors was not appropriate as the design was not fully factorial), with "nutrient addition regime" (i.e. the location and loading combination) as a single factor. The experimental design of two plants per bin meant that individual plants were nested within Nally bins. An alternative approach to the nested analysis would have been to consider the Nally bin the unit of replication and pool the data for the two individual plants within each Nally bin. However, as comparisons were to be made at the plant level, the nested analysis was preferred. Post-hoc analyses were performed using Tukey-Kramer tests. As the data were not normally distributed (Shapiro-Wilk test), a square root transformation was performed which improved the distribution of the data. All statistical analysis was performed using JMP software (SAS Institute Inc. 1989). Tables of ANOVA results can be obtained from the author upon request.

4.3. Results:

4.3.1. Leaf Production and Number:

All plants were similar (i.e. approximately four leaves per pot across all treatments $P = 0.9585$ Table 4.2) at the beginning of the experiment (Day = 0, three weeks after planting). The number of leaves per plant remained relatively constant over time for treatments W0S0 and W0S300, whereas the number of leaves for plants in the other treatments increased during the experiment. A significantly greater number of leaves ($P = 0.0312$) was recorded in W0S100 (8.9 ±2.17) than W0S0 (4.6 ±1.51) by Day 67. By Day 112, the number of leaves recorded for W0S0 and W0S300 was significantly ($P < 0.0001$) less than for all other treatments. Additionally, the highest loading delivered in the water, W300S0, recorded significantly ($P < 0.0001$) greater number of leaves (25.7 ± 6.13) than the lower loading in the water, W100S0 (16.5 ± 5.29).

The number of new leaves produced over the entire experimental period was greater ($P < 0.0001$) in treatments W0S100, W150S150, W50S50 and W300S0, than treatment W0S0. The total number of leaves lost over the experimental period per plant was the same across all treatments ($P = 0.1173$) and was therefore independent of nutrient regime and leaf production (Table 4.2). At Day 0, area of longest leaf was similar across all treatment ($P = 0.5419$) with the area ranging between 108.1 and 130.8 cm^2. By Day 112, the area of the longest leaf for treatments W100S0, W0S100, W50S50, W300S0 and W150S150 was significantly greater ($P < 0.0001$) than that recorded for W0S0 and W0S300 (Table 4.2).

Table 4.2: Parameters of leaf number and production for *Triglochin* under 7 nutrient supply treatments (loading and delivery) at various observation intervals (n = 8 ± standard deviations).

	W0S0	W100S0	W0S100	W50S50	W300S0	W0S300	W150S150
Number of leaves							
Day = 0	4.0	3.8	4.1	4.1	3.9	4.0	4.4
	(±1.07)	(±1.03)	(±0.99)	(±0.99)	(±0.99)	(±0.76)	(±2.15)
Day = 15	4.0	4.3	4.3	4.3	4.0	4.8	4.6
	(±0.75)	(±1.49)	(±1.39)	(±1.98)	(±1.31)	(±1.04)	(±1.60)
Day = 28	4.3	4.5	5.4	4.1	4.1	5.4	4.3
	(±1.39)	(±1.69)	(±1.85)	(±1.89)	(±1.89)	(±1.62)	(±1.91)
Day = 42	4.8	5.0	5.6	4.5	5.1	5.3	5.3
	(±1.16)	(±1.85)	(±1.69)	(±2.00)	(±2.47)	(±2.43)	(±1.75)
Day = 57	5.1	6.7	7.9	5.8	8.1	6.6	6.5
	(±1.81)	(±2.87)	(±2.36)	(±1.90)	(±2.91)	(±2.15)	(±2.33)
Day = 67	4.6	7.4	8.9	7.0	8.7	6.0	7.4
	(±1.51)	(±3.20)	(±2.17)	(±2.27)	(±2.56)	(±2.31)	(±3.46)
Day = 86	4.8	11.9	12.8	10.3	14.6	5.1	10.6
	(±1.75)	(±4.58)	(±2.25)	(±3.41)	(±3.64)	(±4.97)	(±4.31)
Day = 99	5.4	14.8	16.3	15.3	18.7	6.1	14.6
	(±1.85)	(±4.62)	(±3.81)	(±4.68)	(±3.64)	(±5.98)	(±4.69)
Day = 112	4.9	16.5	22.0	18.3	25.7	6.0	20.4
	(±2.00)	(±5.29)	(±4.99)	(±5.15)	(±6.13)	(±6.74)	(±6.83)
Total number of leaves produced							
	10.4	21.0	30.8	25.7	34.1	15.9	27.0
	(±4.03)	(±6.98)	(±6.54)	(±3.15)	(±6.67)	(±8.57)	(±10.07)
Total number of leaves Lost							
	9.5	9.3	12.9	10.4	12.6	13.0	14.7
	(±2.45)	(±2.50)	(±2.90)	(±3.10)	(±3.87)	(±3.65)	(±6.43)
Area of Longest Leaf							
Day = 0	120.7	130.8	118.2	108.1	112.6	111.8	143.5
	(±46.48)	(±29.92)	(±33.10)	(±35.57)	(±22.77)	(±45.02)	(±40.12)
Day = 112	106.7	268.7	238.3	254.69	262.8	40.8	206.2
	(±20.92)	(±83.80)	(±39.15)	(±70.11)	(±67.56)	(±37.92)	(±50.69)

4.3.2. Total Biomass:

Total plant biomass (final dry weight) was similar between the nutrient addition treatments W100S0, W0S100, W50S50, W300S0 and W150S150, and ranged from 46.56 g to 86.24 g (Table 4.3). The total biomass of W0S0 (25.55 g) and W0S300 (10.37 g) was significantly (P < 0.0001) lower than the other treatments (except between W0S0 and W150S150).

4.3.3. Above Ground Biomass:

The total above ground biomass (shoots plus flower) was the same for plants of treatments W100S0, W0S100, W50S50, W300S0 and W150S150, with a range of 32.00 to 57.78 g (Table 4.3). Total above ground biomass of W0S0 (6.33 g) and W0S300 (6.98 g) were similar to each other, but significantly (P < 0.0001) lower than that of the other five nutrient addition treatments. Flowering occurred in all treatments except W0S0 and W0S300 and generally made up less than 10% of the above ground biomass (Table 4.3). Biomass of the shoots for W0S0 and W0S300 was significantly (P < 0.0001) lower than that of the other treatments (Table 4.3).

Table 4.3: Final biomass (g) of various plant components of *Triglochin* grown for 112 days under 7 nutrient supply treatments (loading and delivery) (n = 8 ± standard deviations)).

	W0S0	**W100S0**	**W0S100**	**W50S50**	**W300S0**	**W0S300**	**W150S150**
Above ground	6.33 (±1.82)	48.22 (±22.45)	53.02 (±15.21)	43.55 (±22.34)	57.78 (±20.29)	6.98 (±5.62)	32.00 (±17.36)
Shoots	6.33 (±1.82)	43.65 (±20.04)	49.38 (±12.40)	41.12 (±19.39)	53.97 (±16.54)	6.98 (±5.62)	29.98 (±15.51)
Flower	0 (±0)	4.58 (±4.89)	3.65 (±4.92)	2.42 (±4.49)	3.82 (±5.51)	0 (±0)	2.02 (±3.41)
Below ground	19.22 (±7.38)	36.23 (±15.66)	21.78 (±7.66)	28.11 (±15.33)	28.46 (±7.79)	4.67 (±2.72)	14.56 (±9.57)
Storage roots	6.67 (±3.45)	13.33 (±6.29)	7.15 (±2.37)	10.47 (±5.79)	8.68 (±2.02)	1.62 (±1.14)	4.85 (±3.08)
Tubers	1.17 (±1.26)	2.73 (±4.07)	0.44 (±0.68)	1.94 (±2.75)	1.71 (±2.34)	0.08 (±0.16)	0.61 (±0.99)
Rhizome	5.49 (±2.92)	11.99 (±6.86)	10.70 (±4.19)	11.94 (±8.19)	14.64 (±4.87)	2.93 (±1.84)	8.38 (±5.95)
Fine roots	5.89 (±2.38)	8.18 (±4.28)	3.49 (±3.59)	3.76 (±1.45)	3.43 (±1.23)	0.04 (±0.09)	0.71 (±0.43)
Total	25.55 (±8.83)	84.45 (±35.46)	74.80 (±21.03)	71.66 (±34.96)	86.24 (±24.24)	10.37 (±8.05)	46.56 (±24.52)

4.3.4. Below Ground Biomass:

The total below ground biomass recorded for W0S300 (4.67 g) was significantly ($P < 0.0001$) lower than that recorded in other treatments, except W150S150, (14.56 g). The below ground biomass was similar between the other treatments with a maximum total below ground biomass of 36.23 g recorded in W100S0 nutrient addition (Table 4.3).

Tuber biomass made up only a small proportion of the total below ground biomass (<8%) and exhibited high variation within treatments. Consequently, no differences in tuber biomass were detected between treatments (Table 4.3).

Rhizome biomass ranged from 2.93 g (W0S300) to 14.64 g (W300S0) ($P = 0.0004$). Storage roots biomass ranged from 1.62 g (W0S300) to 13.33 g (W100S0) ($P < 0.0001$).

The greatest biomass of fine roots (8.18 g) was recorded for treatment W100S0 and observations at the time of harvest suggested that the majority of these roots were at or near the water-soil interface, implying the roots were of an adventitious nature. This high fine root mass for W100S0 was significantly greater than that recorded for all other nutrient addition treatments ($P < 0.0001$), except W0S0. The fine root biomass was similar between treatments W0S100, W50S50 and W300S0 and the lowest fine root biomass was recorded in W0S300.

4.3.5. RGR:

The relative growth rate (RGR) was calculated for the total plant, and separately for the above and below ground components of each plant (Table 4.4). The RGR for the total plant was significantly lower ($P < 0.0001$) in treatments W0S0 and W0S300 than all other treatments (except W150S150). The RGR recorded for W0S300 was only slightly greater than zero, demonstrating very little net gain of biomass over the course of the experiment. In contrast, the RGR recorded for treatments W100S0, W0S100, W50S50 and W300S0 (15-16 mg g^{-1} d^{-1}) represented considerable net biomass gain.

The RGR of the below ground component for treatment W0S300 was significantly lower than all other treatments ($P < 0.0001$). Treatments W100S0, W0S100, W50S50, W300S0, W150S150 and W0S0 all recorded similar RGR of below ground components (in addition W150S150 was significantly lower than W100S0, W50S50 and W300S0).

The RGR of the above ground component was significantly ($P < 0.0001$) lower in treatments W0S0 and W0S300 than for all other treatments (except between W0S300 and W150S150). The RGR for W0S0 was negative, indicating a net loss of above ground biomass over the experimental period.

Table 4.4: Relative growth rate (RGR; mg g^{-1} day^{-1}) of *Triglochin* under 7 nutrient supply treatments (loading and delivery) after 112 days ($n = 8$). Calculated for total plant and above and below ground components separately.

	W0S0	W100S0	W0S100	W50S50	W300S0	W0S300	W150S150
Total Plant							
	8.0	16.3	15.5	15.8	16.5	1.2	10.4
	(±0.96)	(±5.10)	(±3.44)	(±3.51)	(±2.26)	(±8.52)	(±3.07)
Above Ground							
	-0.2	13.4	14.6	13.7	15.1	2.9	9.2
	(±1.91)	(±6.86)	(±3.78)	(±3.74)	(±2.75)	(±3.13)	(±3.08)
Below Ground							
	17.6	22.1	18.5	20.8	20.4	7.3	13.9
	(±1.13)	(±4.01)	(±2.79)	(±3.88)	(±2.20)	(±6.23)	(±3.76)

4.3.6. Nutrient concentration and content:

The concentration (mg g^{-1}) of carbon, nitrogen and phosphorus was determined in the above ground tissue from plants of treatments W100S0, W0S100 and W300S0 (Table 4.5). Carbon concentration in the above ground tissue ranged between 416 mg g^{-1} and 422 mg g^{-1}, but did not differ between the three treatments ($P = 0.3496$). Nitrogen concentration was significantly lower (P = 0.0035) in treatment W100S0 (21 mg g^{-1}) than the same loading added to the soil (W0S100; 41 mg g^{-1}) and the higher loading added to the water column (W300S0; 35 mg g^{-1}). Phosphorus concentration ranged between 2.2 mg g^{-1} and 2.8 mg g^{-1} and also did not differ between the three nutrient addition treatments.

Table 4.5: Carbon, nitrogen and phosphorus concentration (mg g^{-1}) and total above ground nitrogen and phosphorus storage (g) in above ground portion for *Triglochin* grown for 112 days under 7 nutrient supply treatments (loading and delivery) (n = 8).

		W100S0	W0S100	W300S0
Concentration (mg g^{-1})				
	Carbon	418 (±5.9)	422 (±4.0)	416 (±5.2)
	Nitrogen	21 (±3.7)	41 (±6.4)	35 (±3.3)
	Phosphorus	2.2 (±0.3)	2.8 (±0.7)	2.6 (±0.4)
Above ground Storage (g)				
	Nitrogen	1.01 (±0.47)	2.07 (±0.77)	2.04 (±0.72)
	Phosphorus	0.11 (±0.05)	0.15 (±0.05)	0.15 (±0.05)

Nitrogen and phosphorus storage of the above ground fraction were determined by multiplying concentration by biomass, for those treatments analysed for nutrient concentration (Table 4.5). Nitrogen storage was significantly (P = 0.0064) greater in treatments W0S100 and W300S0 than treatment W100S0. Phosphorus storage ranged between 0.11 and 0.15 g per plant and was not significantly (P = 0.1908) different between nutrient addition treatments.

4.4. Discussion:

4.4.1. General Plant Responses:

The similar above ground biomass and above ground RGR recorded for *Triglochin* from all treatments except W0S0 and W0S300, suggested that the potential harvestable biomass of *Triglochin* plants receiving a loading of at least 100 g N m^{-2} y^{-1} was independent of the location of nutrient addition (i.e. surface vs. sub-surface). However, differences in tissue nutrient concentration and biomass allocation across treatments demonstrated that plants responded differently to nutrient location and loading. The number of leaves lost over the course of the experiment was the same for all treatments despite the higher rate of leaf production in W300S0 compared to W100S0, and suggested that plants retained their leaves to the same degree independent of nutrient addition strategy.

The availability of nutrients for plant uptake and assimilation will depend on the location of nutrient delivery and the subsequent movement of nutrients between other biota, water and soil as regulated by parameters such as concentration gradients, redox, pH and substrate binding capacity and uptake rates of the other biota.

Plants subjected to the nutrient regime W0S300 (300 g N m^{-2} y^{-1} to the substrate) experienced poor growth, due possibly to a toxic load of micronutrients. This was not anticipated, as the same Osmocote product had been used previously to induce substrate loading of 350 g N m^{-2} y^{-1} for *Triglochin* and optimum growth rates were recorded (Bald 1994). Micronutrients in Osmocote are chelated and even though the actual dose could not be determined, a slow, nontoxic release was anticipated. Nutrients such as iron and sulphur become more available and more toxic to plants under the anoxic conditions typical of constructed wetlands (Kadlec and Knight 1996). Nutrient analysis was not conducted for treatment W0S300, however, the levels of Fe and S in plants used in ICP analysis (W100S0, W0S100 and W0S300, Fe 85.3–191 μg g^{-1}; S 0.41 mg g^{-1}; data not shown) were within the range considered normal for plants receiving effluents (Fe up to 3,709 μg g^{-1}; S up to 1.58 mg g^{-1}, Kadlec and Knight 1996).

If treatment W0S300 had suffered from toxicity effects, excessive micronutrient accumulations may have appeared in the other treatments.

Ye *et al.* (1997) investigated the growth response of adult and seedling *Typha latifolia* to mine tailings high in Pb and Zn. Seedlings demonstrated a high degree of susceptibility to the heavy metal concentrations, whereas adult plants had an apparent tolerance. This tolerance in adult plants was attributed to one of two possible mechanisms; more developed arenchymatous tissue in adult plants which facilitated greater oxygen transport and favourable rhizosphere modification, or more effective internal systems to restrict metal translocation from root to shoot. The *Triglochin* plants used in the current study were less than 12 months in age and therefore may have also been highly susceptible to micronutrient toxicity. As adult plants at the Willunga wetland system did not show signs of micronutrient toxicity (see section 3.2.3.1), it may be suggested that *Triglochin* also adopts mechanisms of tolerance which develop with age.

An apparent lag period (67 days) prior to any significant treatment response (number of leaves per shoot) was observed for all regimes. Data from section 2.3.2.1. suggested *Triglochin* could support active growth over that period (July to September). However, the limited initial below ground biomass (a function of plant age) may have suppressed the ability to recover from the experimental set up under winter conditions.

4.4.2. Resource Allocation:

A preferential allocation of resources to the below ground organs was demonstrated in treatment W0S0 with a greater RGR in the below ground component compared to the RGR of the total plant and above ground component. This result is consistent with the observation by Chapin *et al.* (1987) that plant resources are allocated to the organ which acquires the resource in short supply, i.e. in W0S0 plants resources were allocated to the below ground portion responsible for acquiring nutrients, in particular to the fine (resource acquiring) roots. However, sufficient nutrients were not available (in the soil or water) to allow plants to adequately respond to the limitation.

W100S0 (100 g N m^{-2} y^{-1} to the water) plants also preferentially allocated biomass to the below ground component, particularly the resource acquiring fine roots (greater allocation than all other treatments except W0S0). It is unknown whether the roots at the soil-water interface were accessing the nutrients directly from the water column via adventitious roots such as reported for other macrophytes (Kozlowski 1984, as cited by Kadlec and Knight 1996), or at the interface where nutrients may have been associated with the clay particles. In contrast to W0S0, plants of treatment W100S0 had sufficient nutrients available to respond to the deficiency and acquire sufficient nutrients to ultimately achieve above ground biomass equivalent to the other treatments.

4.4.3. Nutrient Storage Potential:

The storage potential of an emergent plant is a function of total harvestable (i.e. above ground) biomass and the nutrient concentration of that biomass (see section 1.5.2.). The phosphorus concentration of the above ground tissue and total phosphorus storage were similar between the three treatments W0S100 W300S0 and W100S0, suggesting a similar degree of either phosphorus acquisition or distribution between treatments. The concentration was approximately half that of *Triglochin* tissue at Willunga (see section 3.2.2.1.). However, the population within this experimental system had only been established for a few months compared to the several years of the field population. Despite the similar total above ground biomass between the nutrient addition treatments, the greater nitrogen concentration in W0S100 and W300S0 led to a greater nitrogen storage potential, in those treatments compared to W100S0. This suggests that an excess uptake of nutrient (luxury uptake), or greater allocation of nutrients to the above ground fraction occurred when the delivery of nutrients was in the substrate or at a high loading in the water, despite the greater allocation to fine roots in treatment W100S0.

Nutrient storage (in the above ground fraction) expressed as a percentage of the nutrient supply (loading) was used as a measure of storage efficiency. The efficiencies were calculated using Equation 4.4 and displayed in Table 4.6.

$$Efficiency = \frac{Capture}{Supply} \times 100$$

Capture = content (g) of above ground biomass per bin
Supply = nutrient release (from Equation 4.2)

Equation 4.4

Net nitrogen and phosphorus stored (two plants per bin) was calculated using the storage in the standing above ground biomass at end of the experiment (Table 4.5). Gross nitrogen and phosphorus accumulated was calculated from net biomass accumulation plus biomass lost over the experimental period (assuming that leaves lost were of same weight (calculated by dividing final weight by final number of leaves) and nutrient concentration as leaves at the end of the experiment).

Triglochin processed nitrogen more efficiently than phosphorus, with net efficiency ranging from 23 - 71 % for N and 8 - 25 % for phosphorus. Gross reached 119% of the nitrogen loading in W0S100. Nutrients, additional to those supplied via Osmocote, may have been available from the clay and sand substrates, however, this is still unlikely to account for the efficiency value exceeding 100%. The assumption that leaf weight and nutrient concentration were constant over time may have over-estimated the nutrient accumulation. The calculation also ignored recycling and translocation of nutrients from senescing leaves. Regardless of these assumptions, it is apparent that a significant proportion of the nutrient loading (especially for nitrogen) would be retrievable via harvesting, and that nutrients were processed most efficiently in treatment W0S100.

This measure of efficiency differed to that for the four species at the Willunga system (section 3.2.3.2). The current calculation expresses nutrient storage as a percentage of the actual amount (g) of nutrient released over the experimental period, as opposed to expressing storage as a percentage of annual load. The net efficiency calculated in this experiment for *Triglochin* with a loading of 300 $g\,N\,m^{-2}\,yr^{-1}$ supplied to the water column (23% for nitrogen and 8% for phosphorus) were similar to that calculated for

Triglochin at the Willunga system (21% for nitrogen and 8% for phosphorus), despite the lower phosphorus concentration in the above ground tissue of the pond experiment.

In addition to nutrient deficiency relative to other resources influencing plant performance, the deficiency of one nutrient relative to other nutrients may also influence plant performance and nutrient assimilation efficiency. Fertilization studies, which vary the relative proportions of nutrients supplied to plants have been used to identify specific nutrient limitations of particular system. Disadvantages associated with cost and time often render such experiment unviable. Koerselman and Meuleman (1996) developed a model of N:P ratio in plant tissue to determine the relative limitation of nitrogen and phosphorus in wetland systems. They suggested the following nutrient concentration ratios: N:P < 15, nitrogen limited; N:P > 16, phosphorus limited, N:P between 14 & 16, no limitation. The N:P ratios for treatments W100S0, W0S100 and W300S0 were 9.5:1, 14.7:1 and 13.7:1 respectively. Therefore, applying the (Koerselman and Meuleman 1996) N:P ratio model, W0S100 was not limited by either N or P (N:P between 14 & 16), whereas both W100S0 and W300S0 were limited by nitrogen (N:P < 14). This suggests that nutrient supply to the water at both loadings (100 and 300 g N m^{-2} y^{-1}) induced a relative nitrogen deficiency, perhaps due to competition for nitrogen from other storage and removal components within the system, but this deficiency was not observed when nutrients were supplied to the substrate at a loading of 100 g N m^{-2} y^{-1}.

Table 4.6: Efficiency calculations and total (g) nutrient supplied over the course of the experiment (via Osmocote) and estimates of Net (in standing biomass at end of the experiment) and Gross (standing biomass plus biomass lost via leaf turnover) accumulation.

			W100S0	**W0S100**	**W300S0**
Nitrogen	**In Osmocote (g)**		5.8	5.8	17.5
	Plant Tissue (Above Ground)	Net Accumulation (g)	2.02	4.14	4.08
		Net Efficiency	35%	71%	23%
		Gross Accumulation (g)	3.14	6.92	6.08
		Gross Efficiency	54%	119%	35%
Phosphorus	**In Osmocote (g)**		1.21	1.21	3.61
	Plant Tissue (Above Ground)	Net Accumulation (g)	0.22	0.30	0.30
		Net Efficiency	18%	25%	8%
		Gross Accumulation (g)	0.33	0.47	0.44
		Gross Efficiency	27%	39%	12%

4.5. Conclusion:

The hypothesis that biomass allocations within plants would be influenced by nutrient supply and location was supported. The hypothesis that greater yield of *Triglochin* would be achieved when nutrients were delivered to the substrate compared to the delivery to the water column was not fully supported in this experiment. Above ground biomass and above ground RGR were similar for all nutrient addition treatments regardless of total loading and location (except when no nutrients were added; W0S0 and when levels were seemingly toxic; W0S300). This was achieved by the plants responding to the relative nutrient limitation (W100S0) by allocating resources to fine roots which acquire nutrients. More specifically, those roots were located at the water-soil interface to access nutrients either directly from the water column or as soon as they entered the clay layer hence improving the efficiency of uptake.

Despite the similarities in above ground biomass, the overall nitrogen storage and potential removal differed between treatments due to the higher nitrogen concentration in W0S100 and W300S0 compared to W100S0. When the nutrient storage was expressed as a proportion of the total nutrient supplied, nitrogen accumulation was more efficient than phosphorus accumulation and treatment W0S100 demonstrated the most efficient processing of supplied nutrient. The implication of this finding may be that in order to maximize nutrient capture in the above ground tissue of *Triglochin* a constructed wetland should provide a subsurface supply of nutrients at a moderate loading.

CHAPTER 5: GROWTH AND YIELD OF *TRIGLOCHIN* AND *TYPHA* WHEN ESTABLISHED IN VARIOUS PLANTING COMBINATIONS:

5.1. Introduction:

Typha and *Triglochin* were the only species to successfully colonise new regions within the Willunga wetland system (see section 2.3.2.2.). Colonisation occurred across two environmental zones; the shallow gravel based subsurface zone and the deep (60cm) clay based surface flow section. Growth and performance of the two species differed between the environmental zones (see section 2.3.2.3.). However, as depth and substrate type were confounded in the design of the system (i.e., shallow depth was also associated with gravel substrate and deep depth with clay substrate), it was difficult to determine the specific response to each factor. Responses to water depth have been widely investigated for a variety of macrophyte species (Blanch *et al.* 1999; Grace 1989; Lieffers and Shay 1981; Rea and Ganf 1994a; Siebentritt and Ganf 2000) whereas plant responses to substrate type have received limited attention. Substrates with small particle size and high organic content, such as clay, tend to have a greater oxygen demand than large particle sized, low organic substrates such as sand and gravel (see Lenssen *et al.* 1999). Oxygen availability to the below ground organs influences plant growth and reproductive ability. For example, White and Ganf (1998) found that the ability to maintain oxygen supply to below ground organs influenced rhizome extension in *Typha domingensis.* Other substrate characteristics (such as nutrient binding ability and resistance to water flow) may also influence plant growth and performance (Lenssen *et al.* 1999; Peverly *et al.* 1995).

Nutrient storage capacity differs between macrophyte species (chapter 3). Therefore, the total nutrient storage and removal from wetlands may be influenced by the relative distributions of species such as *Typha* and *Triglochin*. The influence of floristic composition on nutrient storage and removal can be quantified via studies of biomass yield under a variety of planting combinations. Investigations into the mechanisms of plant growth that control these responses will further enhance this understanding. Growth analysis (i.e. RGR, and its components of LAR and NAR) is often used for investigations of plant growth and function (Lambers and Poorter 1992). Relative growth rate (RGR) is defined as an increase in plant weight per unit of plant weight already present. RGR is a function of two parameters, leaf area ratio (LAR) and net assimilation rate (NAR). LAR is defined as the amount of leaf area per unit total plant weight, and NAR the rate of increase in plant weight per unit leaf area (Evans 1972).

Space may become available in wetlands through seasonal population declines or as a result of management protocols such as harvesting. Insight into the ability of various species to preempt space (i.e. the ability of one species to colonise available space over another species) will aid in achieving desirable floristic compositions within wetlands. *Triglochin* and *Typha* at the Willunga system exploited sexual and asexual colonisation strategies respectively (section 2.3.2.2.). *Triglochin* seedlings successfully established in shallow zones of the wetland. In the deep zone, where conditions were not favourable for seed establishment, *Triglochin* exhibited "phalanx" style (Lovett Doust 1981) rhizome extension. In contrast, *Typha* adopted an opportunistic "guerrilla" strategy (Lovett Doust 1981) and despite a high incidence of flowering and fruiting, no seedling establishment was recorded for *Typha*. Under a situation where colonisation by both *Typha* and *Triglochin* was via rhizome extension, it may be expected that *Typha,* with the guerrilla strategy, would pre-empt available space more effectively than *Triglochin,* with the more conservative phalanx strategy.

Species interactions can be investigated through a variety of controlled experimental conditions. Replacement series designs have been used since the 1960s to explore a diversity of processes, such as species coexistence, exclusion, coadaptation, niche differentiation, abundance, distribution, productivity and diversity (Jolliffe 2000). The design of the standard replacement series uses two or more populations in monoculture and mixtures, with a constant combined density (Harper 1977). Recently, many limitations inherent to the design of replacement series experiments have been highlighted, and the validity of the method questioned (Connolly 1988; Silvertown 1989; Snaydon 1991; Snaydon 1994). However, many authors maintain the validity of the design and stress that interpretation is restricted to the identification of an interaction, not determination of the cause of the interaction (e.g., inter- and intra- specific competition) (Firbank and Watkinson 1985; Firbank and Watkinson 1990). In a recent review of the replacement series design by Jolliffe (2000), it was proposed that the design was valid for comparisons of yield and productivity between monocultures and mixtures, however, biases resulting from the choice of density, initial plant size and time of observation need to be recognised.

Preemption of space and yield comparisons between *Typha* and *Triglochin* were investigated using a replacement series design experiment.

The aims of this chapter were to:

- Suggest outcomes of preemption of the available space by *Triglochin* and *Typha*.
- Determine the resulting biomass of *Typha* and *Triglochin* under varying initial planting proportions (at a constant combined shoot density).
- Investigate the influence of substrate type on the resulting biomass.
- Use RGR and its components (NAR and LAR) to investigate the mechanisms of the responses.

A number of hypotheses were tested in this chapter. Firstly, it was expected that *Typha*, with the aggressive guerrilla style colonisation strategy would preempt space more effectively than *Triglochin*, and as a result *Typha* would dominate when the two species were grown in mixture populations. The other major hypothesis was that plant performance, as determined by biomass and RGR (and components NAR and LAR), would be greater under substrates which provide potentially more favourable oxygen environment (i.e. plant performance would be greater under gravel than clay).

5.2. Methods:

Single-shoot *Triglochin* plants with attached rhizomes were collected from Bool Lagoon, south east of South Australia (37°08′S, 140°41′E) in September 1996. Single-shooted *Typha* and associated rhizomes were collected from the Barker Inlet Wetland site, South Australia (34°49′S, 138°34′E) in September 1996. Total fresh weight was recorded for all individuals and was similar between shoots of the two species. An initial harvest of eight representative plants from each species was conducted to establish the starting condition of the experimental plants. Physical parameters (number of leaves, number of shoots and length of longest leaf) were measured before separating the plants into various plant components; shoots, rhizomes, roots (and tubers, *Triglochin* only). This material was dried at 80°C for 96 hr for dry weight determination.

All treatments were supplied a nutrient loading of 100 g N m^{-2} yr^{-1} to the substrate through the addition of a slow release fertilizer (Osmocote Plus 8-9 months). The quantity of Osmocote required to deliver this loading was determined via the release rate of Osmocote (Equation 4.2) and the nitrogen content (NPK 15+3+9.1).

Series 2000 Nally bins (product of Nally Ltd.; inside dimensions: 502 x 388 x266 mm; surface area: 0.19 m^2; approximate volume 52 L) were used for individual replicates.

Typha and *Triglochin* were grown in a number of planting combinations using a replacement series design, with a constant density of three shoots per bin (Table 5.1).

Table 5.1: Plant proportions of *Triglochin* and *Typha* in replacement series design.

Triglochin	*Typha*
3	0
2	1
1	2
0	3

Three substrate treatments were imposed using distinct media of three particle sizes, "cricket clay" (< 1/256 mm), low nutrient sandy loam (1/8 – 1 mm), and white gravel (10 mm). All Nally bins were filled to approximately 20 cm deep with the appropriate substrate and the Osmocote allocation.

A total of 48 bins were used, with four replicates of three substrate types by four planting combinations. All bins were distributed between three experimental outdoor ponds (4.5m x 3.5 x 1.2m deep) in a semi-random design (i.e. one replicate bin of each treatment in two ponds, with the last two replicate bins in the third pond). Treatments were randomly distributed within the ponds. Water level within the ponds was set at 0.15 m above the substrate surface (approximately 0.1 cm over the top of each bin), therefore all replicates shared a common water column. The experiment began in December 1996, ran for 260 days and was harvested late August/early September 1997.

As the main response of interest was the final yield resulting from the initial planting and substrate conditions and not necessarily short term changes, measurements of the number of leaves per bin were made on an infrequent basis, with five measurements over the 260 day period.

At the end of the experiment, plants were removed from the bins and separated into various plant components; above ground components of shoots and flowers; below ground components of rhizomes and roots (plus tubers in *Triglochin* only). All material was labelled and dried at 80°C for 96 hours for dry weight determination.

The relative growth rate (RGR) was calculated for each treatment using the total dry weight at the beginning and the end of the experiment using equation from Harper (1977) (equation 5.1). The two components of RGR: net assimilation rate (NAR) (equation 5.2 from Harper 1977) and leaf area ratio (LAR) (equation 5.3 from Harper 1977) were also calculated in order to investigate the mechanisms of the observed growth responses. Leaf area was calculated for a subsample of plants using a Delta T meter.

$$RGR = \frac{\ln W_2 - \ln W_1}{(t_2 - t_1)}$$

(mg g^{-1} day^{-1})

(Equation 5.1)

$$\mathrm{NA}R = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\log eL_2 - \log eL_1}{L_2 - L_1}$$

($g\ m^{-2}\ day^{-1}$)

(Equation 5.2)

$$LAR = \frac{L_2 - L_1}{W_2 - W_1} \times \frac{\log eW_2 - \log eW_1}{\log eL_2 - \log eL_1}$$

($m^2\ kg^{-1}$)

(Equation 5.3)

W_1 = Initial dry weight
W_2 = Final dry weight
t_1 = time one (beginning of experimental period)
t_2 = time two (end of experimental period)
L_1 = Initial leaf area
L_2 = Final leaf area

Initial dry weight was estimated for all plants using a fresh:dry weight ratio determined from the destructive harvest of eight representative plants (*Triglochin* represented in equation 5.4 and *Typha* in equation 5.5).

$$(Triglochin)dwt = (0.08278 \times (Triglochin)fwt) - 0.1364$$

$$r^2 = 0.87$$

(Equation 5.4)

$$(Typha)dwt = (0.1011 \times (Typha)fwt) + 0.50028$$

$$r^2 = 0.89$$

(Equation 5.5)

Data were analysed using a model 1, two-tailed two way analysis of variance with substrate type and planting combination the factors. Post-hoc analyses were performed using Tukey-Kramer tests. Log transformations were applied to dry weight data to correct the skewed distributions. Statistical analysis was performed using JMP software (SAS Institute Inc 1989). P values stated in the text were from the highest level interaction where a significant result was recorded. For example, if the two-way interaction was not stated then that level of interaction was not significant, and therefore the P values of the single factors were stated. Tables of ANOVA results can be obtained from the author upon request. Chi squared analysis was performed using the equations from Zar (1987) on the observed and expected values from Figure 5.1.

5.3. Results:

The number of leaves per bin was measured at various intervals over the experimental period (Table 5.2). The number of leaves for *Typha* under all substrates peaked at 93 days and then declined to the end of the experiment. The number of leaves for *Triglochin* generally increased over the course of the experiment, and peaked at or just prior to the end of the experimental period.

All dry weight (Table 5.3) and RGR (Table 5.5) data are considered for the two species separately, and reference made to the initial planting proportion, i.e. the original number of shoots of that species per bin (total density held constant at 3 shoots per bin).

The total dry weight of *Triglochin* per bin was influenced by the original planting proportion ($P < 0.0001$), with a significant increase in biomass between each increment in planting proportion, i.e. three shoots > two shoots > one shoot (Table 5.3). Substrate type did not significantly influence ($P = 0.2165$) the total dry weight of *Triglochin* per bin. The increased total biomass was a result of a significant increase in both the above ($P < 0.0001$) and below ($P < 0.0001$) ground components with planting proportion. Flowering only occurred in *Triglochin* at a planting proportion of three shoots, i.e.

Triglochin monoculture, and substrate type did not significantly (P = 0.1294) influence the dry weight of the inflorescence.

Table 5.2: Average number of leaves per bin at four intervals over the course of the 260 day experimental period for *Typha* and *Triglochin* grown in replacement series (constant density of 3). (n=4).

	3 *Triglochin*	**1 *Typha***	**2 *Triglochin***	**2 *Typha***	**1 *Triglochin***	**3 *Typha***
Sand						
Day 0	22.0	17.0	14.8	53.3	6.8	59.8
Day 59	21.3	55.0	15.5	135.3	8.0	130.3
Day 93	22.3	85.5	15.0	168	7.0	155.5
Day 131	27.5	89.0	11.8	150.5	4.8	138.8
Day 260	32.0	36.0	18.8	43	5.5	60.0
Gravel						
Day 0	22.0	25.8	15.3	59.8	4.3	74.8
Day 59	28.0	61.0	18.5	129.5	7.3	156.5
Day 93	34.0	95.3	18.3	147.5	6.5	177.5
Day 131	42.5	88.0	17.3	137.5	6.0	154.8
Day 260	32.5	41.8	12.3	50.0	4.0	37.5
Clay						
Day 0	65.0	23	13.8	43	5.5	65.0
Day 59	168	62.8	18.5	126.8	7.0	168.0
Day 93	195.7	103.8	16.0	164.0	7.3	195.7
Day 131	180.0	105.8	15.3	167.5	6.3	180.0
Day 260	81.7	73.8	13.5	61.3	4.0	81.7

Total dry weight of *Typha* was greater under an initial planting proportion of two or three shoots than a planting proportion of one shoot (P = 0.0033) (Table 5.3). Significantly greater (P = 0.0156) below ground biomass was recorded for a planting proportion of two and three shoots than a single *Typha* shoot. The final above ground biomass was independent of planting proportion, with dry weight the same (P = 0.4478) across the three planting proportions. The total dry weight per bin of *Typha* was significantly (P = 0.0333) greater in sand than the other two substrates. Flowering did not occur in *Typha* at any planting proportion or substrate type.

The total dry weight for the two species in mixtures was expressed as proportions of the biomass achieved for each species in monoculture in a DeWitt style diagram (Figure 5.1). The expected relationship on the graph represented a linear relationship between planting proportion and final biomass (i.e. 1 and 2 plants per pot were expressed as 33% and 66% respectively, of the final biomass observed in monoculture treatments). This relationship indicated the expected biomass if no interactions, either within or between species, were acting. The total biomass of *Triglochin*, when grown in a species mixture, was always significantly less than would be expected from a density-dependent relationship ($P < 0.001$ for clay, sand and gravel). The biomass of *Typha*, when grown in the presence of *Triglochin*, was significantly greater than would be expected from a density dependant relationship ($P < 0.001$ for clay, sand and gravel). Therefore, biomass of *Triglochin* was suppressed in the presence of *Typha*, whereas biomass of *Typha* was to some degree, independent of initial planting proportion.

Table 5.3: Dry weight (g per bin) of various plant components for *Typha* and *Triglochin* after 260 days under various planting combination (n=4) (mean ± standard deviations).

	3 *Triglochin*	1 *Typha*	2 *Triglochin*	2 *Typha*	1 *Triglochin*	3 *Typha*
Sand						
Above Ground	56.1 (±19.7)	351.4 (±174.2)	10.3 (±9.4)	351.2 (±57.2)	2.18 (±1.34)	382.9 (±69.9)
Below Ground	51.2 (±35.8)	494.8 (±38.8)	36.0 (±27.7)	983.3 (±310.4)	9.71 (±5.06)	911.5 (±432.6)
Flower	15.5 (±15.3)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Total	122.7 (±67.7)	846.2 (±206.5)	46.2 (±36.4	1334.5 (±320.7)	11.9 (±6.2)	1294.4 (±406.8
Gravel						
Above Ground	46.9 (±18.0)	214.6 (±94.2)	7.40 (±3.34)	340.0 (±115.4)	2.73 (±0.83)	290.6 (±34.6)
Below Ground	83.7 (±35.0)	423.5 (±144.5)	31.4 (±8.1)	744.8 (±423.3)	10.0 (±1.74)	683.4 (±204.2)
Flower	16.1 (±11.7)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Total	146.6 (±61.4)	638.1 (±91.4)	38.8 (±11.0)	1084.7 (±208.3)	12.8 (±2.51)	973.9 (±85.4)
Clay						
Above Ground	94.7 (±43.3)	341.2 (±122.2)	28.4 (±36.0)	343.0 (±74.3)	3.35 (±2.28)	384.2 (±99.4)
Below Ground	117.9 (±38.8)	435.1 (±94.9)	30.6 (±12.5)	463.5 (±141.0)	9.95 (±3.60)	595.0 (±112.3)
Flower	34.5 (±13.0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)
Total	247.0 (±64.6)	776.3 (±216.5)	59.0 (±34.0)	806.4 (±114.8)	13.3 (±4.63)	979.2 (±13.0)

In order to gain a approximate comparison between the final yield achieved under the controlled conditions to that observed under the field condition (Table 2.5), the final above ground biomass was expressed per square metre using the surface area of the experimental unit (the bin = 0.19 m^2) (Table 5.4).

Table 5.4: Dry weight (g) of above ground biomass expressed per square meter for *Typha* and *Triglochin* after 260 days under various planting combinations (n=4) (mean ± stdev).

	3 *Triglochin*	1 *Typha*	2 *Triglochin*	2 *Typha*	1 *Triglochin*	3 *Typha*
Sand	295.3	1849.5	54.2	1848.4	11.5	2015.3
	(±103.7)	(±916.8)	(±49.5)	(±310.1)	(±7.1)	(±367.9)
Gravel	246.8	1129.5	38.9	1789.5	14.4	1529.5
	(±94.7)	(±495.8)	(±17.6)	(±607.4)	(±4.4)	(±182.1)
Clay	498.4	1795.8	149.5	1805.3	17.6	2022.1
	(±227.9)	(±643.2)	(±189.5)	(±391.1)	(±12)	(±523.2)

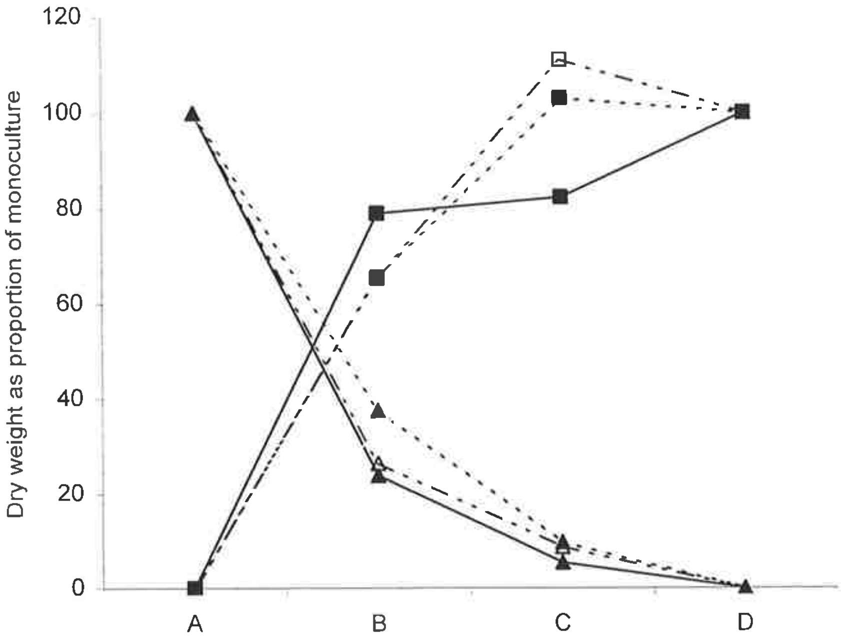
Relative growth rate (RGR) of *Triglochin* was similar across all substrate types ($P = 0.8339$). *Triglochin* RGR was greater at the higher planting proportions ($P < 0.0001$), i.e., RGR of three shoots > two shoots > one shoot (Table 5.5). The components LAR and NAR responded in a similar way to RGR with significant ($P = 0.0164$; $P < 0.0001$, respectively) increases with planting proportion (Table 5.5).

The RGR of *Typha* per bin was significantly ($P = 0.0301$) greater in sand than the other two substrates. The greatest RGR for *Typha* was recorded from the single shoot initial planting proportion, and decreased significantly ($P < 0.0001$) with increased planting proportion. LAR and NAR in *Typha* both decreased significantly ($P < 0.0001$) with increased planting proportion, hence the response of RGR was a function of both components. LAR was the same ($P = 0.1248$) across substrate types, whereas NAR was significantly ($P = 0.0301$) greater in sand than the other substrates, and therefore it was the changes in NAR which drove the RGR response to substrate.

Table 5.5: Relative growth rate (RGR) and its components (net assimilation rate (NAR) and leaf area ration (LAR) of *Typha* and *Triglochin* after 260 days under various planting combinations (n=4) (mean ± standard deviation).

	3 *Triglochin*	1 *Typha*	2 *Triglochin*	2 *Typha*	1 *Triglochin*	3 *Typha*
Sand						
NAR g m^{-2} d^{-1}	0.41	1.76	0.34	1.60	-0.01	1.39
	(±0.18)	(±0.14)	(±0.16)	(±0.14)	(±0.06)	(±0.18)
LAR m^{2} kg^{-1}	11.05	9.40	11.08	9.16	11.23	9.03
	(±0.537)	(±0.089)	(±0.521)	(±0.065)	(±1.02)	(±0.090)
RGR mg g^{-1} d^{-1}	4.60	16.6	3.78	14.7	-0.14	12.5
	(±2.17)	(±1.50)	(±2.04)	(±1.41)	(±0.69)	(±1.77)
Gravel						
NAR g m^{-2} d^{-1}	0.53	1.62	0.21	1.50	-0.13	1.28
	(±0.16)	(±0.10)	(±0.21)	(±0.14)	(±0.14)	(±0.05)
LAR m^{2} kg^{-1}	11.46	9.35	10.82	9.13	10.31	8.99
	(±0.497)	(±0.093)	(±0.727)	(±0.069)	(±0.673)	(±0.018)
RGR mg g^{-1} d^{-1}	6.14	15.1	2.35	13.7	-1.29	11.5
	(±2.04)	(±1.01)	(±2.37)	(±1.40)	(±1.33)	(±0.43)
Clay						
NAR g m^{-2} d^{-1}	0.64	1.68	0.20	1.34	-0.12	1.31
	(±0.09)	(±0.09)	(±0.23)	(±0.09)	(±0.15)	(±0.15)
LAR m^{2} kg^{-1}	11.69	9.34	10.58	9.06	10.35	9.01
	(±0.289)	(±0.041)	(±0.580)	(±0.057)	(±0.536)	(±0.101)
RGR mg g^{-1} d^{-1}	7.53	15.7	2.21	12.2	-1.23	11.8
	(±1.19)	(±0.86)	(±2.53)	(±0.88)	(±1.62)	(±1.52)

Figure 5.1: Total dry weight of *Typha* (squares) and *Triglochin* (triangles) grown in replacement series (A, 3 *Triglochin*; B, 2 *Triglochin*, 1 *Typha*; C, 1 *Triglochin*, 2 *Typha*; D, 3 *Typha*) expressed as a proportion of the total dry weight recorded in monoculture treatments under sand (dashed line, solid symbol), gravel (dashed line, open symbol) and clay (solid line, solid symbol) (n=4). Expected relationship (line, no symbol) calculated as 3 plants 100%, 2 plants 66% and 1 plant 33%.



5.4. Discussion:

Replacement series designs, such as used in this experiment, have specific limitations inherent to their design (Firbank and Watkinson 1985; Firbank and Watkinson 1990; Jolliffe 2000). Selection of planting density, the initial size of individuals and timing of the experimental observation are the limitations of primary concern (Jolliffe 2000). As the main aim of this experiment was to determine the harvestable biomass and investigate relative growth rates resulting from particular planting combinations, the replacement series design was appropriate. The interpretation of space preemption was more restricted by the replacement series design.

5.4.1. Preemption of Space and Colonisation:

Typha, with the guerrilla style rhizome extension, displayed a greater ability than *Triglochin* to preempt available space and colonise the bin over the experimental period. In contrast, *Triglochin,* which adopted the phalanx style rhizome extension, displayed limited ability to preempt space and was suppressed by presence of *Typha* compared to the presence of its own species.

The replacement series design only gave insight into preemption potential for plants of initial size, planting density and specific timing of this particular experiment. For example, the experimental period was near the end of the peak growing season for *Triglochin* and the beginning of the peak season for *Typha* (see section 2.3.2.1.). Therefore, *Typha*'s dominance may have been due in part to a seasonal advantage. An experimental design encompassing variations in timing, initial plant size and planting density would be required to understand the role of preemption of space on the coexistence of these species. A longer term study would also be required to determine whether this planting combination was dominance-controlled (i.e. preemption exerts only a temporary effect to the community structure), or founder-controlled (i.e. the species able to preempt the space (*Typha*) dominates the community over the long term) (see Grace 1987).

5.4.2. Biomass Response:

The similar above ground biomass achieved by *Typha* under each planting proportion suggested an upper limit of growth. There was visual evidence that *Typha* plants became pot bound, i.e. space became limiting. The fact that differences were still observed in the below ground fraction between planting proportions suggested that the space limitation was experienced in the above ground fraction before it was fully experienced in the below ground fraction of all treatments.

The biomass yield of *Typha* and *Triglochin* did not display a linear correlation with initial planting proportion, suggesting the occurrence of an interaction either within or between species. Growth and biomass production of *Triglochin* was suppressed by the presence of *Typha*. This suppression was further highlighted by the fact that flowering in *Triglochin* plants only occurred in the absence of *Typha*, i.e. in monoculture.

Maximum above ground biomass achieved by *Triglochin* in this experiment (in monoculture) (498 g m^{-2} Table 5.4) was slightly less than that at Willunga for an equivalent season (529-852 g m^{-2} Table 2.5). The above ground biomass of monocultural *Typha* in this experiment (2022 g m^{-2} Table 5.4) was slightly higher than that of an equivalent season at Willunga (1030-1925 g m^{-2} Table 2.5).

When grown in the presence of *Typha*, *Triglochin* only represented a maximum of 7% of the total biomass yield. The maximum above ground yield represented by *Triglochin* in a mixed population was 8% (planting proportion of one *Typha* and two *Triglochin* in clay). Given the higher nutrient concentration in *Triglochin* compared to *Typha* (Table 3.1), the former species may be considered more desirable in terms of potential nutrient storage and removal. However, in this experiment, biomass production by *Typha* was far superior to that of *Triglochin* both in monoculture and mixtures. This was particularly the case in mixtures where the suppression of *Triglochin* in the presence of *Typha* was evident.

Substrate did not have a significant influence on the growth of *Triglochin*, however there was a trend for more prolific growth in clay than in sand. In contrast, *Typha* demonstrated a significant response ($P = 0.0333$) to substrate with a greater below ground biomass in sand compared to clay. This observation was similar to the finding of White (1999), who recorded a greater allocation of biomass to *Typha* roots in sand compared to clay, despite a greater total biomass in clay.

Peverly *et al.* (1995) observed greater below ground biomass for *Phragmites australis* in coarse gravel (4cm) compared to pea gravel (0.5 cm) or a gravel (2cm)/sand mix. However, the overall treatment efficiency was higher in the finer substrate systems. Lenssen *et al.* (1999) found that wetland plant responses to sediment type were related to the organic matter content of the substrate. The greater plant productivity observed in organic rich substrates compared to mineral substrates was attributed to nutrient availability. Anaerobic decomposition of macrophyte litter produces phytotoxic organic acids and therefore plants under flooded conditions performed poorly in sediments rich in litter compared to sediments rich in labile organic matter (Lenssen *et al.* 1999). The experimental period of this study was probably not sufficient in duration for significant litter accumulation and decomposition. However, the long term impacts of litter decomposition on systems such as Willunga need to be investigated, and the role of aerobic decomposition and litter reduction via harvesting could be explored.

The fact that no difference in growth response was observed between clay and gravel substrates for either species may suggest that the difference in performance (for both species) observed across the environmental zones in section 2.3.2.3. were due primarily to water depth and nutrient delivery rather than substrate type (assuming no interaction between the factors occurred in the field).

5.4.3. RGR response:

A greater planting proportion of *Triglochin* resulted in greater RGR of that species. This indicated a suppression of *Triglochin* growth in the presence of *Typha*. A greater planting proportion for *Typha* resulted in a lower RGR of that species, i.e. the greater the initial biomass, the lower the biomass increase per biomass already present. This confirmed the space limitation observed in the above ground biomass and suggested that the limitation ultimately influenced RGR and its components.

Even though the two species had contrasting RGR responses to planting proportion, in both cases, variations in RGR were functions of changes in both NAR and LAR. Additionally, the greater RGR for *Typha* in sand was driven by changes in NAR. These responses in NAR are in contrast to the observations by Lambers and Poorter (1992), who concluded that variations in RGR were due predominantly to changes in LAR. The importance of leaf area to plant growth was further demonstrated by the correlation ($r^2 = 0.979$) between emergent leaf area and final plant biomass measured in *Bolboschoenus medianus* by Blanch *et al.* (1999). In the current study, *Triglochin* displayed a strong relationship between leaf area and total biomass (sand $r^2 = 0.61$; gravel $r^2 = 0.96$;

clay $r^2 = 0.80$; see Appendix B), similar to that observed by Blanch *et al.* (1999), whereas the relationship for *Typha* was poor (sand $r^2 = 0.07$; gravel $r^2 = 0.18$; clay $r^2 = 0.39$; see Appendix B).

As with RGR, the decreased NAR for *Typha* under higher planting proportions was most likely a function of the space limitation. However, in the case of *Triglochin*, the decreased NAR with lower planting proportion was a function of the suppression experienced by *Triglochin* in the presence of *Typha*.

NAR is determined by the rates of photosynthesis, respiration and other losses from exudation and volatilization (Konings 1989). Therefore, greater NAR observed for *Typha* in sand compared to clay was a result of a shift in the balance between these resource gains and losses. The more highly oxygenated condition expected in the sand treatment compared to clay may have reduced the respiratory losses by the plant, therefore, increasing the NAR. Presumably the gravel substrate also provided relatively oxygenated conditions, however, the NAR recorded in gravel was not significantly greater than that of clay.

The maximum RGR (7.53 mg g d^{-1} for *Triglochin*; 16.6 mg g d^{-1} for *Typha*) and NAR (0.64 g m^{-2} d^{-1} for *Triglochin*; 1.76 g m^{-2} d^{-1} for *Typha*) observed in this study were lower than those of other studies. For example, Morris (1998) recorded RGRs of 42 mg g^{-1} d^{-1}, NAR up to 16 g m^{-2} d^{-1} for *Typha domingensis* and Blanch *et al.* (1999) recorded RGRs up to 43.7 mg g^{-1} d^{-1}, NAR up to 17.5 g m^{-2} d^{-1} in *Bolboschoenus*. Lambers and Poorter (1992) recorded RGRs of 19-386 mg g^{-1} d^{-1}, and NARs of 2-25 g m^{-2} d^{-1} in tree species, herbaceous species, perennials and annuals. A correlation exists between high leaf nitrogen content and high photosynthetic capacity (see Konings 1989). Given the high nitrogen concentration (41 mg g^{-1} Table 4.5) of *Triglochin* leaf tissue in a similar experimental condition (100 g N m^{-2} yr^{-1} nutrient addition to substrate, chapter 4), the NAR (and consequently the RGR) recorded in this study was lower than may have been expected. Studies into photosynthetic capacity would be required for both species in order to determine whether the low NAR was due to low photosynthetic capacity or large losses via respiration (and other mechanisms).

The maximum LARs recorded in this study (11.69 $m^2 kg^{-1}$ for *Triglochin* and 9.40 $m^2 kg^{-1}$ for *Typha*) were higher than the maximum 2.7 $m^2 kg^{-1}$ recorded by Blanch *et al.* (1999) and within the range of 2-65 $m^2 kg^{-1}$ reported by Lambers and Poorter (1992). As suggested by the low values for RGR and NAR, the high investment in leaf area by *Typha* and *Triglochin* did not translate into high growth rates. For *Typha*, this may have been due to the space limitation experienced within the experimental unit, whereas the cause for *Triglochin* was not clear. The anatomical arrangements of both *Triglochin* and *Typha* result in a high investment in leaf area relative to structural tissues (such as stems). As discussed in section 2.3.3.3., the leaf area per biomass (considered to be analogous to LAR) for *Triglochin* decreased with depth due to changes in leaf structure and form, whereas the leaf area per biomass (and leaf structure and form) in *Typha* remained constant with depth. Therefore the LAR reported in this experiment may be greater than would be expected for *Triglochin* in deeper conditions.

The RGRs (and components) determined in this experiment were calculated over the entire experimental period even though a space limitation (and therefore, a reduction in RGR) was evident for *Typha*. In order to provide a more realistic comparison, maximum RGR for *Typha* was estimated by assuming that no further increase in biomass was achieved after the recorded peak in leaf number (93 days, Table 5.2). Therefore, the RGR was recalculated maintaining the recorded change in biomass over the entire experimental period, but adjusting the experimental duration from the original 260 days to 93 days. The recalculation was not performed for *Triglochin* as this species did not display the same evidence of space limitation.

The recalculated RGRs (Table 5.6) provide a more realistic comparison of the performance of the two species, with the RGR of *Typha* significantly greater than that of *Triglochin*. The values were also similar to those recorded for *Typha domingensis* by Morris (1998) and for *Bolboschoenus medianus* by Blanch *et al.* (1999). Given that the number of leaves decreased between day 93 and the end of the experimental period, the recalculated RGR may still be an underestimate of *Typha*'s performance over the early period of the experiment. It was not possible to accurately recalculate the NAR and

LAR over this reduced experimental period. However, it may be expected that NAR contributed significantly to the increased RGR over shorter experimental period.

Table 5.6: Recalculated relative growth rate (RGR) for *Typha* (experimental period from 260 days to 93 day) under a replacement series design (with *Triglochin*), with three substrate types (clay, gravel and sand). (n=4, ± standard deviations).

	1 *Typha*	2 *Typha*	3 *Typha*
clay	43.9 (±2.4)	34.1 (±2.5)	33.0 (±4.2)
gravel	42.3 (±2.8)	38.2 (±3.9)	32.0 (±1.2)
sand	46.3 (±4.2)	41.0 (±3.9)	35.1 (±5.0)

5.5. Conclusion:

Growth and biomass accumulation of *Triglochin* was suppressed by the presence of *Typha,* as was predicted in the original hypothesis. Such interactions may influence nutrient storage and removal within constructed wetland systems. Further investigations are required to determine the longer term interactions from such planting combinations, using a variety of species.

Growth responses to substrate and planting proportion were due to changes in both NAR and LAR. The importance of LAR in growth analysis has been well documented, however, the mechanisms of changes in NAR are not as well understood. The relative contribution of photosynthetic gains and respiratory losses (plus other losses) to the overall NAR was not determined. However, the high leaf nitrogen content previously recorded for these species (especially *Triglochin*) would suggest that large losses account for the comparatively low NARs. The response of *Typha* to substrate type was due primarily to changes in NAR. It was expected from the hypothesis that plant growth in gravel substrates would exceed that in clay substrates, however the only significant response to substrate was the greater below ground biomass for *Typha* in sand compared to clay.

CHAPTER 6: GENERAL DISCUSSION, PREDICTIONS AND FUTURE DIRECTIONS:

The primary aim of this study (section 1.9.) was to quantify potential nutrient storage and removal of the plant component within a constructed wetland treating wastewater and this was achieved predominately in chapter 3. Secondary aims investigated the mechanisms of plant responses to conditions within the Willunga constructed wetland system. The information obtained from the experimental investigations and the field observations has been consolidated to form comments of overall plant performance in constructed wetlands as well as to suggest the role of plants in nutrient storage and removal.

6.1: Growth and performance of aquatic macrophytes at the Willunga wetland system:

Emergent macrophytes at Willunga recorded large biomass (chapter 2) and high nutrient storages (chapter 3) compared to other studies (Adcock and Ganf 1994; Breen 1990; Ennabili *et al.* 1998; Greenway and Woolley 1999; Hocking 1989; Mars *et al.* 1999; Oki 1992; Surface *et al.* 1993; Tanner 1996). The two species not commonly used in constructed wetlands (*Triglochin* and *Bolboschoenus*) recorded the maximum nitrogen and phosphorus storage, respectively.

6.1.1. *Triglochin:*

Triglochin displayed advantages for use in constructed wetlands over the other three species. High biomass and nutrient storages were maintained throughout the year (chapter 3) due to the lack of a seasonal senescence Adcock and Ganf (1994), and chapters 2 & 3). *Triglochin* also displayed the ability to access nutrients supplied in the water column (probably via adventitious roots) (section 4.4.2.). Nutrient storage capacity per shoot of *Triglochin* was increased with inundation, even though the leaf area and therefore potentially photosynthetic capacity was reduced (2.3.2.3.). Nutrient removal via *Triglochin* was enhanced by a multiple harvest strategy. However, further investigations into the long term influences of harvesting on plant performance are required.

The major disadvantage associated with the use of *Triglochin* was poor establishment and colonisation. The phalanx style (Lovett Doust 1981) rhizome extension limited colonisation of deep sections in the Willunga wetland (section 2.3.2.2.) and establishment (at a total density of 3 plants per 0.19 m^2) was suppressed by the presence of *Typha* (section 5.3.). Evidence of successful *Triglochin* colonisation observed within the Willunga systems was restricted to small regions of seedling growth. Providing conditions favourable for seed production and establishment (i.e. deep water to induce flowering followed by a period of drawdown to allow establishment) may be an effective method of achieving widespread colonisation of *Triglochin*. Deep water would not only induce flowering, but would also provide conditions favourable for increased above ground nutrient storage (see section 2.3.2.3., increased storage at shoot level).

A management strategy could be implemented to incorporate both increased storage and seedling establishment. An extended period (approximately 12 months) of inundation (60cm) would induce flowering/seed production and increase above ground nutrient storage. Peak nutrient storage at Willunga coincided with the end of the flowering season in late spring/early summer. Therefore, the imposition of a drawdown (dry phase) at this period, followed by a complete harvest of existing above ground material, may allow widespread seed establishment, and achieve maximum nutrient removal. Advantages other than the optimal establishment and nutrient storage may result from such a management strategy. Firstly, harvesting may be logistically and physically easier under a drawdown than a flooded state. Secondly, the drawndown state would allow the original population to recover from the harvest without a risk of "drowning" (see Sale and Wetzel 1983 for detrimental responses of *Typha* species to repeated cutting treatment below water level).

The installation of a fourth wetland basin within the Willunga system, and the ability to bypass flow to any wetland, would provide the flexibility to induce a drawdown condition without compromising the volume of water able to be treated within the system. However, manipulation of water levels and the extra area required may put such a management strategy beyond the capability of many systems. In such cases, the initial planting density within the system would need to be high and subsequent planting to replace an aging cohort may be necessary.

Given the advantages and disadvantages stated above, *Triglochin* may be best suited to small scale wetlands where high initial planting densities can be implemented, and a high degree of maintenance can be achieved. The suppression of growth may also suggest that *Typha* should be excluded from these small scale wetlands in order to obtain maximum productivity from *Triglochin*. Investigations into the interactions with other species such as *Bolboschoenus* would recommend whether *Triglochin* is ultimately suited to polyculture (i.e. more than one species) or monoculture wetlands.

6.1.2. *Bolboschoenus:*

Not only did *Bolboschoenus* display high phosphorus storage in spring and summer, but the potential nutrient removal was enhanced by the implementation of a multiple harvest regime. Not only did the plant respond by replacing above ground material (except over the winter period), but high concentrations of nitrogen and phosphorus were recorded in the regrown above ground tissue.

Bolboschoenus exhibited the most favourable response to multiple harvesting of the four species at Willunga. *Bolboschoenus* responded more favourably to the multiple harvest regimes of this study than that imposed on *Phragmites* by Suzuki *et al.* (1989b). Complete winter senescence (section 2..3.2. and 3.2.2.1.) and the associated potential nutrient pulse was the major disadvantage associated with the use of *Bolboschoenus*.

6.1.3. *Typha:*

Even though the total nutrient storage of *Typha* was less than the maxima recorded for *Bolboschoenus* and *Triglochin*, it may still be the species of choice in some wetland systems. Unlike *Bolboschoenus* and *Phragmites*, *Typha* shoots did not undergo complete winter senescence, and maintained a small degree of active growth throughout the year. As a consequence, *Typha* responded to multiple harvest regimes during the winter period.

Observations of rhizome rafting (i.e. rhizome extension above the substrate surface) at Willunga (2.3.2.2.), suggest that *Typha* may have the ability to access nutrients supplied to the water column in a similar way to that observed for *Triglochin* (section 4.4.2.). *Typha* demonstrated an ability to colonise new areas with aggressive, guerrilla style rhizome extension (Lovett Doust 1981), which may be advantageous for achieving widespread colonisation from a small number of original individuals. Advantages (such as increased nitrification rates) associated with *Typha*'s ability for rhizosphere oxygenation may also favour the use of *Typha* over the species with higher storage capacity but limited ability for rhizosphere oxygenation (i.e. *Bolboschoenus* or *Triglochin*).

The aggressive colonisation strategy observed in *Typha* may be a disadvantage under certain circumstances. For example, the ability of *Typha* to preempt space may result in a floristic composition other than that intended from the initial wetland planting, i.e. preempt and dominate. Comprehensive, long term, studies need to be conducted on a variety of planting combinations to identify combinations which avoid *Typha* founder-controlled systems (i.e. *Typha* preempts space and dominates the community over the long term).

6.1.4. *Phragmites:*

Phragmites was not extensively studied at the Willunga system. Numerous other studies have focused on growth and performance aspects for *Phragmites* in constructed wetlands. As with *Typha, Phragmites* has the advantage of rhizosphere oxygenation via convective flow. However, nutrient removal potential of *Phragmites* at Willunga was restricted by the complete winter senescence. The poor response to harvesting observed for *Phragmites* was in contrast to the positive harvesting responses reported for *Phragmites* in other studies (e.g., Suzuki *et al.* 1989b). Therefore, a more comprehensive study investigating the response of *Phragmites* to a variety of harvesting regimes is needed.

6.1.5. Species combinations in wetlands:

Mitchell (1978, as cited by Hocking 1985) suggested five basic criteria for selecting desirable species for wastewater amelioration; (1) rapid and fairly constant growth rate, (2) ease of propagation, (3) reasonable capacity for absorbing nutrients, (4) tolerance of hyper-eutrophic conditions and (5) ease of harvesting. Chambers and McComb (1994) suggested that macrophytes selected for constructed wetlands should be of a robust habit, maintain high biomass throughout the year and be readily available in the local area.

Each of the four species at Willunga displayed advantages and disadvantages for use in constructed wetlands. A combination of species (i.e. a polyculture) may achieve a greater overall nutrient removal than a single species (monoculture). For example, *Bolboschoenus* could achieve high storage of phosphorus, but was limited to the spring and summer seasons. The addition of *Typha* or *Triglochin* to the system may enable storage of phosphorus to continue over the winter period.

Additional information on rates of photosynthesis/respiration, leaf turnover, decomposition and nutrient retention rates, could be used to develop nutrient budgets and models for each of the species at Willunga similar to those developed by Asaeda and Karunaratne (2000) for the growth of *Phragmites australis* and by Asaeda *et al.* (2000) for the growth and decomposition of *Potamogeton pectinatus*. Such models allow greater insight into the sink/source/transformer capacities of these plants.

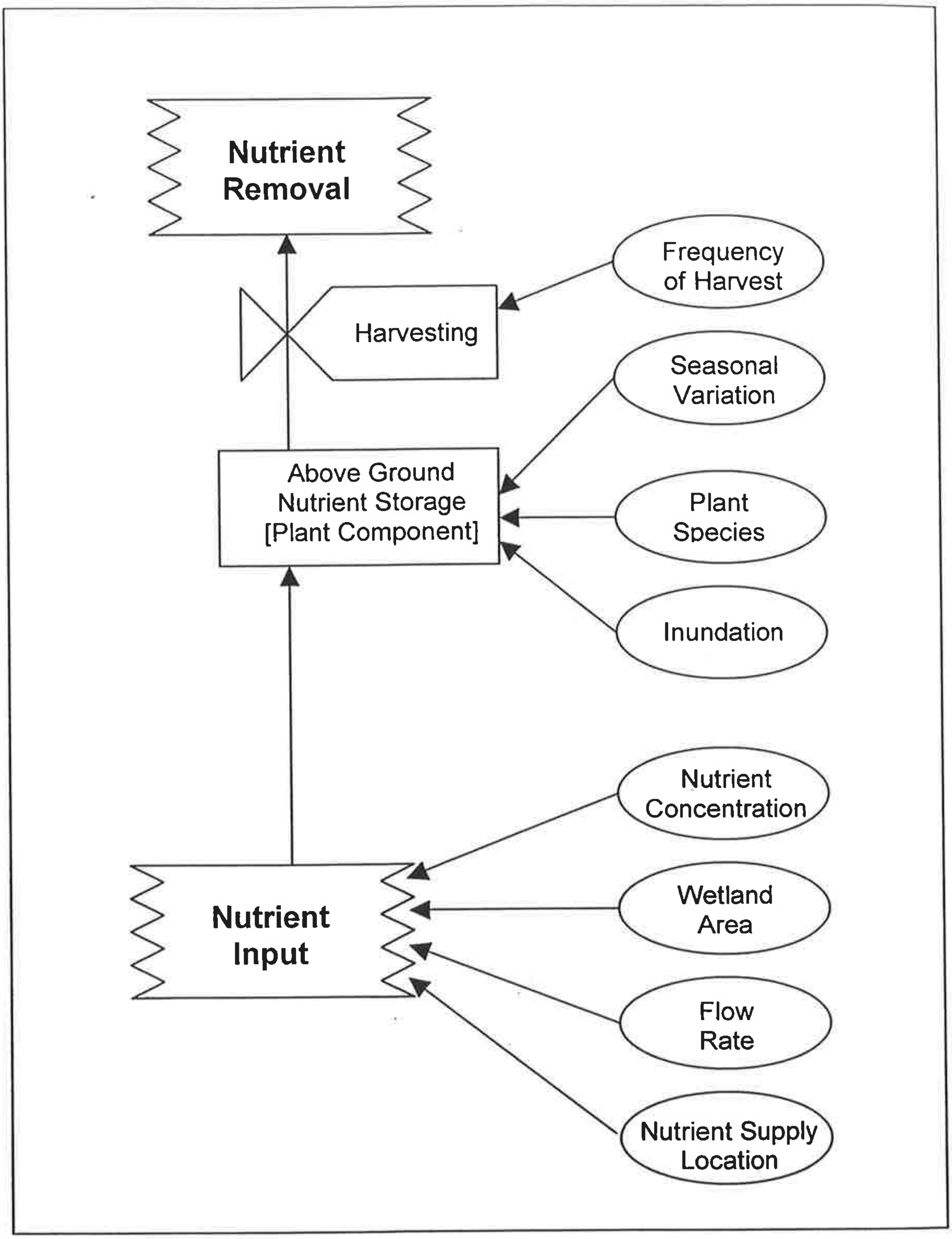
6.2: Harvesting as a Nutrient Removal Mechanism in Constructed Wetland Management: Observations and Predictions:

Harvesting plants for nutrient removal will be most successful when above ground nutrient storage is seasonally predictable. Nitrogen and phosphorus storage in the emergent species at Willunga were relatively high and seasonally predictable, therefore indicating high potential for nutrient removal via harvesting. In contrast, above ground storage of iron, manganese, copper and zinc was seasonally unpredictable, hence the potential for micronutrient removal via harvesting may by limited. All species, except *Phragmites*, at the Willunga wetland system responded favourably to harvesting (i.e., most shoots regrew and after approximately 3 months achieved biomass of greater than 50% of unharvested shoots).

6.2.1. Model of nutrient removal via harvesting the plant component:

A model was created to estimate potential nutrient removal from the Willunga wetland system, and predict nutrient removal upon various alterations to wetland design. The model incorporated parameters of plant performance, nutrient storage and nutrient removal established from the results of this study (chapters 2, 3, 4 and 5) (see Appendix C (back cover) for a working version of the model in Microsoft Excel 2000). Figure 6.1 shows a schema for the model with the various parameters described below.

Figure 6.1: Schema of model for estimating nutrient removal represented by the plant component.



Nutrient storage data from the four species (Table 3.3), as well as physical parameters of nutrient loading (Table 2.3) and wetland area (Table 2.6) formed the basis of the model. Nutrient storage and removal were calculated for wetland 1 and 3 separately, and estimated for wetland 2 as the average of the other two wetlands. From observations in section 5.3 it was assumed that substrate type (clay versus gravel) had no influence on nutrient storage. No data were available on potential interactions between wetland components (i.e. plants, sediment, microbiota), and therefore, plant responses within the model were considered independently of other processes acting with the system.

In order to estimate nutrient removal under the current configuration of the Willunga system, the model user specifies which month of the year to impose a single annual harvest. Nutrient storage data were collected on a seasonal rather than a monthly basis, however, any month of the year can be selected for harvest within the model input. Therefore, the nutrient storage of plants was calculated to be the same either side of each month of measurement (e.g. nutrient storage in April and June was assumed to be the same as that recorded in May). Predictions of nutrient removal under other configurations can be made by adjusting the appropriate parameters within the model input, i.e. set the annual nutrient load, the percentage of the total area that each zone (i.e. barrage, perimeter, surface flow and open water) comprises and the species composition within those zones.

The model output expresses nutrient removal via harvesting all above ground plant material from all four species as a percentage of the total annual nutrient input and total nutrient (kg) removed calculated as wetland area multiplied by annual loading. The *Triglochin* portion of the removal calculation was expressed using two population densities, nutrient storage (g m^{-2}) represented by the current population density (Table 2.5), and that potentially achieved by a maximum population density, i.e. the "clump" density (Table 2.5).

A multiple harvest factor was used to determine the increase in nutrient storage for a multiple harvest regime compared to a single harvest. This factor was estimated for *Bolboschoenus*, *Phragmites* and *Triglochin* as the percentage increase in nutrient accumulation under regime 1 (November harvest followed by a February harvest) compared to regime 2 (single November harvest) from the three species harvest experiment (Table 3.14). *Typha* was not included in the three species experiment, hence no data were available for the (November followed by February) harvest regime of the model. Nutrient storage for *Typha* under the multiple regime was estimated to be 1.5 times that of a single November harvest (for both nitrogen and phosphorus) from observations of the 12 month harvest experiment (Table 3.11). Nutrient storage and removal for all species was calculated in the model by multiplying the nutrient storage per square metre achieved in November by the multiple harvest factor for the respective species (Table 6.1.). No flexibility in the timing of the multiple harvest was built into the model, but with further investigations into harvest responses, such flexibility could be incorporated.

Table 6.1: Multiple harvest factor applied to the four species within the model to estimate nitrogen and phosphorus removal via a multiple harvest regime: first harvest in November followed by harvest in February.

	Nitrogen	Phosphorus
Bolboschoenus	1.57	1.64
Phragmites	1.05	1.03
Triglochin	1.99	1.95
Typha	1.50	1.50

A set of correction factors were configured from plant trait information and incorporated into the model to provide more accurate predictions of nutrient removal. A depth correction factor was incorporated into the model to account for the response to water depth observed per shoot for *Triglochin* and *Typha* (section 2.3.2.3.). The correction factors (Table 6.2) were determined as the increase in above ground nitrogen and phosphorus storage (g m^{-2}) per shoot in the deep zone compared to the shallow zone (Table 2.10). Even though the observations of Table 2.10 were collected for a single season, the model assumed that this response to inundation was valid for all seasons.

As no information was available on the performance of *Phragmites* and *Bolboschoenus* in deep zones, the model excludes their growth in those zones.

Table 6.2: Depth correction factor applied within the model to the two species able to grow in deep and shallow zones within the Willunga wetland system to estimate nitrogen and phosphorus removal per shoot in the deep zone compared to the shallow zone.

	Nitrogen	Phosphorus
Triglochin	2.17	2.93
Typha	1.75	1.34

The final correction factor incorporated into the model compensated for nutrient storage under varying nutrient loadings (Table 6.3). These factors were based upon the responses of *Triglochin* to nutrient supply location (chapter 4). It was assumed that nutrient response of *Typha* to nutrient loading and location was similar to that observed for *Triglochin*. The nutrient loading selected by the model user determines the correction factor used within the model to predict nutrient removal. With further investigations into plant responses to a range of loadings a more complete response curve could be created and incorporated into the model.

Table 6.3: Nutrient loading correction factor applied within the model to all species within the Willunga wetland system to estimate nitrogen and phosphorus removal at various nitrogen and phosphorus loadings.

	Loading (g m^{-2} yr^{-1})	Correction Factor (both N & P)
Nitrogen	< 50	0.5
	< 150 for surface flow only	0.5
	> 150	1.0
Phosphorus	< 19	0.5
	< 57 for surface flow only	0.5
	> 56	1.0

Even though the model was constructed within the conditions and assumptions of the Willunga wetland system, it could be applied to other systems or hypothetical systems as a tool to optimise wetland design and management. If harvesting is to be part of the management strategy within a wetland, it is important to incorporate design features which optimise nutrient removal prior to the construction of the wetland. This model may aid in selection of such design features. The incorporation of other storage and removal processes into the basic model presented from this study, complete with interactions between the processes, would create a powerful tool for wetland designers and managers.

6.2.2. Estimates and predictions of nutrient removal from the model:

The current Willunga wetland configuration (zones, species, area, nutrient concentrations and inflow) was applied to the model and the output displayed in Figure 6.2. The nutrient removal via a single November harvest was 6.1% of total nitrogen input and 2.2% of total phosphorus input. Removal was not substantially increased under a multiple regime (8.7% N and 3.3% P). Therefore, under the current configuration and densities, harvesting would not constitute a substantial nutrient removal mechanism. This observation was consistent with that of Mann (1990) who reported that harvesting accounted for less than 10% of annual nutrient input.

Given the low overall storage and removal represented by the plants under the current configuration at Willunga, it may be assumed that other processes such as abiotic storage and biological removal (via denitrification) accounted for the majority of the nutrient reduction observed by Piranti (1995) (Table 2.3).

As well as estimating the contribution of harvesting to nutrient removal under the current configuration, the model is able to predict nutrient removal under hypothetical alterations to wetland design. If a optimum density of *Triglochin* could be achieved within the wetland system, the removal increased to 9.0% N and 3.3% P under a single November harvest and 14.5% N and 5.4% P under a multiple harvest regime (Figure 6.2). These increased removals still only represent a small percentage of the total nutrient input.

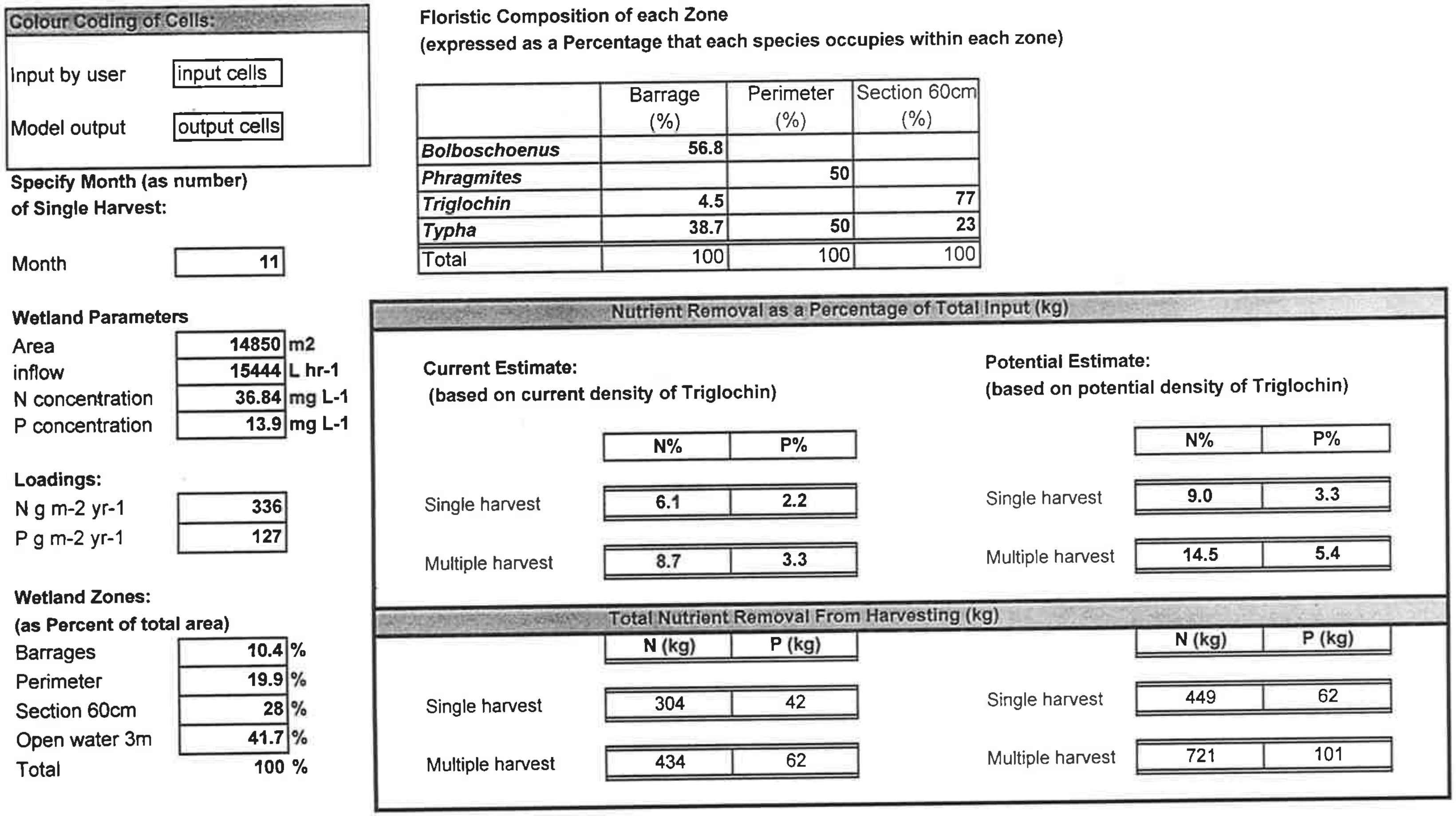
The wetland design, in particular the large area (41.7%) of unplanted open water section (3m deep), appears to be a major contributor to the low predicted nutrient removal. The wetland configuration and floristic composition were altered within the model (Figure 6.3) so that the barrage zone made up half the total wetland area and surface flow section (60cm deep), the other half. The barrage area was configured with 100% *Bolboschoenus* and the surface flow section with 100% *Triglochin*. The nutrient removal predicted from this design (with optimum *Triglochin* density) was 15.3% N and 8.4% P under a single November harvest and 27.3% N and 14.7% P under a multiple harvest. The elimination of open water sections would lead to a reduction in wetland volume capacity and would therefore need to be taken into account during wetland design and construction.

The introduction of floating plants to the open water sections of the systems may significantly contribute to nutrient storage of the plant component while still maintaining a high volume capacity. For example, Reddy and DeBusk (1985) estimated nutrient storage capacity from *Lemna minor* in a microcosm (1.7 m^2) experiment of up to 106 g N m^{-2} yr^{-1} and 32 g P m^{-2} yr^{-1}. Nutrient removal data for a variety of floating plants under local conditions could be incorporated into the existing model.

High annual nutrient load also contributed to the low predicted nutrient removal from harvesting observed in figure 6.2. By increasing the wetland area to 30000 m^2, and maintaining a constant inflow and nutrient concentration, the loading decreases to 166 g N m^{-2} yr^{-1} and 63 g P m^{-2} yr^{-1}. The percentage of the total nutrient input potentially removed with a multiple harvest regime (with species configuration and wetland zones set to the current configuration, i.e. same as Figure 6.2), was 29.2% N and 10.8% P (Figure 6.4).

The model input was altered to incorporate both the revised loading (166 g N m^{-2} yr^{-1} and 63 g P m^{-2} yr^{-1}) and wetland composition (50% barrage, 100% *Bolboschoenus*; 50% surface flow section, 100% *Triglochin*) (Figure 6.5). Under an optimum density of *Triglochin*, the potential removal was 31.0% N and 17.0% P for a single November harvest and 55.2% N and 29.7% P under a multiple harvest regime. Nutrient removals of this size would make harvesting a viable management strategy.

Figure 6.2: Estimate of nutrient removal from the model under the current configuration at the Willunga wetland system.



Colour Coding of Cells:

Input by user	input cells
Model output	output cells

Specify Month (as number) of Single Harvest:

Month	11

Wetland Parameters

Area	14850	m2
inflow	15444	L hr-1
N concentration	36.84	mg L-1
P concentration	13.9	mg L-1

Loadings:

N g m-2 yr-1	336
P g m-2 yr-1	127

Wetland Zones:
(as Percent of total area)

Barrages	10.4	%
Perimeter	19.9	%
Section 60cm	28	%
Open water 3m	41.7	%
Total	100	%

Floristic Composition of each Zone
(expressed as a Percentage that each species occupies within each zone)

	Barrage (%)	Perimeter (%)	Section 60cm (%)
Bolboschoenus	56.8		
Phragmites		50	
Triglochin	4.5		77
Typha	38.7	50	23
Total	100	100	100

Nutrient Removal as a Percentage of Total Input (kg)

Current Estimate:
(based on current density of Triglochin)

	N%	P%
Single harvest	6.1	2.2
Multiple harvest	8.7	3.3

Potential Estimate:
(based on potential density of Triglochin)

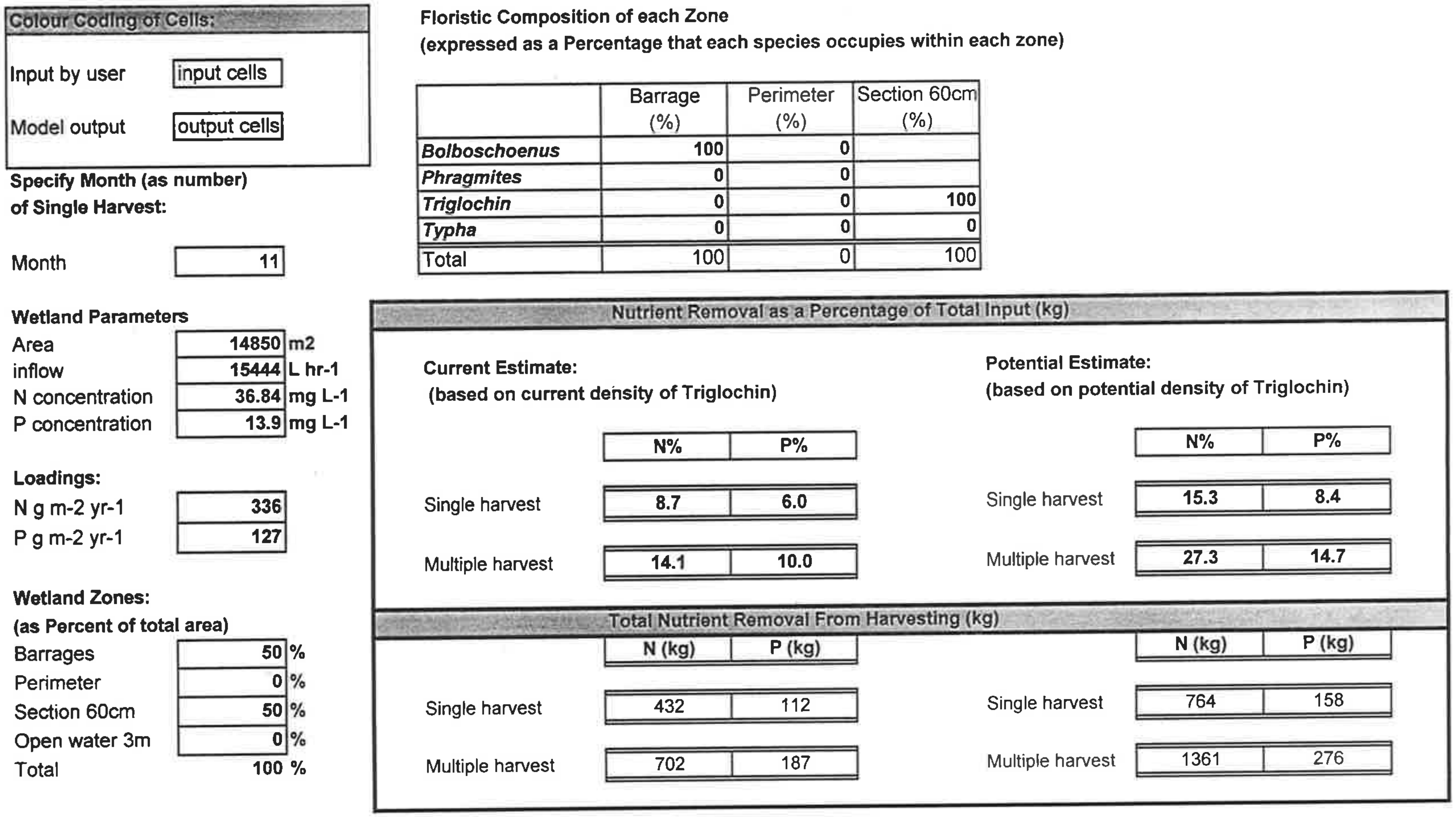
	N%	P%
Single harvest	9.0	3.3
Multiple harvest	14.5	5.4

Total Nutrient Removal From Harvesting (kg)

	N (kg)	P (kg)
Single harvest	304	42
Multiple harvest	434	62

	N (kg)	P (kg)
Single harvest	449	62
Multiple harvest	721	101

Figure 6.3: Estimate of nutrient removal from the model under alterations to the planting and zone configurations at the Willunga wetland system.



Colour Coding of Cells:

Input by user	input cells
Model output	output cells

Specify Month (as number) of Single Harvest:

Month	11

Wetland Parameters

Area	14850	m2
inflow	15444	L hr-1
N concentration	36.84	mg L-1
P concentration	13.9	mg L-1

Loadings:

N g m-2 yr-1	336
P g m-2 yr-1	127

Wetland Zones:
(as Percent of total area)

Barrages	50	%
Perimeter	0	%
Section 60cm	50	%
Open water 3m	0	%
Total	100	%

Floristic Composition of each Zone
(expressed as a Percentage that each species occupies within each zone)

	Barrage (%)	Perimeter (%)	Section 60cm (%)
Bolboschoenus	100	0	
Phragmites	0	0	
Triglochin	0	0	100
Typha	0	0	0
Total	100	0	100

Nutrient Removal as a Percentage of Total Input (kg)

Current Estimate:
(based on current density of Triglochin)

	N%	P%
Single harvest	8.7	6.0
Multiple harvest	14.1	10.0

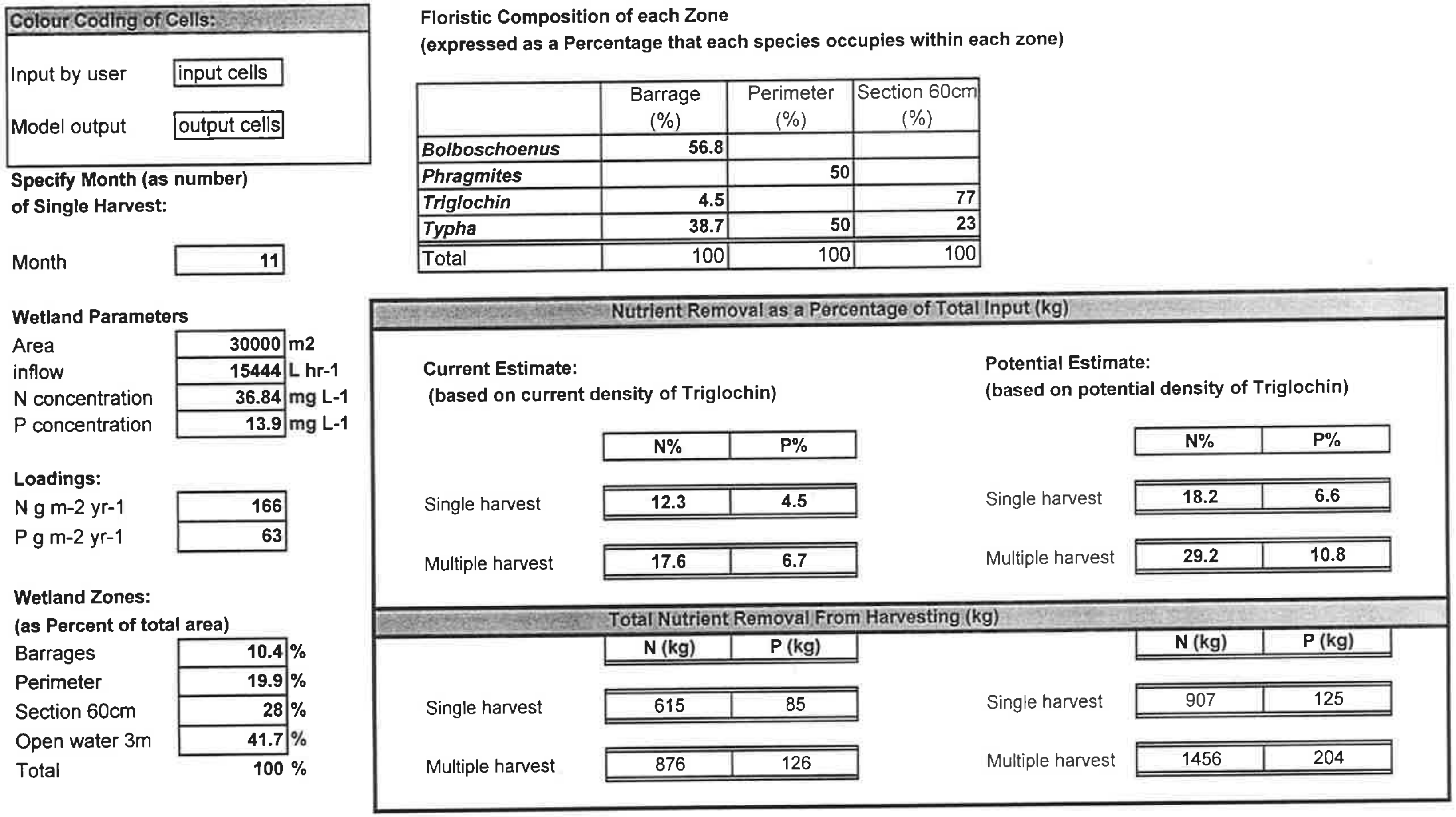
Potential Estimate:
(based on potential density of Triglochin)

	N%	P%
Single harvest	15.3	8.4
Multiple harvest	27.3	14.7

Total Nutrient Removal From Harvesting (kg)

	N (kg)	P (kg)
Single harvest	432	112
Multiple harvest	702	187

	N (kg)	P (kg)
Single harvest	764	158
Multiple harvest	1361	276



Colour Coding of Cells:

Input by user	input cells
Model output	output cells

Specify Month (as number) of Single Harvest:

Month	11

Wetland Parameters

Area	30000	m2
inflow	15444	L hr-1
N concentration	36.84	mg L-1
P concentration	13.9	mg L-1

Loadings:

N g m-2 yr-1	166
P g m-2 yr-1	63

Wetland Zones:
(as Percent of total area)

Barrages	10.4	%
Perimeter	19.9	%
Section 60cm	28	%
Open water 3m	41.7	%
Total	100	%

Floristic Composition of each Zone
(expressed as a Percentage that each species occupies within each zone)

	Barrage (%)	Perimeter (%)	Section 60cm (%)
Bolboschoenus	56.8		
Phragmites		50	
Triglochin	4.5		77
Typha	38.7	50	23
Total	100	100	100

Nutrient Removal as a Percentage of Total Input (kg)

Current Estimate:
(based on current density of Triglochin)

	N%	P%
Single harvest	12.3	4.5
Multiple harvest	17.6	6.7

Potential Estimate:
(based on potential density of Triglochin)

	N%	P%
Single harvest	18.2	6.6
Multiple harvest	29.2	10.8

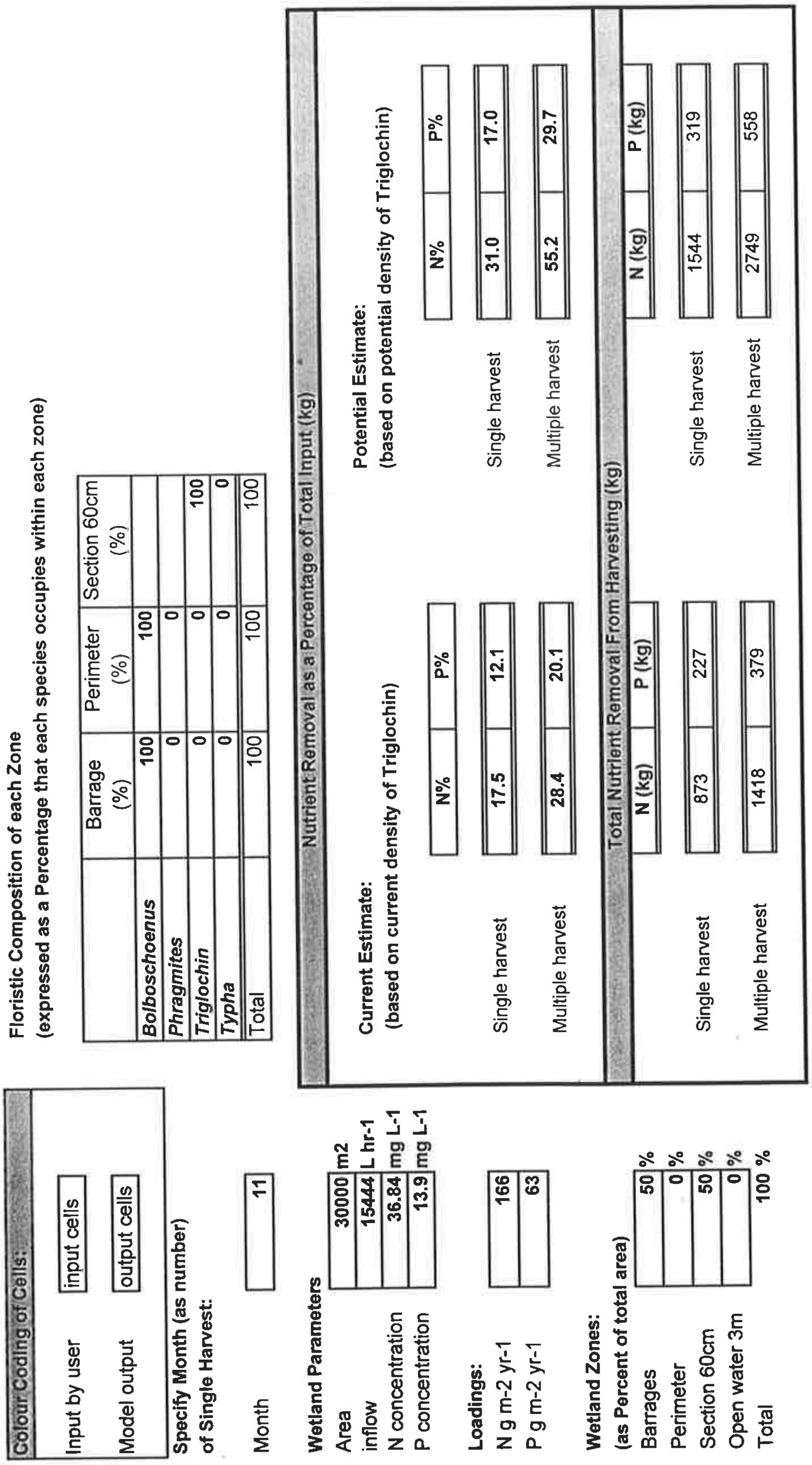
Total Nutrient Removal From Harvesting (kg)

	N (kg)	P (kg)
Single harvest	615	85
Multiple harvest	876	126

	N (kg)	P (kg)
Single harvest	907	125
Multiple harvest	1456	204

Figure 6.4: Estimate of nutrient removal from the model under alterations to the wetland area and nutrient loading at the Willunga wetland system.

Figure 6.5: Estimate of nutrient removal from the model under alterations to planting and zone configurations and wetland area and nutrient loading at the Willunga wetland system.



Colour Coding of Cells:

Input by user	input cells
Model output	output cells

Specify Month (as number) of Single Harvest:

Month	11

Wetland Parameters

Area	30000	m2
inflow	15444	L hr-1
N concentration	36.84	mg L-1
P concentration	13.9	mg L-1

Loadings:

N g m-2 yr-1	166
P g m-2 yr-1	63

Wetland Zones:
(as Percent of total area)

Barrages	50	%
Perimeter	0	%
Section 60cm	50	%
Open water 3m	0	%
Total	100	%

Floristic Composition of each Zone
(expressed as a Percentage that each species occupies within each zone)

	Barrage (%)	Perimeter (%)	Section 60cm (%)
Bolboschoenus	100	100	
Phragmites	0	0	
Triglochin	0	0	100
Typha	0	0	0
Total	100	100	100

Nutrient Removal as a Percentage of Total Input (kg)

Current Estimate:
(based on current density of Triglochin)

	N%	P%
Single harvest	17.5	12.1
Multiple harvest	28.4	20.1

Potential Estimate:
(based on potential density of Triglochin)

	N%	P%
Single harvest	31.0	17.0
Multiple harvest	55.2	29.7

Total Nutrient Removal From Harvesting (kg)

	N (kg)	P (kg)
Single harvest	873	227
Multiple harvest	1418	379

	N (kg)	P (kg)
Single harvest	1544	319
Multiple harvest	2749	558

Predictions of nutrient removal over a variety of nutrient loadings and hypothetical wetland configurations can be investigated using the model. The model was configured for a single November harvest with a planting of 100% *Triglochin* for five wetland zone configurations; 100% barrage zone, 75% barrage-25% surface, 50% barrage-50% surface, 25% barrage-75% surface, 100% surface. The predicted nutrient removal (percentage of inflow) achieved under these five wetland configurations for a variety of nutrient loadings (constant N:P ratio) was graphically represented (Figure 6.6)

Greatest nutrient removals were predicted at low loadings. Greater removal was predicted in the surface flow system (for a wetland planted exclusively with *Triglochin*) than for a gravel subsurface flow system. The influence on predicted removal of the loading correction factor within the model can clearly be seen at loading of 50 and 150 g N m^{-2} yr^{-1}. Data on nutrient storage and removal for a variety of loadings could be quantified and incorporated into the model.

Another hypothetical relationship of nutrient removal versus loading was constructed within the model for three configurations; a planting of 100% *Triglochin* in a wetland with 100% barrage zone, 100% *Triglochin* in a wetland with 100% surface flow zone, and 100% *Bolboschoenus* in a wetland with 100% barrage zone (Figure 6.7).

Figure 6.6: Removal (% of inflow) vs. nitrogen loading predicted from the model by a single November harvest (wetland planted 100% *Triglochin* for a variety of configurations: 100% barrage zone (A), 75% barrage-25% surface (B), 50% barrage-50% surface (C), 25% barrage-75% surface (D), 100% surface (E).

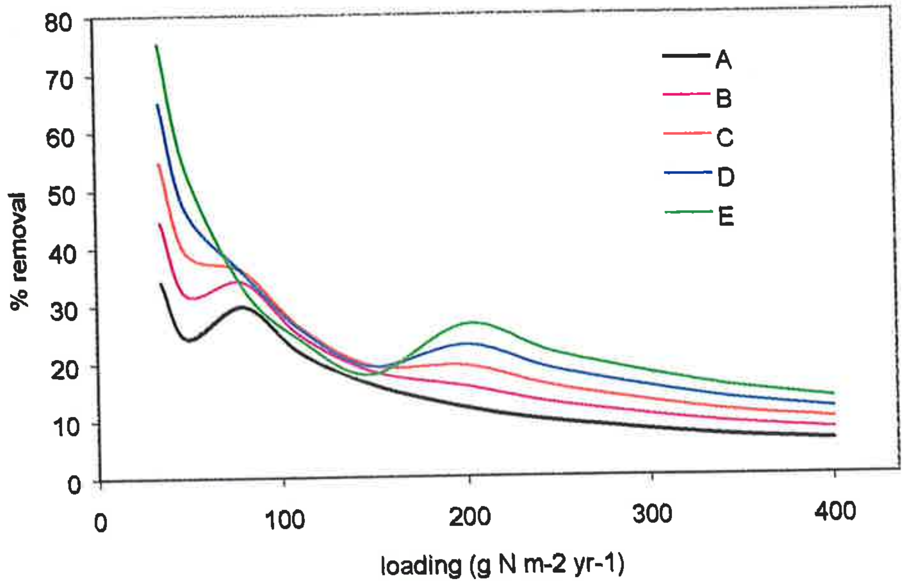
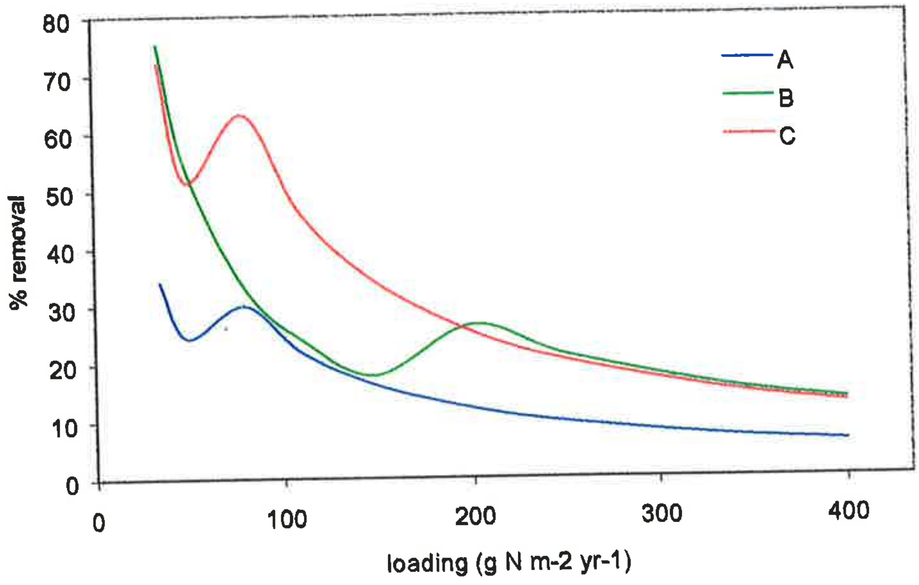


Figure 6.7: Removal (% of inflow) vs. nitrogen loading predicted from the model by a single November harvest for three wetland configurations: Planting of 100% *Triglochin*, configuration of 100% barrage zone (A), planting of 100% *Triglochin*, configuration of 100% surface flow zone (B), planting of 100% *Bolboschoenus*, configuration of 100% barrage zone (C).



As with the previous predictions, greatest removal was predicted at the lower loadings. Predicted removal was similar for either *Bolboschoenus* in barrage zone and *Triglochin* in surface flow at both low and high loadings. Removal was greater from the *Bolboschoenus* system than the *Triglochin* system for loadings between the critical loadings (50 and 150 g N m^{-2} yr^{-1}) used in the correction factors.

The importance of parameters such as loading, wetland design and species selection to nutrient removal from wetlands were highlighted within the model. Further refinements to the model, such as improvements to the relationship between loading and removal would further enhance the power of the model.

6.3. Significance of this study:

Quantification of nutrient storage and removal represented by the plant component in this study contributes to the knowledge of nutrient processing within constructed wetland systems. The model demonstrated that nutrient storage within the plant component was largely dependent on the design of the wetland. This may account for some of the discrepancies observed between the plant storage in small- and large-scale systems (Mitchell et al 1995).

It was also demonstrated that nutrient storage potential varies between species, highlighting the importance of species selection. Moreover, the species that exhibited the greatest nutrient storage potential were the two species not traditionally used in constructed wetland systems.

6.4. Future Directions of Study:

Future investigations into the relationship between wetland design and plant storage would further enhance the understanding developed in this study. An important aspect of long-term plant nutrient storage that was not included in this study, is an understanding of the factors controlling the rates of decomposition. Stored nutrients are potentially released back into the water as plant material decomposes. Investigations into decomposition could include the relationship between the rates of decomposition and wetland design. For example, wetlands which encourage highly anoxic conditions would be expected to support lower rates of decomposition than oxygenated systems (Brinson et al. 1981 as cited by Mitsch and Gosselink 1993). Rates of decomposition may also differ between plant species, particularly between those with high lignin versus low lignin content. Investigations into the relationship between plant structure and rates of decomposition would give insight into the duration of nutrient storage within plants.

Additional information is also required on the long term impacts of high nutrient loads and processes such as plant harvesting have on wetland function. Evidence of long term change was already evident with lower than anticipated nutrient removals achieved from the multiple harvest experiment conducted in 1999/2000 compared to that conducted over 1996/1997 (section 3.3.2.2.). Long term investigation of biomass and nutrient concentrations would determine whether such changes were due to long term changes or seasonal variations.

Investigations into long term recovery from harvesting of a variety of species, particularly from multiple harvest regimes, are essential for validation of harvesting as a viable management tool. Sundblad and Robertson (1988) found that rhizome biomass of *Glyceria maxima* was reduced by a harvest treatment, however, it was suggested that high external supply of nutrients, such as experienced in constructed wetlands, may overcome potential nutrient limitations in the shoot. The study by Sundblad and Robertson (1988) was still relatively short term, spanning a single growth season and further investigations into possible enhancements to long term performance, such as recovery periods are required.

Given the ability of some species to alter their distribution (via colonisation, section 2.3.2.2.), significant changes in floristic composition may be expected over time. These changes may be influenced by stresses induced by rigorous multiple harvest regimes or changes in nutrient supply regime over the long term.

Interactions between wetland components and all storage and removal processes could be incorporated into the model with more intensive investigation into wetland processes.

APPENDIX A

Table A.1: Dry weight per shoot (g) for four species of aquatic macrophyte in wetland 1 (W1) and wetland 3 (W3) at the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

	Bolboschoenus		*Phragmites*		*Triglochin*		*Typha*	
	W1	W3	W1	W3	W1	W3	W1	W3
February 96	12.37	9.73	24.47	26.27	91.54	27.92	83.17	94.04
	(±4.88)	(±5.58)	(±11.26)	(±10.15)	(±28.89)	(±13.60)	(±36.07)	(±51.95)
March	11.07	13.47	42.41	45.43	61.77	21.97	88.54	97.66
	(±4.43)	(±9.12)	(±11.83)	(±14.50)	(±30.95)	(±8.25)	(±28.73)	(±45.48)
April	11.75	12.60	33.38	34.02	69.24	27.01	56.60	54.67
	(±4.74)	(±2.25)	(±13.36)	(±6.28)	(±30.65)	(±9.96)	(±18.76)	(±27.41)
May	8.83	9.75	31.73	31.27	49.22	17.78	79.23	61.20
	(±2.79)	(±2.56)	(±14.24)	(±7.64)	(±29.69)	(±6.07)	(±21.41)	(±22.07)
June	6.34	9.54	28.99	25.06	57.60	21.26	63.39	50.74
	(±2.03)	(±2.36)	(±8.67)	(±12.64)	(±37.03)	(±5.24)	(±21.34)	(±10.74)
July	4.15	6.59	38.31	21.28	59.63	23.77	58.92	47.45
	(±1.02)	(±3.59)	(±8.85)	(±3.36)	(±21.99)	(±10.64)	(±20.94)	(±22.30)
August	5.55	5.83	27.43	22.63	55.14	46.29	52.31	33.90
	(±1.19)	(±1.33)	(±4.40)	(±8.67)	(±13.04)	(±26.66)	(±10.70)	(±19.52)
September	1.52	1.18	9.25	9.11	49.16	44.07	15.33	10.07
	(±0.38)	(±0.46)	(±4.19)	(±4.62)	(±17.12)	(±24.48)	(±5.76)	(±7.00)
October	3.61	4.32	12.32	7.46	59.73	68.20	26.61	24.75
	(±1.34)	(±2.76)	(±6.65)	(±2.63)	(±14.69)	(±34.51)	(±8.23)	(±11.28)
November	14.76	13.31	29.07	22.69	82.11	80.56	33.38	47.80
	(±1.67)	(±5.04)	(±19.39)	(±13.91)	(±25.68)	(±26.16)	(±12.37)	(±17.26)
December	14.58	13.82	40.96	38.82	84.96	83.77	46.07	76.30
	(±5.89)	(±6.84)	(±8.69)	(±9.20)	(±33.95)	(±31.34)	(±16.34)	(±22.65)
January	15.97	11.89	32.13	42.37	76.41	55.26	38.01	60.77
	(±5.09)	(±5.25)	(±14.65)	(±13.51)	(±24.88)	(±23.53)	(±19.74)	(±36.54)
February 97	17.37	3.06	45.54	3.49	93.95	34.41	64.06	6.69
	(±1.11)	(±1.18)	(±25.29)	(±1.93)	(±22.91)	(±7.94)	(±19.40)	(±2.18)

Table A.2: Dry weight per square metre (g) for four species of aquatic macrophyte in wetland 1 (W1) and wetland 3 (W3) at the Willunga wetland system over a 12 month period (n=5-10 ± standard deviations).

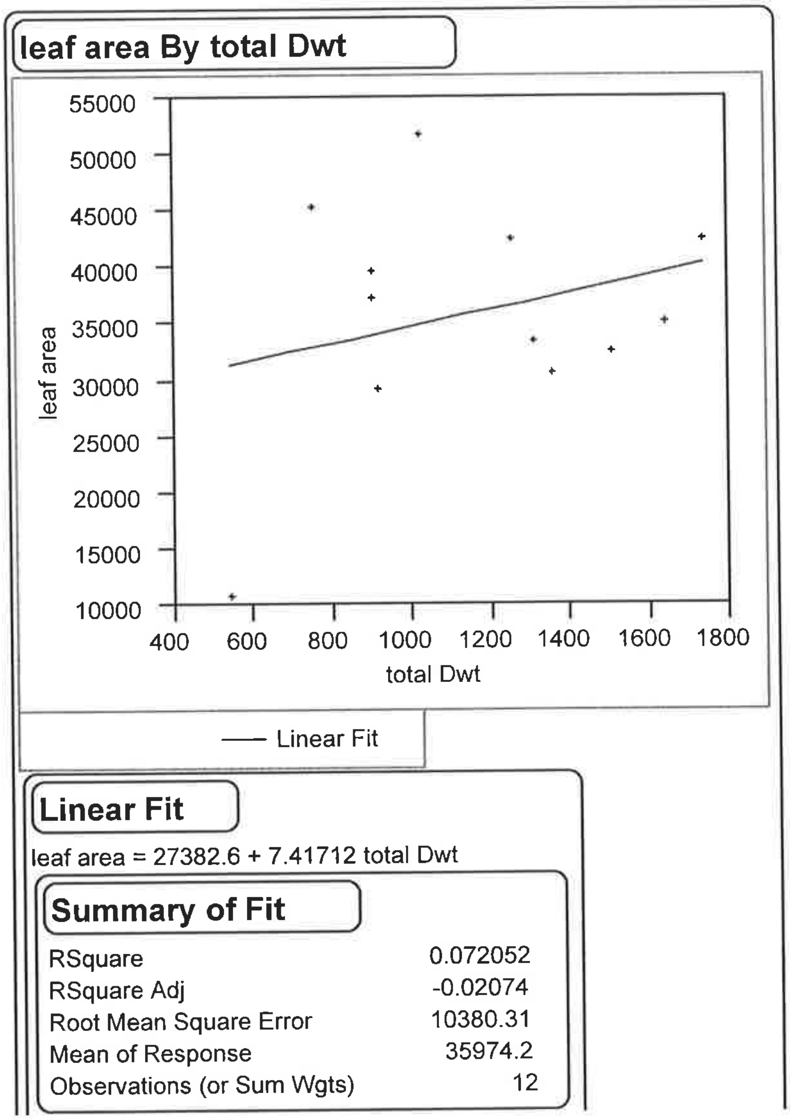
	Bolboschoenus		*Phragmites*		*Triglochin*		*Triglochin "clump"*		*Typha*	
	W1	W3	W1	W3	W1	W3	W1	W3	W1	W3
February 96	4145	2024	3699	2669	118	24	902	203	3593	3762
	(±1635)	(±1161)	(±1702)	(±1032)	(±61)	(±24)	(±471)	(±207)	(±1558)	(±2078)
March	3709	2801	6412	4615	86	17	658	142	3825	3906
	(±1484)	(±1896)	(±1789)	(±1473)	(±75)	(±18)	(±574)	(±155)	(±1241)	(±1819)
April	2049	1563	2109	1551	126	26	949	212	1902	1837
	(±827)	(±279)	(±845)	(±286)	(±124)	(±26)	(±913)	(±241)	(±630)	(±921)
May	834	804	2335	2251	85	14	642	119	2852	1763
	(±263)	(±211)	(±1048)	(±550)	(±85)	(±10)	(±654)	(±91)	(±771)	(±636)
June	563	748	2134	1804	116	14	883	113	2282	1461
	(±180)	(±185)	(±638)	(±910)	(±118)	(±9)	(±921)	(±57)	(±768)	(±309)
July	296	469	2697	1447	133	27	995	202	2168	1442
	(±72)	(±256)	(±623)	(±229)	(±100)	(±30)	(±731)	(±187)	(±771)	(±678)
August	311	326	1449	1195	105	62	732	356	1925	1030
	(±67)	(±75)	(±232)	(±458)	(±67)	(±96)	(±473)	(±526)	(±394)	(±593)
September	34	23	732	736	122	91	852	529	135	169
	(±9)	(±9)	(±332)	(±373)	(±100)	(±119)	(±703)	(±646)	(±51)	(±118)
October	286	359	1281	902	195	223	1355	1302	1149	970
	(±106)	(±229)	(±692)	(±318)	(±112)	(±242)	(±772)	(±1288)	(±355)	(±442)
November	2350	1682	3465	2451	241	200	1674	1247	1496	2256
	(±266)	(±638)	(±2312)	(±1502)	(±139)	(±206)	(±976)	(±1089)	(±554)	(±815)
December	2321	1747	4883	4193	315	228	2184	1451	2064	3601
	(±938)	(±865)	(±1036)	(±994)	(±253)	(±208)	(±1756)	(±1085)	(±732)	(±1069)
January	2542	1503	3830	4576	199	88	1376	563	1703	2868
	(±811)	(±664)	(±1747)	(±1459)	(±115)	(±81)	(±792)	(±505)	(±884)	(±1725)
February 97	2738	113	4992	302	299	45	2145	249	2921	268
	(±174)	(±43)	(±2771)	(±167)	(±270)	(±23)	(±1885)	(±127)	(±885)	(±87)

Table A.3: Nutrient concentration (mg g-1) for four species of aquatic macrophyte in wetland 1 (W1) and wetland 3 (W3) at the Willunga constructed wetland system over 12 month period (n=5-10, ± standard deviations).

		Bolboschoenus		*Phragmites*		*Triglochin*		*Typha*	
		W1	W3	W1	W3	W1	W3	W1	W3
February 96	C	450	449	456	462	431	415	446	440
		(±27.2)	(±18.7)	(±16.4)	(±14.1)	(±17.5)	(±17.4)	(±5.59)	(±33.0)
	N	14.0	17.5	16.7	11.3	31.6	33.6	14.8	13.0
		(±4.68)	(±0.59)	(±2.83)	(±3.60)	(±5.82)	(±3.14)	(±0.64)	(±0.32)
	P	4.0	4.9	1.7	1.2	4.0	4.8	2.1	2.0
		(±1.00)	(±0.42)	(±0.64)	(±0.26)	(±0.90)	(±0.67)	(±0.67)	(±0.31)
May	C	448	442	433	439	412	417	446	447
		(±3.60)	(±5.55)	(±14.3)	(±8.07)	(±4.32)	(±8.12)	(±3.95)	(±1.14)
	N	10.1	9.8	10.0	8.5	32.3	35.1	11.5	12.2
		(±1.47)	(±1.33)	(±2.18)	(±1.41)	(±2.44)	(±6.24)	(±2.49)	(±1.07)
	P	2.2	2.0	1.0	0.7	3.6	3.6	1.8	1.6
		(±0.28)	(±0.56)	(±0.18)	(±0.30)	(±0.30)	(±0.36)	(±0.38)	(±0.15)
August	C	459	452	453	434	424	400	458	449
		(±2.59)	(±4.73)	(±2.31)	(±5.89)	(±10.17)	(±2.22)	(±5.50)	(±10.00)
	N	7.7	7.3	5.6	4.7	36.2	36.6	9.8	10.7
		(±1.53)	(±1.51)	(±2.15)	(±0.85)	(±3.21)	(±4.62)	(±0.50)	(±3.55)
	P	0.9	1.2	0.5	0.4	4.5	4.0	1.4	1.5
		(±0.38)	(±0.52)	(±0.31)	(±0.16)	(±0.08)	(±0.55)	(±0.28)	(±0.64)
September	C	429	425	452	439			448	429
		(±6.25)	(±0.70)	(±10.66)	(±2.22)			(±15.32)	(±3.20)
	N	27.3	29.3	21.6	20.4			20.1	22.1
		(±2.66)	(±1.11)	(±2.57)	(±3.49)			(±2.18)	(±2.14)
	P	6.8	6.7	3.1	2.8			3.7	3.4
		(±0.36)	(±0.80)	(±0.36)	(±0.44)			(±0.62)	(±0.21)
November	C	444	427	457	452	425	421	438	429
		(±10.8)	(±25.2)	(±3.55)	(±10.0)	(±5.76)	(±3.12)	(±5.23)	(±9.24)
	N	21.3	21.4	15.7	16.7	29.1	31.9	19.6	17.8
		(±1.39)	(±2.90)	(±1.63)	(±1.72)	(±1.05)	(±4.18)	(±2.97)	(±3.70)
	P	5.7	6.3	1.5	1.7	3.8	4.6	2.6	2.3
		(±0.16)	(±0.77)	(±0.43)	(±0.43)	(±0.58)	(±0.47)	(±0.56)	(±0.11)
February 97	C	452	450	457	442	421	414	431	437
		(±7.88)	(±4.01)	(±5.37)	(±13.15)	(±3.26)	(±8.79)	(±11.04)	(±6.36)
	N	10.1	11.6	11.2	10.2	33.0	34.7	12.6	12.0
		(±0.87)	(±2.87)	(±0.87)	(±1.77)	(±3.69)	(±8.60)	(±4.00)	(±2.12)
	P	2.0	2.1	1.2	0.8	4.6	5.0	2.5	1.7
		(±1.02)	(±1.11)	(±0.18)	(±0.04)	(±0.47)	(±1.25)	(±0.94)	(±0.17)

APPENDIX B

B.1: Regression of leaf area (cm2) and total dry weight (g) for *Typha* grown in replacement series with *Triglochin* in sand substrate.



leaf area By total Dwt

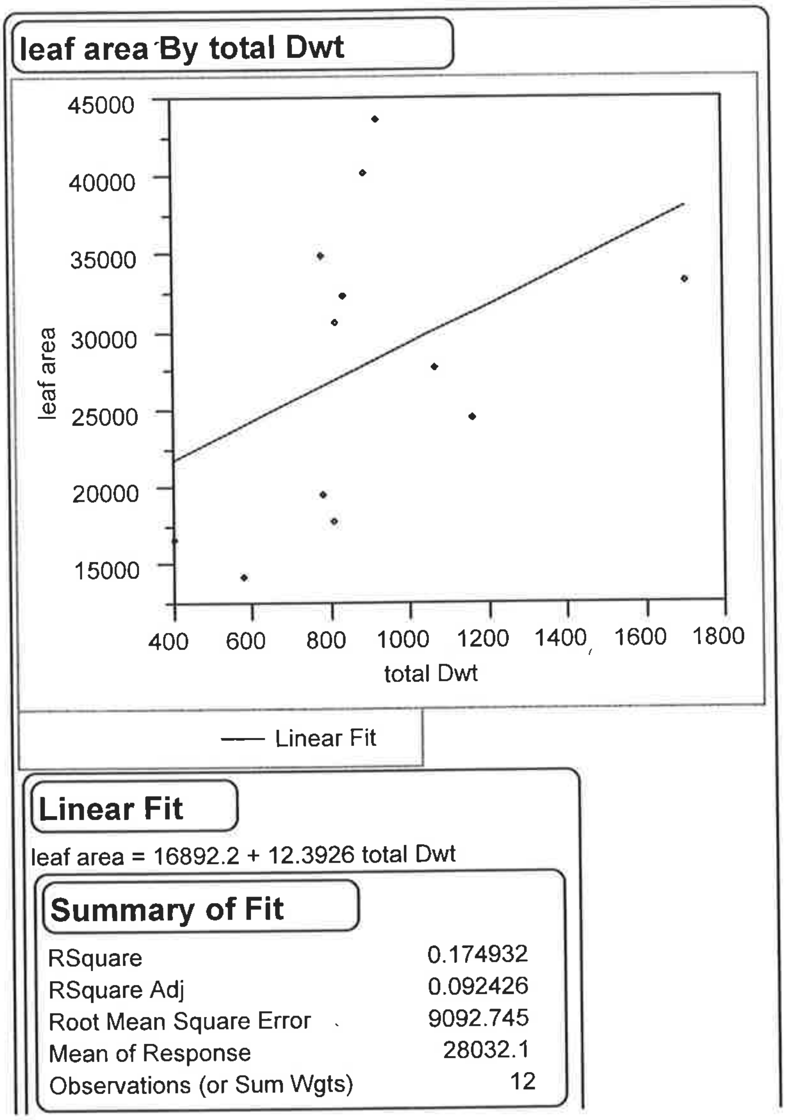
Linear Fit

leaf area = 27382.6 + 7.41712 total Dwt

Summary of Fit

RSquare	0.072052
RSquare Adj	-0.02074
Root Mean Square Error	10380.31
Mean of Response	35974.2
Observations (or Sum Wgts)	12

B.2: Regression of leaf area (cm2) and total dry weight (g) for *Typha* grown in replacement series with *Triglochin* in gravel substrate.



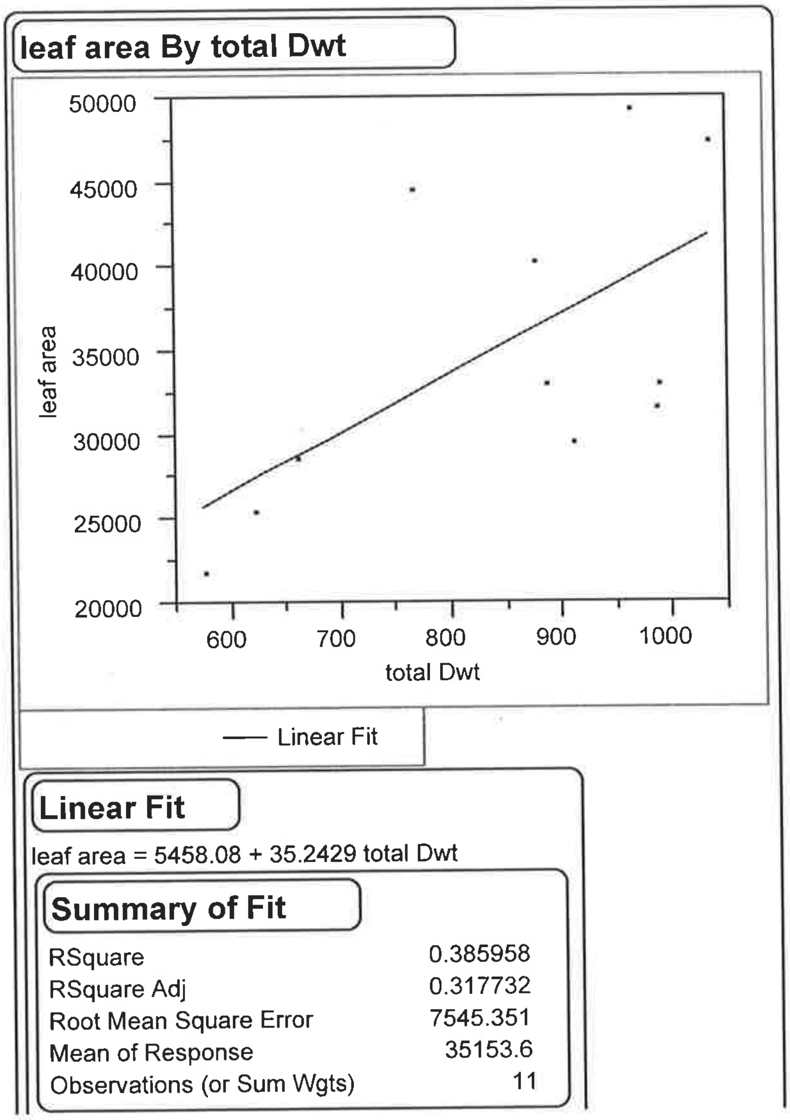
Linear Fit

leaf area = 16892.2 + 12.3926 total Dwt

Summary of Fit

RSquare	0.174932
RSquare Adj	0.092426
Root Mean Square Error	9092.745
Mean of Response	28032.1
Observations (or Sum Wgts)	12

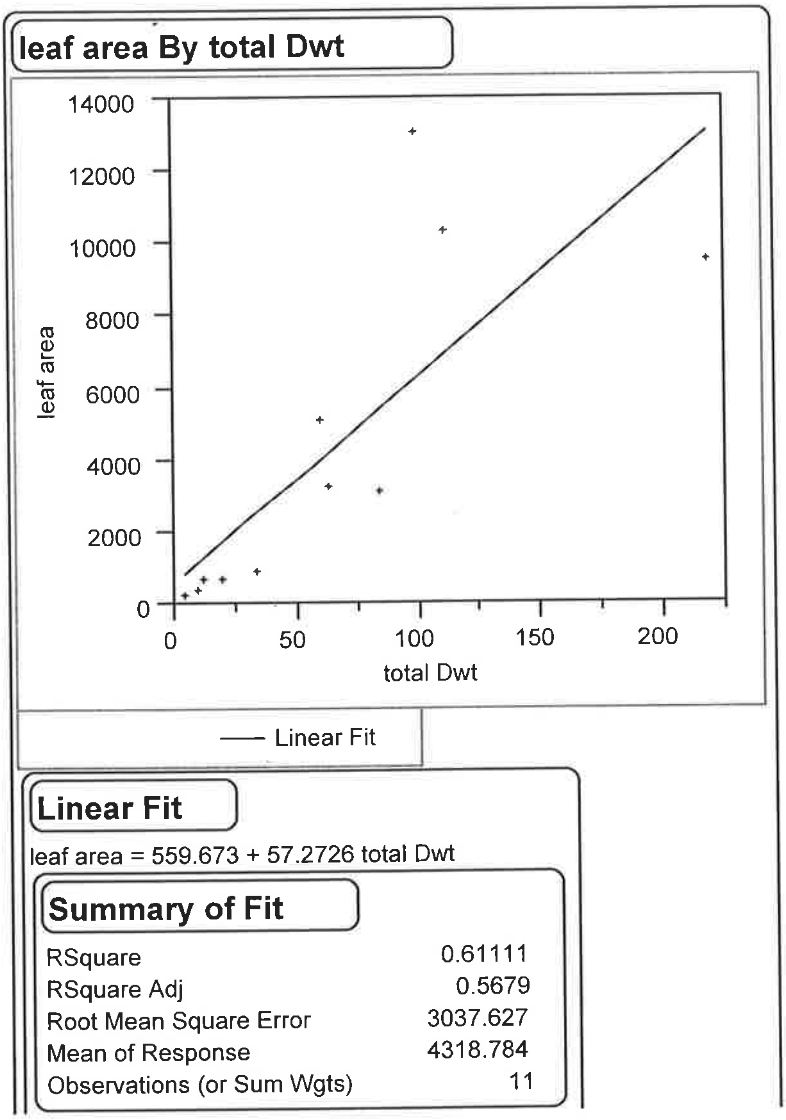
B.3: Regression of leaf area (cm2) and total dry weight (g) for *Typha* grown in replacement series with *Triglochin* in clay substrate.



```
Linear Fit
leaf area = 5458.08 + 35.2429 total Dwt

Summary of Fit
RSquare                          0.385958
RSquare Adj                      0.317732
Root Mean Square Error           7545.351
Mean of Response                  35153.6
Observations (or Sum Wgts)             11
```

B.4: Regression of leaf area (cm2) and total dry weight (g) for *Triglochin* grown in replacement series with *Typha* in sand substrate.



leaf area By total Dwt

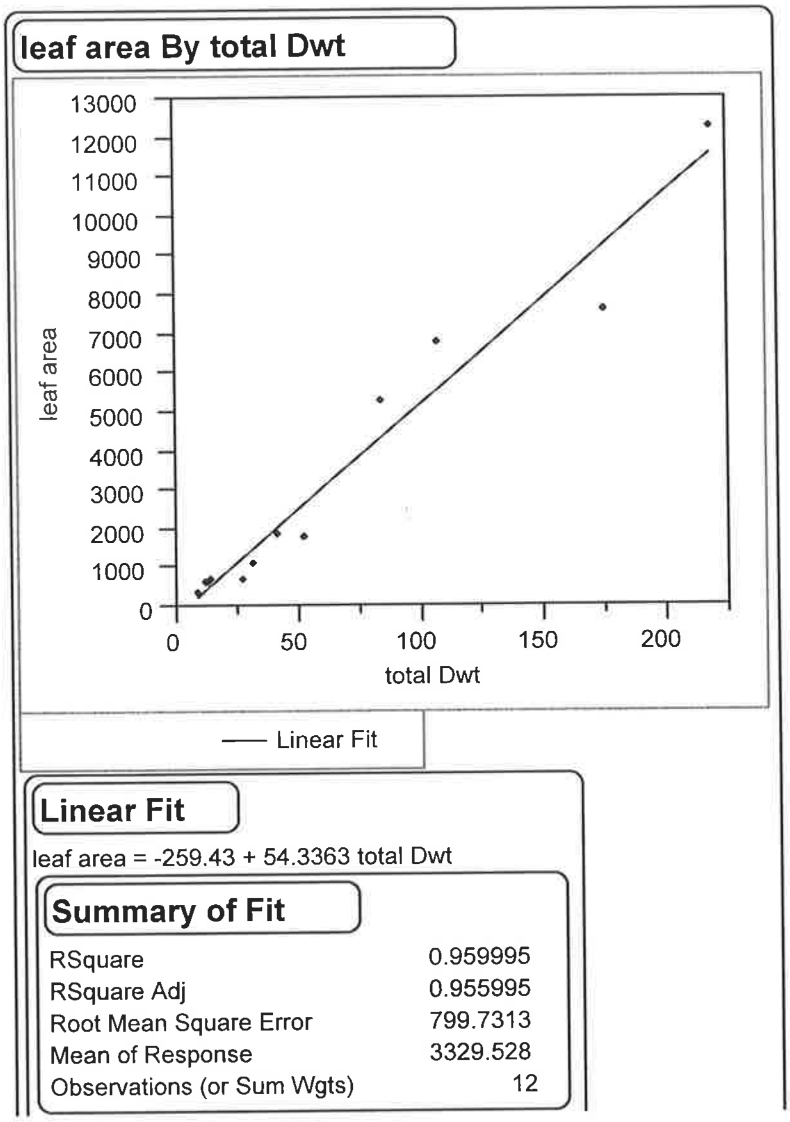
Linear Fit

leaf area = 559.673 + 57.2726 total Dwt

Summary of Fit

RSquare	0.61111
RSquare Adj	0.5679
Root Mean Square Error	3037.627
Mean of Response	4318.784
Observations (or Sum Wgts)	11

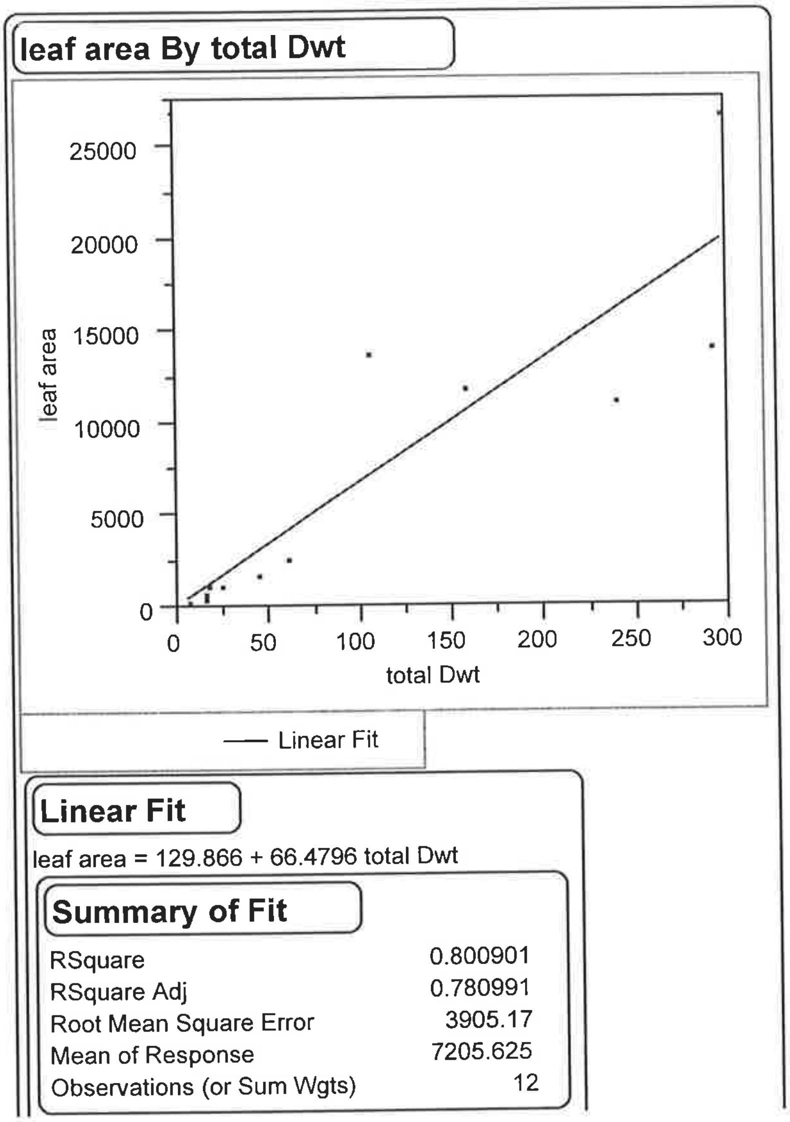
B.5: Regression of leaf area (cm2) and total dry weight (g) for *Triglochin* grown in replacement series with *Typha* in gravel substrate.



```
Linear Fit
leaf area = -259.43 + 54.3363 total Dwt

Summary of Fit
RSquare                          0.959995
RSquare Adj                      0.955995
Root Mean Square Error           799.7313
Mean of Response                 3329.528
Observations (or Sum Wgts)             12
```

B.6: Regression of leaf area (cm2) and total dry weight (g) for *Triglochin* grown in replacement series with *Typha* in clay substrate.



leaf area By total Dwt

Linear Fit

leaf area = 129.866 + 66.4796 total Dwt

Summary of Fit

RSquare	0.800901
RSquare Adj	0.780991
Root Mean Square Error	3905.17
Mean of Response	7205.625
Observations (or Sum Wgts)	12

APPENDIX C

See compact disc enclosed in back cover.

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