# ADAPTATIONS OF AQUATIC MACROPHYTES TO SEASONALLY FLUCTUATING WATER LEVELS

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## Summary

Mediterranean climates have cool wet winters and warm dry summers. Wetlands in these climates generally experience high water levels in winter and spring but dry conditions in summer and autumn. Plants growing in these habitats have adaptations to accommodate alternate periods of high and low water levels. This thesis tested the hypothesis that plant strategies to seasonal flooding can be used to predict their tolerance to variation in water regime.

*Villarsia reniformis* is a floating leaved plant which responds to inundation with rapid petiole elongation. The capacity of petioles to elongate ~~to~~ flooding is gradually lost after the leaf is exposed to the atmosphere. Petioles elongate primarily by cell division before exposure, but by cell elongation when leaves have been exposed to the atmosphere. The growth of new petioles is sustained until the leaves reach the surface. After exposure, elongation is limited by the number of cells present initially and the maximum length to which they can extend. The continual recruitment of new leaves provides responsive tissues which can adapt readily to water level changes.

The close relationship between petiole length and depth in *V. reniformis* means that biomass allocations must be flexible to accommodate the changes in above ground growth. The trade-off between above ground growth and carbohydrate storage was investigated in a pond experiment. Plants were flooded to 2, 20, 40 and 60 cm harvested after three months. Nutrients were held to limiting levels in one treatment to reduce growth and force trade-offs between tissues. Low nutrient plants allocated more biomass to petioles with increasing depth, as petioles grew longer. This allocation of growth was made at the expense of the allocation of biomass to the storage root. At the higher nutrient level, the increase in above ground growth with depth was supported without the diversion of growth from the storage root. These plants developed runners, which made up approximately 50% of biomass. Runner growth was made at the expense of storage root biomass.

*Baumea juncea* is a slow growing lignified sedge with limited responsiveness to flooding, but tolerates flooding and recovers after drawdown. Plant growth was examined after three months of complete, partial or no flooding, and after a further three months at the surface. *B. juncea* survived complete flooding, and while growth stopped underwater, stem and rhizome growth recommenced after drawdown. Shallow plants recruited the most new stems in December, and growth slowed down later in summer. The allocation of biomass was not plastic, and the proportions of biomass allocated to tissues was not affected by flooding.

These studies illustrated contrasting strategies for survival in seasonally fluctuating water levels. These were tested in the field against similar species. Plants were surveyed at four stages of flooding at Bool Lagoon, before flooding in June, during flooding in October and December and after drawdown, in March. The morphologically plastic species, *V. reniformis* and *Triglochin procerum*, responded to flooding with taller shoots and increased investment in photosynthetic tissue. However, reproductive effort was much lower in the field than in the pond experiments, and changes in the population density of both species were not related to water regime. The strategy of *B. juncea* was compared with another sedge, *Baumea arthrophylla*. Both species exhibited reduced shoot recruitment while flooded and renewed growth after drawdown. However, stem length and stem recruitment were more plastic in *B. arthrophylla*, and this species exhibited characteristics of both strategies.

Although *V. reniformis* and *T. procerum* share similar responses to water regime, they tolerate different ranges of flooding depth and duration. Similarly, *B. juncea* and *B. arthrophylla*, have similar responses to flooding and drawdown, but tolerate different water regimes. It is concluded that plant strategies cannot be used to predict the water regimes in which aquatic plants grow. The identification of the components of water regime which affect individual species provides comprehensive and biologically meaningful classifications of water regime.

## Declaration

To the best of my knowledge and belief, this thesis contains no material previously submitted for a degree or any other award, in any university by any person, or any material previously published or written by another person, except where due reference is made in the text. I consent to the thesis being made available for copying and loan if accepted for the award of the degree.

Marcus Cooling

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# Chapter 1

## General Introduction

### Wetlands in mediterranean climates in Australia

Wetlands in Australia are an important but declining natural resource. Surface water is scarce in much of the continent and wetlands are frequently isolated by dry, unflooded country. Consequently, they are the only habitats in which many aquatic and semi-aquatic communities of plants and animals are found. Their value has been acknowledged to some extent in international agreements, such as the Ramsar agreement, which protect migratory bird habitat and other wetlands of international significance (Michaelis and O'Brien 1988). Wetlands are also important economic and recreational resources. The floodplains of the River Murray provide nursery areas for fish, and form the basis for an important freshwater fishery (McComb and Lake 1988; Cadwallader and Lawrence 1990).

Since European settlement there has been widespread loss of wetlands and alterations to their flooding patterns. Water storage for irrigation and hydro-electric power generation has permanently flooded seasonal flooded wetlands (Kirkpatrick and Tyler 1988). Others have been drained to provide land for farming and urban development (Lane and McComb 1988; South East Wetlands Committee 1984). Salinisation of surface and ground water is increasing in many areas and threatening the survival of freshwater flora (McComb and Lake 1988). Weedy species, often invading degraded vegetation, are also a serious problem (Froend et al. 1993; Finlayson et al. 1988).

Some of Australia's major wetland systems are found in mediterranean climates, characterised by cool, wet winters and hot, dry summers. In the lowland areas, water bodies are generally

shallow and store little water from year to year. Consequently water levels change seasonally, generally peaking in winter and spring and recede in summer and autumn. However because of the variability of the climate, wetlands may remain in flood or drawdown for a number of years. The mediterranean climate in Australia is strongly influenced by the Southern Oscillation which increases the duration and intensity of either the wet or dry seasons and superimposes long term variability on rainfall and wetland water levels (Cawood 1996). The Southern Oscillation has two phases, La Nina and El Nino. Strong trade winds over the Pacific indicate La Nina years, which bring higher than average rainfall to eastern Australia. Weak or reversed trade winds occur in El Nino years and result in widespread drought. Phases persist from one to three years and have oscillated 17 times this century. Wetland vegetation in Australia is therefore exposed to both seasonal and aseasonal climatic cycles (Mitchell and Rogers 1985). Three important wetlands which experience these conditions are the wetlands of the south-east of South Australia, the floodplain wetlands of the River Murray and the wetlands of the Swan Coastal Plain.

In South Australia, most of the wetlands which were present in the south-east region before 1900 have been drained to provide farming land. The flat and poorly drained nature of the area meant that seasonal winter rain could once persist as surface water for months. Before 1900, 54% of this region was frequently flooded in winter to a depth of more than 30 cm (Jones 1978). The construction of a systematic drainage system was completed in 1970, leaving less than 8% of the original wetlands.

The largest remaining wetland in the region, Bool Lagoon (2690 ha), has experienced an altered water regime since drainage works were implemented in 1970. Bool Lagoon is used to retain flood water in spring. While the land downstream of Bool Lagoon is drained to the sea, water is retained in the wetland. Water is not released until flow in the downstream drain is low enough to receive the stored water. Levee banks were constructed around the perimeter to increase the water holding capacity of the wetland and to prevent water from spilling into surrounding farm land. Consequently, since the wetland was regulated in the 1960s, Bool

Lagoon has experienced deeper flooding which persists longer after winter (South East Wetlands Committee 1984). The altered water regime has led to changes in the vegetation. The sedges *Baumea arthrophylla* and *B. juncea* were once dominant but have since retreated to the driest remaining locations. In their place, the semi emergent *Triglochin procerum* is spreading, thriving in the deeper water and tolerating longer periods of flooding (Rea 1992).

In Western Australia, the vegetation of seasonally flooded wetlands on the Swan Coastal Plain have also been affected by changes to water regime (Froend et al. 1993). These wetlands are generally surface expressions of groundwater at the surface but experience the seasonal fluctuations in water level typical of wetlands in mediterranean climates. Water depths peak in spring and fall over summer to a minimum in autumn. Water regimes have been altered by groundwater abstraction, land clearing and the redirection of urban run-off. In some wetlands excessive water input has led to the decline of emergent species and fringing vegetation. In others low water levels have reduced wetland size, as the macrophyte vegetation has advanced down the elevation gradient. In addition, reduced flooding frequency has reduced the vigour of the dominant native emergent macrophyte, *Baumea articulata*, and opened up stands to invasion by *Typha orientalis* (Froend et al. 1993; Froend and McComb 1994).

A third important system of mediterranean climate wetlands, the floodplain of the River Murray, has also been affected by changed water regimes. The regulating structures on the river have reduced the frequency of flooding in some areas of the floodplain by retaining small floods. Prior to regulation floodplain wetlands were flooded at much lower flows than at present. In addition, wetlands are inundated later than before regulation because of the lag in water flow produced by the storage of water in reservoirs and weirs (Walker and Thoms 1993). The altered water regime has led to a loss of ephemeral wetlands and the replacement of species adapted to fluctuating water levels by those adapted to permanent flooding (Pressey 1990; Sainty and Jacobs 1990).

On a smaller scale, gradients in water level fluctuation have influenced plant communities between the weirs. Water levels are kept stable above weirs by the use of stop logs to control discharge. Below the weirs water levels fluctuate much more, as excess flow is allowed through and low flows are retained to maintain the weir pool level. Plants which tolerate large water level fluctuations, *Myriophyllum verrucosum* and *Cyperus* sp., are associated with these habitats. Species favouring stable water levels, eg. *Typha* spp., occur near the weir pool (Walker et al. 1994; Blanch and Walker in press).

Although the processes by which these systems are flooded differ, they are all shallow, flooded seasonally, and their vegetation is adapted to high annual and inter-annual variability. They are also all subject to manipulated water regimes. An understanding of the relationship between wetland plant growth and water regime is needed to determine water management practices for the preservation and restoration of these wetland systems.

## Water regime

The concept of water regime is necessary to interpret the growth and distribution of wetland plants because water levels in most wetlands are not static. In mediterranean climates water levels fluctuate seasonally. Under the influence of the Southern Oscillation, flooding patterns in Australia also vary from year to year. Vegetation is influenced both by short term floods in any given year (eg Coops et al. 1996) and by the history of flooding it has experienced in the past (Millar 1973; Squires and van der Valk 1992). Variables which describe components of water regime, such as depth, frequency or amplitude, do not account for the cumulative effects of flooding histories or the interactions between these components. Water regime describes the aspects of flooding histories within and between seasons which influence plant growth.

Flooding and drawdown are the two basic states in which wetlands are found and the way in which these states alternate also influences wetland vegetation. Flooding amplitude (Wilcox

and Meeker 1991), seasonality (Hultgren 1988; Blom et al. 1990) and frequency (van der Sman et al. 1988, 1993b; Walker et al. 1994) all influence the growth and reproduction of wetland plants.

The term water regime encompasses flooding depth, duration, frequency, amplitude and seasonality. Consequently there is confusion as to how water regime should be defined. It is difficult, if not impossible, to formulate a mathematical index which will represent all of these characteristics. The problem can be simplified to some extent when wetland systems are considered in isolation. In any one system most of the effects of water regime on vegetation can be attributed to a subset of these variables and the index used to describe water regime can be more precise. In the riverine wetlands of the Netherlands and the River Murray, flooding frequency is the most important factor (van der Sman 1993a, b; Walker et al. 1994). In the back waters of the Rhine river, seasonal flooding has spread from spring into summer (Blom et al. 1990). Increased or decreased water depth has influenced vegetation in impounded wetlands such as Lake Kerkini in Greece and the Malung Lakes in Sweden (Papasterigiadou and Babalonas 1992; Hultgren 1989).

Wetland water levels in mediterranean climates are dominated by seasonal fluctuations of depth associated with high rainfall in winter and low rainfall in summer and autumn (Rea and Ganf 1994a). This simplifies the definition of water regime in these systems because the seasonal flooding pattern provides a basis from which alternative water regimes may vary. Altered water regimes may have greater mean depths (Rea 1992) or more frequent fluctuations (Walker and Thoms 1993), but they are still dominated by the flooding pattern imposed by the mediterranean climate.

Mean water depth has been used successfully to characterise some aspects of water regime and to interpret plant responses (Froend and McComb 1994; Rea and Ganf 1994a; Lieffers and Shay 1982). Mean water depth accounts for the flooding history of a site because it is calculated from water levels over a number of years. However this variable does not

distinguish between short periods of deep flooding and long periods of shallow flooding. Nor is it influenced by flooding frequency, which is an important factor in the vegetation of Murray River wetlands (Walker et al. 1994).

Brownlow and his co-workers (1994) classified water regimes on the basis of the total number of days at which water is found at any depth at a particular site. This technique also accounts for flooding histories, and can be based on data over a number of years. An advantage of the technique is that the duration of flooding at particular depths is incorporated. This is more descriptive of the range of depths that vegetation experiences over the course of a flood than mean water depth. However the water regime classifications remain arbitrary, making it difficult to make comparisons between water regimes. The Brownlow et al. (1994) classification has not yet been applied to wetland communities and it remains to be seen what biological significance it may have.

Water regime is not a variable which can be described in a linear scale such as depth, duration or rate. Rather, it is a group of variables which are partly inter-dependent. Variation in single factors, such as flooding frequency, also influence other factors, such as mean water depth. Water regime is analogous to other environmental parameters which are made up of a number of components, such as climate or soil type. Both soils and climates can be classified by the nature of their component variables, but neither can be encompassed by a single linear scale.

The most important components of water regime from a botanical point of view are those which affect the growth of plants. The first priority in establishing the relationship between water regime and plant growth is the identification of those components. It is possible that water regimes are best described by the effects they have on plants and the types of vegetation they promote. This is one of the themes explored in this thesis.

## Effects of Drawdown and Flooding on Wetland Plants

Water levels in mediterranean climate wetlands generally reach their minimum in late summer and autumn and expose vegetation to shallow water or complete drawdown. Drawdown can lead to the death of aquatic macrophytes which are not adapted to periodic exposure and which desiccate or collapse without the presence of surface water (van der Valk and Davis 1980; Thomas and Stewart 1969). However the growth of perennial macrophytes which are adapted to both flooded and exposed conditions can increase as water levels fall and a greater photosynthetic surface area is exposed (Hultgren 1988; Froend and McComb 1994; Rea and Ganf 1994d). Drawdown may be the only opportunity for seeds, which cannot tolerate prolonged anoxia, to germinate (Moore and Keddy 1988; van der Valk 1978; Keddy and Ellis 1985; Armstrong et al. 1994).

Emergent macrophytes are adapted primarily to photosynthesis in air rather than water (Maberly and Spence 1989; Neilsen 1993). As light attenuates with depth, photosynthesis becomes light limited with increasing depth (Spence 1982). Epiphytes can colonise the surfaces of submerged leaves, particularly in eutrophic waters, thereby reducing light penetration and gas exchange across the leaf surface (Phillips et al. 1978). Plants which increase shoot height in proportion to depth, however, may continue to photosynthesise in optimal conditions.

Oxygen is rapidly depleted from flooded wetland soils by biological and chemical oxygen consumption (Armstrong et al. 1994). Shoot emergence also facilitates the aeration of the lacunae, which support respiration in below ground tissues in flooded soils. The lacunae are a continuous system of gas spaces throughout the plant through which gas is exchanged between the atmosphere and the roots (Armstrong 1979; Sculthorpe 1967). Anoxic soils are potentially harmful to emergent macrophyte roots through the depletion of oxygen from plant tissues and the toxic effects of the reduced nitrogen and sulphur compounds which accumulate

in waterlogged soils (Brändle 1991; Crawford and Brändle 1996). Roots are partly protected from these conditions by the supply of air from emergent tissues.

Deep flooding can have consequences for plant growth in subsequent years. The shoot height of plants is generally increased in flooded plants by the re-allocation of growth from below ground carbohydrate stores to above ground tissues. Deeply flooded plants accumulate less carbohydrate in below ground storage tissues and have a smaller tuber biomass than plants in shallow water (Rea and Ganf 1994d). Carbohydrate stores supply the energy for new shoot recruitment and vegetative reproduction in spring (Brix 1989; Steinmann and Brändle 1984a, b; Kausch et al. 1981). Deeply flooded rhizomes of some species continue to produce shoots for a number of years until their reserves are exhausted (Wilcox and Meeker 1991; Shay and Shay 1986; Millar 1973). Conversely, plants grown at depths which support abundant growth produce large stores of carbohydrate which lead to increased growth in the following spring (Lieffers and Shay 1982; Squires and van der Valk 1992).

## Water regime strategies

The mechanisms and adaptations by which plants adapt to water regimes can be grouped into strategies (Mitchell and Rogers 1985; Grime 1979). Strategies vary in the degree of morphological plasticity of the shoot, the time of year in which plants are active and the resistance to adverse conditions. Consequently plant strategies have been correlated with tolerances to variation in components of water regime (van der Valk 1981; Sculthorpe 1969; Blom et al. 1990). In this thesis, the term 'water regime strategy' refers to the suite of adaptations by which plants respond to water level fluctuations.

Some wetland species are adapted specifically to flooded conditions, and die or become dormant when water levels recede. Shoots may have little or no strengthening tissue and rely on the water for support. Species such as *Vallisneria spiralis* and *Myriophyllum variifolium*

have little resistance to water loss and readily desiccate in the absence of surface water (Blanch and Walker in press; Brock 1991). These species survive in temporarily flooded wetlands by persisting as seeds or propagules during drawdown (Casanova 1994; Casanova and Brock 1996; van der Valk 1981).

Drawdown provides a habitat in seasonally flooded wetlands to which some plants are specifically adapted. Seeds may persist for many years in the seed bank and germinate in response to the recession of flood water (Blom et al. 1990; Leck and Simpson 1995). In the Delta Marsh, Manitoba, the mud flats provide an environment in which opportunistic species or summer annuals, such as *Chenopodium rubrum,* grow and set seed before they are flooded again in winter (van der Valk 1981). Without the ability to either increase shoot height, photosynthesise under water or resist anoxia, this species dies soon after flooding (Blom et al. 1990; van der Sman et al. 1993a).

Heterophyllous species are adapted to life in both flooded and exposed habitats. Leaf morphology depends on whether development occurs in air or water. Aerial leaves are protected from water loss by a cuticle and a limited surface area to volume ratio, and fix atmospheric carbon dioxide during drawdown. The morphology of submerged leaves facilitates the uptake of dissolved carbon dioxide from the water column. Leaves are generally thin and spidery and have a lower surface area to volume ratio than aerial leaves. The thickness of the cuticle is reduced, further enhancing dissolved gas exchange. Heterophylly is generally found in species with fleshy, unlignified tissues, such as *Ranunculus* spp., *Callitriche* spp. and *Myriophyllum* spp. which are supported by the water column, but also extend some leaves into the air (Sculthorpe 1969; Young et al. 1995).

Perennial emergent macrophytes mainly fix carbon from aerial shoots and are adapted to both flooded and dry conditions. This is the predominant strategy in some of the most widespread wetland families, including the Cyperaceae, Typhaceae and Juncaceae. Emergent shoots are strengthened by lignin (Metcalfe 1971) and support themselves during drawdown and

flooding. Most emergent species can grow in the absence of surface water, but generally grow at faster rates when flooded. The optimal depth differs from species to species and influences both their position on the elevation gradient of wetlands and their competitive ability in different water regimes (Squires and van der Valk 1992; Spence 1982; Grace and Wetzel 1982; Grace 1987). Emergent shoots fix carbon dioxide and supply below ground tissues with air, through the lacunae, to prevent anoxia in roots growing in highly reduced waterlogged soil.

Shoot height generally increases in response to flooding depth (eg Grace 1989; Coops et al. 1996; Lieffers and Shay 1982; Stevenson and Lee 1987). Emergence is a priority in this strategy and, with increasing depth, biomass is reallocated from other tissues to support shoot growth. However emergent plants can be flooded to depths greater than shoots can respond. A substantial below ground carbohydrate reserve and a high resistance to anoxia provide some species with resilience to prolonged deep flooding (Brändle 1991; Crawford and Brändle 1996; Weber and Brändle 1996; Armstrong et al. 1994). It may take a number of years for such resistant plants to be eliminated from wetlands by deep flooding (Millar 1973; Shay and Shay 1986; Wilcox and Meeker 1991). *Scirpus maritimus* becomes dormant when flooded to a greater depth than it can respond to, by not producing a shoot and conserving resources in the rhizome until conditions become favourable. This species is particularly resilient to prolonged deep flooding, and shoots can be produced from dormant, flooded rhizomes after more than 20 years (Squires and van der Valk 1992).

The shoot height of floating leaved species is also closely related to flooding depth. Floating leaved plants fix carbon from the atmosphere in flooded conditions with a minimum of structural tissue, and high rates of biomass production have been recorded in *Nymphoides peltata*, *Nuphar luteum* and *Nymphaea tetragona* (Brock et al. 1983; Twilley et al. 1985; Kunii and Aramaki 1992). Petioles are generally flexible and bend in response to changes in water level, so that leaves remain floating at the surface when water levels fall. A number of semi-emergent and floating leaved species, such as *Nymphoides peltata*, *Rumex maritimus* and

*Raunnunculus pygmaeus*, rapidly elongate their shoots rapidly when water levels rise (Ridge and Amarasinghe 1984; van der Sman et al. 1988; Horton 1992). Petiole or shoot growth accelerates when leaves become submerged and continues until leaves are again exposed to the air (Arber 1920; Funke and Bartels 1937; Voesenek and van der Veen 1994). This mechanism can restore contact between leaves and the atmosphere in a matter of hours.

## Hypothesis

Water regime is the primary environmental factor governing the composition and distribution of wetland vegetation (Spence 1982; van der Valk 1994; Boon et al. 1994; van Groenandael et al. 1996). The effects of natural and manipulated water regimes on wetland plants are consequently a fundamental consideration in wetland management. The characterisation of water regime tolerances for individual species is a long term process however, and an impractical approach to the management of diverse wetland communities. Detailed experimentation is required to determine the tolerance of species to the possible variations in water regime components they experience in the field. As illustrated above, the duration of flooding and drawdown, the frequency, amplitude and depth of flooding may all influence plant survival. The tolerances of the vegetation of Bool Lagoon, in South Australia, to a variety of water regimes have been investigated in this manner (Denton and Ganf 1994; Rea and Ganf 1994a, c, d). However such studies cannot be undertaken into the different plant communities of every wetland which is threatened by changed water regimes. A method is needed whereby water regime tolerances can be applied more generally to wetland vegetation to broaden the application of investigations into individual species.

Tolerances to various water regime components may be shared between plants with the same water regime strategies. Plant strategies have frequently been correlated with water regimes, and they may provide a more general and simpler basis to classify species' water regime tolerances. Alterations to water regimes in wetlands frequently lead to the disappearance of

whole suites of species. Shifts to deeper flooding generally lead to the decline of emergent macrophytes and promote submerged or semi-emergent species (Millar 1973; van der Valk 1994). Increased water level fluctuations have replaced emergent macrophytes with floating leaved species (van de Steeg 1984; Brock et al. 1987; Papastergiadou and Babalonas 1992). Changes at a community level can generally be related to shifts in plant strategy types.

In this thesis it is proposed that the responses of perennial emergent macrophytes can be generalised into two alternative flooding strategies: plants which react to flooding and plants which rest while flooded. Reactive plants respond to flooding with increased shoot growth. They are active while flooded and change their morphology to ameliorate flooding effects. Resting plants have a limited capacity to change their morphology. They are relatively dormant while flooded and conserve resources until water levels fall. This strategy would involve adaptations to tolerate prolonged top flooding.

This hypothesis is tested with two species found in seasonally flooded wetlands in southern Australia. *Villarsia reniformis* R. Br. (Menyanthaceae) is a floating leaved perennial species which actively responds to flooding with increased shoot growth. *Baumea juncea* R. Br. Palla (Cyperaceae) is a lignified emergent sedge. These species are found in Bool Lagoon in South Australia at elevations corresponding to similar water regimes. The water regime strategies of these species were compared to similar species in the field. *B. arthrophylla* Nees. Boeckeler is a lignified sedge with a similar water regime strategy to *B. juncea* (Rea and Ganf 1994a). *Triglochin procerum* R. Br. (Juncaginaceae) is a fast growing semi emergent species. The shoot height of *T. procerum* is closely related to water depth, and this species shares a similar water regime strategy to *V. reniformis* (Rea and Ganf 1994a).

## Chapter 2

# The Accommodation of Water Level Changes by the Floating Leaved Plant, *Villarsia reniformis*

## Introduction

Many aquatic plants exhibit accelerated shoot elongation rates in response to flooding. Petioles or stems grow rapidly to the surface when submerged, and restore contact between the leaves and the atmosphere. Once the leaves are exposed, growth returns to a slower rate (Arber 1920; Funke and Bartels 1937; Sculthorpe 1967). The petioles or stems of these species are sensitive to high concentrations of ethylene, which accumulates in submerged tissues and triggers cell elongation (Osborne 1984; Voesenek and van der Veen 1994). In many of these species, coincident with the rapid elongation response is the continual recruitment of leaves, as for *Rumex* spp. (Voesenek and Blom 1989), *Ranunculus pygmaeus* (Horton 1992), *Sagittaria* spp. (Funke and Bartels 1937; Suge and Kusanagi 1975) and *Nymphoides peltata* (Ridge and Amarasinghe 1984).

In particular, nymphaeid plants of the Menyanthaceae and Nymphaceae elongate rapidly when flooded (Funke and Bartels, 1937; Ridge and Amarasinghe 1984) and recruit leaves at high rates but with lifespans of only 20 - 30 days (Tsuchiya 1991; Kunii and Aramaki 1992). These species have high rates of annual production for their relatively small standing biomasses (Brock et al. 1983; Twilley et al. 1985; Kunii and Aramaki 1992). While the physiological triggers for accelerated elongation are well documented (Osborne 1984; Jackson 1990; Voesenek and van der Veen 1994), the ecological implications of how this response interacts with the recruitment and lifespan of leaves are not well understood.

A consequence of high recruitment rates is that young leaves and petioles are always present in the canopy of the plant. Young petioles are more sensitive to submergence and potentially grow to a greater length than old petioles (Voesenek and Blom 1989; Horton 1992; Rijnders et al. 1996; van der Sman et al. 1991). Leaf recruitment may contribute to the readiness of aquatic plants to respond to fluctuating water levels. In addition, leaves die prematurely if they fail to reach the surface (Brock et al. 1983, 1987; Tsuchiya 1991), which would make leaf recruitment a priority when plants are flooded rapidly. There is an interaction between leaf recruitment and petiole elongation which is yet to be clarified.

If the responsiveness of petioles to flooding is dependent upon their age, then leaf recruitment rates and life spans are fundamental parameters in models to describe how floating leaved plants survive in habitats with fluctuating water levels. Seasonally adverse conditions are particularly prevalent in mediterranean climates, as in the south-east of South Australia. In these climatic zones wetland plants are subjected to very rapid water level changes. During the winter water levels may rise at rates in excess of 10 cm per day to reach a depth of up to 1m (South East Water Conservation and Drainage Board data). Similarly, during the hot summer months when evaporation exceeds precipitation by a factor of 10 (Specht 1972), low water levels leave the plants exposed to an inhospitable environment.

The role of rapid petiole elongation in the flood accommodation strategy was investigated in *Villarsia reniformis* R. Br. This species is a floating leaved macrophyte which tolerates a wide range of water levels. *V. reniformis* is found in standing water up to 60 cm but also persists when stranded by falling water levels (Aston 1973). In standing water the plant has numerous leaves and elongate petioles (up to 68 cm) but in exposed condition the petioles are usually less than 2 cm. This species was chosen to examine how it responded to various flooding rates, experienced by this plant in wetlands in the south east of South Australia (Brownlow et al. 1994). In this chapter it is proposed that the combination of the three co-incident characteristics -- accelerated elongation, reduced elongation with increasing age and

continual leaf recruitment -- permit this species rapidly to adapt to changes in surface water depth.

## Methods

*Villarsia reniformis* plants were collected from Bool Lagoon (37°08'S, 140°40'E) with permission from the National Parks and Wildlife Service. Plants were washed, weighed and potted in sealed 3.4 litre pots in 80/20 sandy loam. When branched or joined by runners, plants were separated into individuals. The surface of the soil was sealed with 2 cm of clay to limit the infusion of oxygen and reproduce the anaerobic conditions of wetland soils (Muller et al., 1994).

Experiments were conducted in outdoor ponds (4.5 m * 3.5 m * 1.2 m deep). Plants were acclimatised to pond conditions at 2 cm depth for 3 weeks prior to the commencement of the experiments. Pond surface temperature ranged between 19°C and 27°C, pH from 7 to 7.4, conductivity from 600 to 800 $\mu Scm^{-1}$, dissolved oxygen at the surface from 74 to 105 %. The water was usually clear with a light attenuation coefficient, **k**, of 0.7 $ln(units)m^{-1}$ (ducks disturbed the soil in pots in one week and the **k** rose to 2.5 $ln(units)m^{-1}$).

*Petiole Elongation Rates*

The influence of leaf exposure on the elongation rates of *V. reniformis* petioles was investigated. Six *V. reniformis* plants were exposed to three flooding rates: two control plants remained at 2 cm depth and four plants were flooded at 12 cm every 6 days to 60 cm, where they remained for 14 days. Of these, two plants were left at 60 cm and two were gradually re-exposed at the rate of -12 cm every 6 days.

Petioles were produced too rapidly for the growth of every petiole to be followed. Instead, the length of every alternate petiole was repeatedly measured to the nearest millimetre. Measurements were made just before every depth change and on the following two days. Plants growing at a constant depth were measured once every 6 days.

Elongation rates (cm $day^{-1}$) for new, floating and reflooded petioles were calculated as plants were gradually flooded. All values were based on a linear rate of two or more petiole length measurements. Averages were calculated from replicate petioles on each plant. Plants did not have significantly different rates for each elongation type, and pooled rates are shown.

New petiole elongation rates were calculated for submerged petioles longer than 10 cm, with leaves which had never been exposed to the atmosphere. Surface elongation rates were calculated for leaves floating in depths of 12 cm or more at the leaves' first contact with the surface. Reflooded petiole rates were measured when leaves were resubmerged by rising water after a single exposure to the atmosphere.

*Cell Division and Elongation*

An experiment was designed to determine whether the cellular mechanism for petiole elongation changed after leaves were exposed to the atmosphere. Plants were potted in 500 mL of sandy loam, with the addition of 2 g 6-9 month Osmocote. They were grown in glass tanks, 20 x 30 cm (base) x 60 cm (tall), in a glasshouse at 26°C. Plants were acclimatised to the initial flooding depth (20 cm) for three weeks before the water level was manipulated.

Water depth was increased from 20 to 40 cm in the tanks. Comparisons were made between the flooding responses by young petioles with unexposed leaves (approximately 16 cm long), and older petioles whose leaves had been floating at the surface for six days. Four replicate petioles were sampled before flooding, at 20 cm depth, and just before leaves reached the surface after the depth was increased to 40 cm. In total 16 plants were used. Petioles were marked into five equal sections with spots of petroleum jelly mixed with charcoal powder

(Funke and Bartels 1937). Section lengths were measured before and after flooding. Epidermal cell lengths were measured under a microscope on excised petioles after flooding. The length of 15 randomly selected epidermal cells were measured in the middle part of each section using an eye-piece graticule.

Petioles with unexposed, submerged leaves were harvested when approximately 16 cm long. Petioles with leaves which had been exposed at the surface for 6 days were approximately 28 cm long. Petioles which were subsequently flooded to 40 cm were between 35 and 40 cm long when harvested.

*Response of a Population to Gradual Flooding*

The interaction of petiole recruitment and petiole elongation was investigated in a population of plants flooded at four rates. The rate of petiole recruitment was manipulated by supplying plants either with abundant nutrients or growing them in nutrient poor soil. Twelve replicate plants were randomly assigned to eight treatments and an initial harvest group, with the condition that there were no significant differences in fresh weight of the groups, as measured at potting.

Two nutrient levels and four flooding treatments were applied in a factorial design. The experiment was split between two replicate ponds. Six replicates from two nutrient levels were mixed on four fibreglass trays in each pond, enabling depth adjustment for the water treatments.

At the beginning of the experiment, 7 g of 6-9 month Osmocote Plus controlled release fertiliser was added to the soil of the + Nutrient pots by inserting pellets to the soil with a cut-off syringe. This technique overcomes the problem of nutrient toxicity from a single dose of nutrients, as the minerals were released at a constant rate which is dependent only on temperature (Grace Sierra Inc., personal communication). The nutrient addition gave a loading rate of 60.8 g N $m^{-2}$ $year^{-1}$.

Four flooding treatments were applied to the plants. Control plants remained at 2 cm depth for the entire experiment. Flooded plants were gradually lowered to final depths of 20 cm, 40 cm and 60 cm over 30 days at rates of 4 cm per 6 days, 8 cm per 6 days and 12 cm per 6 days, respectively.

Recruitment and petiole length data were collected at least once a week. The length of the longest leaf and petiole was the combined length of the leaf and petiole, measured to the nearest millimetre. The length of the longest leaf-petiole was recorded for each pot. The relative lengths of the leaf and petiole is static and leaf-petiole length is referred to as petiole length throughout this chapter. Leaf recruitment was measured by tagging the youngest leaf with a plastic coated wire ring around the base of the petiole, and counting the leaves which subsequently formed. Leaf death, longevity and canopy were calculated from the total number of leaves per pot and recruitment.

*Statistics*

Unless otherwise stated, tests for the significance of differences and interactions between two treatments were made with two-way anovas. Variability estimates are standard deviation. All statistical tests were performed using SAS (The SAS Institute, 1992). Probabilities for F values are referred to in the text, and full F tables are given in appendix A.

The analysis of the survival of original leaves in four water treatments (section 3) involved an unbalanced design because of missing values. Plants with multiple rosettes produced leaves at particularly high rates and records of petiole recruitment were not always accurate, so the data were excluded. General Linear Models were fitted to the data (SAS Institute, 1992), with separate models fitted for Weeks 4 and 5.

## Results

Figures are found at the end of the thesis.

### Petiole Elongation Rates

At 2 cm depth, leaves were exposed to the atmosphere one or two days after they emerged. Petioles grew continuously over a long period, reaching a final length of approximately 10 cm (figure 2.1a) after 40 days. Petioles which were recruited in deeper water grew at a faster rate until their leaves reached the surface. Once the leaf was exposed at the surface, petiole growth slowed down. When depth was increased again, elongation was re-initiated, and some leaves reached the surface more than once (figure 2.1 b,c). However after leaves had been exposed at the surface, petiole elongation was limited. Petioles elongated once or twice in response to gradual flooding, but were eventually submerged by the rising water. Newly recruited petioles had a greater potential to reach the surface than petioles which had been exposed to the atmosphere.

**Table 2. 1**
Rates of daily elongation of *V. reniformis* petioles at different stages of exposure. Control plants (n = 2) remained at a constant water level of 2 cm. Flooded plants (n = 4) were gradually lowered to 60 cm depth. Standard deviation is in brackets. Submerged rates were measured on petioles growing through the water column leaves which had never been exposed to the air. Surface rates are from petioles with leaves floating on the surface. Leaves of reflooded petioles were exposed to the air once and then resubmerged by a depth increment.

| Treatment | Leaf Exposure | Elongation Rate (cm $day^{-1}$) |
|---|---|---|
| Control | Surface | 0.24 |
| Flooded | Submerged | 10.4 (1.2) |
| | Surface | 0.65 (0.1) |
| | Reflooded | 6.01 (0.8) |

Petiole age also influenced the maximum length to which petioles could grow when flooded. The elongation of alternate petioles from one plant flooded in stages from 2 cm to 12 cm and

24 cm depth are shown in figure 2.2. Before flooding, petiole length depended only on age (white bars). When the depth was increased to 12 cm, all petioles lengthened until their leaves reached the surface. After further flooding to 24 cm, only the youngest petioles continued to respond (hatched bars). At the beginning of the experiment, the flooded plants had an average of 21 leaves each (n=4, sd=1.7). Of these, 92 % (sd=11) were unable to reach the surface at 24 cm depth, 12 days later, and 100% were submerged or dead after the third depth change to 36 cm, after 18 days. Petioles remained submerged apparently because the limit of their responsiveness had been reached.

Petiole elongation rates were closely related to leaf exposure. Rates at 2 cm were low, but were sustained over a long period of time, which left space for new leaves in the centre of the rosette. Petioles elongated more rapidly when submerged, until the leaves reached the surface (table 2.1). Once the leaves floated on the surface, petioles continued to lengthen, but at less than 10% of the submerged rate. Rising water flooded floating leaves and stimulated faster elongation until they were exposed again. Rates were slower after exposure, but petioles were more than able to match the overall flooding rate of 2 cm $day^{-1}$. Not all leaves reached the surface after repeated or prolonged exposure (including the petioles initially present on the plants), and submerged rates gradually slowed to a halt.

When the water level was gradually lowered (fig 2.1c), petiole growth slowed when the leaf reached the surface. Petioles of exposed leaves continued to slowly lengthen, even when the water level was falling.

**Table 2.2**
The length of petiole sections and epidermal cells in five sections from the base to the apex of the petiole. Values are the means of 4 petioles; standard deviations are in parentheses. Cells were measured in petioles with submerged leaves at 20 cm depth or flooded from 20 to 40 cm, just before leaves reached the surface. Petioles with floating leaves were measured after leaves had been exposed for 6 days at the surface, or after they were subsequently flooded from 20 to 40 cm but leaves had not yet reached the surface.

| Leaf Exposure | Petiole Section | | Cell Length (μm) | | Section Length (mm) | |
|---|---|---|---|---|---|---|
| | Depth → | | 20 cm | 20-40 cm | 20 cm | 20-40 cm |
| Leaves Unexposed | Basal | 1 | 328 (89) | 480 (53) | 34 (4.8) | 49 (5.4) |
| | | 2 | 300 (76) | 534 (49) | 28 (6.5) | 56 (15) |
| | | 3 | 254 (73) | 443 (17) | 30 (4.1) | 71 (12) |
| | | 4 | 184 (48) | 364 (34) | 33 (2.9) | 96 (7.5) |
| | Apical | 5 | 139 (35) | 233 (10) | 33 (6.3) | 107 (19) |
| Leaves Floating | Basal | 1 | 483 (135) | 438 (41) | 56 (7.5) | 64 (6.3) |
| | | 2 | 424 (108) | 514 (52) | 58 (5.0) | 68 (2.9) |
| | | 3 | 341 (90) | 510 (68) | 56 (4.8) | 93 (11) |
| | | 4 | 253 (73) | 405 (56) | 60 (5.8) | 98 (2.9) |
| | Apical | 5 | 183 (44) | 316 (68) | 53 (10) | 81 (17) |

The initial 21 leaves present on the flooded plants were all exposed and, as shown above, elongation in these petioles was limited. The life span of these leaves was severely curtailed by submergence, even though many still survived in the control. None of the leaves initially present on the gradually flooded plants were still alive after 30 days (one week after the plants reached the final depth of 60 cm) but 60% of the original 12.5 leaves (n=2 plants) were still living on plants at 2 cm depth. Leaves which lived in exposed conditions lived longer than those which were submerged by rising water.

Continual recruitment provided petioles which could grow through deeper water and re-extend when flooded. All plants recruited leaves throughout the experiment, at 5.4 leaves per week (n=2) for control plants and 3.7 leaves per week (n=4 sd=0.4) for flooded plants. New leaves grew directly to the surface, regardless of the depth, and replaced leaves which had lost the capacity to accommodate rising water.

## The Elongation Mechanism

Cell elongation is the primary mechanism by which ethylene sensitive species lengthen stems and petioles when they are flooded (Osborne 1984; Voesenek and van der Veen 1994). An experiment was designed to test whether the mechanism of petiole elongation in *V. reniformis* changed after leaves were exposed to the surface. It was proposed that petioles grew primarily by cell division before leaves were exposed to the atmosphere. After leaves were exposed petiole elongation becomes limited, and it was proposed that cell elongation becomes the primary mechanism in these petioles.

Submerged petioles in 20 cm of water were marked in five sections, each approximately 30 mm long (table 2.2) Cells were longest in the basal section (328 μm), and were progressively shorter towards the apex (139 μm). This suggests that the number of newly divided, young cells increased towards the top of the petioles. All sections of these petioles lengthened when the depth was increased from 20 to 40 cm. The length of the apical section increased the most, by approximately three times, and sections elongated progressively less towards the base of the petiole. Cell elongation contributed to the lengthening of these petioles, and cell length increased in all sections.

Petioles with floating leaves in 20 cm of water were also marked in five sections, each approximately 56 mm long (table 2.2). The cells in these petioles were approximately one third longer than in the petioles with submerged leaves at 20 cm depth. This demonstrates that in six days cells increased in length while leaves floated at the surface.

All petiole sections lengthened when the depth was increased to 40 cm. The basal section elongated the least (8 mm), and most of the elongation took place in the upper three sections. The change in cell lengths corresponded to the elongation in each section. The length of cells

in the basal sections changed little, but the cells in the upper three sections had elongated by between 50 and 78 %.

Cell elongation contributed to petiole elongation in petioles with exposed or unexposed leaves. Petiole cells lengthened while leaves floated at 20 cm depth and also lengthened when the depth was increased from 20 cm to 40 cm. Cells also lengthened when petioles with unexposed leaves grew to the surface through 40 cm of water. However, cell elongation does not account for all of the elongation of all of the petioles (Table 2.3). For example, the apical section of petioles with unexposed leaves increased by more than three times when the depth was increased from 20 to 40 cm, but the cell length increased by less than 2 times. Clearly, cell division also contributed.

**Table 2.3**
The proportion of petiole elongation contributed by cell elongation. The actual elongation is the change in length, in millimetres, of each section from 20 to 40 cm depth. The predicted elongation is the calculated increase in petiole length based on the change in cell length in table 2.2. Predicted elongation is the initial section length multiplied by the proportional increase in cell length from 20 to 40 cm.

| Leaf Exposure | Petiole Section | | Actual Elongation (mm) | Predicted Elongation (mm) |
|---|---|---|---|---|
| Leaves Unexposed | Basal | 1 | 16 | 15 |
| | | 2 | 29 | 21 |
| | | 3 | 41 | 23 |
| | | 4 | 64 | 32 |
| | Apical | 5 | 76 | 21 |
| Leaves Floating | Basal | 1 | 8 | -5 |
| | | 2 | 10 | 12 |
| | | 3 | 37 | 28 |
| | | 4 | 38 | 37 |
| | Apical | 5 | 29 | 40 |

Cell elongation accounted for 100 % of the length increase in the basal section of petioles with unexposed leaves. Cells also elongated in the more distal sections, but accounted for progressively less of the increase in petiole length. Hence cell division must have contributed

to petiole elongation in sections 2 to 5, and became the predominant petiole elongation mechanism towards the petiole apex.

The basal section of petioles with floating leaves barely changed in length when the depth was increased. The cell lengths were also approximately equal and predicted a minimal section length change. The increase in cell length in sections 2, 3 and 4 was closely approximated by the increase in section length. In these sections cell elongation was the predominant mechanism for petiole elongation. Cell elongation overestimated petiole elongation by a factor of 0.3 in the apical section. This may be due to an inaccuracy in the measurement of apical section lengths. The exact position of the junction of the leaf and petiole may not have been located as accurately in the tanks, on intact petioles, as it was on harvested petioles, which were cut into sections and then measured. However the general trend that cell elongation accounted for most petiole elongation also held for the apical section.

### The Elongation Response of a Population to Gradual Flooding

Leaf age and exposure emerged as important factors in the capacity of *V. reniformis* to respond to a gradually increasing water level. This species continually recruits new leaves, at a rate which can be controlled by the supply of nutrients. It was hypothesised that a high rate of leaf recruitment increases the responsiveness of *V. reniformis* to gradual flooding by replacing old and unresponsive petioles with new petioles capable of greater growth. Consequently, the importance of leaf recruitment in the response of a population of plants to gradual flooding in plants grown in nutrient poor sand was compared with plants grown with additional nutrients.

To follow the influence of gradual flooding on a population of plants the length of the longest petiole was measured in populations with and without added nutrients. The results of the enriched plants are considered first and then compared with the nutrient poor population.

*Flooding*

Petioles responded rapidly to inundation and plants maintained the capacity to respond over the whole flooding period (figure 2.3a). At constant 2 cm, petioles grew to a maximum length of approximately 15 cm throughout the first 30 days of the experiment. Plants which were gradually flooded responded to inundation with rapid petiole elongation, which restored contact between the leaves and the atmosphere after every depth increment. At each depth increment petioles elongated until leaves reached the surface. Longer petioles were produced by more deeply flooded plants.

**Table 2.4**
Leaf abundance and leaf recruitment of *Villarsia reniformis* at stable final depths, following four weeks in four flooding rates. The number of leaves was affected by an interaction between flooding and nutrient supply ($p = 0.0238$). Nutrient enriched plants had more leaves than nutrient poor plants, and depth reduced the number of leaves in high nutrient plants. Depth had no effect on nutrient poor plants. Leaf recruitment was reduced by both flooding rate ($p < 0.001$) and nutrient supply ($p < 0.001$). These factors did not interact ($p = 0.053$). Plants with runners are included. $n = 12$, and standard deviations are in brackets.

| Flooding Rate (cm / 6 days) | Final Depth (cm) | Nutrient Level | Number of Leaves | | New Leaves per Week | |
|---|---|---|---|---|---|---|
| 0 | 2 | + | 28.3 | (6.7) | 6.4 | (2) |
| 4 | 20 | + | 23.3 | (2.9) | 4.5 | (1) |
| 8 | 40 | + | 22.1 | (7.1) | 4.3 | (2) |
| 12 | 60 | + | 19.9 | (5.8) | 3.6 | (1) |
| 0 | 2 | - | 12.5 | (4.4) | 1.6 | (1) |
| 4 | 20 | - | 13.4 | (3.6) | 1.0 | (0.4) |
| 8 | 40 | - | 12.6 | (2.7) | 1.0 | (0.6) |
| 12 | 60 | - | 12.2 | (2.7) | 1.0 | (0.6) |

Flooding intensity reduced the number of leaves, but this effect only developed as plants approached their final depths and the four flooding treatments became more distinct. Plants at all depths accumulated leaves through the flooding phase of the experiment up to week 4 (figure 2.4a). Having reached their final depths the number of leaves was reduced by increasing flooding intensity (table 2.4). The accumulation of leaves slowed in all flooded

treatments, and only at 2 cm did plants continue to accumulate leaves at an uninterrupted rate. After week four, when depths were stable, plants continued to have less leaves with increasing depth

Recruitment rates gradually increased at the beginning of the experiment, following the addition of slow release fertiliser (figure 2.5). The effects of flooding became stronger as the depth treatments diverged and by week 4 depth reduced the development of new leaves (table 2.4). At week 4 plants at 2 cm depth recruited leaves at a faster rate than flooded plants (figure 2.5a) and continued to do so for the remainder of the experiment. Rates did not differ between flooded plants.

Runners began to develop in nutrient enriched plants at all depths in weeks 4 and 5. Leaf abundance measurements were interrupted by runners, which produce their own leaves and are difficult to distinguish from the leaves of the parent plant. Runners led to the formation of multiple rosettes on many plants, and recording leaf recruitment became impossible after week 7. Runner formation may account for the drop in leaf recruitment in plants flooded to 20, 40 and 60 cm after week 4. Fewer runners were produced at 2 cm , and plants continued to recruit leaves at a high rate. Only one out of the 48 nutrient poor plants produced runners. Vegetative reproduction is explored fully in Chapter 3.

The majority of the original leaves, present on the plants at the start of the experiment, were submerged by the flooding treatments. As noted in the earlier experiment on nutrient enriched plants, only 8% of leaves which had developed in 2 cm depth were able to regain the surface at 24 cm depth, when flooded at 12 cm / 6 days. This effect was less severe at lower flooding rates where the depth increments were not as great. However, old petioles in the 20, 40 and 60 cm treatments were inundated by rising water.

Leaf life-span was reduced by submergence, and after four weeks of gradual flooding, the original leaves died (table 2.5). At Week 4 approximately 50% of the original leaves were still

living at all depths. Depth had no effect on leaf survival. One week later, leaf survival was clearly reduced by flooding intensity. The lowest leaf survival was at 60 cm depth, where nutrient enriched plants lost approximately 40% of their original leaf population between Weeks 4 and 5. Survival at shallower depths was still relatively high.

**Table 2.5**
Leaf survival through flooding at two nutrient levels. The percentage of leaves present at the beginning of the experiment which survived to Week 4 and Week 5 is shown. Survival was affected by an interaction between flooding treatment and nutrient level in week 4 ($p = 0.0074$). Depth was not significant ($p = 0.126$). In week 5 there was no interaction ($p = 0.121$) and depth became significant ($p < 0.001$). Standard deviation is in parentheses, n following.

| Nutrient Level | Final Depth | Percent Leaf Survival | | | | | |
|---|---|---|---|---|---|---|---|
| | | Week 4 | | | Week 5 | | |
| + | 2 | 65.5 | (18) | 10 | 60.4 | (21) | 9 |
| + | 20 | 50.0 | (11) | 11 | 37.0 | (17) | 11 |
| + | 40 | 49.3 | (17) | 8 | 40.1 | (26) | 9 |
| + | 60 | 45.6 | (19) | 11 | 21.3 | (31) | 11 |
| - | 2 | 53.0 | (12) | 11 | 45.5 | (15) | 11 |
| - | 20 | 66.0 | (13) | 12 | 49.6 | (15) | 11 |
| - | 40 | 64.4 | (13) | 12 | 35.7 | (14) | 12 |
| - | 60 | 53.4 | (13) | 12 | 11.7 | (11) | 11 |

*Nutrient Level*

The response of *Villarsia* petioles to flooding was reduced by low nutrient conditions, but petioles at both nutrient levels restored contact between the leaves and atmosphere after every depth increment (figure 2.3). Initially the length of petioles at both nutrient levels was the same ($p = 0.67$), but after the first depth increase, petioles of low nutrient plants were significantly shorter at all depths except the control ($p < 0.001$). When plants reached their final depths, petioles in the nutrient poor treatment were 11% shorter at 2 cm, 18 % shorter at 20 cm, 8 % shorter at 40 cm and 4 % shorter at 60 cm.

Low nutrient levels resulted in low leaf abundances (figure 2.4). Flooding intensity reduced the number of leaves on nutrient poor plants as well as nutrient enriched plants. However, this

effect did not become apparent until the death of submerged leaves after week four. Again, differences between flooding treatments developed as the gradual flooding levels became distinct. Leaves slowly accumulated on plants at all depths during the first 4 weeks, but having reached their final depths, leaf abundances fell markedly between weeks 4 and 5, in proportion to flooding depth (figure 2.4b). The number of leaves on flooded plants did not recover after week 5, and only plants at 2 cm continued to accumulate leaves.

Low recruitment rates left nutrient poor plants particularly disadvantaged when flooded. Recruitment rates were always lower than in nutrient enriched plants (figure 2.5), and only plants at 2 cm depth increased leaf abundance over the experiment. Young leaves are more responsive to flooding. Less young leaves were produced while the plants were gradually flooded, and this may have contributed to the slower petiole length response of nutrient poor plants to depth changes (figure 2.3). Having reached their final depths, recruitment failed to replace leaves which had died after submergence (figure 2.4) and only maintained a constant, low number of leaves per plant. While flooding intensity reduced leaf recruitment in enriched plants, low nutrient plants were less affected by depth (table 2.2). However more leaves were recruited overall in shallow plants than deep ones (figure 2.5b).

The survival of the original leaves in nutrient poor plants at week 4 was similar to enriched plants (table 2.5). After the death of submerged leaves which predominantly took place after week four, leaf survival was clearly reduced by flooding intensity. Survival at shallower depths was still relatively high.

Plants with high rates of leaf recruitment were less affected by the death of submerged leaves. While recruitment rates slowed after week 4 in nutrient enriched plants (figure 2.4a), leaf numbers did not suffer the drop which occurred in nutrient poor plants (figure 2.4b). After reaching 60 cm depth, nutrient poor plants had less leaves than at the start of the experiment, whereas leaf abundance of enriched plants at the same depth had almost doubled. Low recruitment had much less effect on plants in 2 cm of water, where leaves were not submerged

and therefore lived much longer (table 2.5). At this depth, leaves continued to accumulate at a steady rate. Less young leaves were produced while the plants were gradually flooded, and this may have contributed to the slower petiole length response of nutrient poor plants to the depth changes (figure 2.3).

## Discussion

*Villarsia reniformis* is one of a number of aquatic plants which initiate rapid petiole growth when leaves become submerged. Ethylene has been considered the primary factor responsible for the accelerated shoot growth response since the rapid elongation of rice coleoptiles underwater was mimicked by the application of exogenous ethylene in air (Ku et al. 1970). Musgrave et al. (1972) demonstrated that *Callitriche platycarpa* also elongates in response to ethylene and that sufficient endogenous ethylene accumulates in the air spaces of submerged petioles to account for the enhanced elongation. They proposed that the much lower rate of diffusion of the gas in water ($D_{ethylene}$ in water $1.69 \times 10^{-5}$ $cm^2$ $s^{-1}$) in comparison to air ($D_{ethylene}$ in air 0.15 $cm^2$ $s^{-1}$) could account for its accumulation in submerged tissues. However the application of ethylene alone to petioles in air does not entirely replicate the growth stimulated by flooding. Interactions with other hormones and the physical effect of buoyancy may also contribute to elongation (Cookson and Osborne 1978; Samarakoon and Horton 1983; van der Sman et al. 1991; Horton 1992; Rijnders et al. 1996).

The growth of new *V. reniformis* leaves through the water column occurred primarily by the formation of new petiole tissue. As has been reported to occur in *Nymphoides peltata* and *Victoria amazonica* (Funke and Bartels 1937; Ridge and Amarasinghe 1984; Ridge 1985), most cell division took place in the apical region of the petiole. The newly formed cells lengthened as they matured, which contributed to petiole growth (table 2.2). When the leaves reached the surface the petiole elongation mechanism changed. Voesenek et al. (1993) have shown in *Rumex palustris* that the concentration of ethylene in submerged petioles and leaves rapidly decreases when leaves reach the surface and the gas can diffuse into the atmosphere.

The exposure of *V. reniformis* leaves to the atmosphere triggered a reduction in cell division and consequently in the growth rate of the petiole. Petioles continued to slowly lengthen while leaves floated on the surface, but primarily from the elongation of existing cells and not by the production of new tissue.

Petioles of *V. reniformis* rapidly lengthened when floating leaves were flooded by rising water. Ethylene accumulates when the leaves of *Callitriche platycarpa* and *Rumex palustris* are submerged, triggering rapid petiole elongation (Musgrave et al. 1972; Voesenek et al. 1993). Petiole cells of these species elongate when they are exposed to the high concentrations of ethylene which accumulate in flooded tissues, and the petiole length consequently increases. Many studies have focussed on cell elongation as the principle elongation mechanism in flooded petioles (Musgrave and Walters 1974; Suge and Kusanagi 1975; Cookson and Osborne 1978; Samarakoon and Horton 1984). However in *V. reniformis, Nymphoides peltata, Victoria amazonica* and *R. pygmaeus* petiole elongation is a combination of both cell elongation and renewed cell division (table 2.3; Funke and Bartels 1937; Ridge and Amarasinghe 1984; Ridge 1985; Horton 1992). Thus the importance of cell division may have been neglected to some extent.

*V. reniformis* exhibits the petiole elongation characteristics of species which regulate the length of their shoots by ethylene within their tissues. Petiole growth in *V. reniformis* slows when leaves reach the surface, elongation recommences when leaves become resubmerged, and cell elongation becomes the primary mechanism for the elongation of resubmerged petioles. Although the effects of ethylene on petiole growth in *V. reniformis* have not been studied, previous studies provide a basis to interpret petiole elongation in this species. The regulation of petiole growth by flooding can be attributed with some confidence to the accumulation and release of ethylene.

Despite the renewal of petiole elongation after leaves were submerged, reflooded *V. reniformis* petioles had limited potential to increase their length. Petioles elongated once or

twice when the water level was gradually raised, but they eventually reached a maximum length and remained submerged. A reduced elongation potential after leaf exposure has also been found in other species (Ridge and Amarasinghe 1984; Voesenek and Blom 1989; van der Sman et al. 1991; Horton 1992; Rijnders et al. 1996). In *V. reniformis*, this was due to the reduced capacity for petiole cell division and the limited elongation potential of cells after leaves had been exposed to the atmosphere. Cell division was reduced after leaves had been exposed to the atmosphere. Instead, the elongation of existing cells became the principal mechanism by which reflooded petioles lengthened (table 2.3). This is also the case in *Ranunculus pygmaeus* and *Nymphoides peltata* (Ridge and Amarasinghe 1984; Horton 1992). The maximum cell length in *V. reniformis* was approximately 500 um and this may be an upper limit.

The maximum length petioles can achieve by cell elongation is limited by the number of cells initially in the petiole and the length to which they can elongate. After leaves are exposed the petiole elongation potential is gradually lost, as the capacity for cell division declines and existing cells increase in length. Cells may retain some capacity to elongate even after prolonged leaf exposure at a constant depth. Old petioles (greater than 20 days leaf exposure) were observed elongating when flooded in preliminary experiments. The potential for cells to elongate is gradually reduced as petioles grow older, but may not be entirely lost.

Leaves which failed to reach the surface died prematurely. Leaves began to decay and break off after four to five weeks of complete submergence (table 2.5), but lived for more than eight weeks when they were continually exposed to the atmosphere (figure 2.1). Leaf death is an unavoidable consequence of gradual flooding in *V. reniformis,* because petioles have a limited capacity to elongate after repeated leaf exposure. Rapid flooding in wetlands such as the Oude Waal in The Netherlands results in the death of the older leaves of the floating leaved plants. The petioles of young leaves of *Nymphaea alba*, *Nymphoides peltata* and *Nuphar lutea* are capable of rapid elongation in these circumstances, but old leaves become submerged and decompose soon after they are submerged (Brock et al. 1987).

Premature death of submerged leaves has been reported in other floating leaved plants (see Tsuchiya 1991).

Leaves submerged by rising water were replaced by the sustained recruitment of new leaves. The two nutrient levels used in this study demonstrated the importance of leaf recruitment in gradually flooded plants. When plants with low leaf recruitment rates were flooded, the number of leaves dropped. Fewer leaves were recruited in these plants than were submerged by the rising water. Plants with higher recruitment rates were less affected by the rising water level. New leaves were recruited at a higher rate than leaves were submerged and these plants continued to accumulate leaves as the water level rose. Sustained leaf recruitment is a widespread characteristic of floating leaved plants (Voesenek and Blom 1989; Horton 1992; Funke and Bartels 1937; Suge and Kusanagi 1975; Ridge and Amarasinghe 1984) and appears to be an important component of this depth accommodation strategy.

The recruitment of young leaves also increased the rate at which *V. reniformis* responded to gradual flooding. Young, submerged petioles potentially grow to greater lengths than petioles with exposed leaves. They also elongate at a faster rate (table 2.1). In the nutrient experiment, plants with a low rate of leaf recruitment had significantly shorter petioles at all depths when plants were gradually flooded. These plants had fewer new petioles to grow directly to the surface, and depended to a greater degree on the elongation of existing petioles to accommodate flooding. At the higher rate of leaf recruitment, petioles were younger and could accommodate the depth increments faster. It is not known whether the addition of nutrients increased the elongation or growth rates of individual petioles but this may have also affected the rate at which petioles responded to flooding.

*V. reniformis* and other floating leaved plants accommodate flooding by a combination of petiole elongation and sustained petiole recruitment. Petiole elongation has been reported in a number of floating leaved plants and appears to be a common characteristic in this growth form (see Voesenek and van der Veen 1994). The cellular mechanism by which these plants

elongate petioles is limited and leaves cannot elongate indefinitely. Submerged leaves are replaced by new leaves, which are continual recruited. Consequently, floating leaved plants have a high rates of leaf turnover. Leaves are produced continuously and generally have a short lifespan (Kunii and Aramaki 1992). Their lifespan is shortened further when they are flooded. Floating leaved plants adapt quickly and continually to water level fluctuations, but must have high rates of biomass production to do so (Brock et al. 1983; Twilley et al. 1985; Kunii and Aramaki 1992).

## Chapter 3

# Reproduction and Carbohydrate Storage in *Villarsia reniformis*

## Introduction

Wetland plants predominantly reproduce vegetatively (Sculthorpe 1967; Grace 1993). Vegetative reproduction can establish new plants in flooded, anoxic soils which are not conducive to seedling establishment. Parent plants can support propagules with energy, nutrients and oxygen, and enable them to establish roots in anoxic soil and to grow shoots through the water column to the surface where they can fix their carbon and supply oxygen for internal aeration (Grace 1993; Armstrong et al. 1994).

Vegetative reproduction has a greater metabolic cost per propagule than sexual reproduction, which must be borne by the parent plant (Bartley and Spence 1987). Plants which produce new tillers in spring accumulate substantial carbohydrate stores in the summer, which are later mobilised to support the heterotrophic growth of vegetative propagules (Kausch et al. 1981; Steinmann and Brändle 1984a; Haldemann and Brändle 1986). The cost of propagule establishment is greater in deep water, where taller stems must be grown and ramet production is negatively correlated with depth (Thomas and Stewart 1969; Lieffers and Shay 1981; Stevenson and Lee 1987; Grace 1989). The size of the carbohydrate store is likely to be an important factor in the success of vegetative reproduction (Steinmann and Brändle 1984b).

A major influence on the size of storage tissue is flooding depth. Flooded emergent and floating leaved plants increase shoot height in response to flooding, in order to expose leaves at the surface (Lieffers and Shay 1981; Osborne 1984; Stevenson and Lee 1987; Chapter 2).

While exposed leaves are able to photosynthesise at higher rates than when submerged, the greater biomass of supporting tissue reduces the net return of carbon to the plant (Maberly and Spence 1989; Grace 1989; Nielsen 1993). As available carbon declines, growth is redirected from below ground storage to above ground tissues to increase carbon acquisition (Grace and Wetzel 1982; Squires and van der Valk 1992; Coops et al. 1996). In addition, total plant biomass can decrease if the costs of carbon acquisition in deep water exceed the amount of carbon fixed. This includes a reduction of storage tissue biomass (Lieffers and Shay 1981; Rea and Ganf 1994d).

In this chapter it is proposed that by diverting growth from storage roots to above ground growth, flooding influences asexual reproduction in the floating leaved macrophyte *Villarsia reniformis*. Nutrient supply was manipulated in order to examine responses to flooding at two levels of growth limitation. By reducing growth, low nutrient levels reduce the responsiveness of aquatic plants to flooding (Chambers and Kalff 1987; Neill 1992). Nutrient limited plants have insufficient resources to increase biomass when flooded and flooding makes the nutrient limitation more acute. Any extra allocations to resource acquiring tissues must come from the re-allocation of growth from elsewhere in the plant (Tilman 1988). Taller shoots can be produced, but the extra above ground biomass is redirected from other tissues in the plant. On the other hand, depth tolerance is increased by nutrient additions (Grace 1988; Neill 1990). Flooding promotes growth in many species, and a high nutrient supply supports the additional shoot biomass developed by plants grown in deep water.

*V. reniformis* reproduces vegetatively in summer by the development of runners which radiate from the centre of the rosette. When the plant is not reproducing, the storage roots make up approximately 70% of plant biomass. Carbohydrate storage has been implicated in both the flooding tolerance and vegetative reproduction of aquatic macrophytes. In this experiment the relative importance of these roles was explored at four depths from 2 to 60 cm and at two levels of mineral nutrition.

## Materials and Methods

The reproductive and storage responses of *V. reniformis* were examined at two levels of nutrient supply and four flooding regimes. The nutrient level was controlled by potting plants in nutrient poor sandy loam with or without the addition of slow release Osmocote fertiliser at a rate of 60.8 g N $m^{-2}$ $year^{-1}$ (Courtesy of Grace Sierra). Flooding regimes consisted of an initial gradual flooding stage and a final stable water depth. Gradual flooding took 30 days: plants were flooded at rates of 0, 4, 8 and 12 cm per day to final depths of 2, 20, 40 and 60 cm where they remained for a further 2 months. The experiment began on October 18 1993 and ended on January 17 1994 (a total of 13 weeks over spring and summer).

Further details of the experiment design are given in Chapter 2.

*Biomass*

An initial random sample of 12 plants was harvested at the start of the experiment before nutrient or flooding regime treatments had begun. The experiment concluded after twelve weeks, and plants were harvested over 10 days. Runners consisted of a stem, which sprouted from the centre of the meristem on top of the corm, and propagules which formed along their length. The length of the longest runner was measured, and the number of propagules on all runners counted. The length of the longest petiole, from the corm to the junction with the leaf, was recorded. Plants were washed of all sand, clay and Osmocote pellets, and separated into component tissues: leaves, petioles, corm, roots and runners. Runner biomass includes all the stem tissue and propagules produced by each plant. Tissues were dried at 80° C for 3 days and weighed. Because it was more convenient than working with fresh tissue, fine roots were separated from storage roots when dry.

*Carbohydrate*

Duplicate carbohydrate samples (100 mg) were taken from each tissue as plants were harvested, and four replicate plants were sampled from each treatment. Samples were weighed and macerated in 2 mL 80% ethanol in 10 mL centrifuge tubes. After rinsing the grinding stick into the tube with an additional 2 ml, the samples were heated at 80° C for 10 min to prevent enzymic degradation of carbohydrate, made up to 10 mL and stored at -10° C until analysis.

*V. reniformis* tissues do not stain for starch but test positively for fructan with thymol and sulphuric acid (Johansen 1940). Accordingly, fructan was extracted from samples in hot water (Pontis 1990). Analysis of samples followed the phenol-sulfuric acid technique of Dubois et al. (1956). Ethanol was evaporated from the samples at 90° C, the sample reduced to less than 2 mL and the volume made back up to 10 mL with water. Immediately before analysis, samples were heated to 100° C, centrifuged at 2500 G for 10 minutes and returned to the waterbath to keep the sample temperature over 70° C. A 25µL aliquot was taken quickly, to minimise the effects of convection and mixing of tissue fragments, and added to 1 mL deionised $H_2O$ and 1 mL phenol solution (see below) in boiling tubes. Samples were mixed thoroughly with a vortex before 5 mL concentrated sulfuric acid was added in a single stream, and were immediately mixed again. After waiting 10 minutes for the colour to develop, samples were transferred to 1 cm path length cuvettes and read at 488 nm with a Bausch and Lomb spectrophotometer, Spectronic 21.

Inaccuracies in the concentration of the phenol reagent initially generated some misleading results. At rest, phenol solutions at concentrations of higher than 6% (saturation) separated out into a syrup at the bottom with concentrated solution above. Consequently, solutions were always mixed thoroughly by swirling to form a 'white-water' solution (Dubois et al. 1956) before any solution was pipetted out.

After solubilisation and hydrolysis, it was expected that the fructose would make up the greatest proportion of carbohydrate in storage tissues, where fructan is accumulated. Other carbohydrates, in particular glucose and sucrose, were expected to be more abundant in leaves and fine roots (Hawker 1985). The absorbance of carbohydrate at a given concentration of phenol depends upon the composition of sugars in the sample. For fructan, absorbance increases with phenol concentration, but for glucose, absorbance decreases with phenol concentration (Buysse and Merckx 1993). Therefore, in order to correctly interpret absorbances as carbohydrate concentrations of tissue samples, the ratios of these carbohydrates in the samples must first be determined.

The change in absorbance between 12% and 24% phenol was determined in a replacement series of glucose and fructan. This provided the relationship between carbohydrate composition and phenol concentration at these two concentrations. Regressions were plotted for 12% and 24%. The difference of these lines was the relationship between the absorbance at 12% and 24% phenol and the ratio of fructan and glucose.

Carbohydrate composition was determined in two plants from each treatment. Duplicate samples of each tissue were analysed at 12% and 24% phenol, and the ratio of the absorbances was equated to the ratio of fructan to glucose. The ratio of absorbances at 12% and 24% phenol indicated the proportions of the carbohydrates in the sample. These ratios were applied to total carbohydrate calculations.

Total carbohydrate concentration was determined in four plants from each treatment. Duplicate samples were analysed with 12% phenol, together with standards of glucose and fructan (0 to 100 $\mu g\ ml^{-1}$). Concentrations were calculated by adding the quantities of fructan and glucose to give total carbohydrate on a dry weight basis.

*Statistics*

The mean relative growth rate (RGR) was calculated according to Hunt (1978):

$$RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

where

$W_1$ was the initial biomass

$W_2$ was the final biomass and

$T_2 - T_1$ was the duration of the experiment.

The structural cost of propagules was calculated as the number of propagules per plant divided by the biomass of runner tissue.

The effects of depth and nutrient level on final plant biomass and relative growth rates were analysed in a two way anova with interaction. Missing values were accounted for by a general linear models procedure, Proc GLM (SAS Institute 1992). The ratios of fructan to glucose and total carbohydrate concentration were analysed in a similar model, but with the duplicate tissue samples nested within nutrient level and depth. None of the nested factors were significant ($p < 0.05$), and the nested MS was used as the denominator to calculate the F ratio for the main effects and interaction. Probabilities for F values are referred to in the text and full F tables are presented in Appendix B.

Carbohydrate concentration is expressed as percent w $w^{-1}$ dry weight. The fresh weight of the analysed sample was converted to dry weight using the fresh to dry weight ratio of the biomass samples for each treatment.

## Results

### Carbohydrate

The corm and the storage root had the highest concentrations of carbohydrate. These tissues also had the highest proportions of the storage polymer fructan (table 3.1). The ratio of fructan and glucose in other tissues was approximately 1:1, indicating that carbohydrate storage was not their primary role. The proportion of carbohydrate ascribed to fructan in these tissues may also represent some fructose and sucrose. Fructose has very similar absorbance qualities to fructan (data not presented), and therefore contributed to the measured fructan concentration. Sucrose molecules, when hydrolysed by the phenol-sulfuric acid technique, are equivalent to equal numbers of glucose and fructose molecules (Buysse and Merckx 1993). Therefore the sucrose in the samples would have contributed equally to the measured fractions of glucose and fructan.

**Table 3.1**
The ratio of fructan to glucose in *V. reniformis* tissues. The petiole and root were affected by an interaction between depth and nutrient level (p = 0.033 and 0.020, respectively). The effect of depth on these tissues is shown for each nutrient level. Other tissues were not significantly affected by the treatments (p > 0.05), and the responses of the nutrient levels (+,-) and depths are combined.

| Tissue | Nutrient Level | 2 | 20 | 40 | 60 | **Mean** | **sd** |
|---|---|---|---|---|---|---|---|
| Leaf | +,- | . | . | . | . | **0.438** | **(0.120)** |
| Petiole | + | 0.38 | 0.45 | 0.49 | 0.44 | **0.459** | **(0.059)** |
| | - | 0.50 | 0.48 | 0.44 | 0.51 | | |
| Corm | +,- | . | . | . | . | **0.774** | **(0.28)** |
| Storage Root | +,- | . | . | . | . | **0.914** | **(0.21)** |
| Root | + | 0.28 | 0.42 | 0.45 | 0.44 | **0.436** | **(0.071)** |
| | - | 0.48 | 0.46 | 0.45 | 0.44 | | |
| Runner | + only | . | . | . | . | **0.479** | **(0.085)** |

**Table 3.2**
Concentration of carbohydrate (percent $w.w^{-1}$) in *V. reniformis* tissues. The corm and storage root concentrations were affected by an interaction between depth and nutrient level ($p = 0.014$ and 0.026, respectively). The petiole was affected by depth only ($p = 0.015$). Treatments did not significantly affect the leaves, roots or runners ($p > 0.05$). Non-significant nutrient levels (+,-) or depths are combined.

| Tissue | Nutrient Level | 2 | 20 | 40 | 60 | **Mean** | **sd** |
|---|---|---|---|---|---|---|---|
| Leaf | +,- | . | . | . | . | **19.5** | **(6.9)** |
| Petiole | +,- | 20.0 | 16.2 | 11.9 | 15.0 | **15.9** | **(5.4)** |
| Corm | + | 37.4 | 59.5 | 53.8 | 52.7 | **52.1** | **(11)** |
| | - | 52.0 | 60.7 | 54.0 | 49.2 | | |
| Storage Root | + | 71.2 | 81.3 | 83.3 | 74.7 | **78.4** | **(15)** |
| | - | 94.1 | 79.2 | 69.2 | 72.3 | | |
| Root | +,- | . | . | . | . | **33.1** | **(13)** |
| Runner | + only | . | . | . | . | **33.9** | **(13)** |

The storage root had the highest concentration of carbohydrate, where it made up 78% of dry weight (table 3.2). The concentration was influenced by an interaction between depth and nutrient level, mainly due to the high concentration in the storage root in low nutrient plants at 2 cm depth. However, storage root concentrations in other treatments were similar, and neither depth nor nutrient level greatly influenced concentrations of carbohydrate. The corm also had similar concentrations in all treatments, except 2+ (see below). The storage root was the largest tissue in the low nutrient plants and represents a large pool of carbohydrate. The storage root was also a large proportion of biomass in nutrient rich plants.

The concentration of carbohydrate in petioles was higher at 2 cm than other depths. Petioles at this depth were exposed to the atmosphere and had a greater potential for photosynthesis than submerged petioles. The carbohydrate concentration in petioles at 2 cm was similar to the leaves, indicating that they may have been photosynthesising and increasing the carbohydrate concentration to the same level.

The fine roots and runners had higher concentrations of carbohydrate than the leaves and petioles (table 3.2). It appears that little carbohydrate is stored in the leaves, and it is instead translocated to carbohydrate stores and sinks. The low proportion of fructan in these tissues indicates that there is little carbohydrate storage in the roots and runners, and the higher concentration may reflect their demand for energy.

Nutrient enriched plants at 2 cm depth show a different pattern of carbohydrate storage to the other treatments (tables 3.1, 3.2). The proportion of glucose in the petiole and root was higher than other treatments, and the corm had a lower carbohydrate concentration. These responses are difficult to interpret and may reflect an inconsistency in technique. If samples from these plants were left too long before preservation in ethanol carbohydrate may have been respired and the concentration of glucose increased.

## Morphological Responses

*V. reniformis* plants adapted their petiole length to the flooding regime in which they grew. The petiole length required to expose leaves at the surface increased with water depth, and at 60 cm, petioles on nutrient enriched plants were more than 5 times longer than petioles at 2 cm (table 3.3). Nutrient poor plants also increased petiole length with depth, and leaves were exposed at the surface at all depths. However, the low nutrient supply reduced petiole length, which was shorter at all depths than high nutrient plants.

**Table 3.3**
The petiole length, leaf width and number of propagules of plants growing for 3 months in 4 flooding regimes at two nutrient levels. Petiole length was affected by depth ($p < 0.001$) and nutrient level ($p < 0.001$). The treatments did not interact ($p = 0.88$). There was an interaction between these effects on leaf width ($p = 0.003$). Values are the means of 12 plants, except for the number of propagules per plant, where only eight plants out of 12 produced runners (a) or 1 plant out of 12 (b).

| Nutrient Level | Flooding Regime (cm) | Petiole Length (cm) | Leaf Width (cm) | Number of Propagules per Plant |
|---|---|---|---|---|
| + | 2 | 13.2 (2.1) | 5.2 (0.6) | a 61 (20) |
| + | 20 | 30.4 (3.1) | 6.9 (0.5) | 92 (23) |
| + | 40 | 52.3 (2.5) | 8.1 (0.8) | 77 (14) |
| + | 60 | 72.2 (3.0) | 9.0 (1) | 56 (11) |
| - | 2 | 10.5 (1.5) | 3.5 (0.4) | |
| - | 20 | 27.3 (2.1) | 4.5 (0.5) | b 28 |
| - | 40 | 49.9 (2.3) | 5.6 (0.6) | |
| - | 60 | 70.2 (3.1) | 5.7 (0.5) | |

Leaves became broader with increasing depth (table 3.3). Leaves were larger at the high nutrient level and increased in size from 40 to 60 cm. Smaller leaves were produced at all depths by low nutrient plants, and leaf size did not increase in size from 40 to 60 cm.

## Total Biomass

All plants, irrespective of treatment, increased in biomass during the course of the experiment. Nutrient enriched plants had a final biomass four to six times larger than that of nutrient poor plants (table 3.4). Consequently RGRs were markedly higher in the enriched treatment. However, there was an interactive effect of nutrients and depth changes in biomass could only be explained by reference to both nutrient status and depth. For the enriched plants maximum biomass occurred at 40 cm but declined at shallower and deeper depths. In contrast, the nutrient poor plants had a maximum biomass at 2 and 20 cm and a minimum at 60 cm. Statistical analysis showed that the addition of nutrients elevated the growth rate. However, this analysis did not discern any interactive effect of depth and nutrients. Nevertheless, the same pattern as for biomass is evident. The highest RGR occurred at 40 cm in the enriched

plants, whereas $RGR_{max}$ occurred at 2 and 20 cm and declined in deeper water in the nutrient poor treatments.

**Table 3.4.**
Biomass and relative growth rates. An interaction between depth and nutrient level significantly affected biomass (p= 0.0056), but not RGR (p= 0.063; see text). RGR was higher when additional nutrients were supplied (p= 0.0001), but RGR was not affected by depth (p= 0.11). Standard deviations of 12 plants are shown in brackets

| Nutrient Level | Flooding Regime (cm) | Biomass g dry wt | | RGR week$^{-1}$ | |
|---|---|---|---|---|---|
| Initial | | 4.70 | (2.5) | | |
| + | 2 | 50.4 | (9.8) | 0.18 | (0.015) |
| + | 20 | 63.2 | (11) | 0.20 | (0.014) |
| + | 40 | 67.5 | (16) | 0.20 | (0.020) |
| + | 60 | 55.1 | (9.4) | 0.19 | (0.014) |
| - | 2 | 13.4 | (4.5) | 0.76 | (0.027) |
| - | 20 | 13.4 | (3.7) | 0.077 | (0.020) |
| - | 40 | 11.4 | (3.5) | 0.064 | (0.025) |
| - | 60 | 10.6 | (11) | 0.060 | (0.019) |

## Biomass allocation

*Nutrient Enriched Plants*

Nutrient enriched plants invested a large proportion of their biomass in vegetative reproduction, and runners made up the greatest proportion of above ground biomass at all depths (figure 3.1a and b). Runners began to develop after 28 days in all but three plants, all of which were at 2 cm. Runners sprouted from the centre of the growing point on top of the corm, where new leaves were formed, and radiated out into the water to a mean length of 1.5 m (sd = 0.3, n = 21). Propagules formed at the nodes of the runners, and runners sometimes branched at these points as well. Propagules were miniature *V. natans* plants, with up to 5 leaves which grew up towards the surface, and had thin storage roots which drifted down in the water.

**Table 3.5**
The number of propagules per plant per gram dry weight of runner tissue for nutrient enriched plants in four flooding regimes. Standard deviation is in brackets.

| Flooding Regime (cm) | Propagules per g dry wt Runner |
|---|---|
| 2 | 3.4 (0.4) |
| 20 | 2.7 (0.5) |
| 40 | 2.2 (0.2) |
| 60 | 2.0 (0.5) |

Plants produced the most propagules at 20 cm (table 3.3), but a large number of propagules were produced at all depths. Reproductive effort was lowest at 2 cm depth, where runners made up the smallest proportion of total biomass and made up only 23% of total plant dry weight (figure 3.1). Runners made up approximately 50% of plant biomass at 20, 40 and 60 cm. Despite the similar biomass allocation at these depths, fewer propagules were produced with increasing depth.

Fewer propagules were produced with increasing depth in proportion to the biomass of runner tissue. At 2 cm, 1 g dry weight runner tissue was equivalent to 3.4 propagules, but only 2.0 propagules at 60 cm (table 3.5). Propagules developed longer petioles with increasing depth, and petioles accounted for a larger proportion of runner biomass in deeper water. At shallower depths, where the petioles were shorter, this biomass was allocated to the storage tissue.

On the parent plants, the biomass allocation to petioles at the high nutrient level increased only slightly with increasing depth (figure 3.1a). Petioles made up 6% of total dry weight at 2 cm and 11% at 60 cm, even though petiole length increased more than five times. The increase in leaf size with depth (table 3.3) may have compensated for the extra carbon invested in longer petioles. The biomass allocation to the leaves changed very little with depth. Biomass increased from 2 to 40 cm and depth did not limit growth in this range. Biomass decreased

from 40 to 60 cm but plants did not respond with a large increase in the allocation to the petioles.

The storage root made up the greatest proportion of tissue below ground (figure 3.1b). The storage root biomass was most strongly affected by the depth change from 2 cm to 20 cm, and this response complimented the change in runner biomass between these two depths. At the shallowest depth the storage root made up approximately 50% of total plant biomass, but only 30% at 20, 40 and 60 cm depths.

*Low Nutrient Plants*

Flooding reduced biomass and influenced distribution of biomass between tissues in low nutrient plants. Carbon became more expensive to fix with increasing depth as longer petioles were required to expose leaves at the surface. Total plant biomass decreased slightly with depth, while the proportion of above ground biomass increased (figure 3.2a). Longer petioles were required to expose leaves on the surface in deeper water, and they involved a greater investment of carbon in their structure. Petioles made up only 6% of plant biomass at 2 cm, but 22% at 60 cm, double the allocation of high nutrient plants at the same depth (figure 3.2a). The leaf allocation also increased with depth, but not to the same degree.

The low nutrient level led to a greater investment in fine root tissue, as plants increased the biomass allocated to the tissue which acquired the limiting resource (figure 3.2b). Approximately 15% of total biomass was allocated to the fine roots in low nutrient plants, which was 3 times the allocation in nutrient enriched plants.

The storage root was the largest tissue in the low nutrient plants, and the storage root allocation declined with depth from 72% at 2 cm to 57% at 60 cm, complimenting the increasing petiole allocation (figure 3.2b). The corm and fine roots were relatively unaffected by flooding regime and made up approximately 5% and 15% of plant biomass, respectively, at all depths.

The relative proportions of tissues responded to flooding regime more than the total plant biomass. Plant biomass decreased by only 13% from 2 to 60 cm in comparison to the decrease in storage root biomass of 28 % and the increase in petiole biomass by 390 % (table 3.3; figure 3.2). The storage root provided a buffer from which growth could be diverted to the tissues which acquire resources, the petiole, leaves and roots.

Only one low nutrient plant developed runners. It produced 27 propagules, well below the average number of propagules per plant produced in the nutrient enriched treatment (table 3.3).

Allocation triangles (Tilman 1988) demonstrate the trade-offs between the storage root and the petiole in low nutrient conditions, and the storage root and runner under high nutrient supply. The proportion of total biomass allocated to the petioles and storage root of every low nutrient plant is plotted in figure 3.3. The data form a straight line relationship. The sum of the two allocations is approximately 70% at all points along the line, and this proportion of total biomass was allocated between the petiole and the storage root. The distribution of the biomass was controlled by depth. Shallow plants are at the high storage root end of the line, and deep plants with a greater allocation to the petioles are at the low storage root end. One plant does not fit this relationship (petiole 3%, storage root 26%), and this individual formed runners even though it had the low nutrient supply. The petiole allocation was lower than the 20 cm plants, and the storage root allocation was much lower than any of the other low nutrient plants.

Under high nutrient conditions, biomass was not allocated to the petiole at the expense of the storage root (figure 3.4). The allocation to the petioles was approximately 10% and was uninfluenced by depth. The storage root allocation was highest at 2 cm, but was unrelated to depth at 20, 40 or 60 cm. The storage root allocation varied greatly, between 10% and 78%.

The storage root allocation was related to the runner under high nutrient conditions (figure 3.5) and growth was allocated to the runners at the expense of storage root biomass. The storage root made up less than 20% of total biomass in plants with the greatest runner allocations. The trade-off between these two tissues was made between approximately 70% of total biomass, similar to the low nutrient level. Although 2 cm plants had the lowest runner and highest storage root allocations, the tissues were not closely related to depth at 20, 40 or 60 cm. The three plants at the high nutrient level which did not produce runners are shown with the zero runner allocation, and had a storage root biomass approximately 70% of dry weight.

The plants which did not fit the reproductive patterns of their nutrient treatments demonstrate the interchangeability of the reproductive and non-reproductive growth forms. The sole low nutrient plant which produced runners did not fit the relationship between petiole and storage root allocation of other low nutrient plants. It did, however, fit the relationship between storage root and runner allocation formed by at the high nutrient level (figure 3.5). Similarly, the three plants which did not produce runners at the high nutrient level closely fit the relationship between storage root and petiole allocation at the low nutrient level (figure 3.3).

## Discussion

*Carbohydrate*

Carbohydrate storage in below ground tissues is a common feature of aquatic macrophytes. The high concentrations of total extractable carbohydrates in the corm and storage root of *Villarsia reniformis* were similar to the rhizomes of *Typha latifolia*, *Schoenoplectus lacustris* and *Spartina alterniflora* (Kausch et al. 1981; Gallagher et al. 1984; Steinmann and Brändle 1984a, b). In addition, the storage root made up a large proportion of *V. reniformis* biomass, and was between 30% and 72% of total dry weight. The carbohydrate pool in this tissue constitutes a substantial allocation of the plant's energy.

The predominant carbohydrate in the corm and storage root was fructan, which is characteristic of the Menyanthaceae (Cronquist 1988). Other tissues had smaller proportions of fructan and lower total carbohydrate concentrations. The leaves and petioles had the lowest concentrations and stored little carbohydrate. However, low concentrations of carbohydrate do not preclude high rates of fructan turnover (Simpson and Bonnett 1993).

Petioles had higher carbohydrate concentrations at 2 cm depth than other depths and are likely to have photosynthesised more than in deeper treatments. Petioles were green at all depths, but at 2 cm were exposed to the air and were a similar dark shade to the leaves. The carbohydrate concentration of petioles at 2 cm was similar to the leaves at other depths and probably reflects carbon fixation. The fine roots and runners both had intermediate concentrations, but did not store fructan.

The biomass of carbohydrate storing tissues is a better indication of the carbohydrate pool than the tissue concentration. Flooding and nutrient conditions both affected the allocations of biomass to tissues but, with minor exceptions, tissue concentrations of carbohydrate were unaffected. In rice, *Zizania aquatica* and *Rumex* spp., tissue concentrations decline when plants are flooded, as reserves are consumed in the absence of aerial photosynthesis (Raskin and Kende 1984; Pip and Stepaniuk 1988; Laan and Blom 1990). Starch concentrations in the rhizomes of other species also vary seasonally, dropping to a minimum at the onset of asexual reproduction (Kausch et al. 1981; Gallagher et al. 1984; Steinmann and Brändle 1984a; Haldemann and Brändle 1986). However, in *V. reniformis* the production of runners was not related to the carbohydrate concentration of the storage root. Nutrient enriched plants at 2 cm had a lower reproductive effort than deeper plants, but did not have a higher carbohydrate concentration (figure 3.1a; table 3.2). The biomass of the storage root responded to flooding and nutrient levels, and it would appear that carbohydrate storage in *V. reniformis* is regulated by the amount of storage tissue, rather than the concentration of carbohydrate it contains.

*Morphology*

Petiole length increased in proportion to depth, as plants developed petioles long enough to expose leaves on the surface. Petioles of floating leaved plants respond rapidly to flooding by growing longer (Osborne 1984; Horton 1992; Voesenek and van der Veen 1994; Chapter 2). Leaf exposure is important in maintaining a positive carbon balance (Armstrong et al. 1994). The leaves of emergent plants are poor at fixing $CO_2$ from an aqueous source, compared to photosynthetic rates in air (Maberly and Spence 1989; Nielsen 1993). Carbon is respired faster than it is fixed in completely submerged plants, leading to loss of biomass (Laan and Blom 1990). Leaf exposure is also important in preventing anoxia in below ground tissues by aerating the roots via the lacunae (Armstrong et al. 1994).

*Biomass Allocation*

The primary objective of the nutrient manipulation in this study was to explore how biomass allocations are influenced by depth when growth is limited. In plants grown under low nutrient conditions, the increase in petiole and leaf biomass with increasing depth was made at the expense of the storage root growth.

The low nutrient supply severely limited growth at all depths, and relative growth rates were two to three times lower than the high nutrient plants. Carbon fixation required a greater investment in petiole biomass in deep water than in shallow water because petioles were required to grow longer to reach the surface. Because growth was limited by low nutrients, additional biomass could only be allocated to the petioles at the expense of allocations to other tissues (Tilman 1988). In low nutrient plants, the additional petiole biomass was provided by the diversion of growth from the storage root.

The allocation of carbon to the storage root did not cease completely, although the rate of growth was reduced. All tissues, including the storage root, increased in biomass over the course of the experiment, and even nutrient poor plants at 60 cm had a positive carbon

balance. However, storage root *growth* was reduced by the increase in petiole allocation with depth. Flooding reduced the size of the storage resource of low nutrient plants.

Petiole growth did not affect the allocation of growth to storage root in plants at the high nutrient level, and consequently the storage root grew much larger. At the higher nutrient level, plants had a greater potential to fix carbon, and total biomass increased at all depths. Leaf recruitment rates responded rapidly to the addition of nutrients at the beginning of the experiment (Chapter 2). Nutrient enriched plants quickly produced more leaves and increased their potential to fix carbon and increase growth. Plants developed additional petiole tissue with increasing depth, but not at the expense of biomass allocations to other tissues. Instead, biomass was allocated to runners at the expense of the storage root.

Plants grown in high nutrient conditions allocated a large proportion of growth to the production of vegetative propagules. Vegetative reproduction is important in aquatic macrophytes because new plants can be established under water. Plants can reproduce without drawdown, when conditions are more suitable for seeds to germinate. However, vegetative propagules are more expensive to produce than seeds and runners biomass made up approximately 30% of dry weight at 2 cm and 50% of dry weight at 20, 40 and 60 cm. This growth was an overflow of carbon which would have otherwise been allocated to the storage root. The more biomass plants allocated to the runners, the less was allocated to the storage root.

Runner development was reduced at 2 cm and the low water level may have limited vegetative reproduction. *V. reniformis* shoots are vulnerable to desiccation in dry conditions, and plants become dormant soon after drawdown. Petiole length is reduced to a minimum and leaves are produced more slowly and are smaller. In view of the limited growth possible ~~under~~ during drawdown, vegetative propagules are less likely to successfully establish in shallow water when drawdown is imminent. The reduced reproductive effort at 2 cm led to a greater carbohydrate storage in the root. The carbon not allocated to the runners was conserved, and

available to support the plant during drawdown or for vegetative reproduction the following spring.

Runner development appears to be an 'all-or-nothing' response, and the uniformity of runner development was remarkable. Forty five out of 48 nutrient enriched plants produced runners and 47 out of 48 low nutrient plants did not. The one low nutrient plant which did produce runners allocated 28% of total biomass to the runner and produced 27 propagules, which is a major departure in growth strategy from the other low nutrient plants. In this regard, runner development in *V. reniformis* is similar to the bolting response of sexually reproducing species, where a large investment is suddenly made in reproductive tissues in response to environmental or allometric cues (Werner 1975; Harper 1977). In contrast, vegetative reproduction in most wetland plants is gradual. Although it may peak seasonally (Bernard 1975; Haldemann and Brändle 1986; Hultgren 1988; Brix 1989), new shoots are usually recruited gradually over time, as ramets grow big enough to support daughter plants (Lieffers and Shay 1981; Stevenson and Lee 1987; Grace 1993; James and Hart 1993).

The cue for runner development is unclear, but it occurred soon after the addition of nutrients. The importance of the storage root allocation in vegetative reproduction suggests that the trigger may be a minimum allocation of total plant biomass to the storage root (Grace and Wetzel 1982). Leaf recruitment rates responded rapidly to the addition of nutrients at the beginning of the experiment (Chapter 2), and nutrient enriched plants quickly increased their potential to fix carbon and increase growth. In low nutrient conditions, the additional growth potential which was not allocated to the petioles was allocated to the storage root. This suggests that the accumulation of storage root biomass is a priority for this species. In high nutrient plants, where the petiole allocation did not take growth away from the storage root, the storage root biomass accumulated unimpeded by petiole growth. Consequently, the proportion of biomass allocated to the storage root would have been higher in high nutrient plants early in the experiment. Further experiments are required to establish the trigger for

runner development, where the ratio of tissue allocations in different nutrient conditions and depths are followed over time.

In the mediterranean climate of south-eastern Australia, *V. reniformis* frequently experiences prolonged periods of drawdown, ranging from over summer and autumn to a number of successive years. Carbohydrate storage is likely to be important in oversummering, particularly in the corm, in supplying energy to support respiration and to support regeneration of new tissues when plants are reflooded. On drawdown, leaves and petioles rapidly desiccate and die. Smaller, waxy leaves, with very short petioles which barely extend above the soil, are produced to replace them. After extended dry periods these leaves die, and Villarsia produces no above ground tissues at all. The corm is the only woody tissue in *Villarsia reniformis*, and is more resistant to dehydration than the other fleshy tissues, and is protected, to some degree, by the soil. Petioles, leaves, roots and runners all originate from meristems on the corm. If the corm is the only tissue to survive a long drought, its carbohydrate reserves and meristems can develop new tissues and re-establish the plant.

## Chapter 4

# Flood tolerance of the slow growing emergent macrophyte, *Baumea juncea*

## Introduction

In seasonally flooded wetlands, emergent macrophytes may experience extremes of flooding and drawdown beyond their normal range of tolerance. Persistent deep flooding eventually leads to plant death and to changes in community composition (Millar 1973; Wilcox and Meeker 1991). Flood water cuts the supply of atmospheric CO2 to leaves and stems, and reduces the photosynthetic capacity of plants which are normally exposed to air (Maberly and Spence 1989; Nielsen 1993). This can impose a carbon deficit, reducing growth rates and biomass (Pip and Stepaniuk 1988; Laan and Blom 1990). Long term flooding also imposes toxic levels of anoxia on below ground tissues, as access to atmospheric oxygen is reduced (Armstrong et al. 1994).

Morphologically plastic species, including *Villarsia reniformis*, *Triglochin procerum*, *Rumex maritimus* and *Nymphoides peltata*, respond actively to increasing water levels by altering above ground morphology to accommodate deep water (Brock et al. 1983; van der Sman et al. 1991; Rea and Ganf 1994d; Chapter 2). Shoot size and structure of these species is readily adaptable because they have high recruitment rates, a low degree of lignification in shoot tissue and short or seasonal life spans. Resources are re-allocated from below ground to above ground to counter the potential carbon shortage.

However, many emergent species without these characteristics survive long periods of flooding by tolerating, rather than responding to, inundation. Above ground tissues which live for 2 or more years have little potential to elongate when flooded, and must survive the change

from exposure to inundation and back again (Hultgren 1989). In addition, the slow rate at which new stems are recruited provides plants with less opportunity to respond to flooding than to survive sub-optimal conditions until water levels fall. Tolerance is important for species which respond slowly to water level fluctuations (Laan and Blom 1990; Shay and Shay 1986; Squires and van der Valk 1992; van den Brink et al. 1995).

As important as flood toleration are the responses of these species to drawdown. Flooding can inhibit the recruitment of new shoots and stems, and drawdown may provide the only opportunity to recruit new shoots (Hultgren 1988; Rea and Ganf 1994d). If flooding persist for too long through the growing season, plants may miss out on a new cohort of stems altogether (Hultgren 1988). The annual peak in plant production may also be postponed until flood water recedes (Froend and McComb 1994; Hogeland and Killingbeck 1985; Neill 1992). Plants with poor recovery from inundation are particularly vulnerable to flooding (Denton and Ganf 1994; Palada and Vergara 1972).

In this chapter it is proposed that the response of a slow growing emergent plant to fluctuating water levels consists of two complementary adaptations: the toleration of long flooded periods of low carbon supply and anoxia, and the regenerative ability to initiate growth when water levels are lower and conditions more favourable to growth. The tolerance and recovery mechanisms of the slow growing emergent macrophyte *Baumea juncea* are explored. This species is a lignified sedge which produces tall stems (up to 130 cm long) from below ground tissues. Stems, roots and long rhizomes originate from the short rhizome, which branches in small clumps and is structured like an inverted candelabra. Long rhizomes are unbranched and between 5 and 30 cm long. Branching clumps of short rhizomes form at the end of long rhizomes, and these rhizomes colonise new areas of soil. Plants were flooded to three depths, 2 cm, partial flooding to 25 cm or top flooding, and then re-exposed at 2 cm depth.

## Methods

*Plant Material*

*Baumea juncea* was collected from Bool Lagoon in the south east of South Australia (37° 08'S, 140°40'E) with permission from the National Parks and Wildlife Service. Long rhizomes were cut to separate plant material into ramets, consisting of short rhizomes and stems. Individual ramets were washed, weighed and potted in 3.4 litre pots (15 cm diameter) with no holes in 80/20 sandy loam with the addition of 7 g of 6-9 month Osmocote slow release fertiliser. The surface of the soil was sealed with 2 cm of clay to limit the infusion of oxygen and replicate the anaerobic conditions of wetland soils (Muller et al. 1994).

Experiments on plant growth and photosynthetic rates were conducted in an outdoor pond. For details of the pond environment see Chapter 2.

*Flooding and Exposure Experiment*

The growth and reproductive response of *Baumea juncea* to flooding and re-exposure was examined at three depths. Plants were randomly allocated to 7 groups: an initial group, three flood and three exposure groups, each with 7 replicate plants. The regimes of flooding and exposure are shown in table 4.1. The experiment ran from October (spring) to March (autumn). Plants were acclimatised to pond conditions for 4 weeks at 2 cm depth in October, after which the initial group was harvested. Control plants remained at 2 cm, the second group was flooded to 22 cm and the third to 120 cm. After 11 weeks at these depths these plants were harvested, at the end of December. The depth of the remaining plants was gradually reduced to 2 cm over 30 days; the 120 cm was reduced by 4 cm $day^{-1}$ and 25 cm reduced by 0.8 cm $day^{-1}$. These plants remained at 2 cm for 8 weeks and were harvested in March.

**Table 4.1**
Flooding stages of the experiment. Plants were acclimatised to 2 cm depth in the initial stage of the experiment, and then flooded to the treatment depths. After 11 weeks, the depth was gradually reduced to 2 cm in the transition stage, at which depth they remained for the re-exposure stage. Plants were harvested at the end of the initial, flood and exposure stages.

| Treatment Depth | Stage Duration (weeks) | | | | |
|---|---|---|---|---|---|
| | Initial | Flood | Transition | Re-Exposure | Total |
| Control 2 cm | 4 | ... | ... | 23 | 27 |
| 25 cm | 4 | 11 | 4 | 8 | 27 |
| 120 cm | 4 | 11 | 4 | 8 | 27 |

Plants were washed free of soil and fertiliser and separated into stems, buds, roots, short and long rhizomes. Rhizomes were distinguished by length and morphology. Short rhizomes were less than 5 cm in length, and branched in an inverted candelabra to form the bases of stems. Long rhizomes have a larger diameter and grow without branching to more than 30 cm. Buds were the new growing tips of stems, short rhizomes and long rhizomes before they developed into new tissues. The number of buds and long rhizomes was counted. Plant material was dried at 80° C for three days and weighed.

*Stem Elongation*

At the beginning of the experiment, five stems from 4 plants in each treatment were tagged with loops of plastic coated wire, and additional stems were tagged every four weeks. Stem length was monitored every 2 weeks during the experiment. Elongation rates were linear for the most part, but slower soon after emergence and as stems approached their final lengths. Elongation rates were calculated from the linear growth period.

*Lignification*

The effects of flooding on the lignification of stems was examined after flooding and re-emergence. A mature stem and a young stem from each pot were sectioned when plants were harvested. *Baumea juncea* stems have intercalary meristems, and a range of tissue ages can be found along a single stem. New tissue is produced upwards from nodes, and stem tissue gradually ages up the stem towards the next node. Young tissue was sampled 1 cm above the bract, which forms a sheath around new tissue above the node, and old tissue was sampled from the top of the same internode, 1 cm below the node.

Sections were stained for 10 minutes in safranin (1% in 50% ethanol). After rinsing in two changes of 50%, 70% , 90% and absolute ethanol for 30 seconds each, sections were stained in light green-clove oil (4% light green in 50% ethanol, mixed with equal parts clove oil) for one minute. Safranin stained lignin and light green was used to bring out the contrasting non-lignified cell walls (Gurr 1956). After rinsing in clove oil and histolene for 5 minutes each, sections were mounted in Canada Balsam.

The thickness of fibre cell walls was measured using an eyepiece graticule at 500x magnification. The external diameter and internal diameters of cell walls were measured and the proportion of cell cross sectional area taken up by cell wall calculated. Circular cell cross sections were assumed for the calculation. Three cells were measured from separate girders (*sensu* Metcalfe 1971) between the epidermis and the parenchyma, until ten cell measurements were made for each section.

*Photosynthesis*

The capacity for *Baumea juncea* stems to photosynthesise underwater was investigated using aqueous $^{14}C$-bicarbonate. The rates of submerged photosynthesis of plants flooded for 23 days ~~was~~ were compared with unflooded plants.

Carbon fixation was measured as a time course over 5 hours in full outdoor irradiance, which did not fall below 1400 $\mu M\ m^{-2}\ s^{-1}$. Three centimetre sections of stem were cut, blotted and weighed, and the ends sealed with vacuum grease to exclude water from the lacuna. Sections were submerged horizontally in petri dishes under a transparent plastic grid which permitted water to flow around the stems. The bathing solution consisted of

1 mM NaCl

0.1 mM $K_2SO_4$

0.5 mM $CaCl_2$

2 mM MOPS.

Just prior to the experiment, the pH was raised to 7.2 by the addition of NaOH. The concentration of $HCO_3^-$ made up to 0.1 mM by the addition of radioactive $NaHCO_3$ in 1 mM MOPS. This concentration of carbon was chosen because it approximates the dissolved $CO_2$ equilibrium of air with pure water at pH 7. Duplicate samples of each treatment were taken every 2 hours. Stems were rinsed in reverse osmosis water and killed in 1 mL 1 mM HCl overnight. Samples were placed in 7 mL polyethylene vials with 5 mL of scintillation fluid and $^{14}C$ activity was determined on a Beckman L3801 liquid scintillation counter.

Photosynthesis rates of emergent stems in air of plants growing in 2 cm of water were measured using a Li-Cor LI 6200 $CO_2$ Gas Analyser. Two centimetre lengths of between four and six stems with a diameter of approximately 1.5 mm were enclosed in a 250 mL chamber facing the sun. Carbon fixation was determined from the change in carbon concentration in the chamber over 35 second periods. Measurements in which leakage of gas from the chamber or stomatal closure were indicated were discarded.

$^{14}C$ photosynthesis rates were calculated on a fresh weight basis and converted to a unit area rate for one side of the stem for comparison with IRGA measurements. Stems were 2 cm long with a 1.5 mm diameter.

*Statistics*

The mean relative growth rate (RGR) was calculated according to Hunt (1978):

$$RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

where

$W_1$ was the initial biomass

$W_2$ was the final biomass and

$T_2 - T_1$ was the duration of the experiment.

Sum stem length was calculated as the total length (end to end) of all stems per plant, averaged for each treatment.

Biomass and RGR were tested for differences with two way anovas, using proc GLM to account for missing values (see below; SAS Institute 1992). The initial sample was not a factor in the tests, but is included in the tables for reference.

The rates of submerged photosynthesis of stems acclimatised to flooding for 23 days were compared with previously unflooded stems and were tested for a significance difference with an analysis of covariance. Rates of stem elongation were tested for differences with at two tailed t-test. Probabilities for F values are referred to in the text and full F tables are presented in Appendix C.

*Outliers*

Three plants showed symptoms of disease which warranted their exclusion from the analysis. *Baumea juncea* stems are normally green along their entire length, but a number of plants had stems which were dying from the tip downwards. Three plants had more than 20% of stems showing gradual death, and these individuals also had the lowest biomass of any plants in the experiment. Their growth rates were the lowest and formed an outlier outside the distribution of the other growth rates (figure 4.1). Two plants from the flood stage (one at 25 and 120 cm) and one from the exposure stage (120 cm) were excluded on the grounds that their aberrant responses may have more strongly reflected a disease than the treatments applied in the experiment.

## Results

Plants were initially grown in 2 cm of water (table 4.1). In the flooding stage of the experiment, plants remained at 2 cm (control) or were flooded to 25 or 120 cm for 11 weeks. The flooding stage began in spring (October) and finished in summer (December). In the re-exposure stage of the experiment, plants which were flooded to 25 and 120 cm were raised to the surface over 4 weeks and harvested 8 weeks later. Control plants remained at 2 cm depth. The re-exposure stage extended from mid summer (December) early autumn (March).

*Photosynthesis*

Submerged stems of *Baumea juncea* were able to photosynthesise, but only at very low rates (table 4.2). The rate at which emergent stems fixed carbon from the atmosphere was four orders of magnitude higher than that of submerged stems. Submerged photosynthesis increased after stems were acclimatised to flooding for 23 days, but the rate remained negligible compared with photosynthesis in air.

**Table 4.2**
Rates of photosynthesis of *Baumea juncea* stems at irradiances greater than 1400 μE. The emergent rate was determined using an Infra Red Gas Analyser from repeated measurements of 3 plants. Submerged rates were calculated from the uptake of $^{14}C$ by stem sections (n = 8). Sections were from plants pre-treated with either 23 days submergence or emergent growth in air. Values are means with the standard deviation in parentheses.

| Stem Exposure | Rate ($\mu mol\ m^{-2}\ s^{-1}$) | |
|---|---|---|
| Emergent stem | 19 | (1.9) |
| Submerged stem | 0.0086 | (0.0034) |
| Submerged stem pretreated with flooding | 0.051 | (0.018) |

*Stem Growth Rates*

The rate at which stems grew in 2 cm of water did not differ from plants flooded by 25 cm of water (table 4.3). After an initial slower rate, stem growth rates were linear (figure 4.2a, b). Growth slowed as stems approached their final length and stopped completely when they reached their mature height. Top flooding changed stem growth substantially (figure 4.2c). Stem growth began to slow within five days of submergence, and almost completely stopped after 10 weeks of flooding. However stems continued to grow at very slow rates. When the water level was reduced, stems were again exposed to the atmosphere. Those which had survived top flooding increased their growth rate and developed to a mature height similar to the other treatments.

**Table 4.3**
Stem elongation rates during the flooding phase of the experiment. Rates were calculated from four stems or more from four plants at each depth. The rates were not significantly different (two tailed t-test p = 0.14). Values are means, followed by the standard deviation.

| Flooding Depth | Rate | |
|---|---|---|
| | cm day-1 | |
| Control | 0.64 | (0.17) |
| Partial | 0.87 | (0.23) |

*Morphology*

The length of *B. juncea* stems did not increase in proportion to flooding depth (table 4.4). The mean stem length of plants flooded to both 2 and 25 cm increased in the flooding stage of the experiment, but partially flooded plants did not increase stem length any more than the control (2 cm). Approximately half of the total stem length of plants flooded to 25 cm was submerged beneath the water. The sum stem length of the 25 cm treatment was 41.1 m (sd 11.0), and 21.8 m (sd 15.3) of that was exposed above the surface. Given that the rate of photosynthesis in submerged stems is negligible compared to photosynthesis in air, this represents a fifty percent loss in carbon fixation potential. The sum stem length of the controls at the same stage was 59.4 m (sd 12.9) and was more than double the emergent stem length of plants flooded to 25 cm. The mean stem length of plants after 11 weeks of flooding to 120 cm were similar to the initial sample. These stems were only able to increase in length by a few centimetres (figure 4.2c).

**Table 4.4**
Mean total stem length and mean emergent stem length. Values are means of seven plants except in flooding treatments marked 'a' n = 6. Standard deviation is shown in parentheses. A two way anova showed a strong interaction between depth and flooding stage on mean stem length ($p < 0.001$).

| Flooding Stage | Flooding Treatment (cm) | Mean Stem Length | |
|---|---|---|---|
| | | Total Length | Emergent Length |
| Initial | 2 | 23 (5.4) | |
| Flooded | 2 | 43 (9.0) | 43 (9.0) |
| | 25 a | 41 (11) | 22 (10) |
| | 120 a | 28 (13) | 0 (0) |
| Re-exposure (all at 2 cm) | 2 | 33 (9.1) | |
| | 25 | 46 (11) | |
| | 120 a | 33 (33) | |

The mean stem length of control plants decreased from mid summer to early autumn (table 4.4). The mean stem length of plants flooded both 25 and 120 cm increased marginally after they were raised to the surface.

*Stem and Rhizome Recruitment*

At the initial harvest, *B. juncea* was in the process of recruiting new stems (figure 4.3a). The stem lengths were bi-modally distributed, with a peak of stems at 30 to 35 cm and another at 10 to 15 cm. The peak of longer stems represents mature stems, and the shorter stems were newly recruited stems which had not yet reached their mature length. The total number of stems per plant increased from 79 to 143 at the control depth between spring (October) and mid summer (December; table 4.5). Stem recruitment at 2 cm continued through summer and the total number of stems per pot continued to increase from 143 to 205. Although the number of stems increased, the height of mature stems was slightly shorter (figure 4.3). This contributed to the reduction in the mean stem length at this depth from December to March.

**Table 4.5**
The number of stems, buds and long rhizomes per plant. Values are means of seven plants except in flooding treatments marked 'a' n = 6. Standard deviation is shown in parentheses.

| Flooding Stage | Flooding Treatment (cm) | Tissue | | | | | |
|---|---|---|---|---|---|---|---|
| | | Stem | | Bud | | Long Rhizome | |
| Initial | 2 | 79.0 | (37) | 16 | (9.8) | 4.1 | (3.0) |
| Flooded | 2 | 143 | (31) | 33 | (18) | 11 | (2.3) |
| | 25 a | 104 | (32) | 16 | (8.0) | 4.8 | (4.0) |
| | 120 a | 72.3 | (34) | 1.7 | (1.5) | 3.7 | (1.8) |
| Re-exposure (all at 2 cm) | 2 | 205 | (45) | 39 | (11) | 16 | (10) |
| | 25 | 108 | (65) | 29 | (8.5) | 13 | (5.3) |
| | 120 a | 69.3 | (35) | 20 | (13) | 1.8 | (2.6) |

Flooding to 25 cm reduced the number of stems *B. juncea* recruited (table 4.5). However the stem length distribution of plants flooded to 25 cm was similar to the control, and these stems reached a similar height to plants growing at 2 cm (figure 4.3c). No new stems were recruited after the water level was reduced to 2 cm (table 4.5). Stems continued to lengthen and the distribution became negatively skewed as a preponderance of long stems developed.

*B. juncea* did not recruit new stems when top flooded (table 4.5). The stem length distribution after 11 weeks of flooding to 120 cm was similar to plants prior to flooding (figure 4.3a, d) except that the peak of short stems had gone. A number of stems died when top flooded plants were raised to the surface (personal observation) Although there was some stem recruitment after drawdown, there was no net increase in the number of stems (table 4.5).

Buds are an indication of the growth status of *B. juncea.* Plants harvested initially, in spring, had a large number of buds, in proportion to their biomass (table 4.5). These plants were recruiting new stems and growing at a high rate. The number of buds was also high in control plants in summer, and these plants continued to recruit new stems in late summer. Plants flooded to 25 cm had half as many buds as the control plants and their relative growth rate was also approximately half that of the control. Top flooded plants were not growing and had very few buds.

Renewed growth in partially and top flooded plants after drawdown is indicated by the increase in the number of buds per plant (table 4.5). The number of buds in plants which had been flooded to 25 cm increased in proportion to the increase in biomass. The number of buds increased more dramatically in plants raised to the surface from top flooding. The number of buds increased from 1.7 to 20 buds per plant in the re-exposure stage of the experiment.

Long rhizomes colonise new areas by forming clusters of stems. The number of long rhizomes in plants flooded to 2 cm increased mainly in early summer (table 4.5). The recruitment of long rhizomes was delayed in plants flooded to 25 cm until drawdown. The number of rhizomes per plant was lowest in top flooded plants after drawdown. In these plants, the growth of stems and short rhizomes appears to have been a higher priority than the colonisation of new areas.

*Growth*

The biomass of plants with emergent stems increased in spring and early summer (table 4.6). The relative growth rate was highest in plants with the greatest exposed stem length, 2 cm depth, and biomass increased by approximately 3 times. The relative growth rate of plants flooded to 25 cm was approximately half that of the control, which corresponded to the difference in the exposed stem length of these two treatments. Plants flooded to 120 cm had no emergent stems, were unable to fix significant quantities of carbon and did not grow. However, they did not lose biomass either, and the tissues of *B. juncea* are resilient to prolonged top flooding.

**Table 4.6**
Biomass and relative growth rates of *B. juncea* at three stages of flooding to three depths. Values are the mean of seven plants except in flooding treatments marked 'a' n = 6. Standard deviations in parentheses. Anova indicated that biomass was influenced by depth ($p < 0.001$) and flooding stage ($p = 0.007$). These factors did not interact ($p = 0.48$). RGR was influenced by the interaction of both factors ($p < 0.001$).

| Flooding Stage | Flooding Treatment (cm) | Biomass (g dry wt) | | RGR (mg $g^{-1}$ $day^{-1}$) | |
|---|---|---|---|---|---|
| Initial | 2 | 19.6 | (5.8) | | |
| Flooded | 2 | 61 | (13) | 14 | (3.4) |
| | 25 a | 39 | (11) | 8.3 | (3.9) |
| | 120 a | 19 | (5.9) | -0.96 | (4.7) |
| Re-exposure (all at 2 cm) | 2 | 74.1 | (24) | 1.9 | (4.0) |
| | 25 | 68 | (33) | 5.9 | (5.0) |
| | 120 a | 31.1 | (15) | 5.0 | (4.7) |

The relative growth rate of control plants declined in late summer, suggesting that in the absence of a flood, the growth of *B. juncea* peaks in early summer (table 4.6). In contrast, the growth rate of plants flooded to 25 cm remained relatively constant when the water level was reduced to 2 cm. These plants maintained a higher rate of growth in late summer than the control. Although partial flooding had reduced growth in these plants earlier in the season, drawdown may have stimulated growth in late summer and early autumn.

The relative growth rate of top flooded plants increased when stems were exposed to the atmosphere (table 4.6). The response of plants flooded to 120 and 25 cm was similar. The relative growth rate of these treatments was higher than the control in late summer. Flooding reduced growth in *B. juncea*, but plants recovered with renewed growth when the water level fell.

*Biomass Allocation*

Despite the influence of partial and top flooding on growth, the allocation of biomass to tissues was relatively fixed, regardless of the flooding treatment (table 4.7). The proportion of total biomass allocated to the largest three tissues, the stem, the short rhizome and the roots, remained fairly constant. The allocation of biomass to the smaller two tissues, the long rhizome and the short rhizome was more plastic. Buds were the new growing tips of stems, long rhizomes and short rhizomes. Plants flooded to 25 and 120 cm allocated less biomass to the buds than the control. However, the allocation to these tissues increased after the water level was reduced. Top flooded plants allocated less biomass to the long rhizome than plants at 2 and 25 cm, and this allocation decreased further after drawdown.

**Table 4.7**
Percentage of the total biomass allocated to the five main tissues. Values are the mean of seven plants except in flooding treatments marked 'a' n = 6.

| Flooding Stage | Flooding Treatment (cm) | Tissue | | | | |
|---|---|---|---|---|---|---|
| | | Stem | Bud | Short Rhizome | Long Rhizome | Root |
| Initial | 2 | 52 | 2.2 | 20 | 6.7 | 19 |
| Flooded | 2 | 57 | 1.5 | 15 | 7.2 | 18 |
| | 25 a | 60 | 0.99 | 20 | 5.4 | 14 |
| | 120 a | 43 | 0.13 | 23 | 7.4 | 24 |
| Re-exposure (all at 2 cm) | 2 | 52 | 1.3 | 19 | 7.5 | 20 |
| | 25 | 50 | 1.2 | 14 | 10 | 25 |
| | 120 a | 50 | 1.1 | 18 | 3.9 | 27 |

*Lignification*

*Baumea juncea* stems are strengthened by bundles of fibre cells which form girders (*sensu* Metcalfe 1971) around the vascular bundles and inside the epidermis. Lignification was well developed in stems at 2 cm, with more than 95% of the fibre cell cross sectional area made up of lignified cell wall (table 4.8). However, stem tissue produced at 120 cm had weaker fibres with less lignification. Cell walls were thinner, with the cytoplasm making up a greater proportion of cell cross sectional area. Only the young tissue, which was produced at the base of the internodes while the plants were submerged, was weakened by flooding. Stem tissue at the top of the internode was produced before plants were flooded, and was lignified to the same degree as the control plants.

Weakened stems were more prone to collapse after sudden exposure than gradual drawdown. When plants were harvested at the end of the flood stage, they were raised directly to the surface, and many stems were too weak to support their own weight in air, bending over at the weakened tissue above the bract. Plants harvested after the re-exposure stage were less prone to collapsing stems. These plants were gradually exposed to the air, at 2 cm $day^{-1}$, and were supported by the water column as they were exposed. These stems may become lignified while they were brought to the surface.

**Table 4.8**
Fibre lignification. The percentage of the cross sectional area of fibre cells taken up by the cell wall in the stems of top flooded and control (2 cm flooding) plants. Young tissue was sectioned above the bract at the base of the internode and old tissue from the top of the internode. More than 10 measurements were made in any section, from one stem of six replicate plants. Standard deviation is shown in parentheses.

| Flooding Depth | Internode Section | Percent of Cells Fibre Tissue | |
|---|---|---|---|
| Control | Young | 96.1 | (3.2) |
| | Old | 95.5 | (3.2) |
| Top | Young | 65.4 | (20.9) |
| | Old | 90.9 | (11.5) |

## Discussion

In the absence of flooding, the growth of *Baumea juncea* showed a seasonal pattern. The relative growth rate (RGR) was highest in early summer and declined in late summer. A number of aquatic macrophytes have a seasonal recruitment pattern of new tissues. *Baumea articulata, Typha* spp., *Phragmites australis* and *Carex rostrata* initiate the recruitment of new shoots and stems in spring, from the mobilisation of stored nutrients and carbohydrate (Kausch et al. 1981; Bernard and Solsky 1977; Prentki et al. 1978; Steinmann and Brändle 1984a; Hultgren 1989). *B. juncea* growth continued in late summer but at a lower rate. Much of the early growth was allocated to the recruitment of long rhizomes. Long rhizomes established new clusters of short rhizomes, from which stems sprouted. In this experiment long rhizomes were constricted to some extent by the walls of the pots, but in the field long rhizomes radiate out from the parent plant and colonise new areas. In contrast to the early recruitment of long rhizomes, stem recruitment was maintained throughout the summer. The establishment of new clumps appears to occur in spring and these clumps are consolidated by the recruitment of stems, which continues throughout the summer.

Flooded stems of *B. juncea* were unable to contribute to growth. The photosynthetic rates of submerged stems were negligible compared to the rate of stems exposed to the air. Low rates of submerged photosynthesis have been recorded in other aquatic macrophytes which have emergent stems (Nielsen 1993). The morphology of emergent stems is adapted to reduce water loss and facilitate the diffusion and fixation of gaseous carbon dioxide in the mesophyll. These adaptations, such as thick cuticles, thick leaves and non-epidermal chloroplasts hinder the diffusion of dissolved carbon dioxide and make emergent stems poorly adapted to photosynthesis underwater.

The photosynthetic rate of submerged stems measured in this study may have been underestimated. The concentration of dissolved carbon dioxide used to measure photosynthesis was the equilibrium of air with pure water, but carbon dioxide concentrations

in wetlands may be higher (Nielsen 1993; Vadstrup and Madsen 1995). Photosynthesis in *Potamogeton natans* and *Ranunculus fluitans* saturates at concentrations greater than 7 mM $HCO_3^-$ in a range of pH values (Bodner 1994). Photosynthesis in submerged stems of *B. juncea* may have been limited by the concentration of dissolved carbon dioxide. The carbon fixation measured in this study is indicative of a low photosynthesis rate in submerged *B. juncea* stems, but needs further investigation to be fully quantified.

Flooding reduced the area of *B. juncea* stem tissue which could photosynthesise in air, and growth was reduced by flooding to 25 cm. The sum emergent stem length at this depth was less than half that of the control, and the potential to fix carbon from the atmosphere was reduced by the same amount. However, the RGR at 25 cm was slightly more than half the control (table 4.6) which is greater than would be expected if RGR was directly related to the length of exposed stem. These plants may have converted carbon dioxide to biomass more efficiently than the controls. However, caution should be used when relating the emergent stem length to photosynthetic potential. Some plants can fix gaseous carbon dioxide from within the lacunae, generated either from respiration within the plant or from the sediment (Singer et al. 1994). Although the lower half of stems were submerged, irradiances were high enough to support photosynthesis. If the submerged portion of the stems was supplied with carbon dioxide, they may have also been contributing to growth. Top flooded plants did not have access to atmospheric carbon dioxide and did not grow at all. Stem exposure is essential in directly or indirectly (via the lacunae) supporting photosynthesis and growth.

Although plants flooded to 25 cm grew at a slower rate than plants at 2 cm, growth was sustained for longer through the summer after the water level was reduced. The RGR of plants flooded to 25 cm ~~was~~ did not decline in late summer and remained high after drawdown. Consequently these plants grew at a faster rate, and after 8 weeks of exposure had reached a similar biomass to the controls. Top flooded plants did not grow at all during the flooding stage, but like the partially flooded plants, grew at a faster rate than plants at constant 2 cm depth when they were re-exposed. Flooding did not necessarily reduce growth

of *B. juncea* but delayed it until drawdown. The annual peak in biomass production of other emergent macrophytes also occurs after drawdown, and varies according to the duration of flooding (Hultgren 1988; Sjoberg and Dannel 1983; Neill 1992; Froend and McComb 1994). The time at which flood water recedes may be important to the survival of *B. juncea*. Summer provides optimal conditions for growth with high irradiances, long days and high temperatures. The period of time which plants spend in optimal conditions for growth will depend on the time of drawdown. Flooding which continues throughout summer may reduce the opportunity for growth to a brief period before winter.

*B. juncea* did not produce longer stems or increase the allocation of biomass to the stems when it was flooded to a depth which was sufficient to reduce growth. Optimally foraging plants increase the allocation of biomass to the tissue which acquires the limiting resource (Tilman 1988). *V. reniformis* increases the allocation of biomass to the petioles at the expense of the storage root when carbon becomes limiting with increasing depth (Chapter 3). Other species, including *Triglochin procerum* and *Typa latifolia* also increase the allocation of biomass to the shoot with increasing depth to increase the supply of carbon (Grace 1989; Rea and Ganf 1994d). Optimal foraging increases the efficiency of plant morphologies by increasing the supply of limiting resources and reducing the supply of resources which are surplus to growth (Tilman 1988; Mooney 1972). *B. juncea* maintained a fundamentally similar morphology in the three depths and did not forage optimally. The growth of this species is optimal only in a narrow range of depths, because shoot height cannot compensate for the lower supply of carbon with increasing depth.

However *B. juncea* inhabits seasonally flooded habitats, and flooding is only a temporary state. When the water level was reduced growth increased. *B. juncea* has long lived, resilient tissues which survive inundation and re-exposure. Stems which were growing before plants were top flooded survived three months of flooding and began to grow again when they were re-exposed (figure 4.2c). This contrasts with the strategy of the morphologically plastic plant, *V. reniformis* (Chapter 2, 3). Leaves of *V. reniformis* die soon after inundation, but are

replaced by continual recruitment. The morphology of *V. reniformis* continually adapts to water level changes, but does so at the expense of continual leaf recruitment. The stems of *B. juncea* are more resilient to flooding and exposure and therefore are not replaced as frequently.

Top flooded plants were not completely dormant and a number of stems continued to elongate (figure 4.2c). However elongation slowed as top flooding continued. If this growth was supported by stored carbohydrate, the supply of energy was not exhausted after 11 weeks of flooding. Starch grains were still visible when these stems were sectioned and stained. The gradual decline in elongation may be due to oxygen starvation. The lacunae may have provided oxygen for respiration initially and have been gradually depleted.

The growth of long rhizomes was strongly controlled by flooding depth in *B. juncea*. Plants at a constant depth of 2 cm predominantly recruited long rhizomes in early summer. Plants established new clumps of short rhizomes early in the season, and consolidated them with additional short rhizome and stem growth through the summer. Long rhizome recruitment was suppressed by flooding to 25 cm. The supply of carbon dioxide limited growth at this depth and ~~have~~ reduced the allocation to non-resource acquiring tissues. A similar response was found in *V. reniformis*, which does not reproduce vegetatively when nutrients limit growth (Chapter 3). The recruitment of long rhizomes increased after drawdown, when the availability of carbon increased. After 8 weeks of drawdown these plants had a similar number of long rhizomes to the control. Again, temporary flooding did not necessarily reduce the growth of *B. juncea*, but delayed growth until drawdown. Top flooded plants did not recruit long rhizomes after drawdown. The plants were recruiting new stems and short rhizomes and did not have the surplus growth which appears to be necessary for long rhizome recruitment.

## Chapter 5

# Responses of Four Aquatic Macrophytes to Seasonal Flooding in Bool Lagoon, South Australia

## Introduction

The establishment of optimum water regimes for individual species is an important component of wetland plant management (Keddy 1992; van der Valk 1981; Sjoberg and Danell 1983; Shay and Shay 1986). Alterations to patterns of wetland flooding can change the position of species on elevation gradients or lead to local extinctions (Blom et al. 1990; Millar 1973). Established vegetation can be displaced by invasive species which are favoured by manipulated water regimes (Rea 1992; Froend and McComb 1994; Grace and Wetzel 1981, 1982). Regulated wetlands provide an opportunity to manipulate water regimes to favour particular species and communities and to control invasive species. If water regimes are to be developed to achieve these goals, they must be based on detailed information on the optimal water regimes of the target species.

Controlled experiments have been used extensively to determine the water depth tolerances and preferences of aquatic macrophytes. This approach has provided information on the effects of depth on plant growth, reproduction, photosynthesis and morphology in detail which cannot be achieved in field experiments (eg Squires and van der Valk 1992; Stevenson and Lee 1987; Grace 1989; Nielsen 1993). These experiments can explain phenomena observed in the field and provide for predictions about the responses of plant populations to particular flooding conditions. However, their value lies in their application to populations and communities in the field. Their findings should therefore be considered in the light of their limitations in replicating water regimes in the field.

Water depth is only one of the components of water regime. Other components, such as the frequency, duration or seasonality of flooding also interact with depth. For example, plants which survive flooding may die on drawdown (Denton and Ganf 1994; Thomas and Stewart 1969; Palada and Vergara 1972) and species which reduce growth during flooding may increase growth when water levels fall (Chapter 4; Rea and Ganf 1994d). Flooding histories are also difficult to replicate in experiments. Wetland vegetation responds to altered water regimes over a number of years (Millar 1973), and generally has a high resilience to irregular flooding in any one season (Squires and van der Valk 1992). Short term responses to flooding are therefore partly dependent on the flooding history of the vegetation.

The aim here was to determine how well the responses to flooding of *Baumea juncea* and *Villarsia reniformis* in controlled experiments predict the responses of populations to seasonal flooding in the field. Experimentally based predictions have also been made of the flooding responses of two other species which are common in Bool Lagoon (Rea and Ganf 1994a). *B. arthrophylla* Nees. Boeckeler is a lignified sedge and has a similar strategy to *B. juncea.* It is relatively dormant during flooding and increases growth after drawdown. *Triglochin procerum* R. Br. is a fast growing fleshy leaved plant with a similar strategy to *V. reniformis.* Bool Lagoon has experienced deeper flooding since the wetland was regulated in 1970, and this has encouraged the spread of *T. procerum* at the expense of *B. arthrophylla* (Rea 1992). These species provided a comparison of alternate strategies to a similar range of water regimes.

## Methods

*Study Site*

The Bool Lagoon system of wetlands is located in the south east of South Australia (37° 05' S, 140° 42' E) and is made up of a number of linked basins (figure 5.1). Together the wetlands make up a total area of 2883 ha. The mean annual rainfall in the catchment for the ten years preceding this study was 590 mm (Bureau of Meteorology, SA). The highest rainfall season is winter (June, July and August) and the least rain falls in late summer/autumn (January, February, March; Cawood 1996). Water flows into the system from Mosquito Creek, entering Hack's Lagoon before flowing into the Main Basin of Bool Lagoon. The only exit is via the drainage channel, Drain M, in the Western Basin, which is controlled by a regulator. The basins have flat bottoms and gently sloping sides.

The basins generally fill in August and September and are gradually drawn down over summer (December to February) to reach minimum water levels in autumn (March to May; figure 5.2). The lagoon experienced high water levels from 1980 to 1989 and was only completely dry on two occasions, each less than three months long. However, this study succeeded a particularly dry two year period (1993 to 1994) during which most basins were dry for all but four months, in 1993.

*Water Levels*

Water level data were supplied by the South Eastern Water Conservation and Drainage Board (SEWCDB), from depth gauges calibrated to the Australian Height Datum (m AHD). Water regimes differ between the basins of Bool Lagoon, and depths were recorded in each of the three basins. A summary of the water regimes in the Main, Central and Western Basins is given by Brownlow et al. (1994).

Mean water depths were calculated for the three basins for the 36 months prior to the study. Only positive values of water depth were used and the depth to ground water was not included in the calculations.

*Design*

Each species was studied in two replicate transects across the gradient of elevations in the basins of Bool Lagoon, with replicate measurements at each elevation. The vegetation was surveyed in March, October and December 1995 and in March 1996, to correspond with the pre-flood drawdown, the rise and fall of the flood and the post-flood drawdown.

## *Villarsia reniformis* and *Triglochin procerum*

*Shoot Density*

Seasonal changes in the density of *Villarsia reniformis* and *Triglochin procerum* shoots were examined between elevations 47.81 and 48.11 m AHD at two sites (figure 5.1). *V. reniformis* was distributed over a 30 cm elevation range in June 1995, which determined the elevation range of the survey. The lower limit was close to the basal elevation of the Main Basin (Brownlow et al. 1994). Elevations were determined using a staff and dumpy level when the lagoon was dry in June 1995. These were correlated with the Australian Height Datum reference on the SEWCDB gauge when the lagoon was flooded. At 10 cm elevation increments, shoot density was measured in replicate 20 x 20 cm quadrats spread over 30 m on the elevation isocline. Twenty quadrats were measured in June and March when the lagoon was dry, but ten were measured in October and December when water depth limited the number of transects which could be measured at the flooded sites.

When transects were flooded, shoot density could only be assessed by digging up 20 x 20 cm clods and counting shoots on the exposed soil surface. Quadrat size was limited by the area of

soil that could be lifted up intact by a spade. Larger quadrats may have reduced variability, but would have been impossible to measure during the flood.

*Plant Morphology*

Seasonal changes in the morphology of the two species were measured on the elevation gradient. The number of leaves and the length and width of the longest leaf were measured on ten (October, December and March) or 20 (June) replicate shoots at the four elevations. Petiole length was measured to the nearest centimetre and leaf width was measured to the nearest millimetre. For *V. reniformis*, the number of leaves above the surface of the water and the number of runners and propagules per shoot were also recorded.

*Leaf Production*

The production of new leaves was recorded by tagging the youngest leaf of ten *V. reniformis* and *T. procerum* shoots at each elevation in June 1995. Efforts to recover the tags three weeks later and to determine the rate of leaf production proved fruitless. The lagoon had recently flooded and tags were submerged between 15 and 35 cm of highly turbid water. Finding tags proved very difficult, and measuring the number of new leaves became impossible after working for a short time in the cold (<10°C) water.

## ***Baumea juncea* and *B. arthrophylla***

*Stem Density and Length*

The density and distribution of *B. juncea* and *B. arthrophylla* stems were examined across an elevation gradient at two sites for each species. Dense, nearly monospecific stands of *B. juncea* range over 20 cm elevation in the Western Basin, from 47.87 to 48.07 m AHD (figure 5.1). Stem density and length was measured at three elevations, 10 cm apart. Twenty replicate 20 x 20 cm quadrats, spread along 30 m, were measured at each elevation and the length of every stem recorded. *B. arthrophylla* also occurred in many quadrats at the *B.*

*juncea* site, and the height and number of stems per shoot of this species were also recorded. Stem length was measured in 10 cm length classes.

Monospecific stands of *B. arthrophylla* range over 30 cm elevation, from 47.84 to 48.15 m AHD (figure 5.1). Stem density, length and the number of stems per shoot were measured at four elevations in this range, 10 cm elevations apart. Stem density was more uniform in *B. arthrophylla* stands than *B. juncea*, and consequently only ten replicate 20 x 20 cm quadrats, spread 30 m along the elevation isocline, were measured at each elevation.

*Stem Growth*

The growth of individual stems was followed over time by tagging stems at each elevation. Stems of a range of lengths were tagged to test whether young, short stems responded more to the flooding depth than long, old stems. Ten stems were tagged at each elevation and site in June and October and the length recorded. It was intended that the tags would be recovered and the stems remeasured on the following sampling date. However none of the tags set in June could be recovered in October because they could not be located under water. In December the water level had fallen and stems were located and remeasured. Unfortunately, only 30% of the tags set in June and October could be located and some elevations and sites yielded no data at all.
Consequently, these results are not presented.

*Statistics*

The effects and interactions of elevation and time of year were tested using the General Linear Models procedure of SAS (SAS Institute 1992) for unequal sample sizes. Two sites were surveyed for each species to account for spatial variation in species responses to elevation. The differences between sites were not of specific interest and are not investigated. Normally distributed data, including leaf width and mean stem length per quadrat were analysed using this technique. Count data, such as stem density and the number of leaves per shoot, was Poisson distributed and not suitable for parametric analyses. Instead, the effects of date and elevation

were tested using non-parametric one-way anovas (Kruskil-Wallis tests, Zar 1984). Probabilities for test statistics are referred to in the text, and full Kruskil-Wallis and anova tables are given in Appendix D.

## Results

### Water Regime

Bool Lagoon experienced a prolonged period of low water levels before this study took place (1993 to 1995; figure 5.2). The lowest elevation of the *Villarsia / Triglochin* site had been flooded for two months 8 months previously, but the upper three elevations had not been flooded for 18 months. None of the elevations at the *B. juncea* nor the *B. arthrophylla* sites had been flooded for 18 months.

All sites experienced a similar pattern of flooding in 1995 and 1996. The lagoon filled rapidly in July, one month after the initial survey and water reached the different sites within two weeks of each other (figure 5.3). The water level at all sites reached a brief maximum in September and then dropped to a lower level which was stable until the beginning of November. The water level fell rapidly at the *Villarsia / Triglochin* site in December (figure 5.3), and all elevations were exposed within two weeks of each other. Water drained more slowly from the *Baumea* sites (figure 5.3), and the shallowest elevations were exposed five weeks before the lowest elevations. A summary of the flooding characteristics of sites is given in table 5.1.

**Table 5.1**
Flooding characteristics of surveyed sites in Bool Lagoon 1995/1996, and Mean Water Depth calculated for the 36 months preceding the study.

| Site | Elevation | Date Flooded | Max Depth (cm) | Date Exposed | No. Days Flooded | Mean Water Depth (m) |
|---|---|---|---|---|---|---|
| *Villarsia / Triglochin* | 47.81 | 12/7 | 75 | 28/12 | 169 | 0.162 |
| (Main Basin) | 47.91 | 14/7 | 65 | 23/12 | 162 | 0.117 |
| | 48.01 | 15/7 | 55 | 19/12 | 157 | 0.081 |
| | 48.11 | 16/7 | 45 | 16/12 | 153 | 0.058 |
| *Baumea arthrophylla* | 47.85 | 13/7 | 70 | 15/1 | 186 | 0.126 |
| (Central Basin) | 47.95 | 15/7 | 60 | 6/1 | 175 | 0.098 |
| | 48.05 | 16/7 | 50 | 20/12 | 157 | 0.071 |
| | 48.15 | 17/7 | 40 | 16/12 | 152 | 0.048 |
| *Baumea juncea* | 47.87 | 17/7 | 61 | 14/1 | 181 | 0.095 |
| (Western Basin) | 47.97 | 18/7 | 51 | 5/1 | 171 | 0.069 |
| | 48.07 | 19/7 | 41 | 22/12 | 156 | 0.047 |

Mean water depth was near to linearly related to elevation in the 36 months prior to the study (figure 5.4). The flooding characteristics of the basins differ, depending on the elevation of the bottom of the basins and the proximity of the basin to the inlet. The basal elevation increases progressively from the Main Basin (47.85 m AHD) to the Central Basin (47.8) and the Western Basin (47.9), and the mean water depths are correspondingly lower.

## *Villarsia reniformis* and *Triglochin procerum*

The range of elevations over which *V. reniformis* was surveyed encompassed the upper and lower limits of shoots which were visible in June 1995. Shoot densities were highest at 47.91 m AHD and this appears to represent the optimum elevation for this species (table 5.2). New shoots were recruited from vegetative propagules from October onwards (table 5.3) and were produced predominantly at the lower elevations. However, the recruitment of new shoots did

not significantly alter the population and shoot densities did not change through time. Not all shoots produced runners, and fewer flowered. Flowering was recorded in one shoot only, in December, at the lowest elevation.

**Table 5.2**
The density of *V. reniformis* and *T. procerum* shoots per $m^2$ on the elevation gradient. Elevation affected the density of both species ($p < 0.001$ for both). The samples at each elevation are pooled for date, because date was not significant ($p = 0.4$ *V. reniformis*, $p = 0.2$ *T. procerum*). Both sites are pooled, giving a total n = 2 (sites) x (20 (Jun) + 10 (Oct) + 10 (Dec) + 20 (Mar)).

| Elevation (m AHD) | *V. reniformis* | | | *T. procerum* | | |
|---|---|---|---|---|---|---|
| | Density | sd | n | Density | sd | n |
| 47.81 | 19.8 | (32.5) | 100 | 32.2 | (32.5) | 100 |
| 47.91 | 59.9 | (73.8) | 100 | 30.7 | (36.4) | 100 |
| 48.01 | 32.3 | (52.7) | 100 | 12.5 | (70.3) | 100 |
| 48.11 | 27.5 | (47.5) | 100 | 10.2 | (17.0) | 100 |

**Table 5.3**
The abundance of *V. reniformis* propagules in December (n=10).

| Elevation (m AHD) | Shoots with Runners (%) | Propagules per Plant | sd |
|---|---|---|---|
| 47.81 | 30 | 2.4 | (4.3) |
| 47.91 | 20 | 0.50 | (1.1) |
| 48.01 | 25 | 1.0 | (2.2) |
| 48.11 | 20 | 0.75 | (1.9) |

*V. reniformis* became active when flooded, producing longer petioles and larger leaves. In June petioles were short and leaves were small (figure 5.5, table 5.4), but by October, petioles had increased in length in proportion to the depth of flooding. Petioles were approximately 20 cm longer than the shortest length required to expose leaves at the surface. The water level in the Main Basin in August briefly peaked at 20 cm higher than the October water level, and the additional petiole length in October may reflect their adaptation to this former depth. As the water level fell in the period to December, petiole length decreased, but remained

approximately 30 cm longer than the water depth. Again, these petioles were recruited when the water level was deeper, and they reflect a lag in the response of petiole length to a falling water level. These flood-adapted petioles did not persist after drawdown and petioles were much shorter in March. However, a slight increase in petiole length persisted down the elevation gradient.

**Table 5.4**
The leaf morphology of *V. reniformis* and *T. procerum* (n=20). The samples at each date are pooled for elevation, because date was not significant (see Appendix D). Both sites are pooled, giving a total n = 10 samples x 4 (elevations) x 2 (sites).

| Date | *T. procerum* | | | | *V. reniformis* | | | |
|---|---|---|---|---|---|---|---|---|
| | No. Leaves per Shoot | sd | Leaf Width (mm) | sd | No. Leaves per Shoot | sd | Leaf Width (mm) | sd |
| June | 3.3 | (1.1) | 0.60 | (0.23) | 6.9 | (2.6) | 1.25 | (0.25) |
| October | 6.4 | (1.4) | 1.9 | (0.57) | 6.5 | (2.3) | 4.4 | (1.4) |
| December | 5.7 | (1.5) | 2.1 | (0.73) | 4.9 | (1.4) | 5.5 | (1.6) |
| March | 2.7 | (1.0) | 0.51 | (0.44) | 4.0 | (1.9) | 2.6 | (0.9) |

The width of *V. reniformis* leaves was four to five times greater when shoots were flooded, in October and December, than in drawdown in June and March ($p = 0.03$; table 5.4). There was no interaction between elevation and depth ($p = 0.08$). Elevation had a weak effect on leaf width ($p = 0.03$) but the means only varied by 9% between the upper and lower elevations. This result is not considered biologically significant and only temporal effects are shown in table 5.4.

Despite increases in leaf size and petiole length, *V. reniformis* shoots did not produce a greater number of leaves in response to flooding. The number of leaves per shoot was highest in June, after a long period of stable dry conditions. In October, shoots had a similar number of leaves to June. Many of the older leaves were submerged leaves which had not elongated sufficiently to match the rising water level. Shoots had fewer leaves in December, when the longest petiole also tended to be the oldest and the older, submerged petioles had died. The number

of leaves remained low in March. The long, flood-adapted petioles died on drawdown and all the petioles in March had been recruited since the water receded.

The density of *Triglochin procerum* shoots increased down the elevation gradient. This species showed a preference for more frequently and deeply flooded water regimes (figure 5.6). Reproductive characteristics of *T. procerum* were not recorded, but shoots were observed flowering in October and setting seed in December. The recruitment of seedlings or vegetatively reproduced shoots did not produce any seasonal changes in the density of shoots.

Like *V. reniformis*, *T. procerum* became active when flooded. Shoots produced longer, wider leaves and more of them. Leaves grew longer in proportion to flooding depth. In contrast to *V. reniformis*, leaf length already showed a response to elevation in June, even though surface water was then limited to a few isolated puddles. In October and December a large proportion of the leaf length was exposed above the surface (70 cm and 50 cm, respectively). Some of the exposed leaf length may have resulted from high water levels before leaves were measured, but leaves of this species emerge above the surface, even at stable water levels. After surface water receded, *T. procerum* produced short leaves again, regardless of elevation.

*T. procerum* shoots produced more leaves when flooded than when dry (date: $p < 0.001$) but the number of leaves per shoot was not affected by elevation ($p < 0.5$; table 5.4). Leaves were also wider when shoots were flooded than in drawdown in either June or March ($p = 0.03$).

### *Baumea juncea* and *B. arthrophylla*

The presence of *B. arthrophylla* in quadrats at the *B. juncea* site had no effect on the density of *B. juncea* stems (two tailed t test, n=301 p=0.32). The density of *B. juncea* stems was not different in quadrats containing both species (mean = 981 stems per $m^2$ sd = 885 n = 57) to

quadrats containing *B. juncea* alone (mean = 885 stems per $m^2$ sd = 671 n = 244). Consequently, quadrats which contained *B. arthrophylla* were treated the same as monospecific *B. juncea* quadrats.

The density of *B. juncea* stems was always highest at the intermediate elevation, 47.97 m AHD (figure 5.7). Stem density increased between June and October at all elevations, as new stems were recruited and added to the population of older stems. By March the higher and lower elevations had similar stem densities to before flooding. The population of stems only increased at the intermediate elevation.

Stem recruitment showed a seasonal pattern, beginning in early spring and increasing in December as the water level fell. The majority of stem recruitment took place during the flood. Recruitment began in the period between June and October, as is indicated by the increase in the proportion of short stems at all elevations (figure 5.8). The proportion of short stems was lowest at the lowest elevation, 47.87 m AHD, and recruitment may have been reduced by the deeper water. Stem recruitment increased further in the period to December as the proportions of short stems increased and long stems decreased. Recruitment declined in the period before March, after surface water had receded, but remained higher than it was before flooding in June.

*B. juncea* did not respond to flooding by increasing mean stem length. The mean length of *B. juncea* stems was actually lower during flooding in October and December than before flooding in June. Mean stem length increased from December to March, as the newly recruited stems matured and fewer short stems were produced. However, the mean stem length was lower after flooding in March, than it was before flooding, in June. Stems were always tallest at the intermediate elevation, 47.97 m AHD (figure 5.9). Stems were shorter at 47.87 m AHD, where they were flooded more deeply, but this species was not able to adapt to high water levels with longer stems.

*B. juncea* maintained contact between the atmosphere and submerged tissues with emergent stems and a proportion remained exposed throughout the flood.

*B. arthrophylla* did not have an optimal elevation in the range of water regimes studied. The density of *B. arthrophylla* stems was unrelated to elevation prior to flooding (figure 5.10). Stem densities increased up the elevation gradient after the flood in March, but this may reflect delayed recruitment at deeper elevations rather than a difference in the number of stems recruited (see below). There was a net increase in stem density at all elevations after the flood.

*B. arthrophylla*, like *B. juncea*, recruited stems in a seasonal event, from early spring to summer (figure 5.11). The proportion of short stems was highest in October and December, when new stems were recruited, and lower in March as the stems matured and lengthened. Stem density doubled from June to October, as the recruitment of new stems overlapped with the presence of old stems. Older stems began to die as the flood continued and stem densities declined. Continuing recruitment in summer increased stem densities, firstly at the shallow elevations and progressively later down the elevation gradient.

There were two stages to *B. arthrophylla* stem recruitment: shoot recruitment in early spring (September / October) which was independent of flooding depth, and multiple stem recruitment which continued until autumn and was depth dependent. *B. arthrophylla* produces stems within shoots, which can include up to five stems altogether. In June, shoot density was the same at all depths (figure 5.12), about 1000 stems per $m^2$. In the period to October, shoot density doubled at all elevations. New shoots were recruited in this period, and their arrival overlapped with the previous season's shoots. The old shoots died after October and in December shoots returned to the previous density measured in June. There was no further change in shoot density to March. Shoot recruitment took place in early spring, and was independent of depth.

Before flooding, the number of stems per shoot ~~contained~~ increased up the elevation gradient (figure 5.13). The new shoots were recruited in October and had only one stem. After October shoots began recruiting multiple stems, predominantly at the higher elevations. Water receded from the highest elevations first and the recruitment pattern of multiple stems followed the drawdown of water. The gradient in stems per shoot became stronger in December as the water level continued to fall. By March all elevations had been exposed and the number of stems per shoot had also increased at the lower elevations. The recruitment of multiple stems within shoots depended on the recession of flood water.

In contrast to *B. juncea, B. arthrophylla* mean stem length increased in proportion to elevation before flooding (figure 5.14). The relationship was weakened in October by the recruitment of new, short stems and weakened further in December as recruitment continued. After recruitment had slowed and new stems had matured, the relationship was established again, with the longest stems at the lowest elevations and the shortest at the highest. The deepest two elevations had similar mean stem lengths in March, and the stems at 47.85 m AHD may not have yet attained their mature height.

Stem recruitment was sustained for longer in *B. juncea* and was less dependent on flooding than in *B. arthrophylla*. Table 5.5 shows the density of short stems, less than 21 cm long, at comparable elevations (*B. arthrophylla* 47.85, *B. juncea* 47.87 m AHD). The lowest elevations are compared because stems did not reach a mature height of less than 21 cm at these elevations. Therefore short stems are more likely to represent the recruitment of new stems. The densities for both species were lowest in June, when very few new stems were being recruited. Short stem density increased rapidly in *B. arthrophylla* in October, and remained high in December. By March, short stem density had dropped again to a level comparable to the previous June. *B. juncea* recruitment did not increase as rapidly in the period to October, but continued to increase through spring. In addition, short stem density did not return to a low level after flooding and remained at the high level recorded in spring. *B. juncea* stem recruitment was still taking place in spring.

**Table 5.5**
The density of *B. juncea* and *B. arthrophylla* stems less than 21 cm long over the course of the flood at comparable elevations: *B juncea* at 47.87 m AHD and *B. arthrophylla* at 47.85 m AHD. Densities are stems per $m^2$.

| Date | *B. juncea* | | | *B. arthrophylla* | | |
|---|---|---|---|---|---|---|
| | Short Stem Density | sd | n | Short Stem Density | sd | n |
| June | 38.1 | (39) | 20 | 50.0 | (20) | 10 |
| October | 93.4 | (92) | 10 | 245 | (185) | 5 |
| December | 174 | (183) | 10 | 258 | (139) | 5 |
| March | 101 | (111) | 20 | 69.1 | (42) | 10 |

## Discussion

Wetland vegetation responds to water regimes over a number of years, and the density and growth of plant populations at any time reflects a history of flooding (van Groenendael et al. 1996; Shay and Shay 1986; Leck and Simpson 1995; Millar 1973). Bool Lagoon had experienced lower water levels in the two years prior to the 1995 flood than in the previous ten years and was completely dry for much of that time. Despite the uniformly dry conditions over the elevation gradient, growth characteristics both before and after flooding in 1995 indicated optimal elevations for *V. reniformis, B. juncea and T. procerum. B. arthrophylla* did not show a distinct preference for any of the elevations within the range studied.

The distribution of *V. reniformis* and *T. procerum* in 1995 depended on the distribution of the propagules and rhizomes in the soil before flooding. Both of these species are able to withstand long, dry periods with little or no above ground tissues and subsist as below ground storage tissues. Their activity is dependent on flooding, and individuals produce shoots in flooded or damp soil, but remain dormant in dry soil. After the winter rains had fallen, shoots of both species appeared at higher elevations in October than in June, when the soil was dry.

The elevation at which shoot densities are highest in any particular year may only be one possibility of many, depending on the flood characteristics. For example, *Scirpus maritimus* shoot length increases up to a maximum depth, beyond which dormancy is triggered and no shoot is produced at all. The rhizomes of this species remain dormant in the soil over a wide range of elevations but are only active in a subset of this range (Squires and van der Valk 1992). The same principle controlled the presence of *V. reniformis* and *T. procerum* shoots at the upper elevations in Bool Lagoon.

The range of elevations in this study did not indicate a water regime at which the maximum density of *T. procerum* occurred, but densities increased down the elevation gradient. Shoot densities show little response to constant depth gradients, but respond distinctly to elevation gradients in the field (Rea and Ganf 1994a, d). Densities were lower than the highest values previously recorded for this species in Hacks Lagoon, 65 shoots per $m^2$, in 1989 following a period of deep flooding and a mean water depth of 21 cm, calculated on the preceding three years (Rea and Ganf 1994a). This density was recorded at a higher elevation, 48.25 m AHD, which has a corresponding water regime at 48.15 m AHD in the Main Basin (Brownlow et al. 1994). Species can 'migrate' up or down the elevation gradient in response to altered water regime (Wilcox and Meeker 1991). The maximum density of this species migrated to lower elevations, 47.85 m AHD, in response to the lower water levels experienced after March 1993. However, even at the lowest elevation in the Main Basin, the water regime was drier and less suitable for growth than the regime formerly experienced at 48.15 m AHD. Wetter sites are favoured by this species, but the plant is active in depths less than those optimal for shoot density.

The *V. reniformis* shoot densities in Bool Lagoon showed a superficial similarity to responses predicted from the pond. The maximum shoot density occurred at 47.91 m AHD and this elevation experienced similar depths to the optimal depth for biomass in the pond experiment, 40 cm. This elevation was flooded briefly to 64 cm in July, after which water levels fell to between 45 and 35 cm for four months. In this respect the pond experiment faithfully

predicted the optimal flooding conditions for *V. reniformis*. However, the density of shoots on the elevation gradient was a response to the flooding history of the site as well as the flood in 1995. Shoot density did not change with time over the course of this study, even though all elevations were flooded more deeply than they had been in the last two years. The shoot densities were therefore not only a response to the depths experienced in 1995, but densities were related to the distribution of shoots before flooding, and consequently also reflected the previous water history. The optimum elevation, 47.91 m AHD, had not been flooded to depths greater than 40 cm for three years. These conditions were drier than the predicted depths at which *V. reniformis* growth was highest from the pond work, where growth increased with depth up to 60 cm.

Elevations can be related to water regimes only with caution. In the previous four years patterns of flooding and drawdown at particular elevations had changed from year to year depending on the size of the flood. Vegetation responds both to past and present flooding events and reflects a variety of different water regimes which have been experienced in the past (Squires and van der Valk 1992; Millar 1973). The lower elevation of *V. reniformis* shoot densities may have ~~been~~ reflected a change in the optimal water regime to deeper elevations in response to shallower flooding from 1992 to 1994. The maximum density of shoots was at a higher elevation than the highest density of vegetative propagules. The density of shoots reflects the recruitment of shoots in past floods, but the density of propagules reflects production in any given year. Propagule recruitment indicates that growth was highest at 47.81 m AHD, and this may lead to higher shoot densities at this low elevation in subsequent years.

Although *B. juncea* population responses were strongly related to elevation, they were not necessarily related to water regime. The patterns of stem density and length were established before flooding, in June. This followed a long dry period when water depth could have had little influence on growth. None of the elevations had been flooded in the previous 18 months and the lower elevations, 47.87 and 47.97 m AHD had been flooded to 16 and 6 cm,

respectively, for only six weeks in 1993. Although stem densities in this species are affected by depth differences of 25 cm (Chapter 4), it is unlikely that a shallow six week flood would have a strong effect on the population 18 months later.

The pattern of stem densities and length did not change in response to deeper flooding in 1995, but became stronger in March 1996 than it was before flooding in June 1995. It is difficult to explain the stem density and stem length patterns in this species. There was no interspecific competition between *B. arthrophylla* and *B. juncea*, and competition at either the upper or lower elevations does not account for its reduced vigour. This species may be resilient to invasion and resilient to a variety of water regimes. Rather than reflecting an optimum range of elevations, it may simply have persisted at these elevations in spite of variations in water regime or competition from other species. Its distribution may reflect a colonisation event in the past which is somewhat independent of water regime and competition from other species.

The density of *B. arthrophylla* shoots was not influenced by elevation. In June 1995, the density of stems and shoots were unrelated to elevation. This reflects the uniformly dry conditions at this site in the preceding 18 months, when all elevations experienced the water regime. New shoots were recruited in a single seasonal event, independently of depth, at the height of flooding. Stem recruitment, however, was related to water level and the timing of drawdown. Secondary and tertiary stems were recruited at progressively later times down the elevation gradient as the water level fell. However, the timing of stem recruitment did not affect the final stem density. The densities at lower elevations increased as the water level fell and approached the densities which developed earlier at the higher elevations.

These results contrast with the survey of shoot densities made by Rea (1992) in 1989, where a marked decline in the *B. arthrophylla* population down the elevation gradient was found. Densities decreased linearly from 2000 shoots per $m^2$ at 48.30 m AHD to 250 shoots per $m^2$ at 47.65 m AHD. This survey was made after a prolonged period of high water levels, which

had a substantial impact on the vegetation. The 47.85 m AHD elevation had been continuously flooded for three years prior to 1989. The mean water depth at this elevation being 42.5 cm during this time (SEWCDB data). This is 30 cm higher than at the same elevation for the three years preceding 1995 (table 5.1). Prolonged, deep flooding reduces *B. arthrophylla* biomass and stem recruitment (Rea and Ganf 1994d) and eventually leads to population decline. However, flooding in 1995 did not reduce growth because water levels fell to levels which stimulate the recruitment of secondary and tertiary stems within shoots.

In summary, the experiments in which the growth of these species at specific depths was examined provided valuable explanatory information on their responses to components of water regime in the field, but they provided insufficient data to predict the optimal elevations for shoot density. In the field the vegetation at a given elevation can experience a different pattern of flooding and drawdown each year, depending on the size of the flood. Plant growth will reflect past and present water regimes and the response of a perennial plant to a single flood can only be predicted with knowledge of the flooding history of the site.

*V. reniformis* and *T. procerum* share similar growth strategies, but have different tolerances to components of water regime. *T. procerum* favours deeply flooded elevations and achieves a maximum shoot density in a mean water depth of 21 cm (calculated from Rea and Ganf 1994d and Rea 1992). In this water regime in Hacks Lagoon, shoots were drawndown for 10 weeks of the year and flooded to a yearly average maximum depth of 69 cm (calculated at 48.15 m AHD for the three years preceding 1989, from SEWCDB data). Shoot densities decline after prolonged drawdown, and the maximum shoot density migrates to lower, more deeply flooded elevations (Chapter 5). The growth of *V. reniformis* is reduced in depths greater than 60 cm (Chapter 3), but inundation to this depth does not reduce *T. procerum* biomass (Rea and Ganf 1994d). *V. reniformis* shoot densities are highest at a mean water depth of 11 cm, where shoots are drawdown for 31 weeks of the year and the yearly average depth peaks at 44 cm (Chapter 5; calculated at 47.91 m AHD for the three years preceding 1995, from SEWCDB data). Despite the similarities of these species strategies to seasonal flooding, they have

different water regime preferences, with *T. procerum* preferring deeper, more frequently flooded water regimes to *V. reniformis*.

Less precise water regime definitions can be given for *B. arthrophylla* and *B. juncea*. The distribution of *B. juncea* in Bool Lagoon in 1995 did not exhibit a clear relationship with water regime and this species existed only over a narrow range of elevations at any one location. An optimum water regime has not been located for *B. arthrophylla* either. However, a range of water regimes in which the highest recorded stem density can be given. The deepest water regime at which stem density reach 2000 stems per $m^2$ was 48.15 m AHD in 1989, at a mean water depth of 18.6 cm. This elevation was drawndown for 18 weeks of the year and flooded to an average yearly maximum depth of 61 cm. The shallowest water regime was also at 48.15 m AHD in 1995, at a mean water depth of 4.8 cm. This elevation was drawndown for 42 weeks of the year and flooded to an average maximum depth of 24 cm (calculated for the preceding three years from SEWCDB data). Stem densities have not been recorded at shallower water regimes than this, and this may not represent the upper limit to the optimum density of *B. arthrophylla*.

The distribution of species on the elevation gradient can be predicted only with knowledge of the cumulative effects of water regimes. This question could be examined by following migrations in species' shoot density optima on the elevation gradient over successive years. A number of traits need to be determined. Do species persist in unfavourable conditions for a number of years, or does the elevation with the maximum shoot density change every year? Are propagules present which support growth at a range of elevations, or are they limited to the distribution of shoots in the previous year? How is the shoot density related to water regime in a consistent flooding pattern?

In this study, drawdown has emerged as a particularly important aspect of flooding in the life history of these species. Both *B. juncea* and *B. arthrophylla* recruit new stems in spring, when they are most likely to be flooded. This is an energetically expensive response, because

young stems are submerged and cannot fix carbon to support their own growth (Chapter 4). Instead, growth is supported from elsewhere in the plant. Carbohydrate stored in the rhizomes supports the heterotrophic recruitment of new shoots in a number of emergent species (Steinmann and Brändle 1984a; Haldemann and Brändle 1986; Kausch et al. 1981; Gallagher et al. 1984). Both of these species have substantial reserves of starch in their rhizomes; these may be mobilised to support the growth of new stems.

This strategy forms a population of stems which capitalise on the highly productive period of falling water levels in summer. As water recedes, high irradiances and temperatures and abundant water provide conditions for rapid photosynthesis. Both *Baumea* species increase their growth rates in these conditions (Chapter 4; Rea and Ganf 1994d) and increase stem recruitment. This period represents a brief window of opportunity between deep flooding and the dry summer, when conditions become less favourable for growth. The stems which were recruited at high water levels can take advantage of these conditions, replacing the energy consumed in their own development and supporting the growth of additional tissue.

*B. juncea* and *B. arthrophylla* are adapted to seasonal drawdown, and their strategies depend on low water levels for the successful recruitment of stems. The deeper flooding in the 1980s reduced *B. arthrophylla* population densities at elevations which did not experience low water levels. However, flooding did not disadvantage *B. arthrophylla* when water levels were drawndown in summer. Summer drawdown also promotes stem recruitment and biomass production in other wetland species (Froend and McComb 1994; Sjoberg and Dannel 1983; Hultgren 1988). Plants in seasonally flooded habitats may have limited potential to fix carbon in winter, but this is compensated by the highly productive conditions in summer.

Drawdown may also be important in the life-history of *V. reniformis*. The length of petioles increases in proportion to depth, and plants which have limited growth allocate biomass to the petioles at the expense of other tissues (Chapter 3). As water levels fall and shorter petioles are produced, the carbon balance of the plant will improve. This species remains active in the

damp soil after drawdown and can fix carbon with a minimal investment in petiole tissue. Drawdown may provide deeply flooded plants with an opportunity to redress the carbon imbalance imposed by deep flooding.

*T. procerum* is less dependent on drawdown than the other species in this study. The biomass of plants is not lower at 50 or 100 cm depths than at 0 cm. Seed production and seedling recruitment are promoted in flooded elevations, at depths up to 47 cm (Rea and Ganf 1994c). Drawdown may therefore be an effective control for the spread of this species. The deeper water levels in Bool Lagoon since regulation in 1970 have promoted the spread of this species and contributed to the decline of the sedgelands (Rea 1992). This species migrated to lower elevations in response to the 1993 / 1994 drought and shoot densities declined. Periodic drawdown in Bool Lagoon may promote the growth of the sedges and control the spread of *T. procerum,* restoring the balance between these species.

## Chapter 6

## General Discussion

Wetlands in mediterranean climates experience extremes of water availability from very dry soils to deep flooding. Wetlands are generally flooded in late winter and spring and drawdown in summer or, in years of high rainfall, in autumn. However, Australian climates are also subject to the El Nino effect, which superimposes long term variability and instability on the climate. This effect produces climatic phases which increase the duration and intensity of either the dry or wet seasons (Cawood and Cechet 1996). Wetland vegetation is adapted to the seasonal and inter-annual vagaries of these conditions. Perennial, emergent species are adapted to flooded and dry states as well as the transition between the two.

*Water Regimes Defined by Plant Tolerances*

The experimental and survey work in this thesis and in previous work (Rea and Ganf 1994a, b c, d) define water regime classifications for *V. reniformis, T. procerum, B. juncea* and *B. arthrophylla*. The growth of *V. reniformis* is optimal in depths of 20 to 40 cm when nutrients are abundant (Chapter 3). Biomass is reduced by flooding in nutrient poor soils, and water logged conditions are likely to be optimal for growth. The shoot of *V. reniformis* adapts readily to rapid flooding to depths greater than 60 cm (Chapter 2). However a proportion of leaves become submerged by depth increases greater than approximately 24 cm (Chapter 2). This leads to early leaf death and reduces the carbon fixing potential of the plant. Frequent depth changes of this magnitude may deplete the canopy and reduce growth. Although this plant tolerates temporary flooding to depths greater than 70 cm, water levels lower than 40 cm in early summer may promote growth and assist the establishment of vegetative propagules (Chapter 5). Plants survive drawdown and dry soils for more than two years as dormant below ground tissues. Drawdown may be necessary for the establishment of seeds.

The growth and reproduction of *T. procerum* defines a more deeply flooded water regime. The growth of this species is promoted by a maximum annual depth of 70 cm and flooding for 42 weeks of the year (calculated from Rea and Ganf 1994a and Rea 1992; Chapter 5). Shoots adapt readily to water level changes by increasing or decreasing height. Although flooding to more than 50 cm reduces the allocation of biomass to below ground storage tissues (Rea and Ganf 1994d), this may be ameliorated by shallow flooding over summer. Rhizomes are resistant to drawdown for periods of one to two years, but desiccate and die after periods of drought longer than this (Chapter 5). Seedlings establish in depths of more than 40 cm (Rea and Ganf 1994c) and sexual reproduction is the primary mechanism for propagation in this species.

The growth of *B. juncea* is reduced by flooding to depths greater than 25 cm and this species is adapted to temporary or shallow flooding (Chapter 4). Stems are recruited annually, initially in spring. Stem recruitment can be reduced in flooded conditions or delayed until drawdown if plants are flooded deeply. Stems grow to more than 100 cm long, and stem emergence is important in supporting stem recruitment (Chapter 4; Chapter 5). *B. juncea* is resistant to top flooding for more than three months, and will continue to grow and recruit new tissues when water levels fall. Drawdown in early summer promotes the recruitment of new clumps of stems, via long rhizomes. Drawdown later in summer delays and reduces long rhizome recruitment and may lead to sparse stands of this species. *B. juncea* is robust to variations from the optimal conditions of shallow flooding (< 25 cm) in winter and spring and drawdown in early summer and may tolerate deeper or shallower flooding for a number of years (Chapter 5).

The water regime defined by the maximum density of *B. arthrophylla* stems encompasses a wide range of flooding depths and durations (Chapter 5). Stem densities of 2000 $m^{-2}$ are produced in flooding which extends from between 34 and 10 weeks of the year and in maximum yearly depths of between 61 and 24 cm (calculated from Rea and Ganf 1994a and Rea 1992; Chapter 5). Like *B. juncea, B. arthrophylla* initiates yearly stem recruitment in

spring. Stem recruitment is more closely dependent on drawdown, however, and is promoted soon after the recession of water. Flooding for more than 9 months to constant depths of greater than 50 cm reduces below ground biomass and may reduce stem recruitment in spring (Rea and Ganf 1994d). Stems grow to more than 120 cm, and stem emergence is also likely to be important in the recruitment of new stems in this species.

*Water Regime Classifications*

The term water regime encompasses a number of inter-related water level variables which affect the growth of plants. A major difficulty with defining water regime is the identification of the variables which influence plant growth. In the past, water regimes have been classified from a hydrological perspective and then used to explain vegetation patterns. Mean water depth is a hydrological measure which may accurately predict vegetation responses in individual systems (Froend and McComb 1994; Rea and Ganf 1994a; Lieffers and Shay 1982). However, it does not identify the components of water regime which directly affect plant growth. It has limited potential in explaining the direct influence of water regime components on plant growth.

A better approach may be to describe and classify water regimes on the basis of the tolerances of individual species to component variables of water regime. This approach classifies hydrological regimes from a biological perspective and identifies biologically important components of water regime. The above description of the important components of water regime which affect the growth of *V. reniformis, T. procerum, B. juncea* and *B. arthrophylla* describes four water regimes. They have quantitative hydrological limits and include all the components of water regime which affect plant growth. These water regimes have a direct application in wetland water level management.

*Relating Water Regimes to Plant Strategies*

In this thesis it was proposed that the strategies with which wetland plants respond to flooding could be used to predict their water regime tolerances. Two alternative strategies of perennial

emergent species were compared. Two species, *Villarsia reniformis* and *Triglochin procerum* were proposed as 'react' species, which respond to flooding with increased above ground growth. *Baumea juncea* and *B. arthrophylla* were proposed as 'rest' species, which are dormant during flooding and only grow in low water levels or drawdown. This work was intended to assist in water level management in regulated wetlands in two ways. The first goal was to determine the water regimes which are tolerated by these species. The second was to assess how closely plant strategies can be associated with water regime tolerances, and if they can be applied more widely to wetland vegetation.

The morphology of *V. reniformis* readily adapts to changing water levels. Rising water is matched by morphologically plastic petioles which lengthen in proportion to depth. This mechanism exposes leaves to the atmosphere as water levels fluctuate. *V. reniformis* is active while flooded, growing and reproducing. Individual petioles can accommodate flooding by cell elongation or growth. Petioles are flexible and bend under the surface when depth decreases, so that leaves continue to float on the surface. *T. procerum* also increases shoot height in proportion to depth, and exposes leaves to the atmosphere while flooded. Both species continually recruit new leaves from the shoot, enabling them to continually adapt to changing water levels.

The 'react' term applied to these species adequately describes their responses during flooding. However, this category describes only the response to flooding, and does not account for the strategy of these plants in the yearly cycle of flooding. *V. reniformis* and *T. procerum* inhabit seasonally flooded habitats which are usually exposed in late summer and autumn. They become dormant when surface water is absent and soils are dry. Shoot length is minimised and smaller leaves are produced. After prolonged drought no shoot is produced at all, and individuals persist as below ground corms and rhizomes. In terms of the dry phase of seasonally flooded wetlands, these species could be placed in the 'rest' category.

In terms of their strategy in a seasonally flooded habitat, *V. reniformis* and *T. procerum* might be better classified as opportunists. They respond to changing water levels as they occur, and have a flexible growth strategy. Dormancy is not seasonal, and both species will continue to produce shoots throughout the year if water is present. Reproduction is dependent on the presence of surface water, and unless shoots are flooded in winter or spring they neither flower nor produce runners. Therefore these species capitalise on the opportunity for growth provided by surface water. They resist unfavourable dry conditions as tubers and corms in the soil, but are ready to break their dormancy whenever they are flooded.

The morphology of *B. juncea* is relatively inflexible, and does not respond to changing water depth. Stems have a limited capacity to lengthen when submerged and flooding reduces the stem area exposed to the atmosphere. Stems can only fix negligible quantities of carbon from the water column and must be exposed to the atmosphere if they are to support growth. Consequently, growth is reduced by partial flooding of stems, but increases when water levels fall and a greater surface area of stem is exposed.

In these respects, *B. juncea* can be classified as a 'rest' species. It tolerates reduced growth during flooding and depends on drawdown to allow a period of higher growth. However, *B. juncea* is not dormant while flooded and cannot be accurately described as 'resting'. The recruitment of new stems is initiated in spring, when stems are most likely to be flooded with water. Many wetland species initiate stem recruitment early in spring from carbohydrate and nutrients stored in the rhizome (Kausch et al. 1981; Bernard and Solsky 1977; Prentki et al. 1978; Steinmann and Brändle 1984a). This adaptation provides a population of new shoots which can take advantage of improving conditions for growth at the earliest opportunity (Bernard and Gorham 1978). Similarly, *B. juncea* may also recruit stems at the expense of stored resources in spring. As water levels fall in late spring and summer, these new stems are exposed to the atmosphere in ideal conditions for photosynthesis: abundant water, high irradiances, long days and high temperatures. Growth and stem recruitment increase after drawdown (Chapters 4 and 5), and stored resources may be replenished over summer.

A classification of plant strategies should account for the life history of the plants at all stages of the flooding cycle. Harper (1977) identified two types of dormancy in seeds, opportunistic and deterministic. Opportunistic seeds germinate whenever conditions are favourable, regardless of season. Seasonally dormant, or deterministic, seeds germinate in response to a seasonal cue, usually day length. These classifications might also be applied to the growth strategies of *V. reniformis*, *T. procerum* and *B. juncea*. The growth of *V. reniformis* and *T. procerum* is related to the presence of surface water, but the growth of *B. juncea* is dominated by seasonal stem recruitment.

*B. arthrophylla* has both opportunistic and deterministic components to its growth strategy. Stems are recruited in an annual cycle, but this is controlled to some extent by water regime. Stem recruitment begins in spring with the establishment of new shoots, each with one stem. In Bool Lagoon in 1995, where all elevations had experienced similar dry conditions prior to flooding, the density of newly established shoots was independent of depth (Chapter 5). However, additional stems were recruited within shoots as the flood receded. Multiple stems appeared firstly at high elevations and later at lower elevations in response to the falling water level. The initial production of shoots was presumably heterotrophic, from stored resources, and a predictable, seasonal occurrence. The subsequent recruitment of stems was opportunistic, and was related to the time at which flood water receded.

The value of these classifications lies in their application to water level management of wetlands. Species may share the strategies by which they adapt to seasonal flooding (van der Valk 1981). If plants with similar strategies share similar tolerances to water regime, then the flooding characteristics of wetlands can be manipulated to manage the growth and distribution of suites of species. This is a less labour intensive approach than the determination of water regime tolerances of individual species (Keddy 1992).

The opportunistic strategy of *V. reniformis* and *T. procerum* is shared with other semi-emergent species in seasonally flooded and exposed habitats. Species which share this strategy include other floating leaved species, *Nymphaea alba* and *Nymphoides peltata* (Papastergiadou and Babalonas 1992) and submerged species, *Chara australis* (Casanova 1994), *Vallisineria spiralis*, *Potamogeton tricarinatus* and *Myriophyllum vericosum* (Briggs and Maher 1985). However, despite their similarities these strategies do not necessarily equate to the same water regimes. The growth of *T. procerum* is promoted in greater depths than *V. reniformis*. *Nymphaea alba* and *Nymphoides peltata* live in deeper water still, and tolerate depths of up to 3 m (Papastergiadou and Babalonas 1992). The general flooding pattern to which these species are adapted are the same, but they have different tolerances to flooding within that pattern. A single water regime cannot be ascribed to these species, even though they share the same strategy.

The seasonal recruitment strategy of *B. juncea* and *B. arthrophylla* is related to the recession of flood water over summer. Although seasonal stem recruitment is a common adaptation in aquatic macrophytes (eg. Shay and Shay 1986; Froend and McComb 1994), the importance of low water levels over summer to successful stem recruitment has not been reported widely in the literature. *Carex rostrata*, growing in wetlands in Sweden, suffers reduced recruitment from high summer water levels (Hultgren 1988, 1989). This species tolerates similar ranges of depth to *B. juncea* and *B. arthrophylla* and these species might be said to share similar growth and water regime strategies. However, *C. rostrata* lives in a very different climate, with ice cover in winter, and this comparison does not provide the basis for a generalisation of this growth strategy and water regime.

It is concluded that water regimes cannot be applied generally to species on the basis of similarities in their life history strategies. Life history strategies are indicative of climates and water levels, but not of the scale or range over which water levels change. The vegetation of wetlands in mediterranean climates is adapted to a seasonal pattern of flooding and drawdown. Some species will share life-history strategies which adapt them to these conditions.

However, the scale of water level fluctuations differs between wetlands and within wetlands on the elevation gradient. The same strategy which equips one species to tolerate drawdown and flooding to 3 m can also found in species flooded to much lower depths.

*Difficulties with Individual Plant Investigations*

Water regimes can only be ascribed to species after detailed and long term investigations. Wetland vegetation has been altered by manipulations to flooding depth (Millar 1973), frequency (Brock et al. 1987), timing, (van de Steeg 1984), amplitude (Wilcox and Meeker 1991) and duration (Rea 1992), or combinations of these factors. Quantitative limits can be set on the tolerances of species to these factors only after experiments and verification in the field. Plants often persist in unfavourable conditions for a number of years, and long term studies are required to identify their limits to flooding regimes (Squires and van der Valk 1992). This may be a valuable approach when there is a need to know the tolerances of a few important species, such as weeds (eg. Froend and McComb 1994). However, this is a time-consuming process when the water regime tolerances of entire wetland communities are in question.

A further difficulty with assigning water regimes to vegetation is individuality of wetland systems and the lack of correlation between them. The tolerances of wetland plants to particular water regimes are influenced by a number of factors, including the past flooding history, the presence of other species (Grace 1987; Grace and Wetzel 1981) and the fertility of the soil (Chambers and Kalff 1985; Neill 1990; Gallagher 1975; Froend and McComb 1994; Grace 1988). Thus the tolerances of species to water regimes in one wetland can only be applied with caution to wetlands with different plant assemblages and soils.

The detail in which species traits and flooding tolerances are studied will depend on the control which can be exercised over flooding. Keddy (1992) recently called for greater precision and quantification of the succession of wetland communities in changing water regimes. However, there is little to be gained from quantitative models in multiple use water

resources in which little control of water regime can be exercised. Many of the wetlands in which the vegetation has been studied are natural wetlands which have been affected by human demands for water, such as water storage (Denton and Ganf 1994; Papastergiadou and Babalonas 1992), irrigation (Walker and Thoms 1993), ground water extraction (Froend and McComb 1994) and electricity generation (Wilcox and Meeker 1991). Any manipulations of water regime for vegetation must accommodate other water management constraints. In addition, detailed relationships between plant growth and water regime may rarely be applicable in variable climates which are subject to drought and flood. Even since regulation, many of the long term high and low water levels in Bool Lagoon and The River Murray have been due to climate, and beyond the control of the regulating authorities (Walker and Thoms 1993). Research should focus on the important aspects of water regime which can be controlled to benefit vegetation within the constraints of other water resource uses.

*Key Aspects of Water Regimes*

Rather than defining water regimes to manage vegetation, a more useful approach may be to make qualitative descriptions of the effects of particular aspects of flooding. Some components of water regime are important to the survival of species than others. Flooding or drawdown at specific stages of plant growth can reduce stem recruitment, vegetative reproduction, germination and flowering. On the other hand, depth changes at other stages can have relatively little impact (Chapters 3, 4 and 5; Hultgren 1988; Lieffers and Shay 1981; Denton and Ganf 1994; van der Sman et al. 1993a, b; Thomas and Stewart 1969; Stevenson and Lee 1987; Keddy and Ellis 1985). In regulated wetlands, there may be more scope to accommodate flooding requirements at specific stages of plant growth than to apply whole water regimes. Classifications of plant strategies, such as those explored in this thesis, may have a role in this process. Opportunistic species may be more robust to long term flooding. The seasonal strategy of deterministic species may require flooding or drawdown at specific times of the year.

*Future Work*

The most important question to arise from this study is the importance of the window of opportunity provided by the drawdown of water in late spring or early summer. There are good grounds for placing a high value on this period in the life history of the sedges (Hultgren 1988; Lieffers and Shay 1982; Chapter 5). This period may also be important in the life histories of the opportunistic species, *V. reniformis* and *T. procerum*. This is when vegetative propagules and seed become established. Rapid or early drawdown may result in their death. There is a tendency for water regime research to focus on water depth and not cycles of flooding and drawdown. Flooding cycles have received more attention in annuals (Blom et al. 1990), but less is known the responses of perennial species. The importance of drawdown and its timing need to be explored experimentally in other species to establish the importance of this period to shoot and propagule recruitment and if it is in fact the highest period of growth in the annual cycle.

A neglected area of research is the importance of the storage of carbohydrate in the flood responses of emergent macrophytes. Most perennial macrophytes have large reserves of carbohydrate and mineral nutrients in below ground tissues, such as rhizomes or tubers. The importance of these reserves in vegetative reproduction and anoxia tolerance has been described for a number of species (Schluter et al. 1996; Armstrong et al. 1994; Barclay and Crawford 1983; Kausch et al. 1981; Steinmann and Brändle 1984a, b; Haldemann and Brändle 1986). However, the consumption of carbohydrate reserves has also been invoked as a flood resistance mechanism by which plants increase shoot height in deep water (Rea and Ganf 1994d; van der Steeg 1984; Brock et al. 1987). As yet, there is little evidence to support this claim (Laan and Blom 1990; Raskin and Kende 1984), but it remains a tempting explanation for the ubiquity of carbohydrate storage in perennial macrophytes. Although the allocation of biomass to storage tissues tends to decrease with increasing depth (Chapter 3; Squires and van der Valk 1992; Coops et al. 1996; Grace 1989), this does not demonstrate that carbohydrate stores support shoot growth in a negative carbon balance in deep water.

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## Appendix A

Analysis of Variance Tables

The effects of Flooding Treatment and Nutrient Level on the number of leaves per *V. reniformis* plant.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 245 | 81.8 | 3.66 | 0.0155 |
| Nutrients | 1 | 2743 | 2743 | 123 | 0.0001 |
| Flooding*Nurients | 3 | 222 | 74.0 | 3.31 | 0.0238 |

The effects of Flooding Treatment and Nutrient Level on the number of new leaves recruited per week by *V. reniformis*.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 39.7 | 13.2 | 6.68 | 0.0004 |
| Nutrients | 1 | 305 | 304 | 154 | 0.0001 |
| Flooding*Nurients | 3 | 158 | 5.26 | 2.65 | 0.0535 |

The effects of Flooding Treatment and Nutrient Level on the percentage of *V. reniformis* leaves formed at the initial 2 cm depth which survived four weeks in four flooding regimes.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 1271 | 423 | 1.97 | 0.0428 |
| Nutrients | 1 | 913 | 913 | 4.24 | 0.1255 |
| Flooding*Nurients | 3 | 2772 | 924 | 4.29 | 0.0074 |

The effects of Flooding Treatment and Nutrient Level on the percentage of *V. reniformis* leaves formed at the initial 2 cm depth which survived five weeks in four flooding regimes.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 15199 | 5066 | 0.92 | 0.3398 |
| Nutrients | 1 | 349 | 349 | 13.4 | 0.0001 |
| Flooding*Nurients | 3 | 2272 | 757 | 2.00 | 0.1211 |

## Appendix B

Analysis of Variance Tables

The effects of Flooding Treatment and Nutrient Level on the ratio of fructose to glucose in *V. reniformis* tissues.

Leaf

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.0345 | 0.0115 | 1.20 | 0.3702 |
| Nutrients | 1 | 0.0214 | 0.0214 | 2.23 | 0.1736 |
| Flooding*Nurients | 3 | 0.0955 | 0.0318 | 3.31 | 0.0779 |

Petiole

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.00718 | 0.00239 | 1.19 | 0.3748 |
| Nutrients | 1 | 0.00785 | 0.00785 | 3.89 | 0.0841 |
| Flooding*Nurients | 3 | 0.0295 | 0.00983 | 4.87 | 0.0326 |

Corm

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.0145 | 0.0483 | 0.99 | 0.4433 |
| Nutrients | 1 | 0.0830 | 0.0830 | 1.71 | 0.2272 |
| Flooding*Nurients | 3 | 0.0808 | 0.0269 | 0.56 | 0.6591 |

Storage Root

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.277 | 0.0923 | 1.89 | 0.2093 |
| Nutrients | 1 | 0.0490 | 0.0489 | 1.00 | 0.3456 |
| Flooding*Nurients | 3 | 0.321 | 0.107 | 2.19 | 0.1665 |

Fine Root

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.0199 | 0.00662 | 2.26 | 0.1582 |
| Nutrients | 1 | 0.0292 | 0.0292 | 9.97 | 0.0135 |
| Flooding*Nurients | 3 | 0.0551 | 0.0184 | 6.28 | 0.0169 |

Runner

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.00731 | 0.00244 | 0.22 | 0.8791 |

The effects of Flooding Treatment and Nutrient Level on the concentration of carbohydrate in *V. reniformis* tissues.

Leaf

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.0297 | 0.00991 | 3.84 | 0.0569 |
| Nutrients | 1 | 0.000109 | 0.000109 | 0.04 | 0.8424 |
| Flooding*Nurients | 3 | 0.00519 | 0.00173 | 0.67 | 0.5939 |

Petiole

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.0491 | 0.0164 | 15.09 | 0.0012 |
| Nutrients | 1 | 0.000866 | 0.000866 | 0.80 | 0.3975 |
| Flooding*Nurients | 3 | 0.0233 | 0.000775 | 0.72 | 0.5699 |

Corm

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.194 | 0.0646 | 17.84 | 0.0007 |
| Nutrients | 1 | 0.0153 | 0.0153 | 4.20 | 0.0741 |
| Flooding*Nurients | 3 | 0.0737 | 0.0246 | 6.78 | 0.0138 |

Storage Root

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.0791 | 0.0264 | 4.20 | 0.0465 |
| Nutrients | 1 | 0.00123 | 0.00123 | 0.200 | 0.6703 |
| Flooding*Nurients | 3 | 0.240 | 0.0799 | 12.7 | 0.0021 |

Fine Root

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.212 | 0.0708 | 6.35 | 0.0164 |
| Nutrients | 1 | 0.00005 | 0.00005 | 0.00 | 0.9461 |
| Flooding*Nurients | 3 | 0.0551 | 0.0184 | 1.65 | 0.2541 |

Runner

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.0822 | 0.0274 | 0.245 | 0.2033 |

The effects of Flooding Treatment and Nutrient Level on leaf width.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 128 | 42.8 | 86.3 | < 0.0001 |
| Nutrients | 1 | 146 | 146 | 294 | < 0.0001 |
| Flooding*Nurients | 3 | 7.28 | 2.43 | 4.89 | 0.0034 |

The effects of Flooding Treatment and Nutrient Level on petiole length.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 47319 | 15773 | 2521 | < 0.0001 |
| Nutrients | 1 | 154 | 154 | 24.7 | < 0.0001 |
| Flooding*Nurients | 3 | 4.19 | 1.397 | 0.224 | 0.8798 |

The effects of Flooding Treatment and Nutrient Level on leaf width.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 128 | 42.8 | 86.3 | < 0.0001 |
| Nutrients | 1 | 146 | 146 | 294 | < 0.0001 |
| Flooding*Nurients | 3 | 7.28 | 2.43 | 4.89 | 0.0034 |

The effects of Flooding Treatment and Nutrient Level on petiole length.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 47319 | 15773 | 2521 | < 0.0001 |
| Nutrients | 1 | 154 | 154 | 24.7 | < 0.0001 |
| Flooding*Nurients | 3 | 4.19 | 1.397 | 0.224 | 0.8798 |

The effects of Flooding Treatment and Nutrient Level on plant biomass.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 924 | 308 | 4.13 | 0.0089 |
| Nutrients | 1 | 47388 | 47388 | 635 | 0.0001 |
| Flooding*Nurients | 3 | 1013 | 338 | 4.53 | 0.0056 |

The effects of Flooding Treatment and Nutrient Level on Relative Growth Rate.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding | 3 | 0.00245 | 0.000817 | 2.10 | 0.1069 |
| Nutrients | 1 | 0.321 | 0.321 | 825 | < 0.0001 |
| Flooding*Nurients | 3 | 0.00296 | 0.000986 | 2.53 | 0.0629 |

## Appendix C

Analysis of Variance Tables

The effects of Flooding Stage and Depth on the mean stem length of *B. juncea.*

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding Stage | 1 | 343 | 343 | 1.12 | 0.2890 |
| Depth | 2 | 75511 | 37756 | 124 | 0.0001 |
| Flooding Stage*Depth | 2 | 62390 | 31195 | 102 | 0.0001 |

The effects of Flooding Stage and Depth on *B. juncea* biomass.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding Stage | 1 | 3210 | 2210 | 824 | 0.0071 |
| Depth | 2 | 11553 | 5777 | 14.8 | < 0.0001 |
| Flooding Stage*Depth | 2 | 583 | 292 | 0.750 | 0.4811 |

The effects of Flooding Stage and Depth on *B. juncea* relative growth rate.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Flooding Stage | 1 | 0.00439 | 0.00439 | 4.79 | 0.0359 |
| Depth | 2 | 0.0129 | 0.00642 | 7.00 | 0.0029 |
| Flooding Stage*Depth | 2 | 0.0264 | 0.0132 | 14.37 | < 0.0001 |

## Appendix D

Analysis of Variance and Kruskil-Wallis Test tables

The Kruskil-Wallis H statistic is approximated by the $\chi^2$ statistic for samples sizes greater than five (Zar 1984), and the $\chi^2$ statistic is presented here.

The effect of Date and Elevation on the density ($m^{-2}$) of *V. reniformis* shoots, n = 20.

| Test | $\chi^2$ | DF | $P > \chi^2$ |
|---|---|---|---|
| Date | 3.1099 | 3 | 0.3750 |
| Elevation | 28.786 | 3 | 0.0001 |

The effect of Date and Elevation on the number of leaves per *V. reniformis* shoot, n = 20.

| Test | $\chi^2$ | DF | $P > \chi^2$ |
|---|---|---|---|
| Date | 74.6 | 3 | < 0.0001 |
| Elevation | 1.9081 | 3 | 0.5917 |

The effects of Date and Elevation on the width of *V. reniformis* leaves, n = 20.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Date | 3 | 849 | 283 | 227 | < 0.0001 |
| Elevation | 3 | 11.2 | 3.75 | 3.00 | 0.0309 |
| Date*Elevation | 9 | 19.4 | 2.16 | 1.73 | 0.0810 |

The effect of Date and Elevation on the density ($m^{-2}$) of *T. procerum* shoots, n = 20.

| Test | $\chi^2$ | DF | $P > \chi^2$ |
|---|---|---|---|
| Date | 4.6361 | 3 | 0.2005 |
| Elevation | 43.429 | 3 | < 0.0001 |

The effect of Date and Elevation on the number of leaves per *T. procerum* shoot, n = 20.

| Test | $\chi^2$ | DF | $P > \chi^2$ |
|---|---|---|---|
| Date | 204 | 3 | < 0.0001 |
| Elevation | 2.63 | 3 | 0.4515 |

The effects of Date and Elevation on the width of *T. procerum* leaves, n = 20.

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Date | 3 | 167 | 55.5 | 221 | < 0.0001 |
| Elevation | 3 | 5.94 | 1.98 | 7.87 | < 0.0001 |
| Date*Elevation | 9 | 5.02 | 0.447 | 1.78 | 0.0724 |

The effect of Date and Elevation on the density ($m^{-2}$) of *B. juncea* stems, n = 40 (June and March) n = 20 (October December).

| Test | $\chi^2$ | DF | $P > \chi^2$ |
|---|---|---|---|
| Date | 11.210 | 3 | 0.0106 |
| Elevation | 65.350 | 2 | 0.0001 |

The effects of Date and Elevation on the mean length per quadrat of *B. juncea* stems, n = 40 (June and March) n = 20 (October December).

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Date | 3 | 3254 | 1085 | 11.49 | < 0.0001 |
| Elevation | 2 | 4949 | 2475 | 26.22 | < 0.0001 |
| Date*Elevation | 6 | 1054 | 176 | 1.86 | 0.0875 |

The effect of Date and Elevation on the density ($m^{-2}$) of *B. arthrophylla* stems, n = 20 (June and March) n = 10 (October December).

| Test | $\chi^2$ | DF | $P > \chi^2$ |
|---|---|---|---|
| Date | 80.1 | 3 | < 0.0001 |
| Elevation | 2.60 | 3 | 0.4579 |

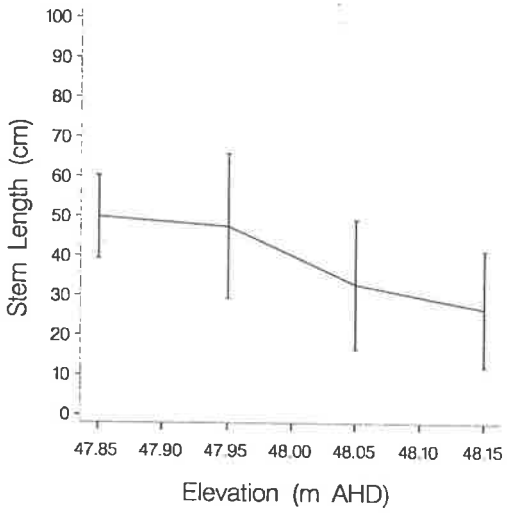
The effects of Date and Elevation on the mean length per quadrat of *B. arthrophylla* stems, n = 20 (June and March) n = 10 (October December).

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Date | 3 | 12828 | 4276 | 64.0 | < 0.0001 |
| Elevation | 3 | 8648 | 2883 | 43.1 | < 0.0001 |
| Date*Elevation | 9 | 4666 | 518 | 7.76 | < 0.0001 |

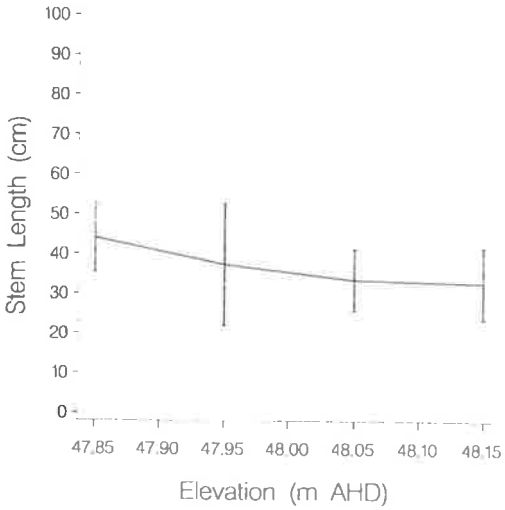
The effect of Date and Elevation on the density ($m^{-2}$) of *B. arthrophylla* shoots, n = 20 (June and March) n = 10 (October December).

| Test | $\chi^2$ | DF | $P > \chi^2$ |
|---|---|---|---|
| Date | 45.4 | 3 | < 0.0001 |
| Elevation | 0.437 | 3 | 0.9324 |

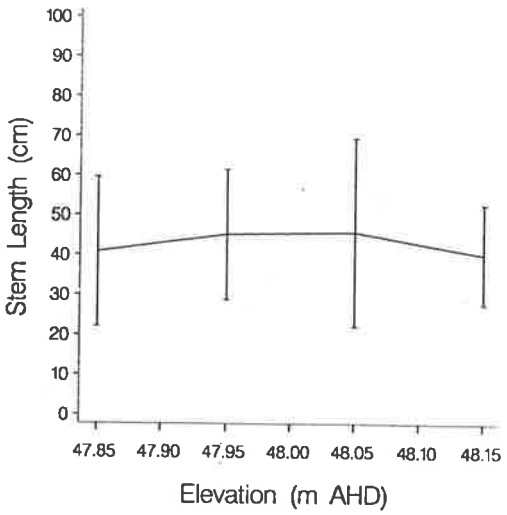
June




October




December




March

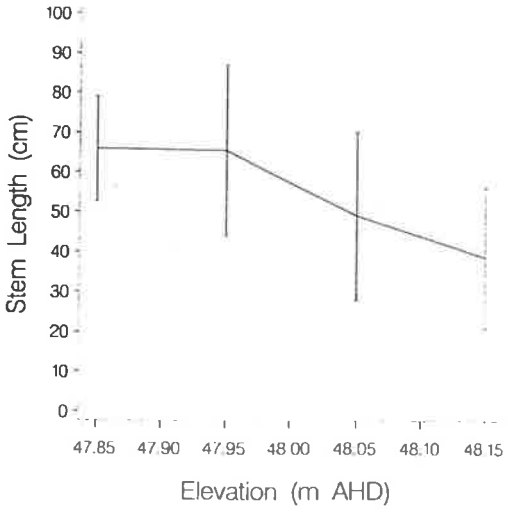
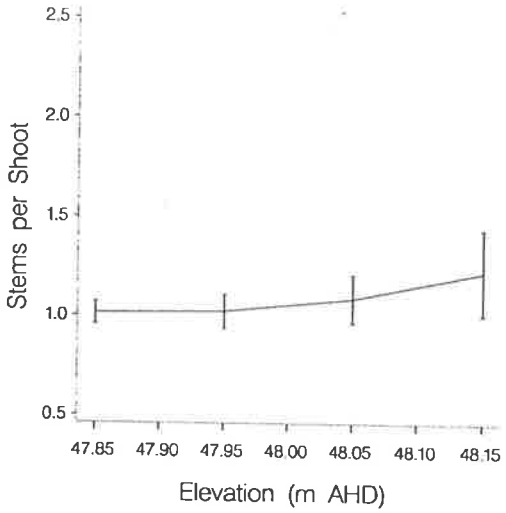


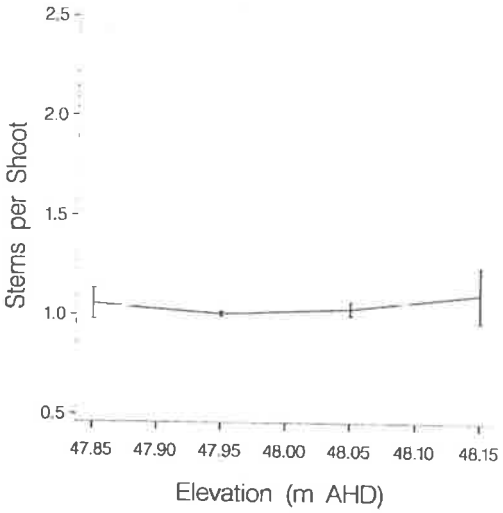
Figure 5.14

The mean length of *B. arthrophylla* stems at four elevations in June, October, December 1995 and March 1996. An interaction between date and elevation influenced mean stem length ($p < 0.001$ two way anova). Error bars indicate +/- 1 standard deviation from the mean of 20 quadrats in June and March and 10 quadrats in October and December.

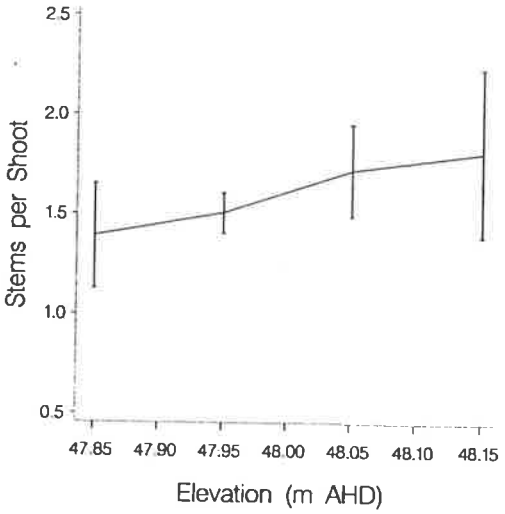
June




October




December




March

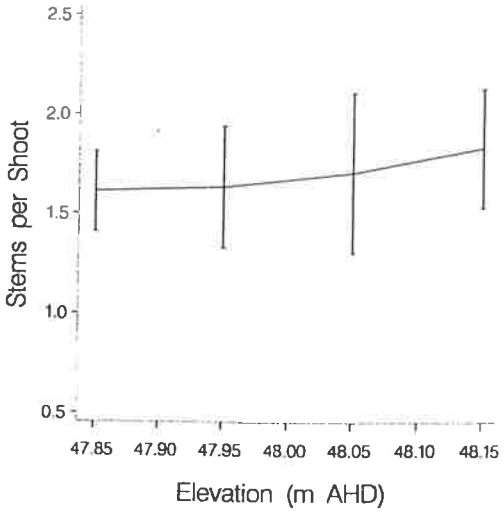
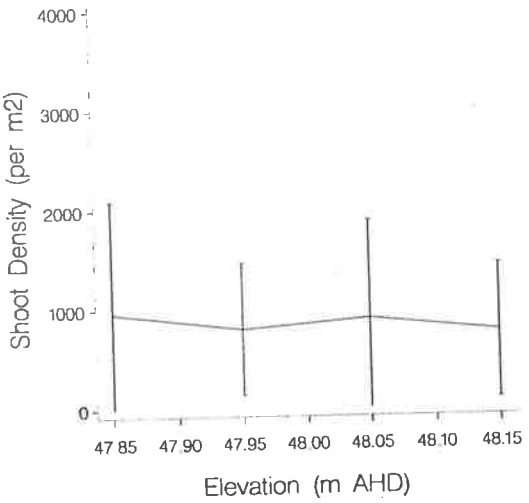
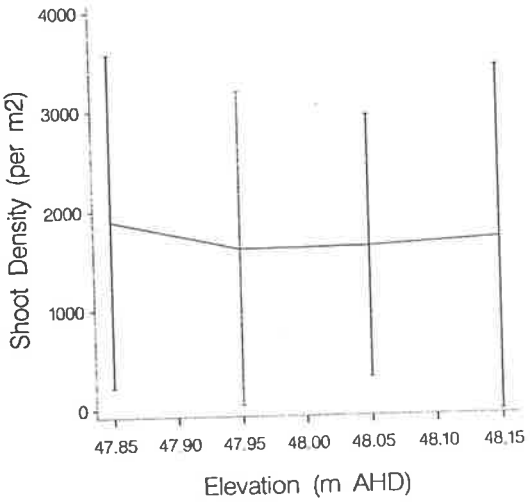


Figure 5.13
The number of stems per shoot of *B. arthrophylla* at four elevations. Stems were counted in June, October, December 1995 and March 1996. Error bars indicate +/- 1 standard deviation from the mean of all the shoots in the quadrats, min=330 shoots, max=931 shoots.

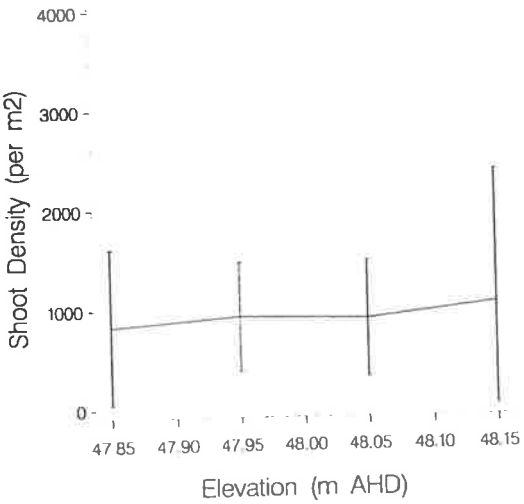
June




October




December




March

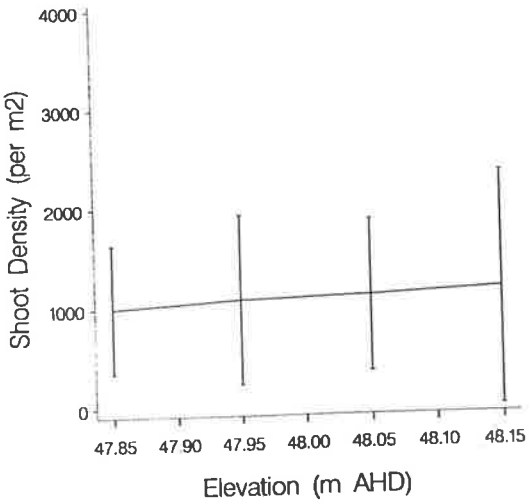


Figure 5.12

The density of *B. arthrophylla* shoots per square metre at four elevations. Shoots were counted in June, October, December 1995 and March 1996. Date ($p < 0.001$) but not elevation ($p = 0.9$) influenced stem density (one way Kruskil-Wallis anovas). Error bars indicate +/- 1 standard deviation from the mean of 20 quadrats in June and March and 10 quadrats in October and December.

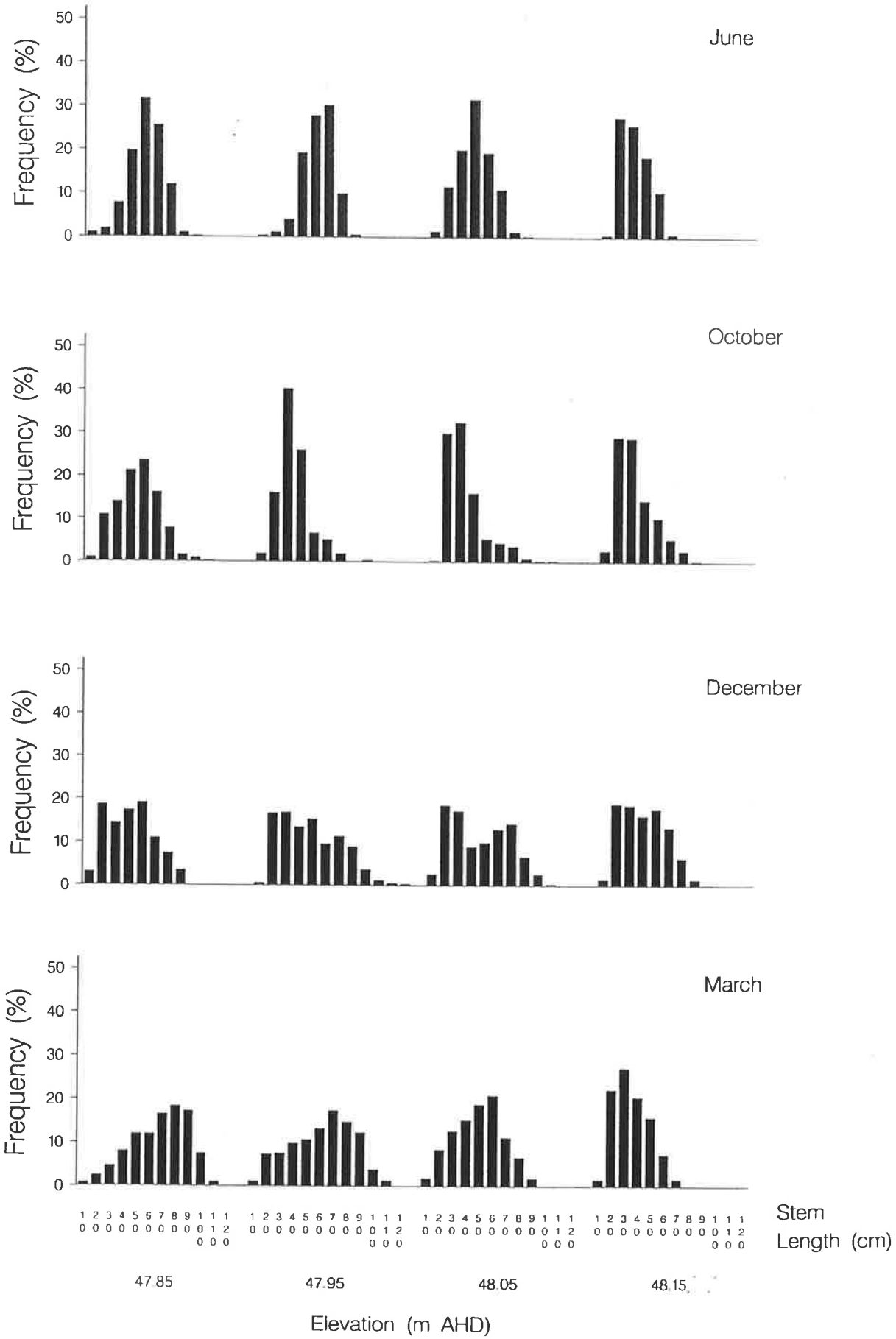
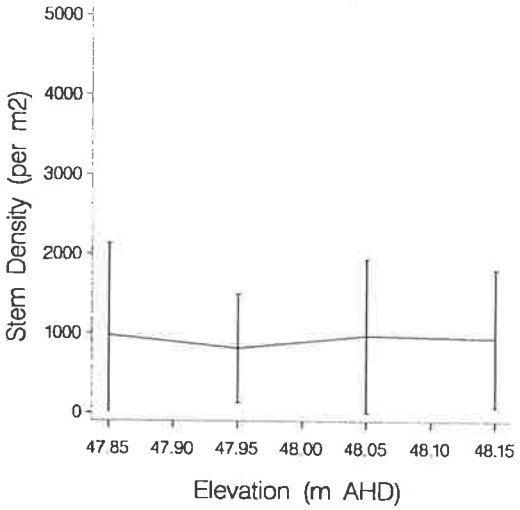
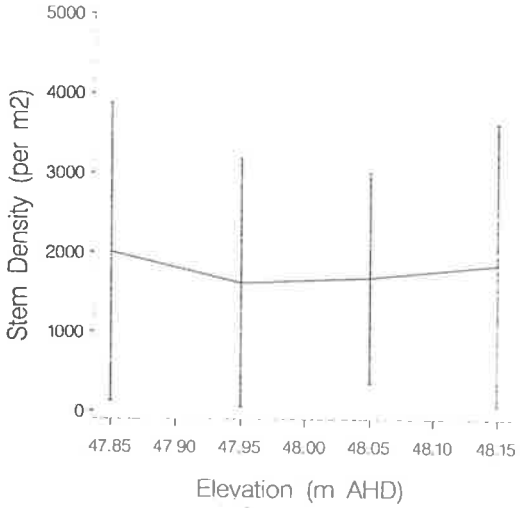

June
October
December
March
Frequency (%)
0
10
20
30
40
50
Stem Length (cm)
47.85
47.95
48.05
48.15
Elevation (m AHD)

Figure 5.11
The percentage frequency distribution of *B. arthrophylla* stem lengths at four elevations in June, October, December 1995 and March 1996. Stem measurements from all quadrats were combined in each distribution.

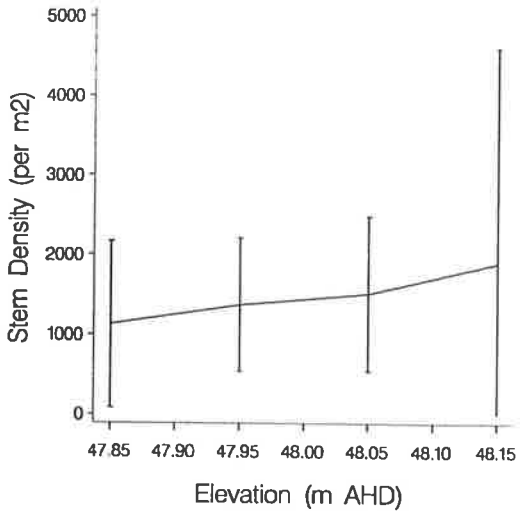
June




October




December




March

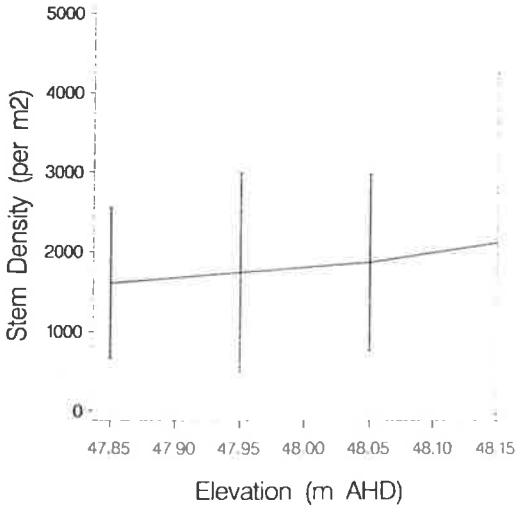
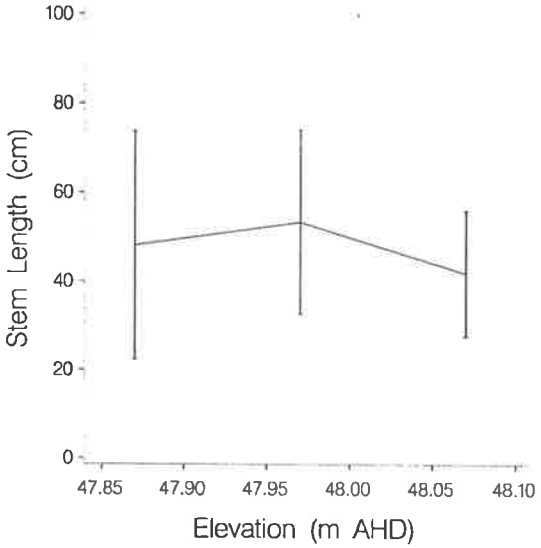


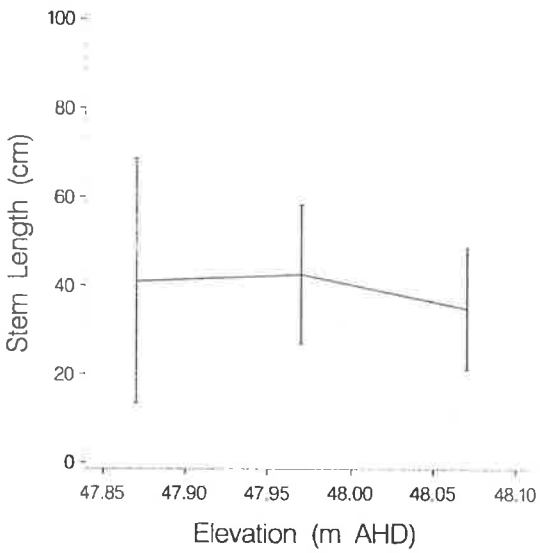
Figure 5.10

The density of *B. arthrophylla* stems per square metre at four elevations. Stems were counted in June, October, December 1995 and March 1996. Date ($p = 0.01$) but not elevation ($p = 0.5$) influenced stem density (one way Kruskil-Wallis anovas). Error bars indicate +/- 1 standard deviation from the mean of 20 quadrats in June and March and 10 quadrats in October and December.

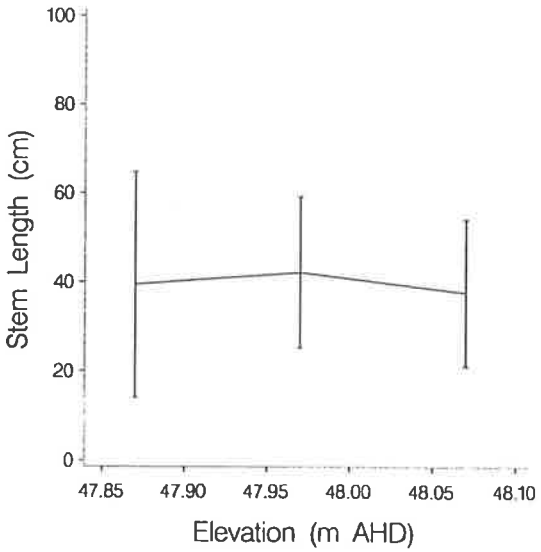
June




October




December




March

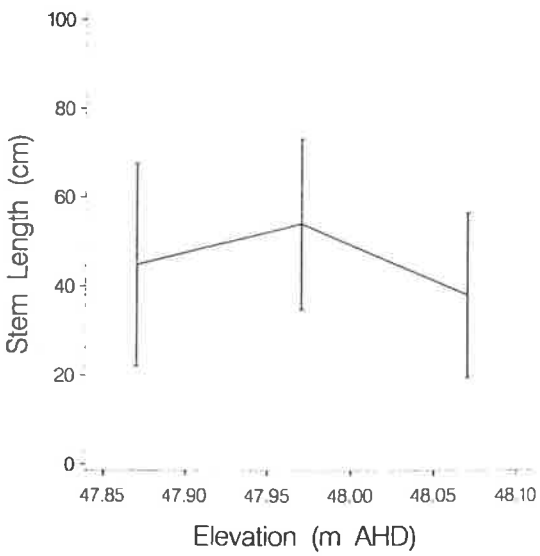


Figure 5.9

The mean length of *B. juncea* stems per quadrat at three elevations in June, October, December 1995 and March 1996. Both date ($p < 0.001$) and elevation ($p < 0.001$) influenced mean stem length. These factors did not interact ($p = 0.09$; two way anova). Error bars indicate +/- 1 standard deviation from the mean of 40 quadrats in June and March and 20 quadrats in October and December.

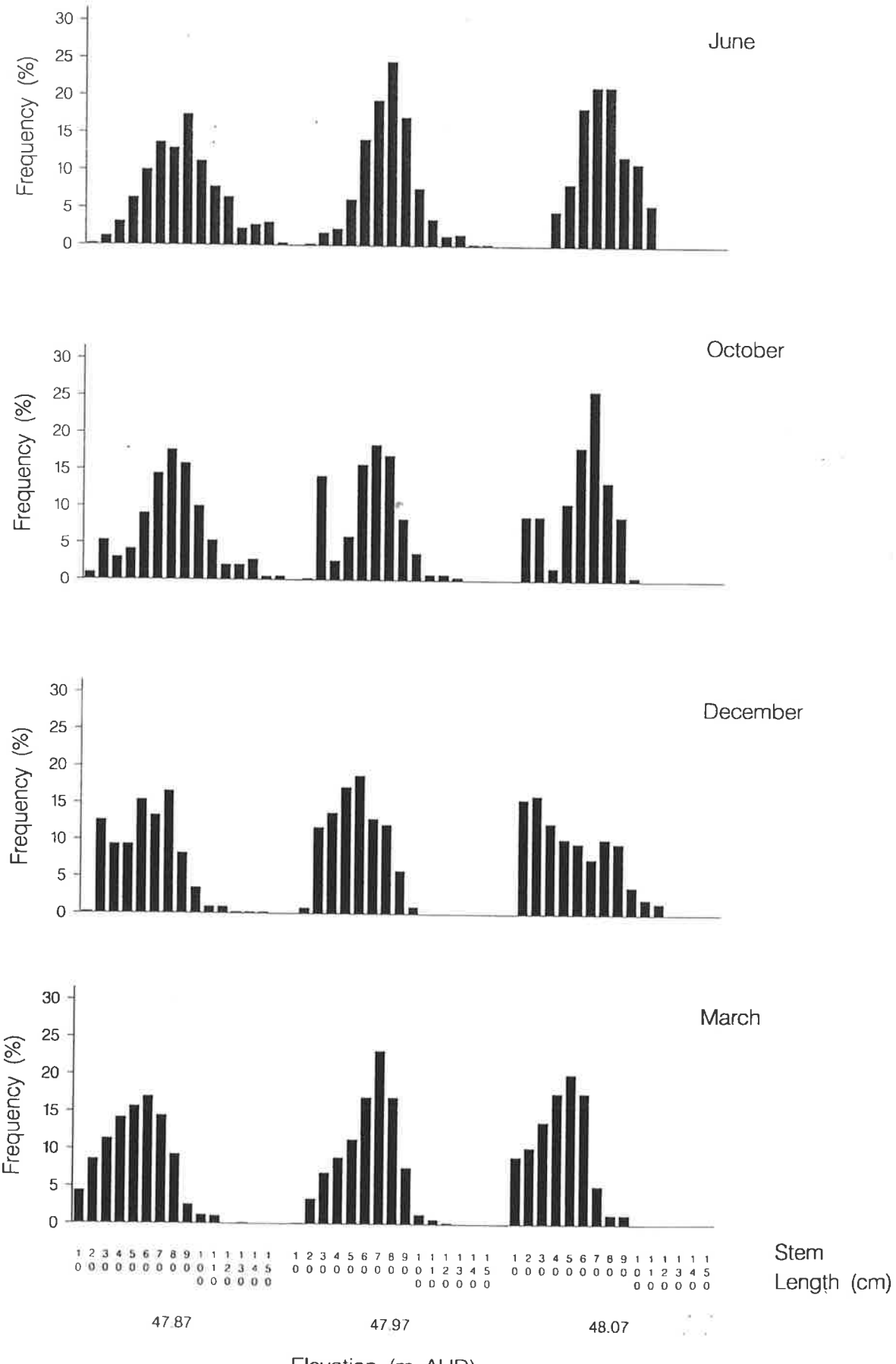
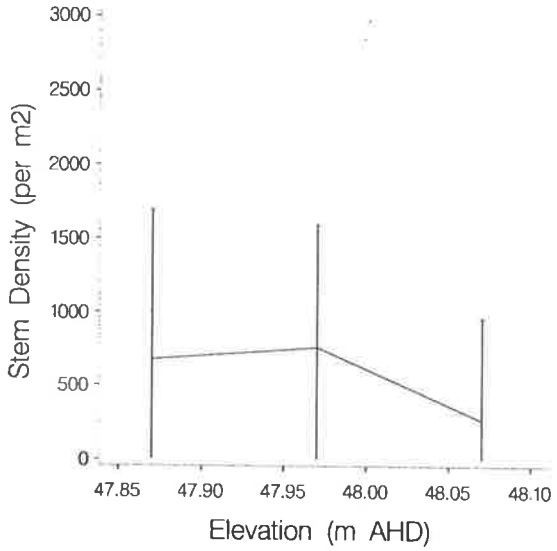
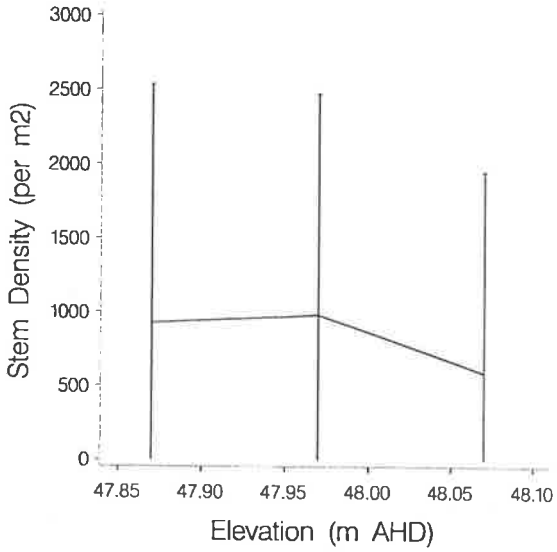

June
October
December
March
Frequency (%)
0
5
10
15
20
25
30
Stem Length (cm)
100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500
47.87
47.97
48.07
Elevation (m AHD)

Figure 5.8
The percentage frequency distribution of *B. juncea* stem lengths at three elevations in June, October, December 1995 and March 1996. Stem measurements from all quadrats were combined in each distribution.

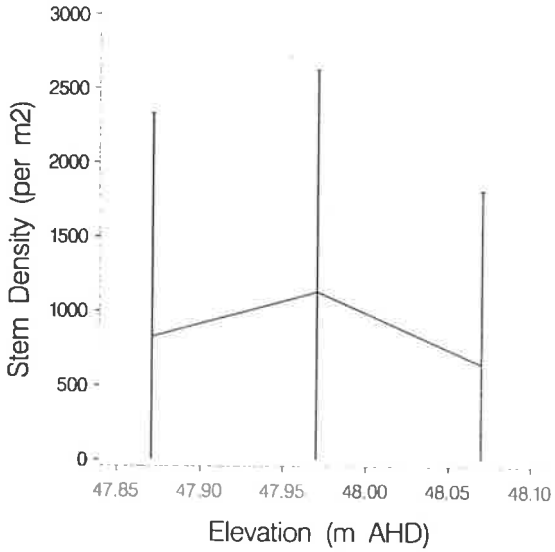
June




October




December




March

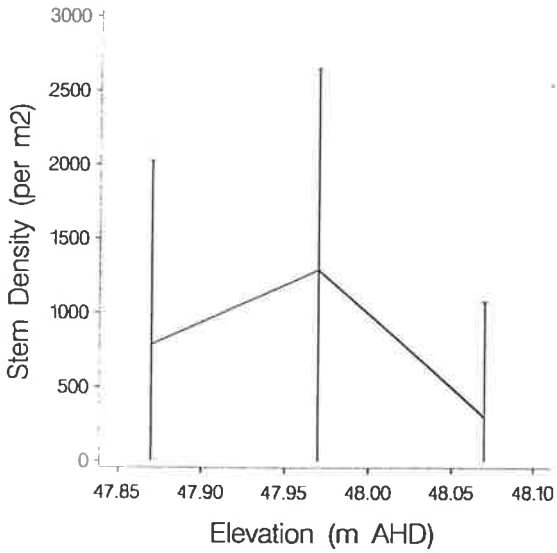
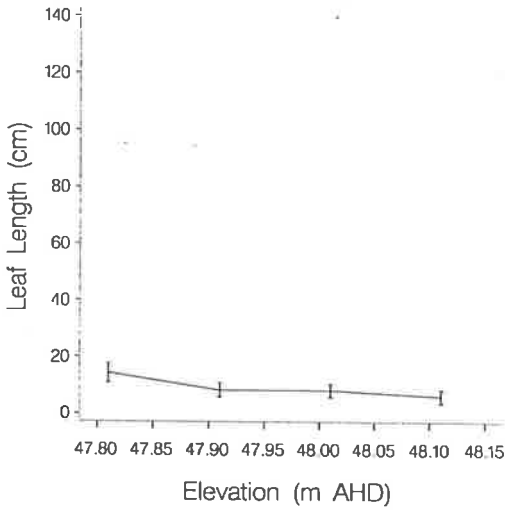


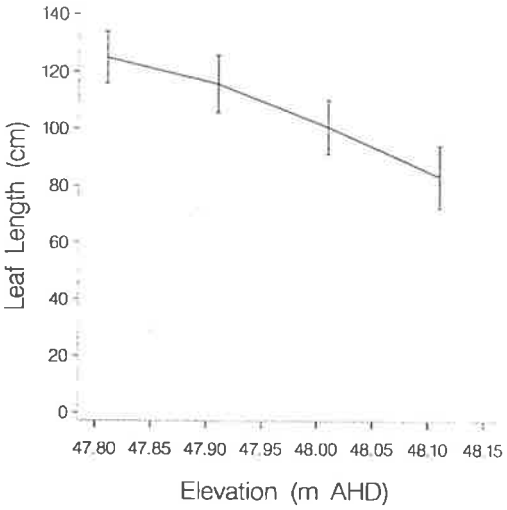
Figure 5.7

The density of *B. juncea* stems per square metre at three elevations. Stems were counted in June, October, December 1995 and March 1996. Both date ($p = 0.01$) and elevation ($p < 0.001$) influenced stem density (one way Kruskil-Wallis anovas). Error bars indicate +/- 1 standard deviation from the mean of 40 quadrats in June and March and 20 quadrats in October and December.

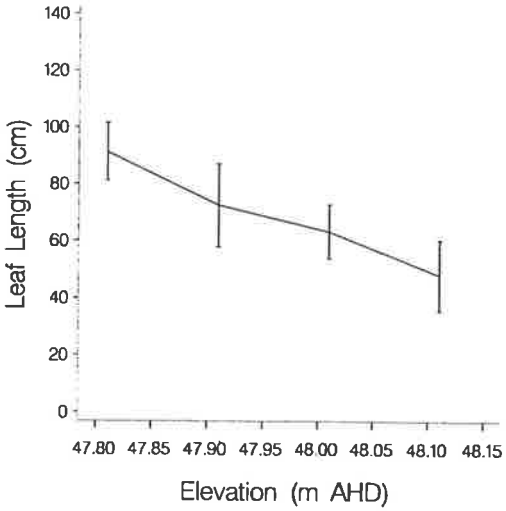
June




October




December




March

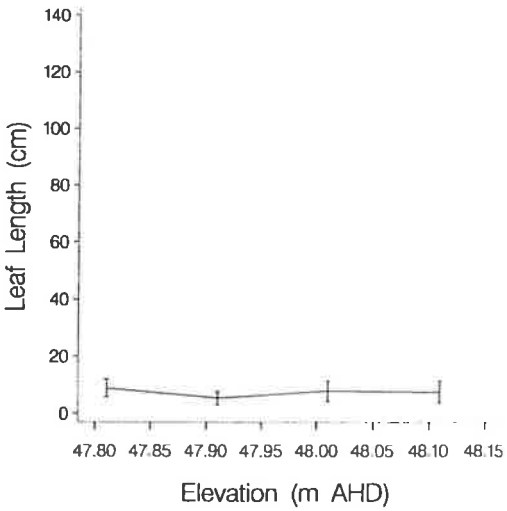
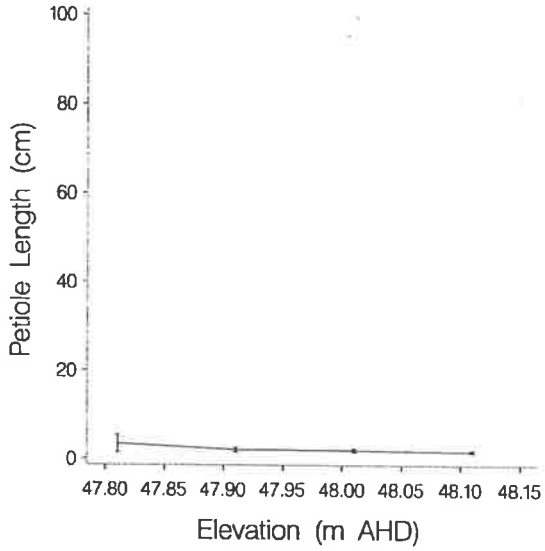
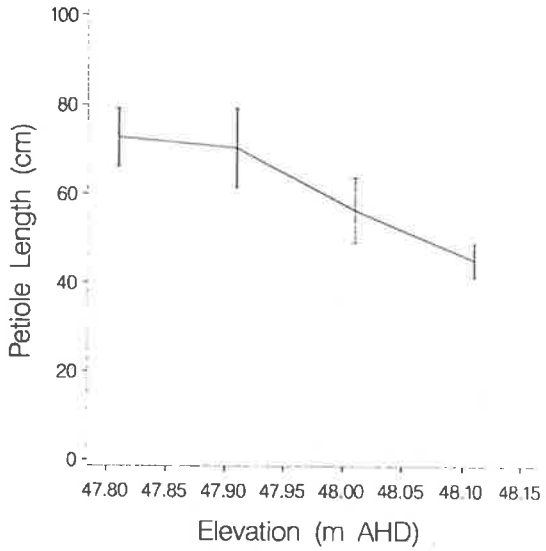


Figure 5.6
The length of the longest *T. procerum* leaf per shoot at four elevations in June, October and December 1995 and March 1996. Error bars indicate +/- 1 standard deviation from the mean of 20 shoots.

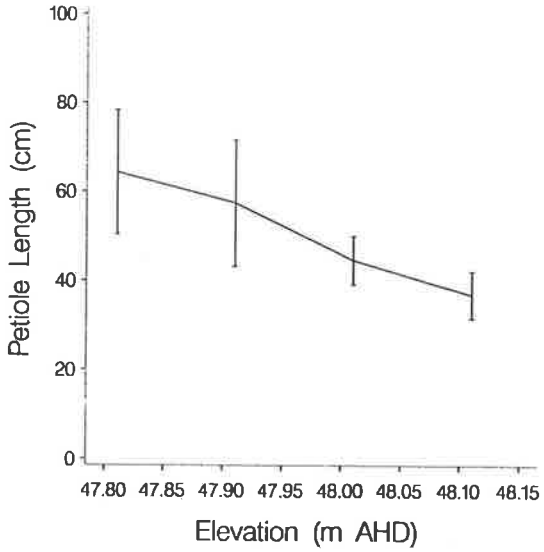
June




October




December




March

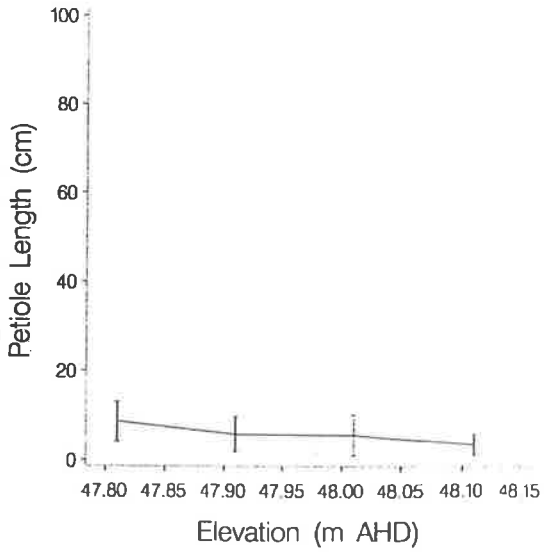


Figure 5.5
The length of the longest *V. reniformis* petiole per shoot at four elevations in June, October and December 1995 and March 1996. Error bars indicate +/- 1 standard deviation from the mean of 20 shoots.

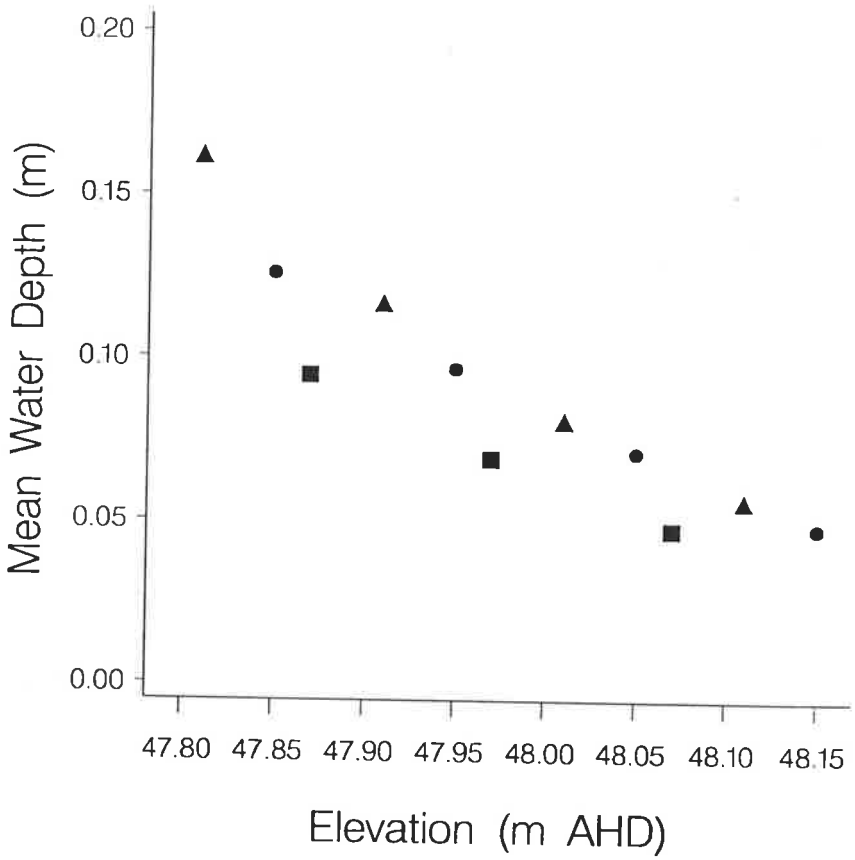
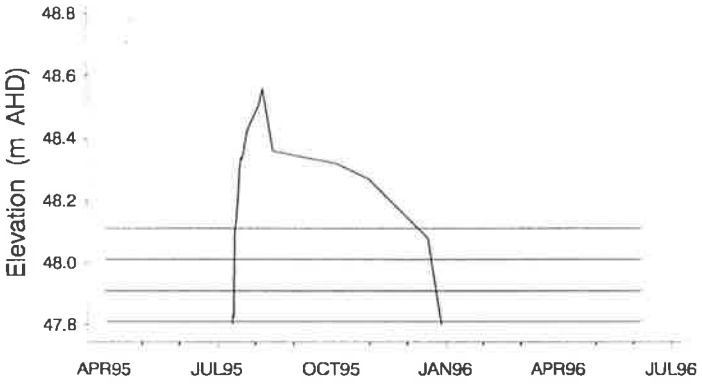
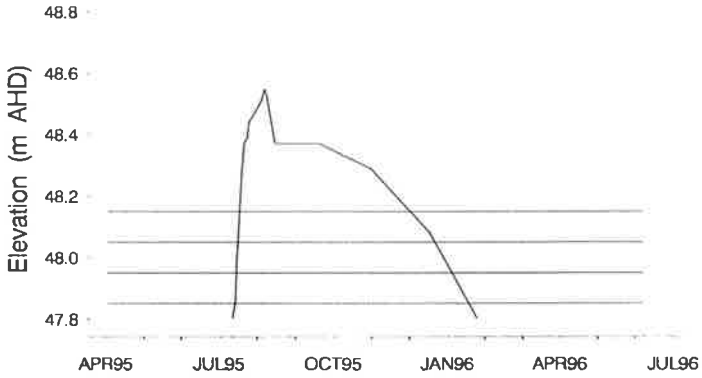

Mean Water Depth (m)
0.20
0.15
0.10
0.05
0.00
47.80
47.85
47.90
47.95
48.00
48.05
48.10
48.15
Elevation (m AHD)

Figure 5.4
The relationship between elevations examined at each site and mean water depth, calculated on positive water depths for the three years preceding the study. The Main Basin is indicated by ▲, the Central Basin by • and the Western Basin by ■.

## Main Basin




## Central Basin




## Western Basin

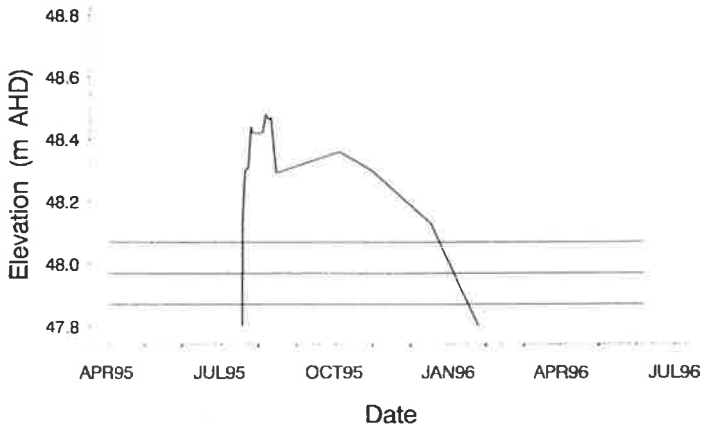


Figure 5.3

The hydrograph of the main basin (*V. reniformis* and *T. procerum*), the central basin (*B. arthrophylla*) and the western basin (*B. juncea*) from April 1995 to July 1996. The elevations at each site are marked on each hydrograph. Main Basin: 47.81 to 48.11 m AHD, Central Basin: 47.85 to 48.15 m AHD and Western Basin: 47.87 to 48.07 m AHD.

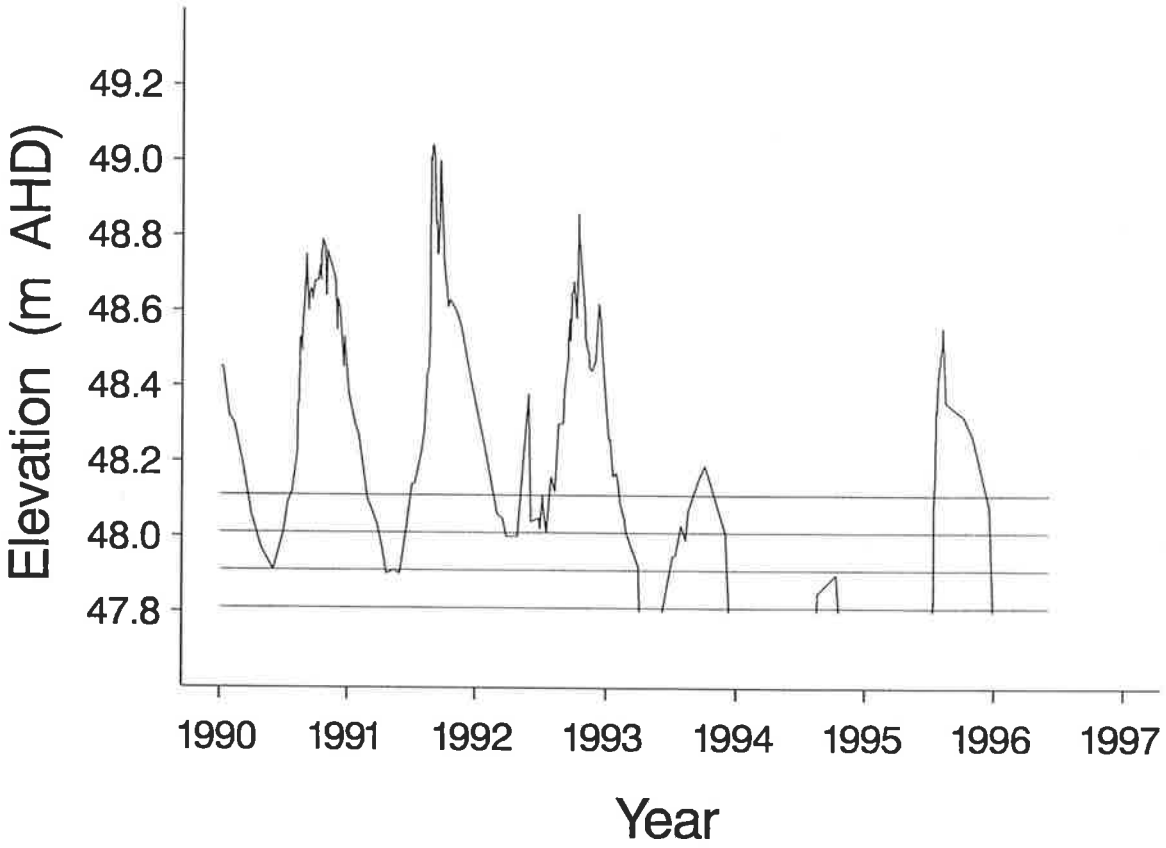

Elevation (m AHD)
49.2
49.0
48.8
48.6
48.4
48.2
48.0
47.8
1990
1991
1992
1993
1994
1995
1996
1997
Year

Figure 5.2
The hydrograph of the Main Basin of Bool Lagoon for the 6 years preceding the study. The elevations at the Villarsia / Triglochin site are marked on the figures: 47.81, 47.91, 48.01 and 48.11 m AHD.

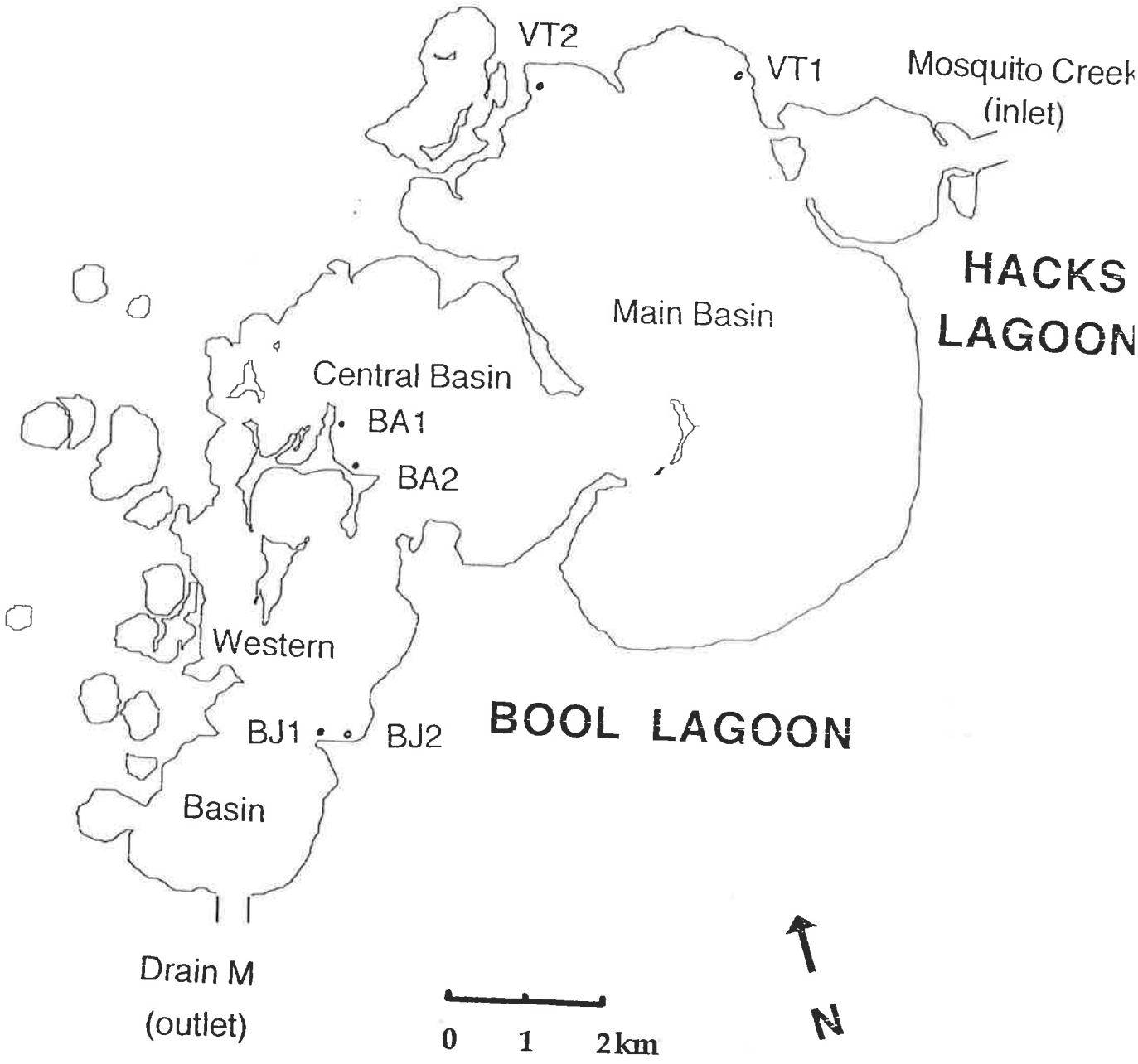

VT2
VT1
Mosquito Creek
(inlet)
HACKS
LAGOON
Main Basin
Central Basin
BA1
BA2
Western
BJ1
BJ2
BOOL LAGOON
Basin
Drain M
(outlet)
0
1
2km
N

Figure 5.1
The Bool Lagoon wetland system, showing points of water inflow (Mosquito Creek) and outflow (Drain M). The site locations are shown: *Villarsia / Triglochin* in the main basin (VT1 and VT2), *Baumea arthrophylla* in the Central Basin (BA1 and BA2) and *B. juncea* in the Western Basin (BJ1 and BJ2).

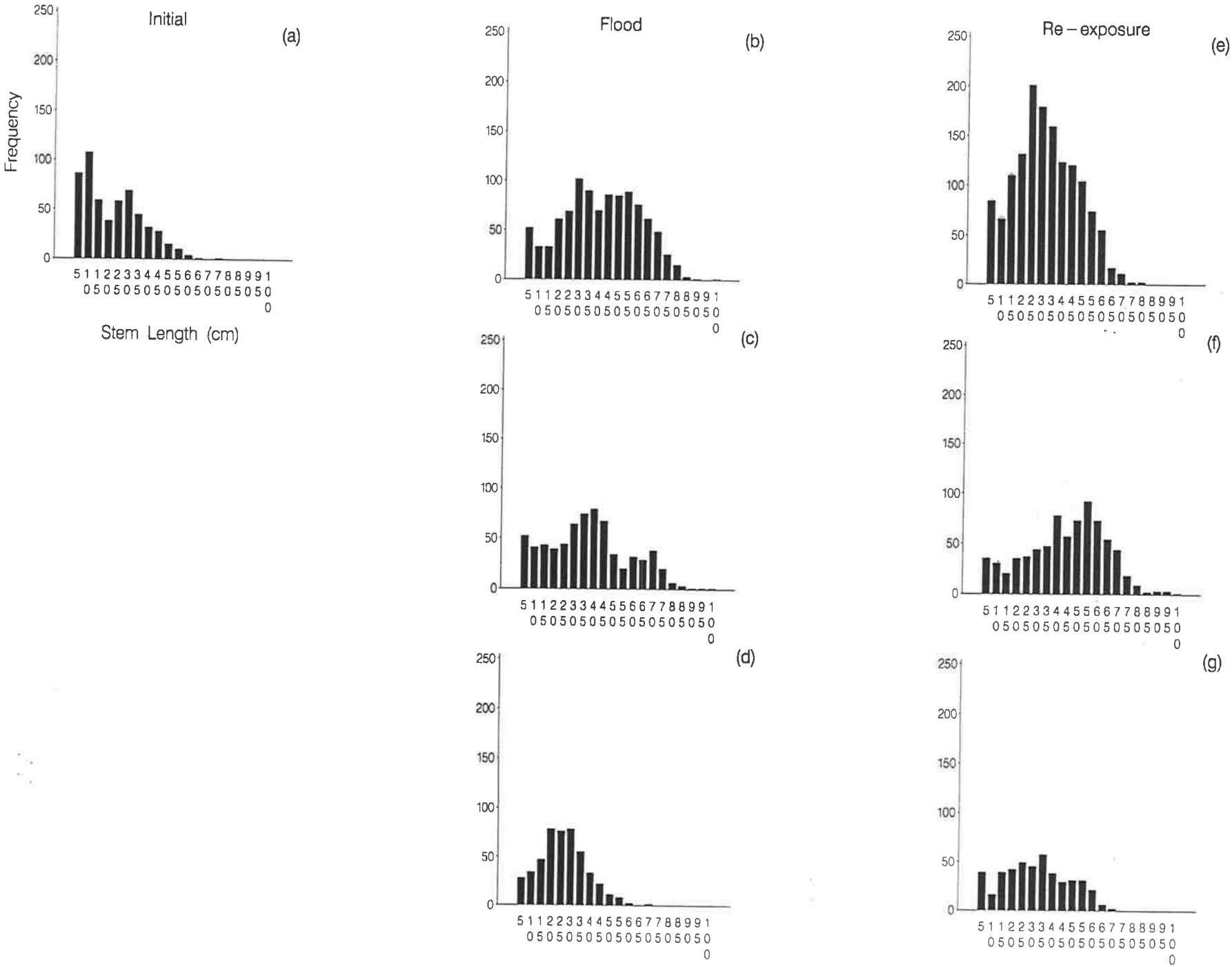

Initial
(a)
Frequency
250
200
150
100
50
0
5 1 1 2 2 3 3 4 4 5 5 6 6 7 7 8 8 9 9 1
0 5 0 5 0 5 0 5 0 5 0 5 0 5 0 5 0 5 0 5 0
0
Stem Length (cm)
Flood
(b)
(c)
(d)
Re – exposure
(e)
(f)
(g)

Figure 4.3

Stem length distribution of *Baumea juncea*. Plants were initially flooded to 2 cm for 4 weeks (a), and sampled 11 weeks later at 2 cm (b), 25 cm (c) and 120 cm (d). Depths were gradually reduced to 2 cm over 4 weeks, where they remained a further 8 weeks before sampling. Plants previously flooded to 2 cm, 25 cm and 120 cm are shown in (e, f and g), respectively.

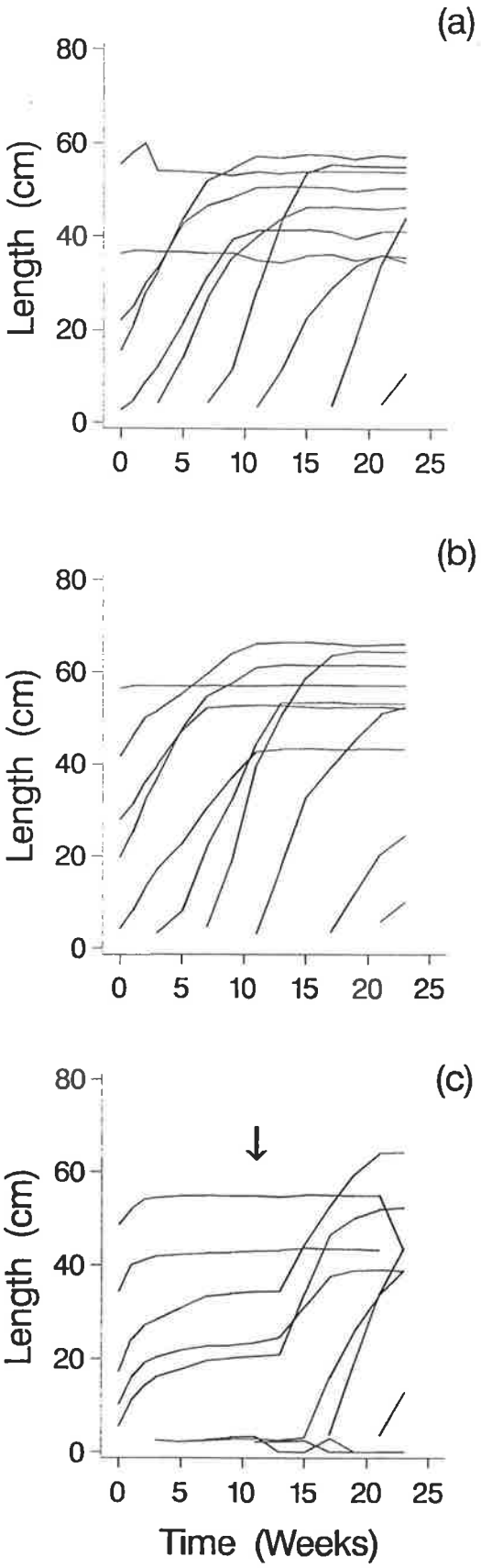

(a)
80
60
40
20
0
Length (cm)
0
5
10
15
20
25
(b)
80
60
40
20
0
Length (cm)
0
5
10
15
20
25
(c)
80
60
40
20
0
Length (cm)
0
5
10
15
20
25
Time (Weeks)

Figure 4.2

Stem growth over time in three water regimes: control plants at 2 cm (a), 25 cm (b) and 120 cm (c). 25 and 120 cm depths were gradually reduced to 2 cm after week 11. Stem elongation was inhibited by top flooding (c). The arrow indicates when depth was reduced, triggering renewed growth in flooded stems.

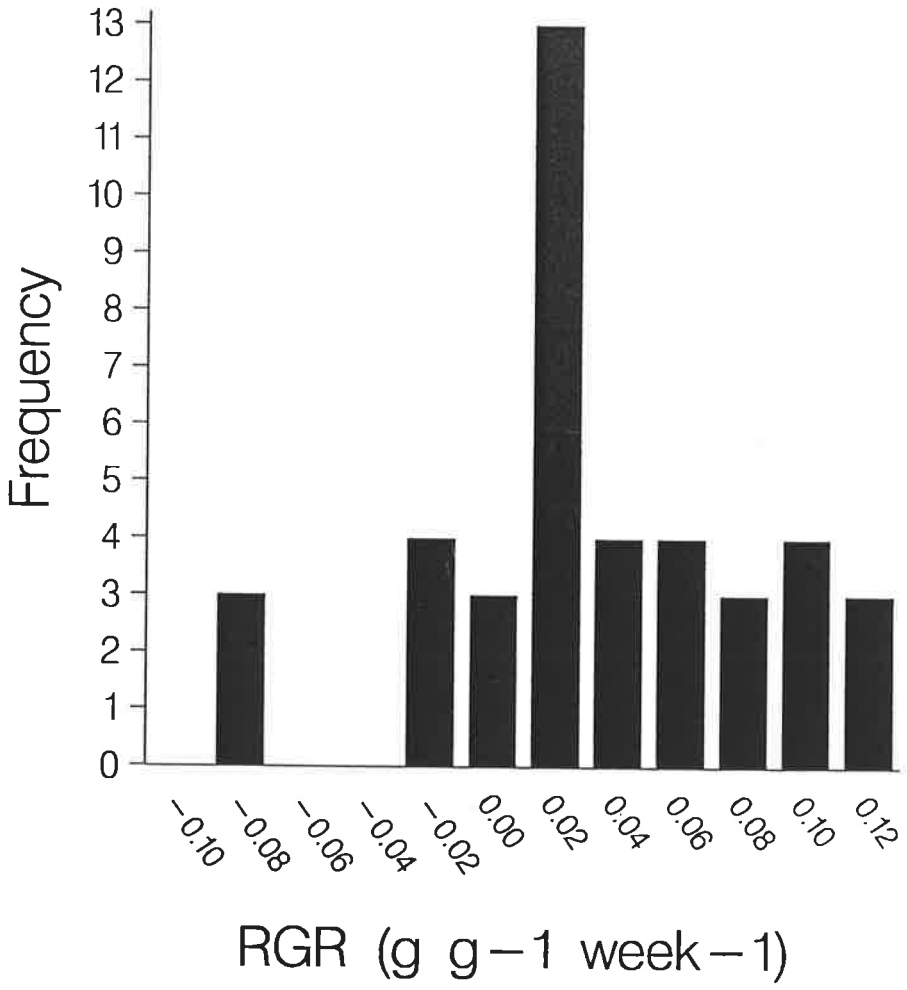

Frequency
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−0.10
−0.08
−0.06
−0.04
−0.02
0.00
0.02
0.04
0.06
0.08
0.10
0.12
RGR (g g−1 week−1)

Figure 4.1

Frequency distribution of relative growth rates of all plants measured in the experiment, showing three outlying individuals with growth rates less than -0.07 $g^{-1}$ $g^{-1}$ week.

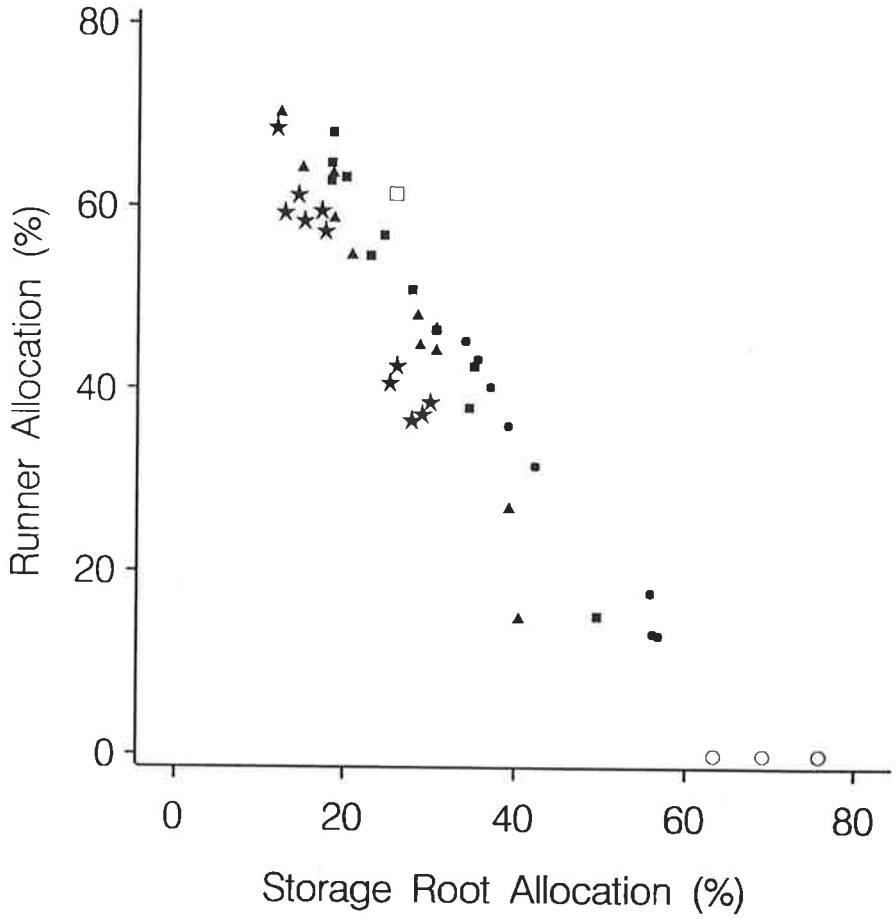

80
60
40
20
0
Runner Allocation (%)
0
20
40
60
80
Storage Root Allocation (%)

Figure 3.5

Allocation of biomass between the runners and the storage root in high nutrient plants as a percentage of total dry weight. Plants at 2 cm are indicated by dot, 20 cm solid square, 40 cm triangle and 60 cm star. Three plants which did not produce runners are indicated by an empty circle. The plant grown in low nutrient conditions which did produce runners is included on this figure and is indicated by an empty square.

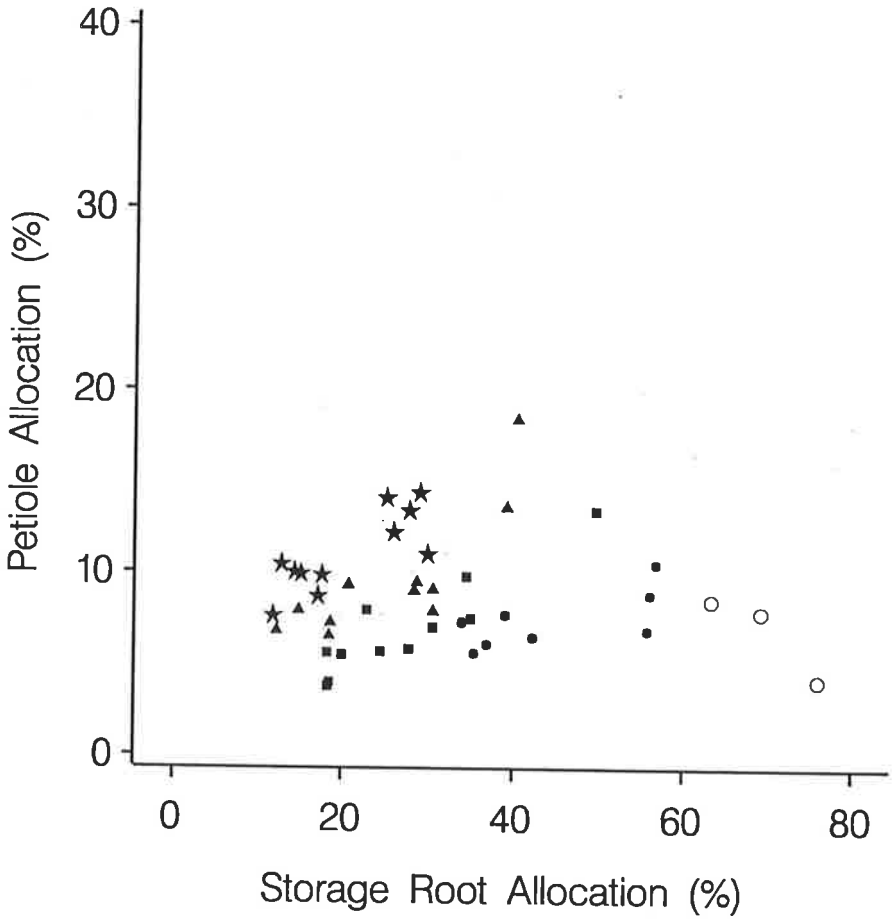

40
30
20
10
0
Petiole Allocation (%)
0
20
40
60
80
Storage Root Allocation (%)

Figure 3.4

Allocation of biomass between the storage roots and petioles in high nutrient plants as a percentage of total dry weight. Plants at 2 cm are indicated by dot, 20 cm solid square, 40 cm triangle and 60 cm star. Three plants which did not produce runners are indicated by an empty circle.

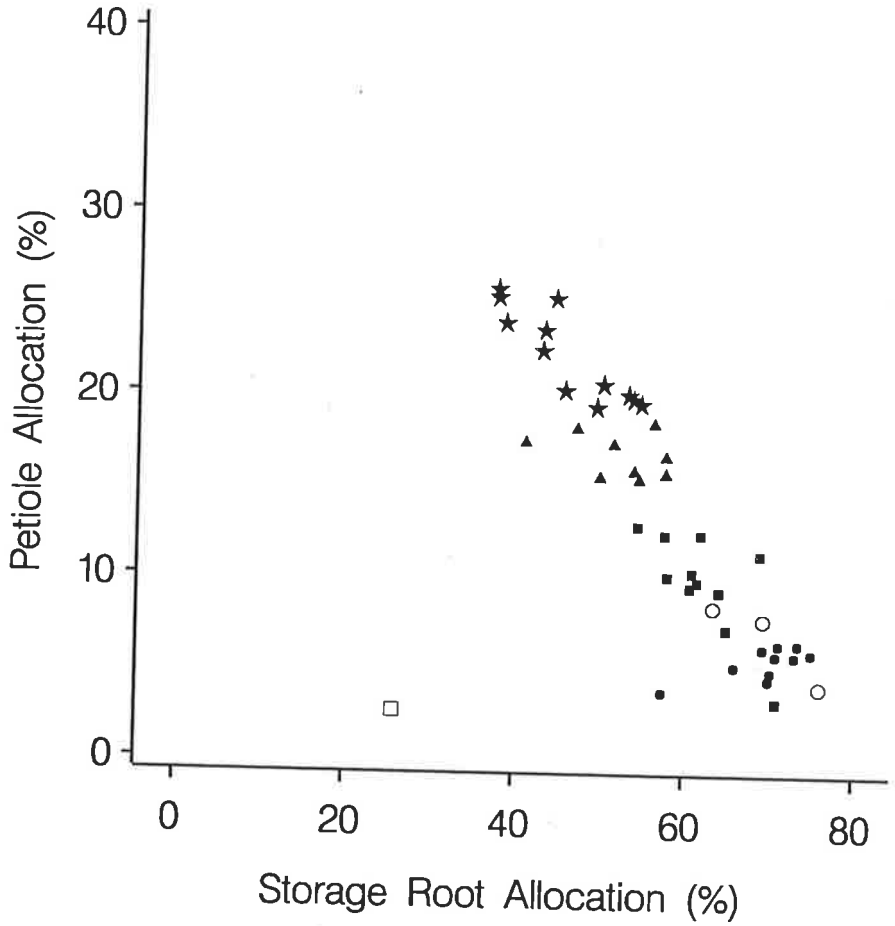
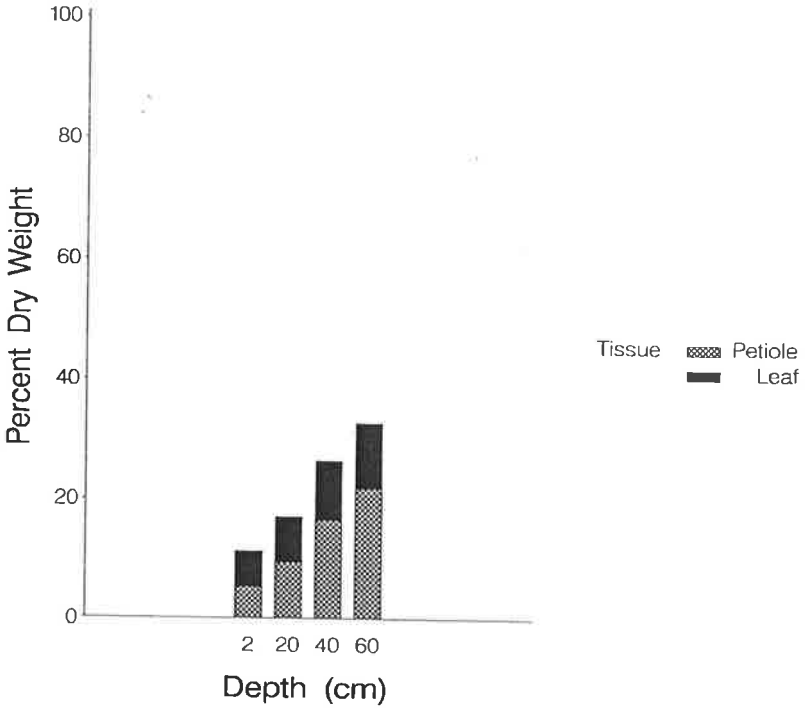

40
30
20
10
0
Petiole Allocation (%)
0
20
40
60
80
Storage Root Allocation (%)

Figure 3.3

Allocation of biomass between the storage roots and petioles in low nutrient plants as a percentage of total dry weight. Plants at 2 cm are indicated by dot, 20 cm solid square, 40 cm triangle and 60 cm star. The single plant in low nutrient conditions to produce runners is indicated by an empty square. Three high nutrient plants which did not produce runners are included on this figure and are indicated by an empty circle.


100
80
60
40
20
0
Percent Dry Weight
2 20 40 60
Depth (cm)
Tissue
Petiole
Leaf

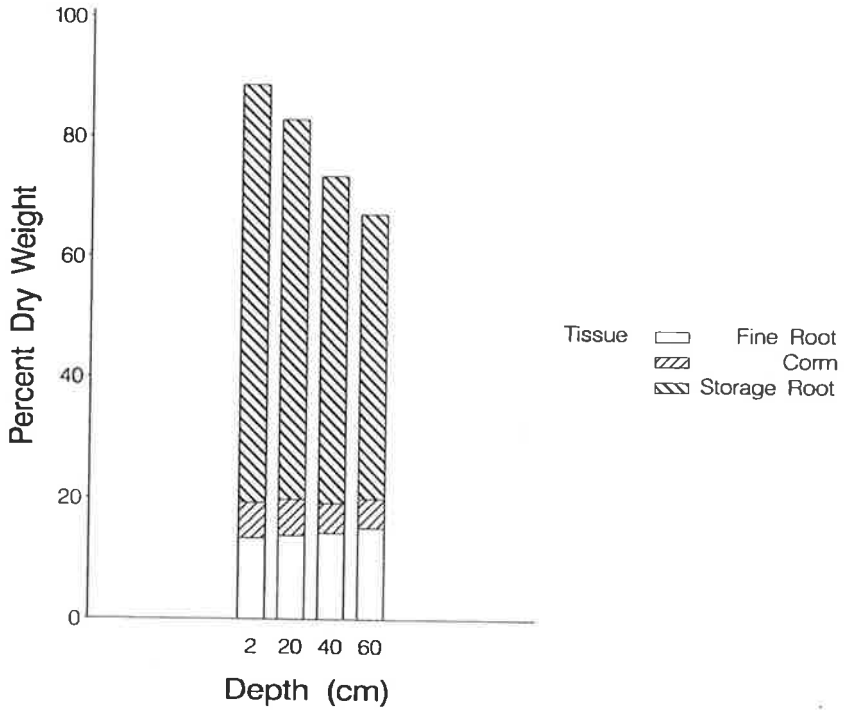
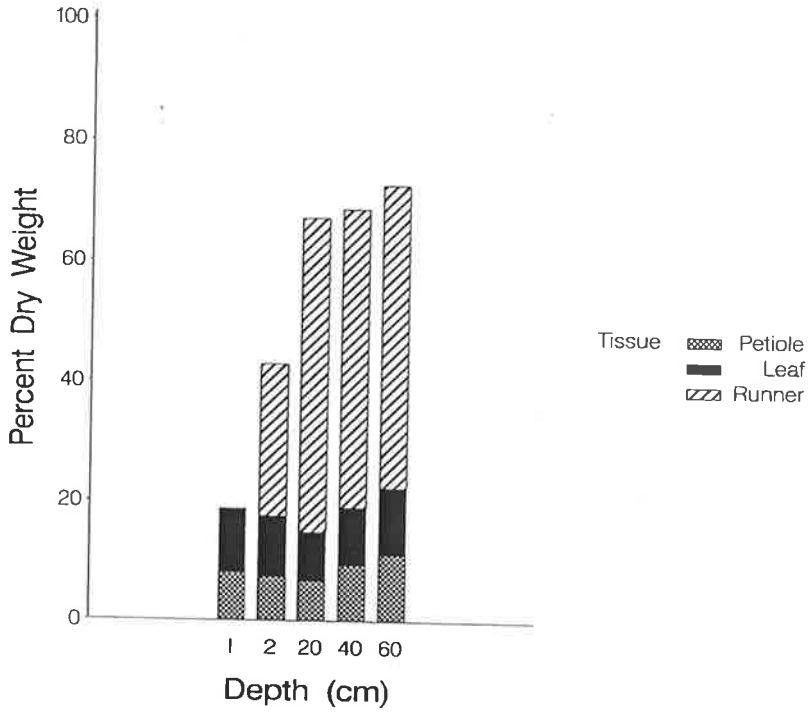

100
80
60
40
20
0
Percent Dry Weight
2 20 40 60
Depth (cm)
Tissue
Fine Root
Corm
Storage Root

Figure 3.2
The proportional allocation of total dry weight to (a) above ground and (b) below ground tissues of *Villarsia reniformis* in low nutrient conditions at four experimental depths.


100
80
60
40
20
0
Percent Dry Weight
1 2 20 40 60
Depth (cm)
Tissue
Petiole
Leaf
Runner

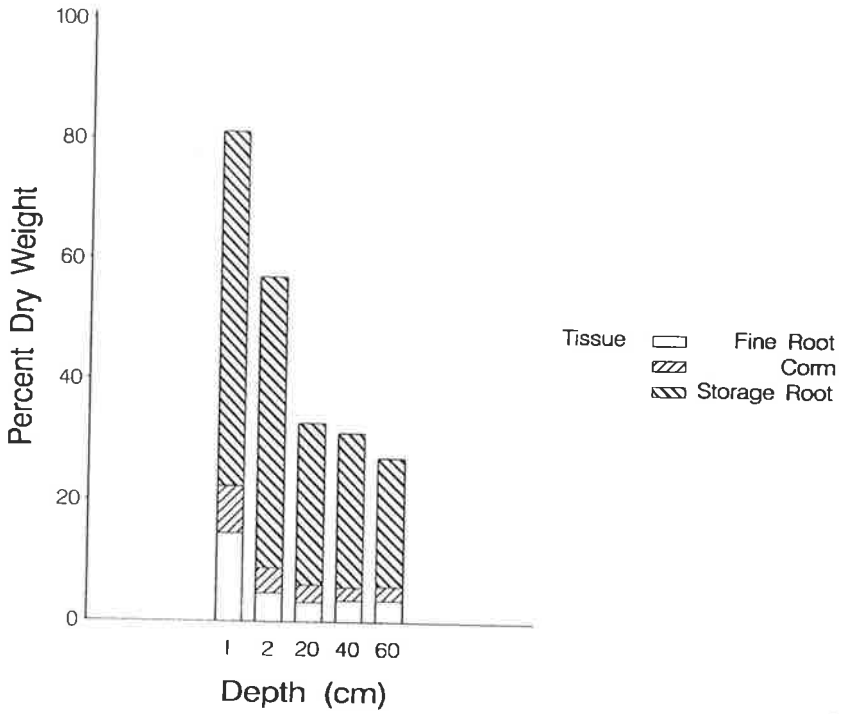
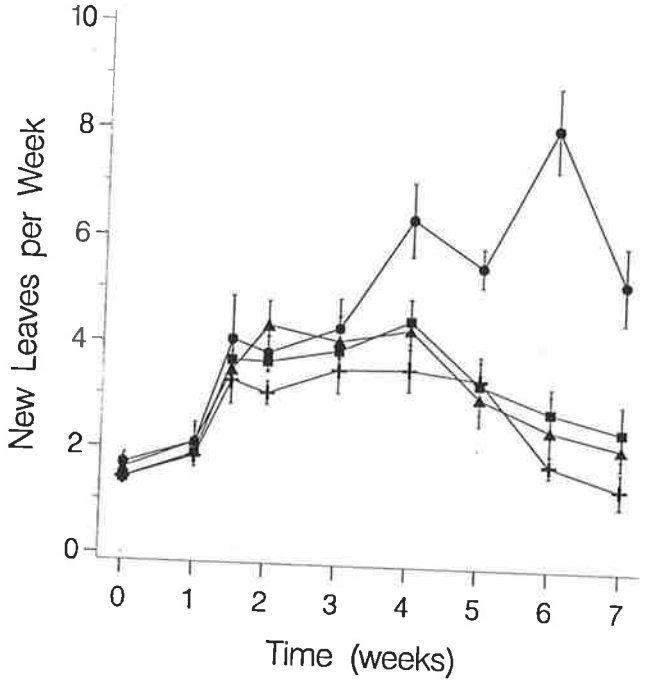

100
80
60
40
20
0
Percent Dry Weight
1 2 20 40 60
Depth (cm)
Tissue
Fine Root
Corm
Storage Root

Figure 3.1
The proportional allocation of total dry weight to (a) above ground and (b) below ground tissues of *Villarsia reniformis* in high nutrient conditions in plants harvested initially (I) and at four experimental depths.


(a)
New Leaves per Week
10
8
6
4
2
0
0 1 2 3 4 5 6 7
Time (weeks)

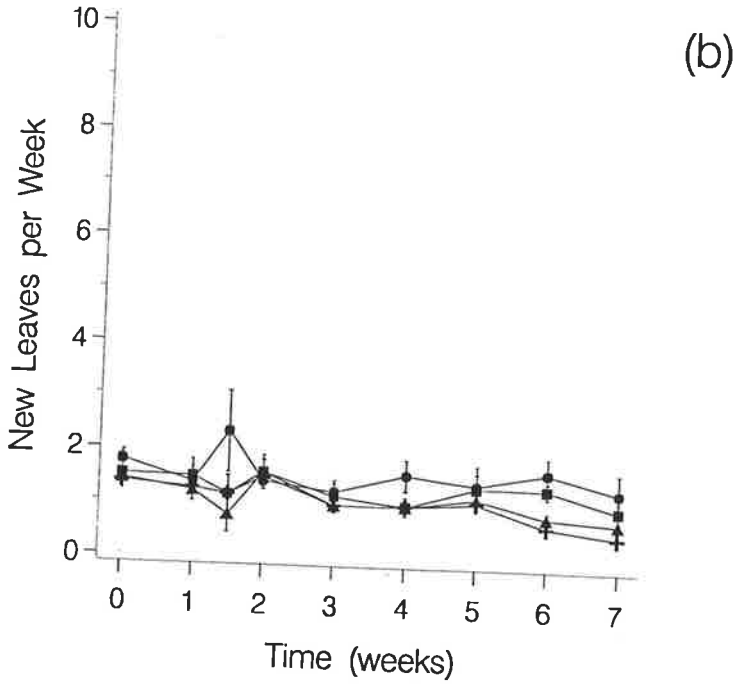
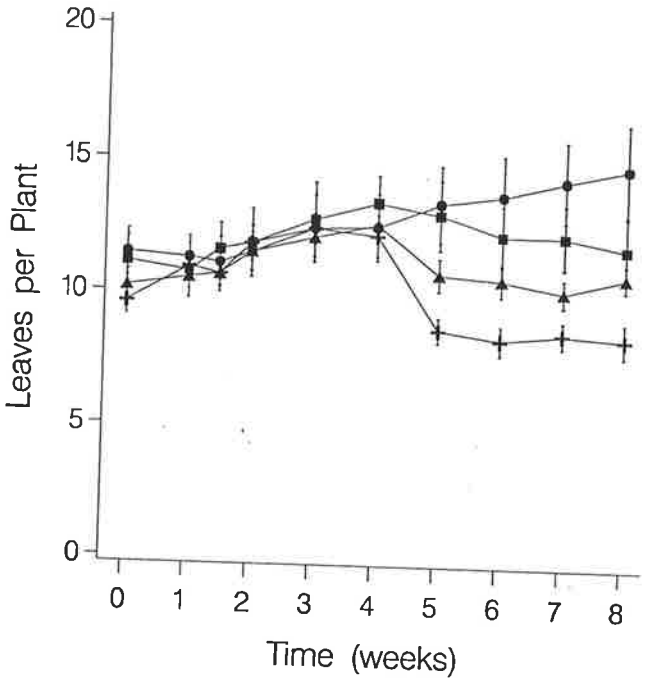

(b)
New Leaves per Week
10
8
6
4
2
0
0 1 2 3 4 5 6 7
Time (weeks)

Figure 2.5

The number of leaves recruited each week in four flooding treatments and at two nutrient levels, (a) high and (b) low. Plants were gradually flooded over the first four weeks to final depths of 2 cm (•), 20 cm (■), 40 cm (▲) and 60 cm (+). Error bars show standard deviation from the mean of 12 plants. Flooding and nutrient treatments began at week 0; plants reached their final depths at week 4. Measurements of the nutrient enriched plants stop at week 7 due to interaction of runner development on growth (see text).


(a)
Leaves per Plant
20
15
10
5
0
0 1 2 3 4 5 6 7 8
Time (weeks)

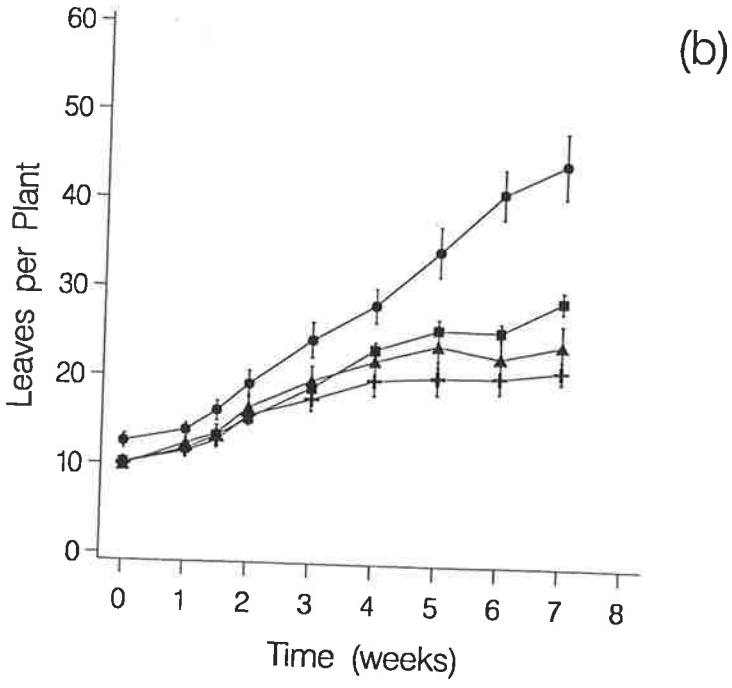
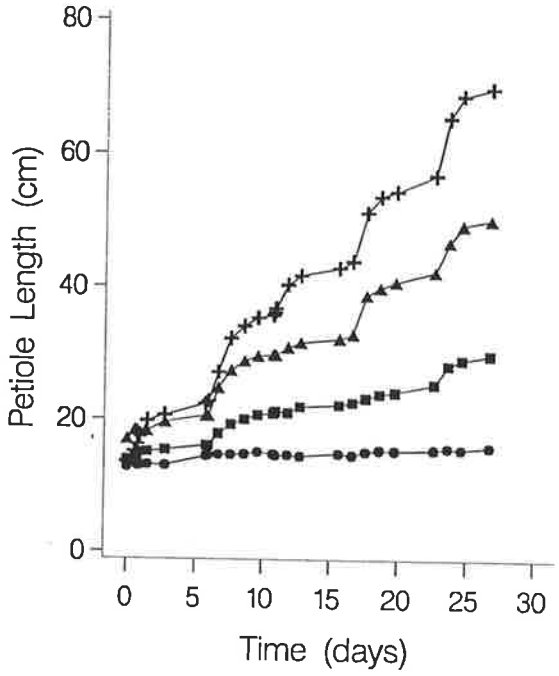
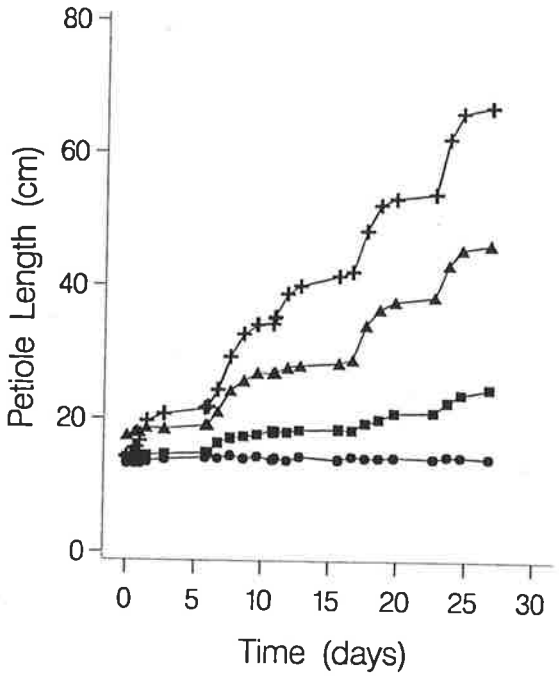

(b)
Leaves per Plant
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8
Time (weeks)

Figure 2.4

The number of leaves per plant at two nutrient levels (a) high and (b) low, and at four flooding treatments. Plants were gradually flooded over the first four weeks to final depths of 2 cm (•), 20 cm (■), 40 cm (▲) and 60 cm (+). Error bars show standard deviation from the mean of 12 plants. Note the different y-axis scales for the two nutrient levels. Measurements of the nutrient enriched plants stop at week 7 due to interaction of runner development on growth (see text).


80
60
40
20
0
Petiole Length (cm)
0
5
10
15
20
25
30
Time (days)

(a)


80
60
40
20
0
Petiole Length (cm)
0
5
10
15
20
25
30
Time (days)

(b)

Figure 2.3

The length of the longest petiole per plant at four flooding rates and at two nutrient levels, (a) plants treated with the addition of nutrients and (b) plants without additional nutrients. Plants were gradually flooded every 6 days by an additional 4 cm to a final depth of 20 cm (■), 8 cm to 40 cm (▲) and 12 cm to a final depth of 60cm (+). Control plants stayed at constant 2 cm depth (•).

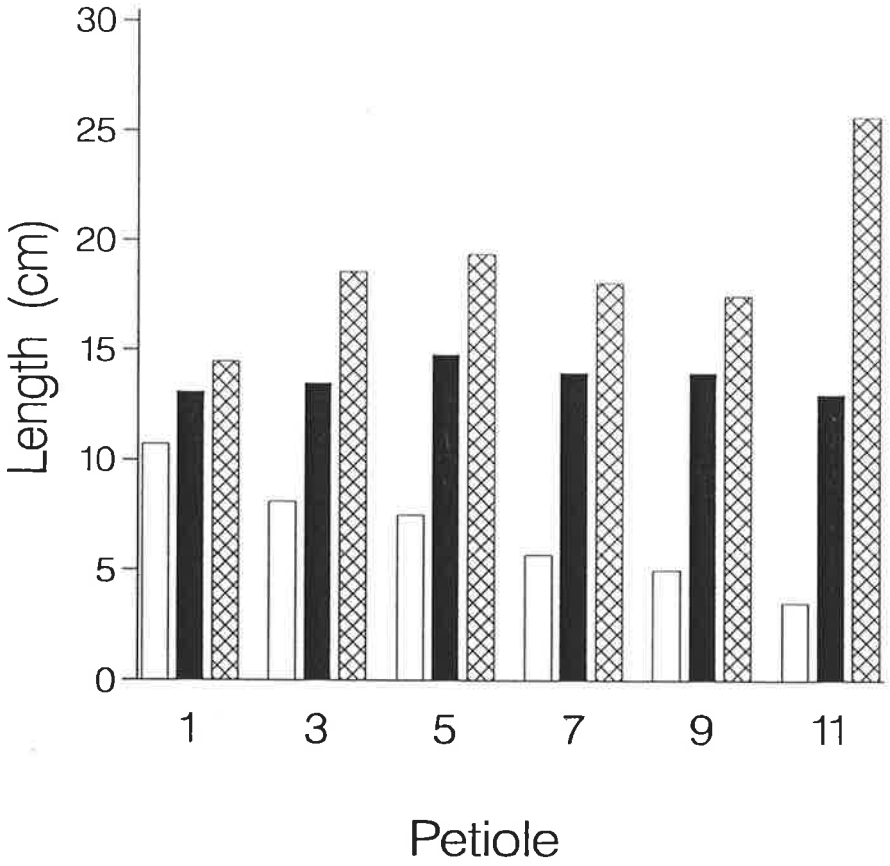
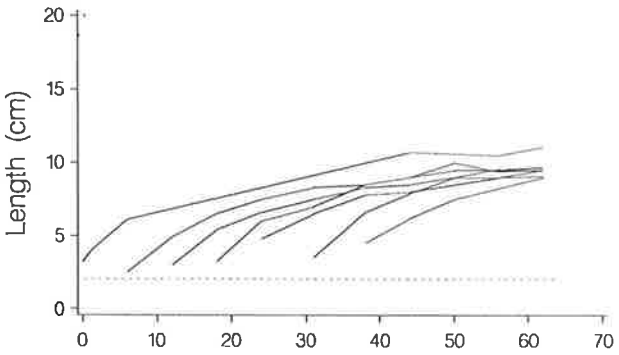

30
25
20
15
10
5
0
Length (cm)
1
3
5
7
9
11
Petiole

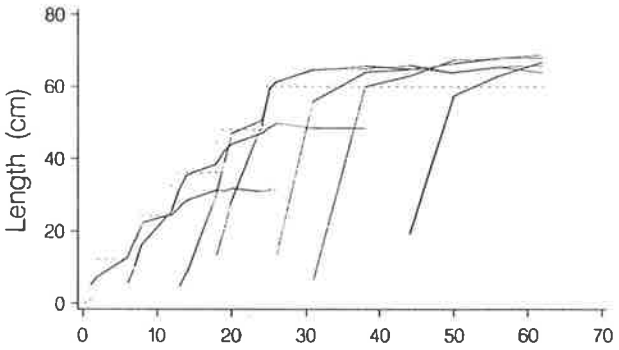
Figure 2.2

The influence of increasing water depth (flooding rate of 12 cm per six days) on petiole length for leaves of different ages (1 = oldest; 11 = youngest). Lengths at the initial depth of 2 cm (white bars), after 6 days at 12 cm, (black bars) and after 6 days at 24 cm (hatched bars) are shown.

(a)


Length (cm)
0
5
10
15
20
0
10
20
30
40
50
60
70

(b)


Length (cm)
0
20
40
60
80
0
10
20
30
40
50
60
70

(c)

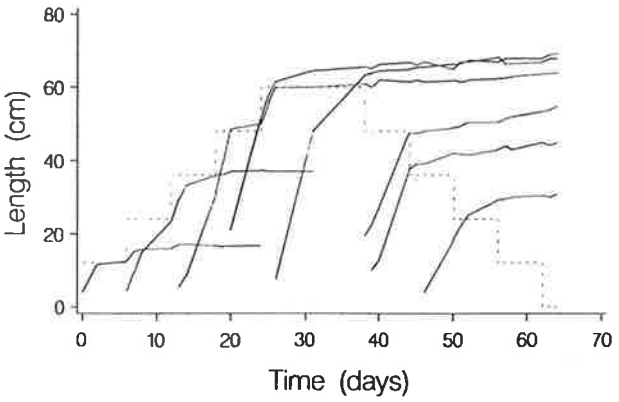

Length (cm)
0
20
40
60
80
0
10
20
30
40
50
60
70
Time (days)

Figure 2.1

Petiole lengths of *Villarsia* leaves recruited under three flooding regimes a) Plants grown at a constant 2 cm depth, b) plants gradually flooded to 60 cm and c) plants gradually flooded to 60 cm and returned to 2 cm. Depths were changed by 12 cm every six days. Petioles recruited every sixth day are plotted in solid lines, and the water depth in the broken line.