Competition between three aquatic macrophytes, *Elodea canadensis* Michx., *Elodea nuttallii* (Planch.) H. St. John, and *Lagarosiphon major* (Ridley) Moss.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

By

Cassandra Sarah James

January 1999

ABSTRACT

Cassandra James

Competition between three aquatic invasive macrophytes, *Elodea canadensis* (Michx.), *Elodea nuttallii* (Planch.) H. St. John and *Lagarosiphon major*(Ridley) Moss.

In some British freshwaters a species displacement has been observed from *Elodea canadensis* to *Elodea nuttallii* and then *Lagarosiphon major*. Competition may play an important role in such displacements. During photosynthesis, submersed aquatic plants create "envelopes" of raised pH and dissolved O_2 concentrations, and depleted dissolved CO_2*. When mass flow of water is low, these stress conditions may extend beyond the plant stand itself, and hence could interfere with the growth and consequently the competitive ability of adjacent plants. It is hypothesised that those species with the best stress generation/toleration mechanisms may be competitively advantaged.

This hypothesis was tested for the three elodeids using a combination of laboratory physiological measurements, laboratory culture experiments under controlled conditions, morphological studies, tissue analyses and field observations. Physiological studies showed that all three species exhibit a high degree of physiological plasticity in response to changing environmental conditions. Their responses to changing light intensities and O_2 concentrations were similar. *E. nuttallii* and *L. major* adapted more readily than *E. canadensis* to changes in bicarbonate and CO_2* availability following acclimation to high and low CO_2* conditions. Results also suggest that *L. major* is more efficient at using bicarbonate as a carbon source than either *Elodea* spp..

The development of stress conditions was measured at different densities in species monocultures and in pair-wise mixtures. While density-dependent differences in increase in pH and decreases in CO_2* concentrations in the growth medium were observed initially these did not perpetuate and within two weeks no significant differences in conditions were found either between densities or species.

Evidence from temperature studies suggests that *L. major* may successfully over-winter without dying back. Higher RGR was observed for this species at 10 °C than either *Elodea* spp., while no significant differences in RGR were observed at 10 °C between *E. canadensis* and *E. nuttallii*. At 15 and 20 °C the RGR of *E. nuttallii* was greatest. Tissue starch concentrations were similar between the three species. None of the species showed chilling injury during short-term exposure to low temperatures (1 and 3 °C), although all three showed freezing injury.

Analysis of plant architecture showed that both *E. nuttallii* and *L. major* form efficient canopies under field conditions with the majority of the biomass in the upper parts of the water column. If canopy production occurs at the water surface, slower growing species such as *E. canadensis* may be shaded out.

Nutrient studies showed the preferences of all three species for ammonium as a nitrogen source. Increases in nitrogen and phosphorus fertilisation did not reveal differences in the responses of the species, although *E. nuttallii* consistently exhibited the highest growth rate. Some luxury uptake of nitrogen was observed. *E. canadensis* had the highest nitrogen and phosphorus standing stocks. Results suggest that a rapid growth rate may be more important than a high nutrient uptake ability in competition between macrophytes and epiphytes.

In a first of two competition studies, intra-specific but not inter-specific competitive effects were found. Results of a following study over a longer duration suggest that the effects of *E. nuttallii* on itself are greater than interspecific effects from either *E. canadensis* or *L. major*. *E. nuttallii* reduced the growth of *L. major* and both *E. nuttallii* and *L. major* reduced the growth of *E. canadensis*.

In conclusion, the rapid growth rate of *E. nuttallii* at summer temperatures, together with formation of a tall, canopy and the development of stress conditions may in combination result in the displacement of *E. canadensis*. The success of *L. major* may in part be explained by its efficient bicarbonate utilisation capacity. This species may tolerate periods of high stress such as during the winter period when temperatures are low with little loss of biomass and a capacity to resume growth at low temperatures, giving it a head start at the beginning of the growing season.

What is a weed? A plant whose virtues have not yet been discovered.

R. W. Emerson (1803-1882)

Acknowledgements

This study was undertaken with the financial support of the Natural Environment Research Council for which I am grateful.

Further thanks are extended to my supervisors Dr. John Eaton, Dr Keith Hardwick and Dr. Peter Gould for all their help and support throughout the project.

I would also like to thank all those in the Jones building for providing moral support and the odd beer when it was needed! For those formerly of the Nicholson building, particularly Dave Hatcher, Hanife Ozbay and Rebecca Farley, who put up with me stinking the place out with canal mud, I thought you may appreciate this quote from Orson Welles:

"They teach you anything in university today. You can major in mud pies."

To my parents who helped particularly when things went wrong, who sorted out computer disasters, helped me move and provided financial support when I was skint…many many thanks!

And last, but by no means least, my thanks to Andy for taking the brunt of my stress and putting up with a lot throughout this Ph.D!

Contents

Chapter 1 INTRODUCTION

In the last 200 years an increase in international travel and trading has resulted in the introduction of many plant species to countries beyond their native ranges. The subsequent colonisation of new habitats with which these species are not in ecological equilibrium has sometimes been extremely rapid. In the aquatic environment, members of the Hydrocharitaceae provide many classic examples of aggressively, invasive species such as *Elodea canadensis*, *Elodea nuttallii*, *Lagarosiphon major* and *Egeria densa*. These invasive species have often been associated with a reduction in the native flora and with the creation of dense monospecific stands (Howard-Williams and Davies, 1988; Howard-Williams, 1993). They may also impede both the recreational and industrial use of water bodies due to their dense growth in some situations (Chapman *et al*, 1974; Johnstone, 1982; Nichols, 1991; Howard-Williams, 1993)

A prime example of spectacular invasion by an introduced species is the spread of *E. canadensis* Michx. in the British Isles. *E. canadensis* is a native of temperate North America and is thought to have been introduced into the British Isles through the logging trade during the early part of the 19th century (Marshall, 1852). It spread rapidly, largely through the canal network, and by the turn of the century had become an extremely common species. A similar species, *E. nuttallii* (also a native of North America), was first recorded in Britain in 1966 (Preston and Croft, 1997). This species has followed a similar period of rapid expansion and this appears to be continuing. A third member of the Hydrocharitaceae, a native of South Africa, *L. major* (Ridley) Moss, was introduced into the UK in 1944 (Belcher and Swale, 1990). This species is now also found naturalised at many locations.

The spread of these species is not confined to the UK, all three species having established on mainland Europe (Preston and Croft, 1997). One of the most likely sources of introduction was through botanists. For example, the curators of the botanical gardens of both Berlin and Hamburg introduced *E. canadensis* locally (Cook and Urmi-Konig, 1985). *E. canadensis* and *L. major* have also presented particular problems in New Zealand. There, the highest known biomass densities of any submerged macrophyte have been recorded, with values as high as 3518 g DW

m^{-2} for *L. major* (Clayton, 1982). The latter species, along with *E. densa* and *Ceratophyllum demersum,* presented particular problems in New Zealand hydro-power lakes (Chapman *et al.*, 1974). *E. canadensis* has also established in Australian irrigation channels, where it is considered a major pest impeding flow in irrigation and drainage channels (Bowmer *et al.*, 1984; Cook & Urmi Konig, 1985). *E. nuttallii* is now established in Japan, where its spread is documented by Kunii (1982).

Mechanism of spread

Although dioecious in nature, with only one exception, only female flowers of both *E. canadensis* and *E. nuttallii* have been found in Europe. *L. major* rarely flowers in Europe, but those flowering plants found are all reported to be female (Preston and Croft, 1997). Consequently, the spread of these three species in Europe has been through vegetative propagation only. They are able to propagate from very small sections of stem provided these have an attached axillary bud or intact apex. It is possible that only a few clones of each species actually exist throughout Europe, although no research so far has been conducted in this area.

Changes in distribution in the UK

Walker (1912) states that following the initial rapid spread of *E. canadensis*, by 1909 it had declined in abundance at many sites, although it was continuing to spread into new localities. It is reported that the introduction of *Elodea nuttallii* in 1966 resulted in the displacement of *E. canadensis* from many situations where the latter had become well established (Simpson, 1990). Observations both on the initial spread of *E. canadensis* and its subsequent decline and replacement with *E. nuttallii* suggest that displacement of *E. canadensis* occurs over a very short period of time (i.e. 1-2 years) (Briggs, 1977; Lund, 1979; Simpson, 1984; Cook, 1990; J. W. Eaton, personal communication). More recent observations in the last 10 years suggest that in some sites in the UK, *L. major* is now displacing *E. nuttallii* (Cook, 1990; J. W. Eaton, personal communication). Again, displacement was observed to occur within a few years. While there are no reports of *L. major* directly outcompeting *E. canadensis* in the UK, this has occurred in New Zealand, where *E. nuttallii* has not been found. Again, displacement was observed to occur within two years (Coffey.

1975). Interestingly, *L. major* itself has now been displaced by *C. demersum* and *E. densa* in some more eutrophic sites in New Zealand (Wells *et al.*, 1997). The speed with which these changes in species composition have taken place suggests a process of competitive displacement. A passive process such as when one species is already receding and the second introduced species simply occupying an already vacated niche, would probably take considerably longer.

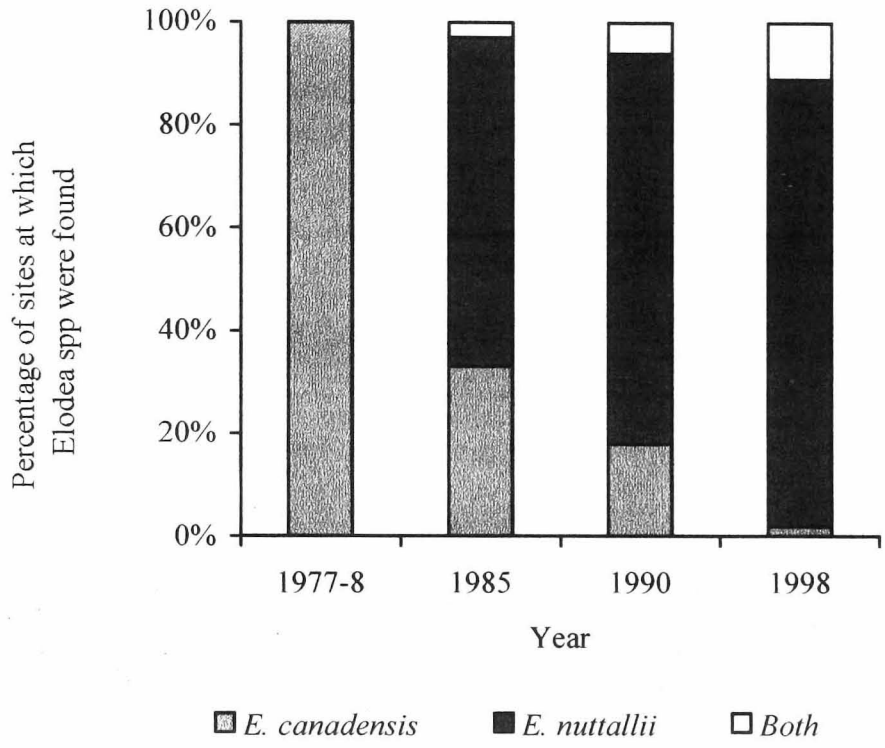


Fig 1.1 Percentage of canal sites at which *E. canadensis* and *E. nuttallii* were found as a percentage of total number of sites with an *Elodea* spp. present. Data compiled from Murphy (1980), Pygott (1987), Willby (1994) and D. Hatcher (unpublished data).

Present distribution maps suggest that *E. canadensis* is an extremely wide spread species in the British Isles. *E. nuttallii* does not appear to be as widespread yet, although Preston and Croft (1997) suggest that its distribution is underestimated. A recent study by Rich and Woodruff (1995) on English and Scottish aquatic vascular plants recorded a significant increase in frequency of *E. nuttallii*, but surprisingly, no decrease in *E. canadensis*. Fig. 1.1, from an examination of a number of previous studies conducted on British canals (Murphy, 1980; Pygott, 1987; Willby, 1994), reveals a decrease in the number of sites at which *E. canadensis* was recorded, and an increase in the number of sites at which *E. nuttallii* was found. A 1998 survey of the British canal network suggests that this trend is continuing,

with a further increase in the percentage of sites containing *E. nuttallii*, a few sites with both species and only a single, isolated site containing only *E. canadensis* (D. Hatcher, personal communication). Canal sites in which *E. canadensis* appears to persist are isolated, tending to be either disconnected parts of the canal or end sections such as in the Nottingham Canal, the Lancaster Canal and the Montgomery section of the Shropshire Union Canal.

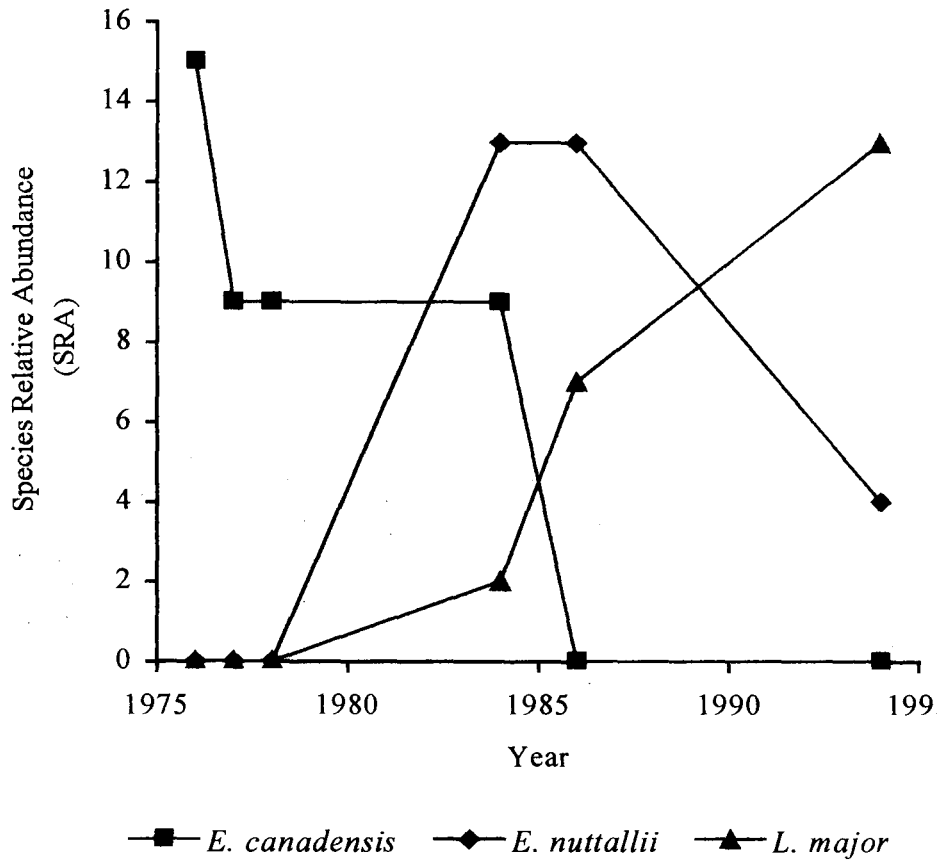


Fig. 1.2 Change in Species Relative Abundance (SRA) (Murphy, 1980) of *E. canadensis*, *E. nuttallii* and *L. major* in the Leeds and Liverpool Canal. Data was collated by H. Whelan from records of species abundance kept through undergraduate research projects and personal observations of J. W. Eaton. Data from several studies on weed control methods (Eaton and Freeman, 1982; Murphy, 1980; Eaton *et al.*, 1981; Murphy and Eaton, 1981) are also included.

One site at which displacement of *E. canadensis* by *E. nuttallii* and the latter species displacement by *L. major* was studied in some detail is the Liverpool end of the Leeds and Liverpool Canal. Fig. 1.2 shows changes in the distribution of the *Elodea* spp. and *L. major* in the canal, with *E. canadensis* declining in abundance

during the late 1970's and early 1980's. During the early 1980's *E. nuttallii* abundance increased rapidly and it became the dominant macrophyte species during the mid-1980's. By the late 1980's however, a third species, *L. major*, had been introduced. This increased rapidly during the early 1990's, becoming the dominant species by the time of the last survey in 1994. A breach in the canal during 1995 and subsequent back-pumping of salt water into the canal to maintain the water level resulted in extremely high salt concentrations (~ 4000 ppm). During this period nearly all the aquatic vegetation died, terminating this series of observations.

The displacement process

In view of both functional and taxonomic similarities, what is driving these displacements? Studies on competition between terrestrial plant species have focused almost exclusively on competition for nutrient resources such as nitrogen and phosphorus (e.g. Tilman, 1986, 1987; Campbell and Grime, 1992). Yet, in the aquatic environment, photosynthesis and growth are often restricted not by nitrogen or phosphorus limitations, but by the availability of carbon (Adams *et al.*, 1978; Madsen and Sand-Jensen, 1987, 1991; Sand-Jensen, 1989; Nielsen and Sand-Jensen, 1991; Schwarz and Howard-Williams, 1993; Jones *et al.*, 1996). Restrictions in carbon availability within the aquatic environment are often equated with the development of stress conditions i.e. a decrease in free CO_2 (CO_2*) and bicarbonate and an increase in pH and O_2, which develop as a result of photosynthesis of the plants themselves.

In the present study it is hypothesised that in neighbouring clumps of competing species, those species that can both create the most stressful conditions, and tolerate those conditions, e.g. high pH, high O_2 and low CO_2, during photosynthesis, will displace less tolerant neighbouring species.

In the aquatic environment, availability of dissolved inorganic carbon, light and macronutrients are critical for photosynthesis and growth of submerged macrophytes. Despite the high concentrations of organic and inorganic substances often found in the water bodies, supply may nevertheless be limiting to plant performance. This is largely due to the slow diffusion rates of substances in water

and through the laminar flow boundary layers that surround submerged surfaces. Resistance to diffusion in water is 10^4 times greater than in air (Losee and Wetzel, 1988). Water movement close to leaf surfaces is reduced as result of friction between the surface and the water flow, with little turbulent mixing, thus exchange of substances through the boundary layer occurs mainly via diffusion. Boundary layers measured in water have been found to be several hundred microns thick (Raven, 1970; Smith and Walker, 1980). This is particularly so in slow moving and static water bodies where turbulent mixing is low. The development of dense plant canopies reduces water flow and consequently turbulence inside and to within 10-15 cm of the plant bed boundary (Losee and Wetzel, 1988, 1993). Slow diffusion rates and reduced turbulence have a great impact upon the uptake and release of substances by submersed plants and limit the transfer of substances between the plant and the environment (Smith and Walker 1980; Black *et al.*, 1981). Uptake of inorganic nutrients and dissolved inorganic carbon may be reduced due to the slow replenishment of depleted resources from the bulk surrounding water body. Conversely, released substances such as oxygen may accumulate within the boundary layers. Dissolved inorganic carbon (DIC) utilisation and the production of oxygen result in a shift in pH towards alkalinity.

Slow diffusion rates and reduced mixing within the plant bed result in the development of distinct physical and chemical partitioning of the water body within a macrophyte stand. Field measurements on both submersed and floating macrophytes show distinct differences in temperature, light, pH, O_2 and CO_2 between waters within and outside plant stands (e.g. Frodge *et al.*, 1990; Jones, 1994). A study conducted by Ultsch (1973) showed that temperature increased while the pH and the dissolved oxygen content decreased under a surface cover of *Eichornia crassipes* (Mart.) compared to open water. Frodge *et al.* (1990) investigated the effects of different macrophyte growth forms on littoral water quality. They found that floating species tended to cause a decrease in dissolved oxygen and pH and an increase in temperature within and beneath the canopy, while submersed species, such as *E. canadensis*, resulted in an increase in temperature, dissolved oxygen and pH within the canopy. Diurnal changes in dissolved oxygen and pH were significant within the canopy of submerged species but diurnal changes were not observed within the canopy of floating species (Frodge *et al.*, 1990).

Similar studies (e.g. Buscemi, 1958) have illustrated that there are changes in water quality and diurnal changes associated with the development of dense canopies of plants. Jones *et al.* (1996) observed diurnal changes within stands of *E. nuttallii*. During the afternoon, pH increased to in excess of pH 9 and oxygen concentrations of almost 20 mg l^{-1} were recorded. This increase was associated with diurnal restrictions in free carbon dioxide (CO_2*). Despite evidence of significant effects of dense macrophyte stands on water quality, few studies have considered how these effects may interfere with the physiology and growth of neighbouring species.

Aims of study

The aim of this study was to determine what was driving the observed displacement of *E. canadensis* by *E. nuttallii*, and the latter species displacement by *L. major*. It was hypothesised that the differential ability of species to generate stress conditions (i.e. high pH, low DIC, CO_2*, bicarbonate and high O_2) and to successfully survive those conditions is instrumental in the ability of one species to displace another. Thus, those species that generate the greatest stress conditions and have the greatest tolerance to stress will be competitively superior.

In this study a combination of laboratory physiological measurements, laboratory culture experiments under controlled conditions, morphological studies, tissue analysis and field observations were used. Chapter 2 describes the standard procedures used for experiments. Chapter 3 is an analysis of the basic morphological features of the three species, concentrating on those which are likely to provide a framework for a future mathematical model of growth (see Chapter 8), such as shoot branching patterns, internode elongation and minimum viable shoot length.

Sculthorpe (1985) emphasising the importance of seasonal timing of growth, suggests that a newly invading species may exert its influence on competition early on in the growing season, before established species have attained their maximum growth. Moen and Cohen (1989) observed that early growth of *Potamogeton pectinatus* may be instrumental to its ability to out-compete *Myriophyllum exxalbescens*. Both a species response to temperature and over-wintering storage

ability are likely to affect the potential of a species for growth in early spring and these aspects are considered for the three species in Chapter 4.

In Chapter 5 studies on the comparative physiology of the three species are presented and discussed. Integral to a plant's ability to outcompete a neighbouring species through the creation of stress conditions, is tolerance of those conditions. This chapter concentrates on physiological tolerance to pH increase, free CO_2 (CO_2*) limitations and bicarbonate usage.

A feature of the current distribution of *E. canadensis* and *E. nuttallii* in the UK is the continuing presence of *E. canadensis* in many Scottish water-bodies. It is unknown to what extent this is simply geographical isolation, or whether this is indicative of an underlying difference in adaptation to the trophic status of waterbodies, as Scottish sites are predominantly oligotrophic. The nutrient studies described in Chapter 6 analyse growth of the species under different trophic conditions, together with possible indirect interactions with algae.

In Chapter 7 all the above aspects were integrated using two experimental competition studies to study intra- and inter-specific competition in pair-wise comparisons between the three species. These studies also provided information under controlled laboratory conditions on the effects of plant density on the creation of stress conditions.

Initially construction of a mathematical model was intended to provide further insight into competition between the three species. However, due to time constraints it was not found to be feasible. Chapter 8 provides a short summary and discussion of plant features important for inclusion in an intended future model.

Finally, in the overall discussion in Chapter 9, the results of this study are considered in relation to both the initial hypothesis of resource-mediated interference, and the ways in which traits such as a rapid growth rate, over-wintering survival and tolerance to increasing nutrient status relate to the competitive success of each of the three species.

Chapter 2 MATERIALS AND METHODS

2.1 Collection and preparation of field material

2.1.1 Plant identification

The displacement of one species by another can go largely unnoticed for a long time. This is particularly so when species are very similar in morphology, as is the case with *E. canadensis* and *E. nuttallii*. Difficulties in identification can sometimes cast doubt on the validity of historical data. A dramatic increase in the percentage of canal sites surveyed containing *E. nuttallii* during the early 1980's (i.e. Fig. 1.2) is probably partially due to increased awareness of the presence of a second species rather than a biological factor. Examination of vegetative material has highlighted problems in using morphological features for identification. In comparing leaf width and leaf length of *E. canadensis* and *E. nuttallii,* Catling and Wojtas (1985) found distinct overlap in the dimensions of these features between the species making tem unreliable for toxanomic identification. *Elodea* spp exhibit extreme plasticity in form. Particular variation can be observed in leaf size, shape, posture, internode length and plant colour (Simpson, 1988). Flower morphology can be used to aid identification, but only pistillate plants of these species are present in the British Isles (Preston and Croft, 1997)) and floral morphology is difficult to discern in the field due to the small size of the flower. Additionally, flowering in these species is infrequent and brief (Catling and Wojtas, 1985). Identification of *L. major* is easier, as the leaves are arranged spirally and not usually in pseudo-whorls as for the *Elodea* spp. For the *Elodea* spp., leaves are actually inserted spirally on the plant stem, however, normally the leaves are grouped in threes and appear to be in whorls, consequently the term pseudo-whorls is used to describe the leaf arrangement in these species. *L. major* can resemble *E. nuttallii* in some habitats. Figures 2.1 a,b and c shows examples of field material of the three species, showing their similarities in form.

The speed with which the species displacements took place led to suggestions by some workers that *E. nuttallii* was a phenotypic variant of *E. canadensis* (Simpson, 1988). These have been disproved by Simpson (1988) on the basis of

Fig. 2.1 Photographs showing examples of the three species, plant material collected from indoor cultures.

(a) ***E. canadensis***

(b) ***E. nuttallii***

(c) ***L. major (over page)***

a)

b)

c)

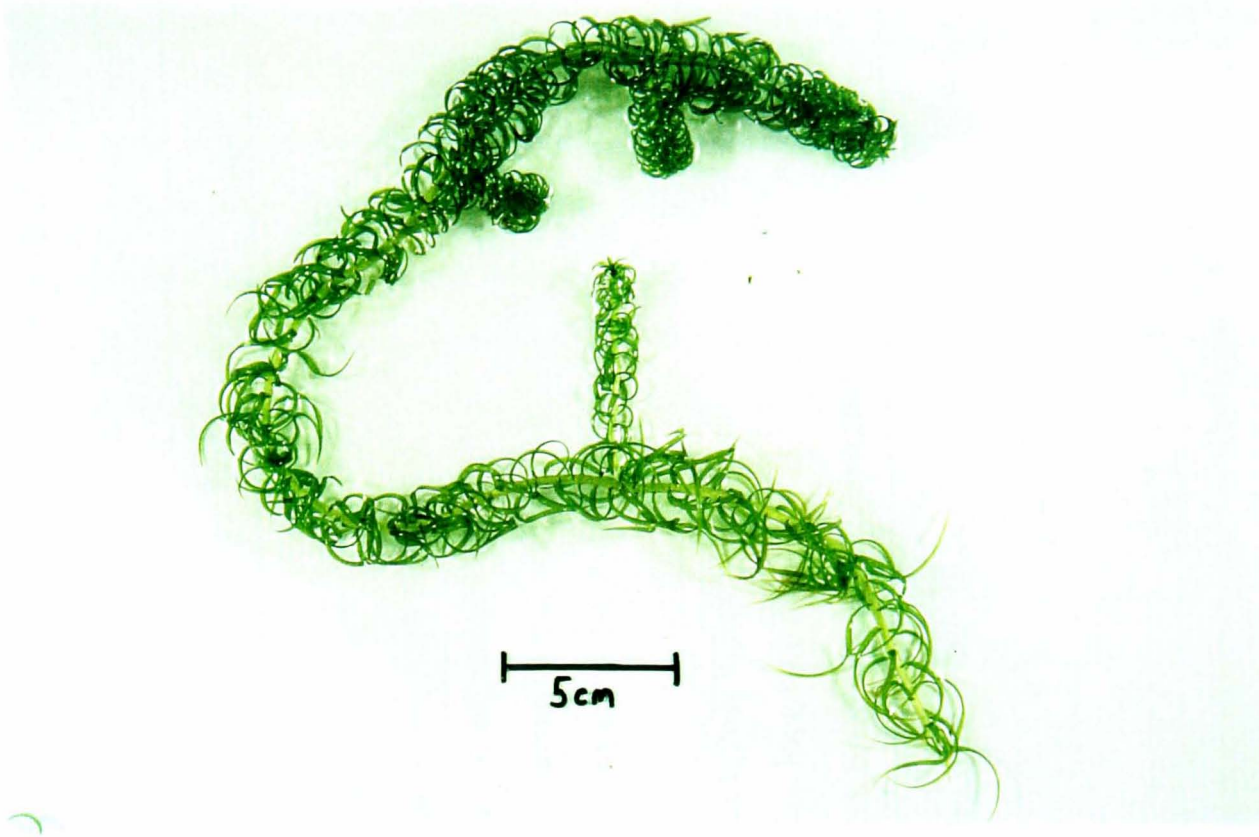


Table 2.1

Origin of plants used in experimental studies.

Species	OS Grid Ref.	Location	Code
Elodea canadensis	SJ 805 325	Pond, near Neston	Ec N
	SJ 262 206	Walls Bridge, Montgomeryshire Canal	Ec WB
	SJ 250 105	Abbey Barns, Montgomeryshire Canal	Ec AB
Elodea nuttallii	SD 384 004	Leeds and Liverpool Canal, near Melling	En M
	SD 460 171	Leeds and Liverpool Canal, Rufford Branch, near Rufford	En R
	SJ 252 145	Burgedin Locks, Montgomeryshire Canal	En BL
Lagarosiphon major	SJ 676 809	Pond at Arley Hall, Cheshire	Lm AH
	SJ 343 922	Stanley Dock Branch, Leeds and Liverpool Canal	Lm SD

distinct morphological differences between the species when grown under a range of environmental conditions. The following species descriptions, compilated from St. John (1965), Simpson (1986) and Stace (1991), are used for the identification of the three species in the field during this study

E. canadensis

A submerged aquatic species with lengthy stems up to 3 m. Leaves positioned in pseudo-whorls of three, linear to oblong lanceolate in shape, 4.5 - 17 by 1.4 - 5.6 mm, leaf apices obtuse or broadly acute (0.7) 0.8 - 2.3 mm wide, 0.5 mm behind the apex of the leaf.

E. nuttallii

Similar to *E. canadensis* in morphology with stems up to 3 m, leaves linear or linear lanceolate in shape, varying considerably in length and width 5.5 - 35 by 0.8 - 3 mm, positioned in whorls of 3 (4), often twisted and strongly recurved. Leaves on the lower stem decussate. Leaf apices acute or narrowly acute 0.2 - 0.7 (0.8) wide 0.5 mm below the apex.

Lagarosiphon major

A submerged species with lengthy branched stems up to 3 m. Linear leaves 6 - 30 by 1.3 mm, arranged spirally, recurved and denticulate.

2.1.2 Plant collection

All plant material used in growth experiments was collected from the locations specified in Table 2.1. Material was collected using a grapnel and returned to the laboratory in large plastic bags filled with canal water. The plants were then cleaned carefully to remove epiphytic algae and marl from their surfaces and stored in large buckets in a temperature-controlled water tank or growth room (For conditions see Section 2.2.2)

2.1.3 Sediment

Sediment for plant culturing was collected using a Petersen Benthic Grab from near Lydiate, Lancashire (Grid reference SD 373 059) on the Leeds and Liverpool Canal. Being fine with few stones, the sediment did not require sieving. Any large pieces of debris were removed by hand before the sediment was stored frozen to a minimum temperature of -18 °C. When needed, the sediment was left to defrost for at least 24 hours before use.

2.2 Experimental protocol

2.2.1 Growth media

For all studies except when otherwise specified, tap water was used as the medium for growth. Some chemical characteristics of the tap water are described in Appendix I. *E. nuttallii* was found by Ozbay (1998) to grow successfully in this medium. The tap water was aerated for 24 hours prior to use to allow dechlorination to occur.

2.2.2 Mass culturing of plant material

To maintain a constant supply of plant material, mass cultures were set up in 50 l plastic containers out doors at the University of Liverpool. Each container was supplied with a layer of canal sediment 10 - 15 cm deep, then filled with aerated tap water. A number of healthy shoots were planted in each container. Regular harvesting of material ensured a constant supply of shoots for experimental work.

2.2.3 Nursery culturing

Preliminary tests showed that growth in cultures was extremely variable. Analysis of the results revealed that some shoots had exhibited no growth and in many cases had disintegrated completely. This was possibly a consequence of collection and handling prior to the culturing, rather than the effects of the treatments themselves.

To avoid confounding the effects of the treatment with the effects of handling, a standard nursery culturing method was developed. For this procedure, which was used before all growth experiments except when stated otherwise, 10 cm lengths of each plant were selected. More shoots than required were selected to allow for death of some replicates and for sub-sampling (see Section 2.3.2) at the start of experiments. Single shoots, unless otherwise stated, were planted in 250 ml circular plastic cups (height 9 cm and average width 6 cm) filled with canal sediment to 0.5 cm below the rim (approximate volume of sediment = 225 cm^3) and placed in large containers (10 or 50 l) filled with previously aerated tap water. The containers were then placed in one of three thermostatically controlled water tanks or growth rooms and plants cultured for a minimum period of two weeks (Growth conditions: 70 – 100 $\mu mol\ m^{-2}\ s^{-1}$, 16:8 light:dark cycle and 15 °C). From the nursery stocks, for each experiment only healthy, established shoots were selected for experimental use.

2.2.4 Experimental conditions

Experiments were carried out using either 3 l glass jars (Fig. 2.2 a) or 10 l green plastic buckets (Fig. 2.2 b) as specified. Container type was determined according to the size of the experiment and the number of replicates needed. All growth experiments took place in three thermostatically controlled water tanks. Temperature was maintained at 15 ± 2 °C unless otherwise stated. Light (PAR 400 – 700 nm) was supplied by fluorescent tubing, with a 16:8 light : dark cycle. Light measurements were taken 1 cm below the water surface in the centre of each container using an underwater light probe (Macam Quantum Radiometer/ photometer Q101). Light levels ranged between 70 $\mu mol\ m^{-2}\ s^{-1}$ and 106 $\mu mol\ m^{-2}\ s^{-1}$ in 3 l glass jars, with an average of 84.6±1.8 $\mu mol\ m^{-2}\ s^{-1}$, and 48.6 $\mu mol\ m^{-2}\ s^{-1}$ to 91.8 $\mu mol\ m^{-2}\ s^{-1}$ in 10 l green buckets with an average of 64.9±3.9 $\mu mol\ m^{-2}\ s^{-1}$. Although statistical analysis revealed a significant difference in light levels between the three tanks, extremely low within tank variability may account for this, as the difference between tank means was less than 10 %.

Fig. 2.2 Photographs showing experimental set up in the three thermostatically controlled water tanks.

(a) 3 l glass jars set up in a tank with the aeration system in place (from Temperature Study 1, Chapter 4).

(b) 10 l green plastic buckets set up in a tank (from Competition Study 2, Chapter 7).

a)

b)

Table 2.2
Physical and chemical monitoring

Measurement	Method	Details
pH	pH electrode	Orion (model SAS20) Camlab (model pH Boy-P2)
Conductivity	Conductivity meter	pHOX 52E (pHOX systems limited, Shefford, Bedfordshire)
Oxygen	Oxygen meter	OXI 126 (Wissenschaftlich Technische Werktatten, Wiellheim, Germany)
Alkalinity	Mackereth et al. (1989)	Titration of 25 ml water sample with 0.01 HCl to pH 4.5
Nitrate-nitrogen	Mackereth et al. (1989)	A colorimetric method in which nitrate is reduced to nitrite using spongy cadmium, and reagents added to form diazomium salt which reacts with an aromatic amine to form red azo-dye.
Ammonium-nitrogen	Chaney and Morbach (1962)	A colorimetric method in which ammonium reacts with phenol and hypochlorite, catalysed by nitroprusside, to form Indophenol-blue
Total Soluble (TSP) and Total Phosphorus (TP)	Mackereth et al. (1989)	A colormetric method in which phosphorus is first hydrolysed in acid and then reacted with molybdate to form molybdo-phosphoric acid which is then reduced to form a blue molybdenum complex.
Soluble Reactive Phosphorus (SRP)	Mackereth et al. (1989)	Same as for TP and TSP except without first hydrolysing in acid.

2.2.5 Monitoring

During growth experiments various chemical water parameters were measured, as shown in Table 2.2. Where necessary, water was filtered through glass fibre filter paper (Whatman GF/C) before analysis. Details of actual monitoring regimes are given in individual experiments.

2.3 Assessment of growth

2.3.1 Introduction

Growth of plants may be measured using various parameters such as dry weight, fresh weight, number of apical meristems and shoot length. In order to calculate growth parameters such as Relative Growth Rate (RGR), some measurement(s) of growth must be made on at least two occasions. Dry weight change is generally used to measure overall growth. However, it is by its nature destructive hence it is impossible to measure the dry weight of the same individuals more than once. Other measurements, while not in themselves destructive e.g. fresh weight analysis or length, often require excessive handling of plant material. This is likely to lead to damage of apical meristems and plant structure and thus may be detrimental to subsequent plant growth. If dry weight change is used in assessing the growth of a plant, it is necessary to approximate the initial weight. This can be done by a) approximating initial dry weight from fresh weight of shoots, b) approximating initial dry weight from length of shoots or c) taking, and dry weighting, a representative sample of the starting material (sub-sampling). In order to determine a baseline for the measurement of growth rates and other growth parameters, measurements were made to assess the accuracy of using length, fresh weight and sub-sampling to approximate initial dry weight. Length to dry weight ratios were only determined in the present study for *E. canadensis* and *L. major,* as in previous studies, Birch (1990) found a highly significant linear relationship between length and dry weight of *E. nuttallii* ($r = 0.95$), which was therefore used in this study. In addition photosynthetic surface area to dry weight relationships were determined for each of the species to be used in subsequent studies.

2.3.2 Methods

2.3.2.1 Fresh weight to dry weight relationship

For determination of fresh to dry weight ratios, twelve shoots each of *E. nuttallii*, *E. canadensis* and *L.major* were selected at random from larger samples. For this study plant material was collected from collected from Ec WB, En M and Lm L (see Table 2.1 for site details), for *E. canadensis*, *E. nuttallii* and *L. major* respectively. Each shoot was spun for 30 seconds in a spin drier and fresh weight was measured immediately afterwards. The shoots were then dried to a constant weight at 70°C and weighed.

2.3.2.2 Length to dry weight relationship

The length to dry weight relationships were determined by selecting 30 random shoots including an intact apical meristem of *E. canadensis* and *L. major*. The total length of each shoot was measured including branch lengths, and the shoot was then dried at 70°C to a constant weight. For *E. canadensis*, this investigation was performed on material collected from two sites (Ec AB and Ec WB), where the species was seen to have distinctly differing morphologies. Plant material collected from Ec AB consisted of shoots with a compact form, where leaves were observed to be very similar in size and morphology, and situated in pseudo-whorls close together on the main stem. Material collected from Ec WB was visually more variable with larger leaves varying greatly in size and morphology and distance between consecutive pseudo-whorls also varying greatly. Material of *L. major* was collected from Lm L. In previous studies (e.g. Birch, 1990; Jones, 1994; Ozbay, 1998) 10 cm shoot lengths with an intact apex and no visible side shoots have been used as starting units for laboratory experiments. For comparisons between the species and as a measure of the variability in dry weight between shoots of the same length (i.e. 10 cm lengths) the dry weight of 20 10 cm lengths of each species was also measured.

2.3.2.3 Sub-sampling

For sub-sampling of plant material at the start of each experiment it is

necessary to know the minimum number of replicates needed to reduce the standard error to an acceptable level. For this, eight 10 cm shoots of each species were grown under the standardised procedures for nursery culturing as described. After two weeks growth the shoots were harvested and dried to a constant weight at 70 °C. The standard error of the dry weight means was then determined with increasing replication.

2.3.2.4 Photosynthetic surface area to dry weight relationship

For calculation of leaf area, a 3 cm length from between 4 and 7 cm below the shoot apex was selected. Leaf area was calculated by removing 9 leaves from the shoot section, 3 from the upper whorl (closest to the apical meristem), three from mid way along the section, and three from the lower whorl. These were then photocopied and cut out under a dissecting microscope using a scalpel, the paper weighed and converted to a surface area using a conversion factor previously calculated from known sized segments of photocopied paper. This value was then multiplied by two to take into account both sides of a leaf. For the calculation of stem surface area, the stem diameter was measured under a standard, binocular microscope using a numerically scaled graticule. Stem surface area was then calculated as the surface area of a cylinder. Leaves from 10 replicate stems of *E. nuttallii* and *L. major* and 13 replicates of *E. canadensis* were measured. Following the photocopying, shoot samples were dried to a constant weight at 70 °C and re-weighed.

2.3.3 Results and discussion

All three species showed a strong linear correlation between fresh weight and dry weight (Fig 2.3), with mean values for dry weight as a percentage of fresh weight of 15.40, 12.45 and 13.87 for *E. canadensis*, *E. nuttallii* and *L. major* respectively. For length to dry weight ratios of the species examined in this study, *E. canadensis* and *L. majo*r, showed significant linear correlations (Fig. 2.4). *E. canadensis,* collected from Abbey Barns (Ec AB) where the plant material was visibly more uniform and compact with a smaller leaf size, showed a stronger correlation (Fig 2.4 a) (with a correlation coefficient (r value) of 0.93) than material collected from Walls

Bridge (Ec WB) (r = 0.52), which displayed distinct variation in morphology (Fig. 2.4 b). Previous authors (e.g. Birch, 1990) have used length as a measure of growth in studies of single species. In the current study where three species are being compared, growth measurements based on increase in length alone do not allow for differences in structure e.g. stem diameter and leaf size, between species. For comparative purposes therefore, measurement of dry weight is more appropriate. However, this introduces the problem mentioned

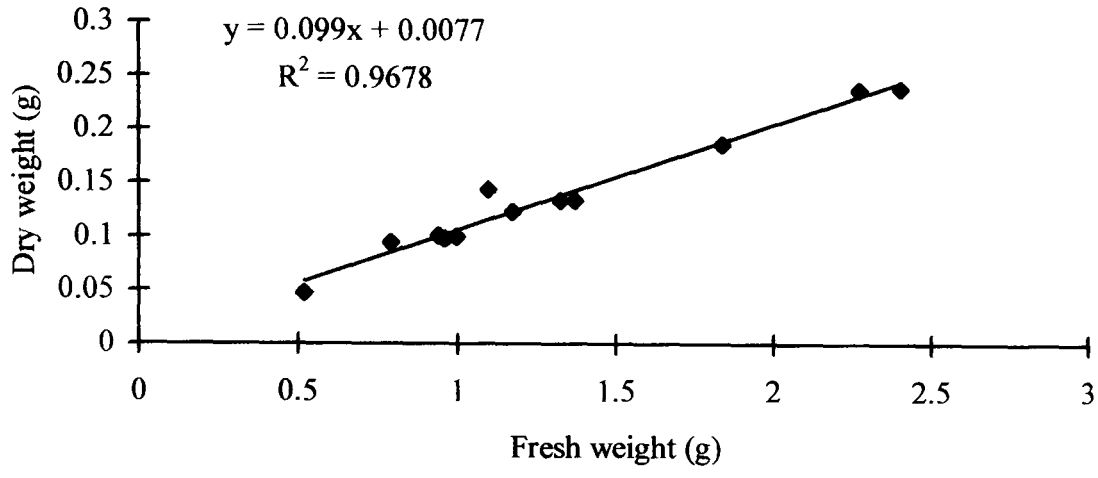
Table 2.3

Showing characteristics of 10 cm lengths of plant material. Error is expressed in brackets as 95 % confidence limits. (n = 20)

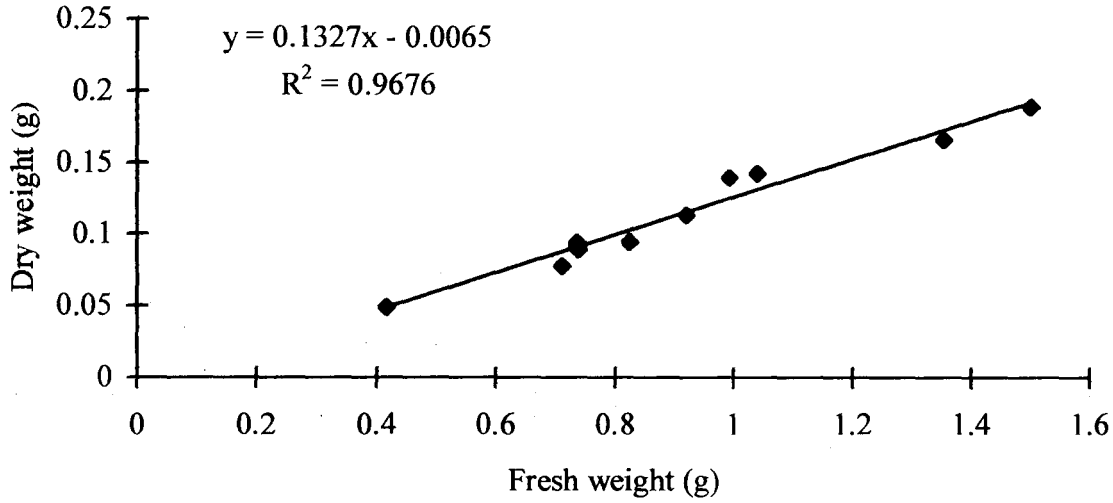
Species	Fresh weight (g)	Dry weight (g)	Dry weight as % of fresh weight
E. canadensis	0.280 (0.027)	0.029 (0.004)	10.268 (0.596)
E. nuttallii	0.186 (0.037)	0.018 (0.004)	9.692 (0.509)
L. major	0.679 (0.079)	0.080 (0.009)	11.839 (0.441)

earlier concerning estimation of initial biomass. Fresh weight may be used as an approximation as a highly significant linear relationship was found between fresh weight and dry weight (Fig. 2.3). However, removal of excess water by shaking manually or, in particular, by using a spin drier is likely to result in damage to the shoot. Length did appear to be an accurate measure of dry weight (Fig. 2.4), particularly for the shorter 10 cm shoot lengths. However, in situ measurements of length following nursery culturing is awkward and may result in damage or breakage of brittle shoots. From the sub-sampling data it was apparent that for all species standard error was reduced to approximately 25 % of the mean with only four replicates (Fig. 2.5). Since increased replication produced no significant reduction in error, 4 replicates was deemed the minimum number of sub-samples needed. However, where possible, due to the sometimes variable nature of the plant material, more replication should be used. From the results of length to dry weight ratio comparisons and sub-sampling data, it was decided that for initial selection of starting units for nursery culturing, a 10 cm shoot length would be used with an intact

(a)



(b)



(c)

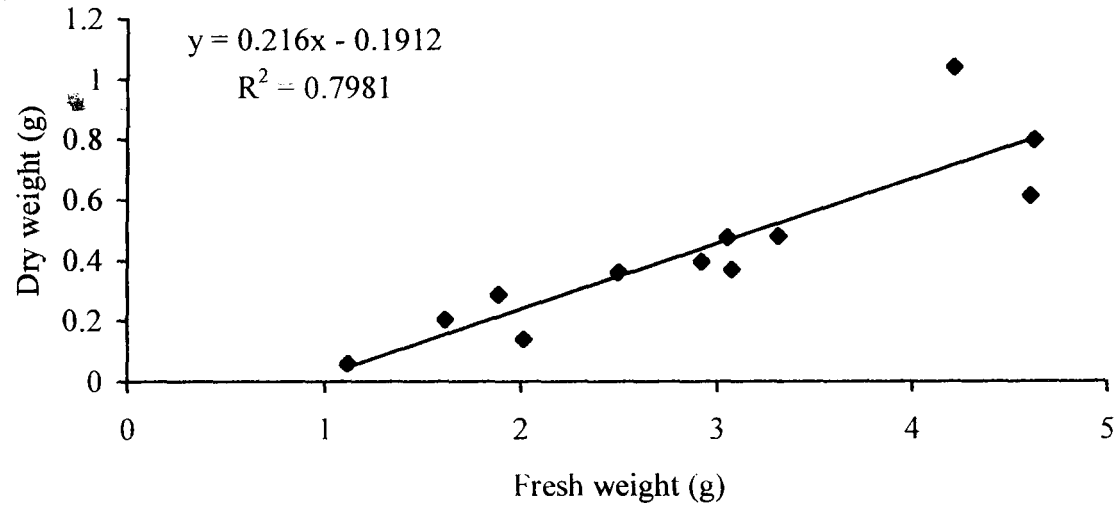
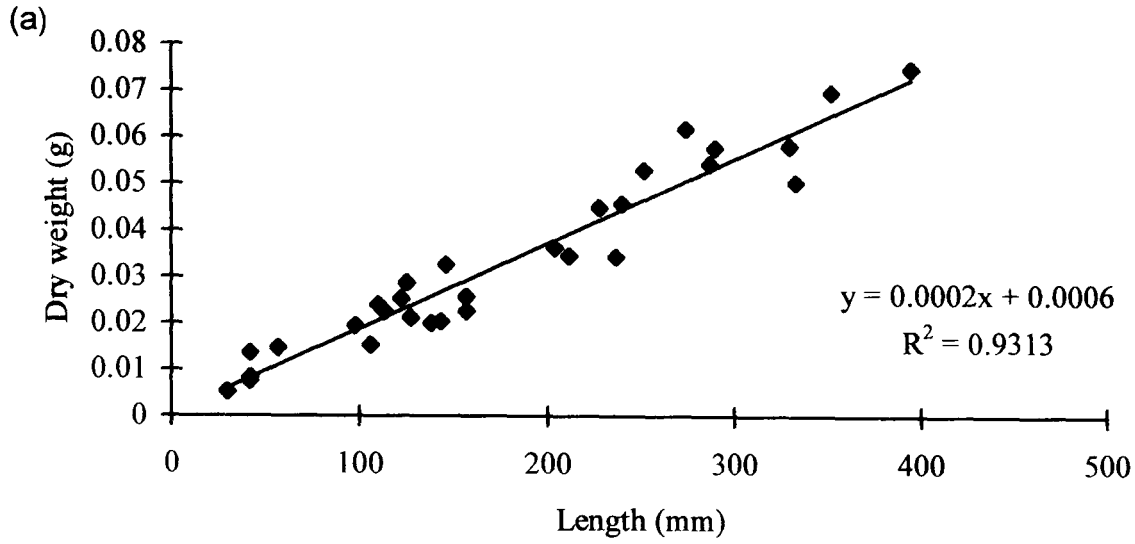
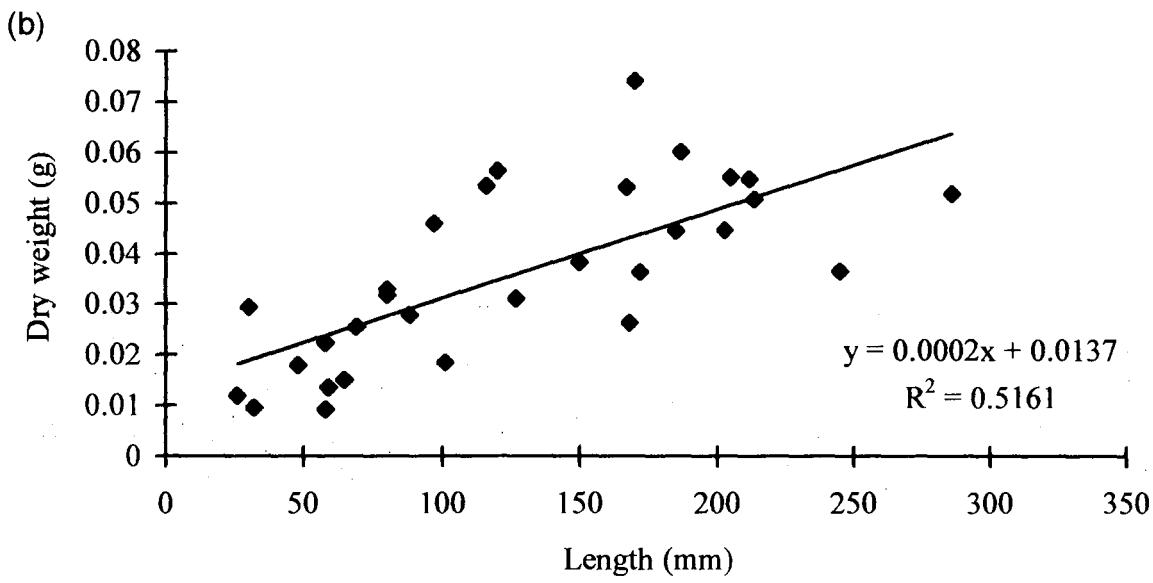


Fig 2.3 The relationship between fresh weight (g) and dry weight (g) of (a) *E. canadensis* collected from Ec WB, (b) *E. nuttallii* collected from En M, and (c) *L. major* collected from Lm L. (n = 12)





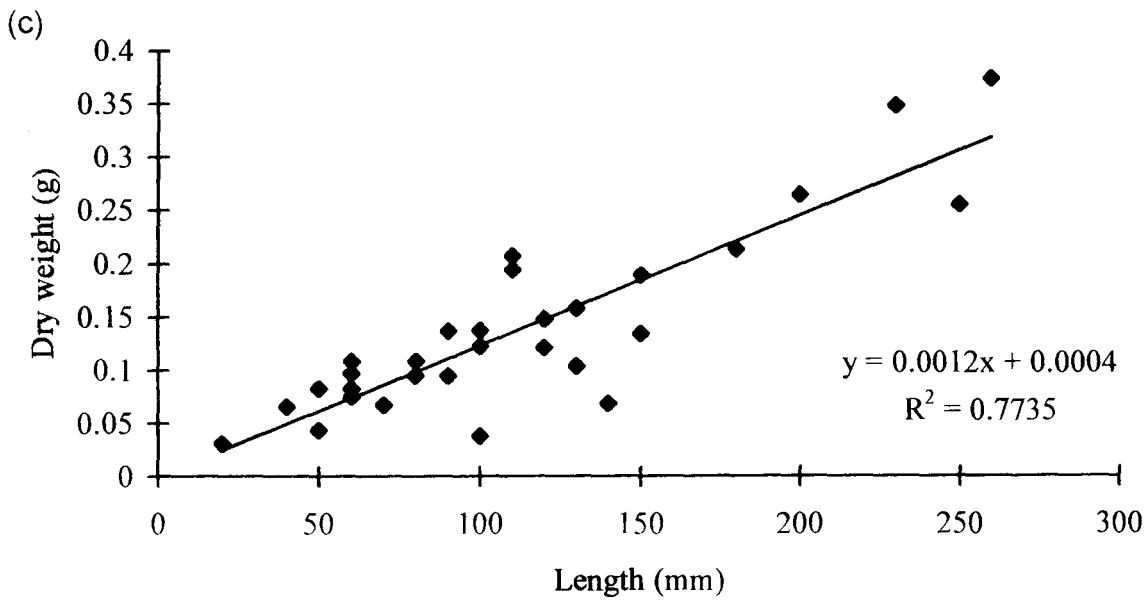


Fig. 2.4 Relationship between length of shoot (mm) and dry weight (g) for (a) *E. canadensis* collected from EcAB, (b) *E. canadensis* collected from EcWB, and (c) *L. major* collected from Lm AH. (n=30)

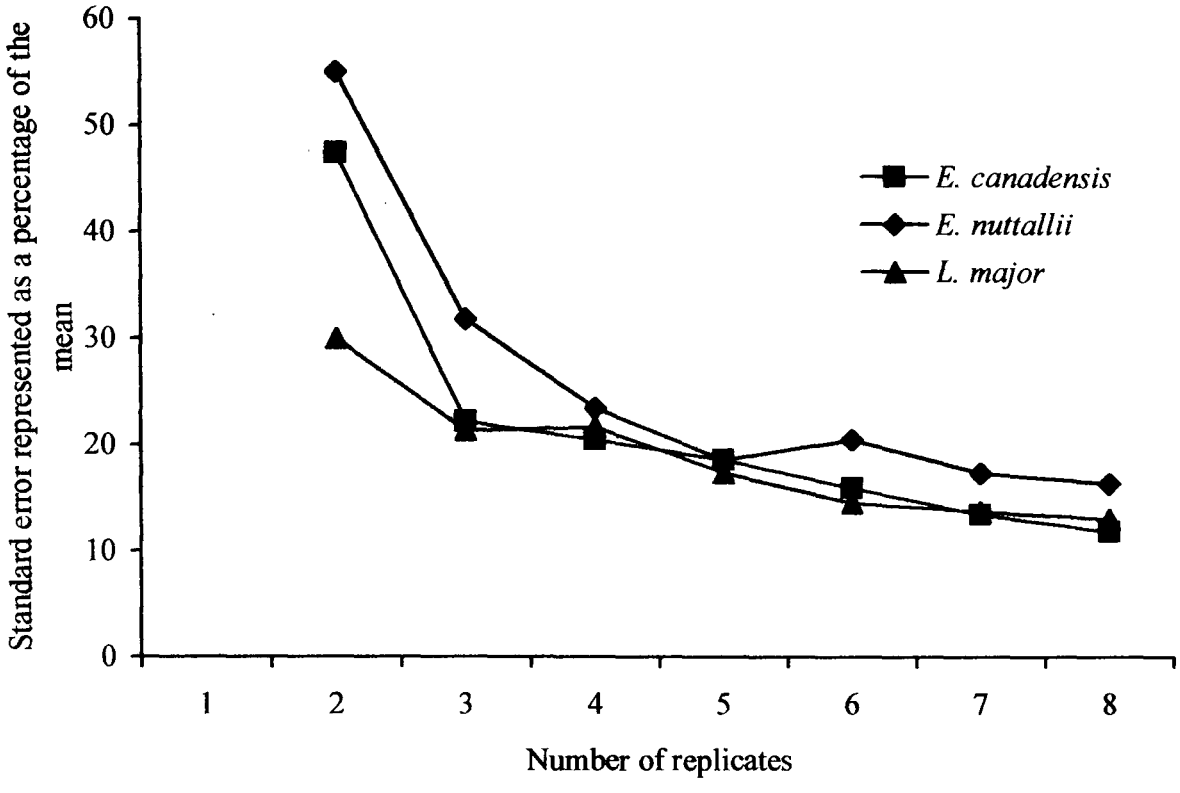


Fig. 2.5 Graph showing standard error as a percentage of the mean dry weight of nursery cultured shoots with increasing numbers of sub-samples (replicates).

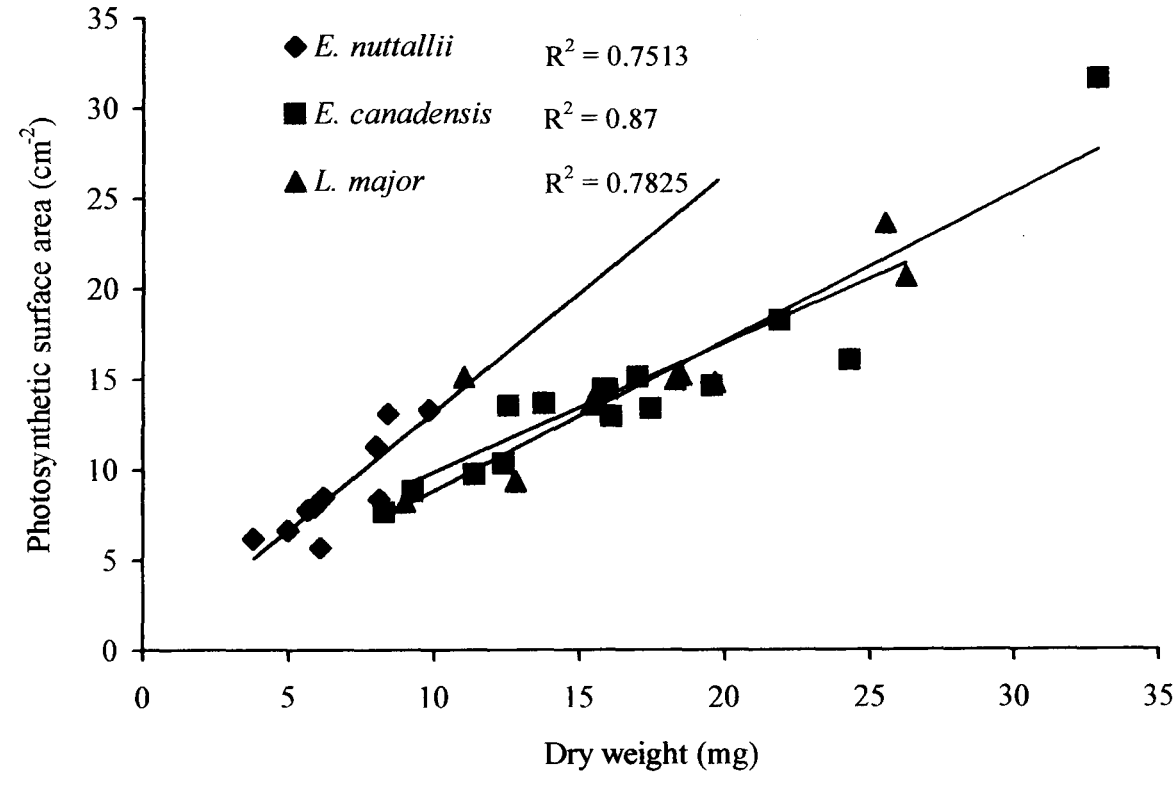


Fig. 2.6 Relationship between dry weight (mg) and photosynthetic surface area (cm^{-2}) of the three species. (n = 10 - 13)

apical tip and no significant branching. Following nursery culturing, a sub-sample of the cultured shoots (minimum of four replicates) would be taken and used to approximate the initial starting weight at the beginning of each experiment.

Photosynthetic surface area (leaf + stem surface area) graphed against plant dry weight revealed a linear relationship between the two parameters (Fig 2.6). Table 2.4 provides equations used for conversions of data in subsequent chapters.

Table 2.4

Estimation of photosynthetic surface area (PSA) (cm^2) from dry weight (mg).

	Conversion factor	Correlation coeff.
E. canadensis	PSA (cm^2) = 0.5724 (mg dw) – 4.2004	$r^2 = 0.79$
E. nuttallii	PSA (cm^2) = 1.3095 (mg dw) – 0.0946	$r^2 = 0.75$
L. major	PSA (cm^2) = 0.7085 (mg dw) – 2.7774	$r^2 = 0.78$

2.4 Growth analysis

Relative growth rates were calculated using the following standard formula (Hunt, 1978):

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

Where W_1 is the starting dry weight (sub-sampled), W_2 the final dry weight, T_1 the starting time (in this case 0) and T_2 the number of hours after the start of the experiment.

2.5 Determination of photosynthetic and respiratory rates

Photosynthetic and respiratory rates were measured as oxygen uptake or evolution using a Clark type O_2 electrode as described by Jones (1994). A tungsten

slide projector bulb provided incident light and temperature was controlled using a water jacket fed from a thermostatically regulated water bath. Unless otherwise stated light and temperature were maintained at 290 μmol m^{-2} s^{-1} PAR, sufficient to saturate photosynthesis, and 15 °C respectively. Previous studies on rates of photosynthesis have shown a decrease in photosynthetic rate and an increase in respiration with increasing distance of leaves from the shoot apex (Simpson, 1981; Birch, 1990). Following the work of Jones (1994), Birch (1990) and Simpson (1981), fully expanded leaves, from 3 cm below the apex were used, unless stated otherwise. These leaves would be of a similar age, although differences in growth rate both between and within the species can not be accounted for. In previous studies with *E. canadensis* and *E. nuttallii*, three leaves have been used to determine rates (Simpson, 1981; Birch, 1990; Jones, 1994). From preliminary studies (Appendix II) on the effect of leaf number on photosynthetic and respiratory rates, it was found that one pseudo-whorl, comprising three leaves of *E. canadensis* or *E. nuttallii*, and in the case of *L. major* two neighbouring leaves were optimal. Above these numbers variability in calculated rates increased and photosynthetic rates appeared to decrease slightly, possibly as a result of self-shading within the electrode chamber. Cumulative means tests, used to determine the appropriate number of replicates, showed that to reduce standard error to within 15 % of the mean for all species, 6 replicates were required. (Appendix II).

2.5.1 Procedure

For measurements of photosynthetic and respiratory rates, the leaves were detached carefully and placed in 1.5 cm^3 of modified Forsberg II solution (Forsberg, 1965). In the preparation of the Forsberg II solution, a similar procedure to that of Jones (1994) was adopted. Unless stated otherwise, the solution was maintained at pH 7 by the addition of Tris Buffer (NaOH) at 0.5 M. To initiate photosynthesis, 0.1 cm^{-3} of $NaHCO_3$ (the carbon source) was added. This gave a final inorganic carbon concentration of 2.4 mM, the measured concentration of dissolved inorganic carbon (DIC) in the Leeds-Liverpool canal (Jones, 1994). Respiratory rates were measured (as dark respiration) by covering the electrode in foil and black plastic to prevent light penetration. Both photosynthetic and respiratory rates were measured over 10 to 20

minute periods. Rates of photosynthesis and respiration were then calculated as oxygen uptake or evolution (mg) respectively, per unit chlorophyll (g), per unit time (minute).

2.5.2 Chlorophyll estimation in leaves

Total chlorophyll content (μg) of leaves was determined following the basic method of Arnon (1949), using acetone extraction, but modified as suggested by Porra (1991) to measure chlorophyll a at a wavelength of 665 nm rather than 663 nm. Leaves were macerated in a few drops of ice-cold acetone and the sample was made up to approximately 5 ml, and was left refrigerated over night. The following day the samples were shaken, made up to exactly 5 ml, and then centrifuged at 3000 rpm for five minutes. The supernatant was then removed carefully and spectrophotometer readings taken at 645 nm, 665 nm and 750nm. Absorption values were then substituted into the following equation:

$$\text{Total chlorophyll a + b} = 5 * [(20.20 * (\text{abs645} - \text{abs750})) + (8.02 * (\text{abs665} - \text{abs 750}))]$$

2.6 Chlorophyll *a* assessment for phytoplankton

Phytoplankton growth was assessed by filtering a known volume of water through a Whatman G/C glass fibre filter paper. The filter papers were then macerated in a few drops of ice-cold acetone, made up to 5 ml and left overnight. The next morning samples were shaken, centrifuged for five minutes at 3000 rpm and the supernatant removed. Spectrophotometer readings were made at 665 and 750 nm and chlorophyll *a* concentration (μg) estimated using the following equation:

$$\text{Total Chlorophyll a} = 5 * [8.02 * (\text{abs665} - \text{abs750})]$$

2.7 Statistical analysis

Unless otherwise stated, all results were analysed using Analysis of Variance techniques. Where appropriate, least significant differences were calculated using Tukey's multiple comparison test. For comparison of regression lines, differences in slope and elevation were analysed as described by Zar (1996).

Chapter 3 GROWTH AND ARCHITECTURE

3.1 Introduction

The basic anatomies of the *Elodea* spp. and *L. major* are similar. The leaves of these species consist of two epidermal layers that form the majority of the leaf thickness. A few mesophyll cells are present, situated around the vascular bundle, which forms a single vein running through the centre of the leaf. Vascular tissue is, as in many hydrophytes, reduced, with a characteristic reduction of xylem in particular. Numerous small air spaces can be found throughout the leaf. The presence of sclerenchyma in the leaves of *L. major,* both on the margin and close to the central vascular bundle, may provide additional support to the leaves of this species (Triest, 1982). The stem has a single layer of epidermal cells with a few layers of collenchyma tissue beneath this. A reduction in supporting tissue such as collenchyma is characteristic of many hydrophytes. The support provided by water alleviates the need for mechanical strengthening and rigidity. Air cavities (lacunae) distributed throughout the cortex provide buoyancy and may facilitate gas exchange. These are regularly spaced in *L. major*, but irregularly in *Elodea* spp.. Diaphragms (cross partitions) block the lacunae at each node, where a leaf is attached. An endodermis surrounds the vascular tissue which consists of a central protoxylem lacuna and close to the endodermis, some metaxylem and phloem elements (Ancibor, 1979). The roots are of a similar construction to the stem, with a suberised exodermis, a cortex consisting of peripheral layers of collenchyma and a central vascular cylinder. Root hairs are produced on contact with the sediment (Ancibor, 1979).

The development of the apical meristem occurs by cell division immediately below the shoot tip. The shoots elongate by extension of the internodes, consequently leaf age increases with distance from the apical tip. Leaf size of *Elodea* spp. varies little down the stem and it is apparent that the majority of leaf expansion occurs within 1 cm of the apical tip (Simpson, 1981; Birch, 1990). Lateral buds are produced approximately every 7 nodes in the case *of Elodea* spp. and every 10 to 12 nodes for *L. major*. The branching of both *E. canadensis* and *L. major* are described by Ancibor (1979) and agrees with observations made in the present study. A bud (B1) develops in the axil of one leaf on the main branch (B0) and this develops into a new branch

(Fig. 3.1). The first three nodes of the new branch have opposite pairs of scale leaves. A lateral bud (B2*) is present in the axial of one of the scale leaves at the first node of the new branch, and subsequently buds (B2) occur approximately every 7th node. For the purposes of this study, if the first bud (B2*, Fig 3.1) produced in the scale leaves of a branch develops, this is termed secondary branching (B2* is also included as a second order branch). The roots are adventitious and unbranched and occur at the same nodes as those that develop axillary buds. One root is produced per axillary bud in the case of *Elodea* spp. and numerous roots in the case of *L. major*. It is unknown to what extent a newly developing apical meristem is dependent

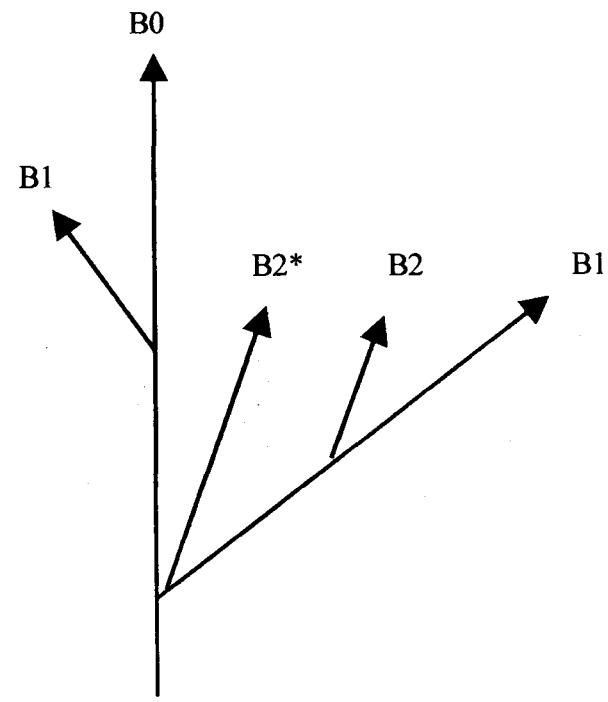


Fig. 3.1 Diagram showing branch ordering of a generalised *Elodea/Lagarosiphon* shoot. From the main branch (B0), two first order branches arise (B1) and from the first order branching, two second order branches arise (B2). Secondary branching (B2*) is also shown in the axial of the first lateral branch (B1). Drawn from personal observations of the author and those of Ancibor (1979).

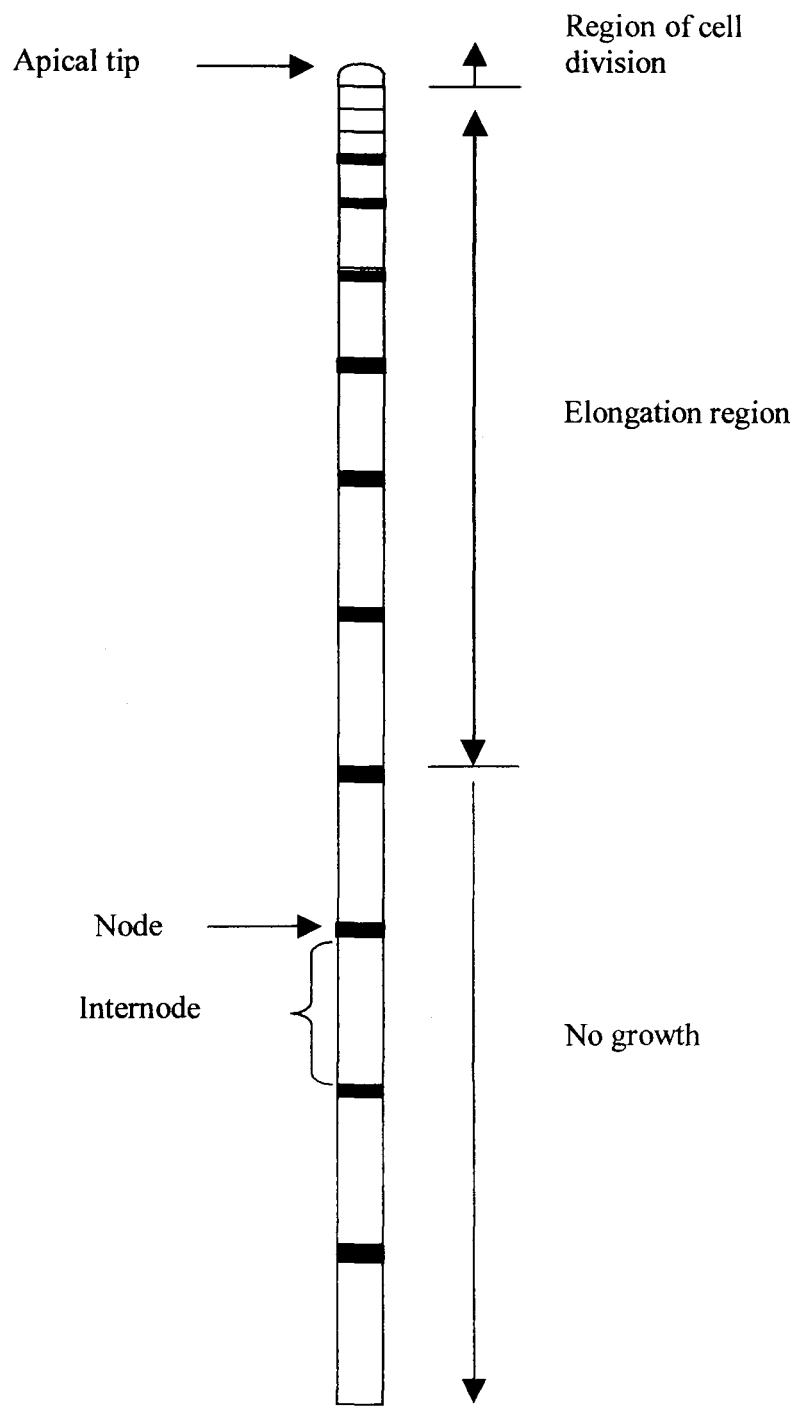


Fig. 3.2 Figure showing activity of different regions of shoot after Birch (1990).

upon internal transport of resources from other parts of the plant. Extremely short sections of shoot (5mm in length) of *E. nuttallii* can, if including an apex or lateral bud, develop into a new plant (Birch, 1990), indicating that newly developing apical meristems of *E. nuttallii* may become self-sufficient very rapidly.

Birch (1990) describes a shoot of *E. nuttallii* as having an active apical tip region below which there is a length of stable mature stem that supplies metabolites to growing regions (Fig. 3.2). Below this is a region of largely older, inactive material that simply holds the upper, active shoots in place. The actively growing part of the plant is characterised by positive net photosynthesis of its leaves. In progressively older parts photosynthesis declines and respiration increases until at some point along the stem respiration exceeds photosynthesis and the leaves begin to die. Once a dense canopy is in place, particularly later in the growing season, the actively growing region will be restricted by self-shading to plant material that is receiving sufficient light for positive net photosynthesis.

Within the aquatic environment, light is described as a major limiting factor on the growth of many submerged macrophytes. Light reaching the water surface may be reflected back, or absorbed by the water and substances in it, the photosynthetic biota (phytoplankton, and macrophytes) and suspended particulate matter (Kirk, 1994). Absorption due to photosynthetic biota is extremely variable and will depend largely upon the growth form and morphology of the species (Kirk, 1994). Floating species and dense stands of submerged macrophytes can virtually block out light altogether. Previous studies (i.e. Van *et al.*, 1977) found that different light wavelengths promoted shoot elongation and branching of *H. verticillata.* While shoot elongation was promoted by green light which penetrates deeper into the water body and inhibited by red, branching was promoted by red light and inhibited by green. Thus, at least in this species, light quality may in part be responsible for the observed growth patterns observed in the field.

Growth forms that maximise light interception and reduce self-shading will obviously be advantageous to a species. The support provided by the water allows the development of such an architecture relatively unfettered by the support requirements

that influence terrestrial plant architecture. In the terrestrial environment, structural support of a canopy is often provided by increasing the diameter of the main axis, placing physical constraints on the growth form. Within the water environment, where the high density of water gives support, a macrophyte can theoretically develop the growth form that would be most efficient for light interception and reduced self-shading. This is synonymous with the idea that "branching and efficiency must go hand-in-hand in the control of space" (Bell, 1986). That is, to maximise resource acquisition, a plant needs not only to produce biomass, but also to produce it in an efficient way that best ameliorates the limitations of the environment.

Models for terrestrial species structure have studied branching of both shoots and roots in attempts to derive systematic descriptions of architecture (Barker *et al.*, 1973; Fitter *et al.*, 1991; Fitter and Stickland, 1992). Just as a systematic root description has been used to describe the exploitation of nutrient deposits within the soil (i.e. Fitter and Stickland 1992), a macrophyte's architecture can be assessed in terms of its ability to exploit its light environment through maximising light interception and minimising self shading. Fig. 3.3 shows the theoretical distribution of the plant biomass for a generalised *Elodea* or *Lagarosiphon* plant. An inefficient growth form may have a large proportion of the biomass concentrated towards the base of the plant, resulting in increased self-shading and therefore inefficient use of available light particularly as the canopy develops (Fig. 3.3, a). A more efficient growth form may concentrate biomass towards the upper parts of the shoot, even when the plant is relatively small in size thereby maximising light interception (Fig. 3.3, b).

Duarte and Roff (1991) emphasised the importance of differences in plant architecture in determining community structure. Different plant growth forms were described in terms of their maximum height and biomass density (BD) (defined as biomass /stand height, units g m^{-2}). They predicted that in productive habitats those species with the highest biomass densities and maximum height would dominate the

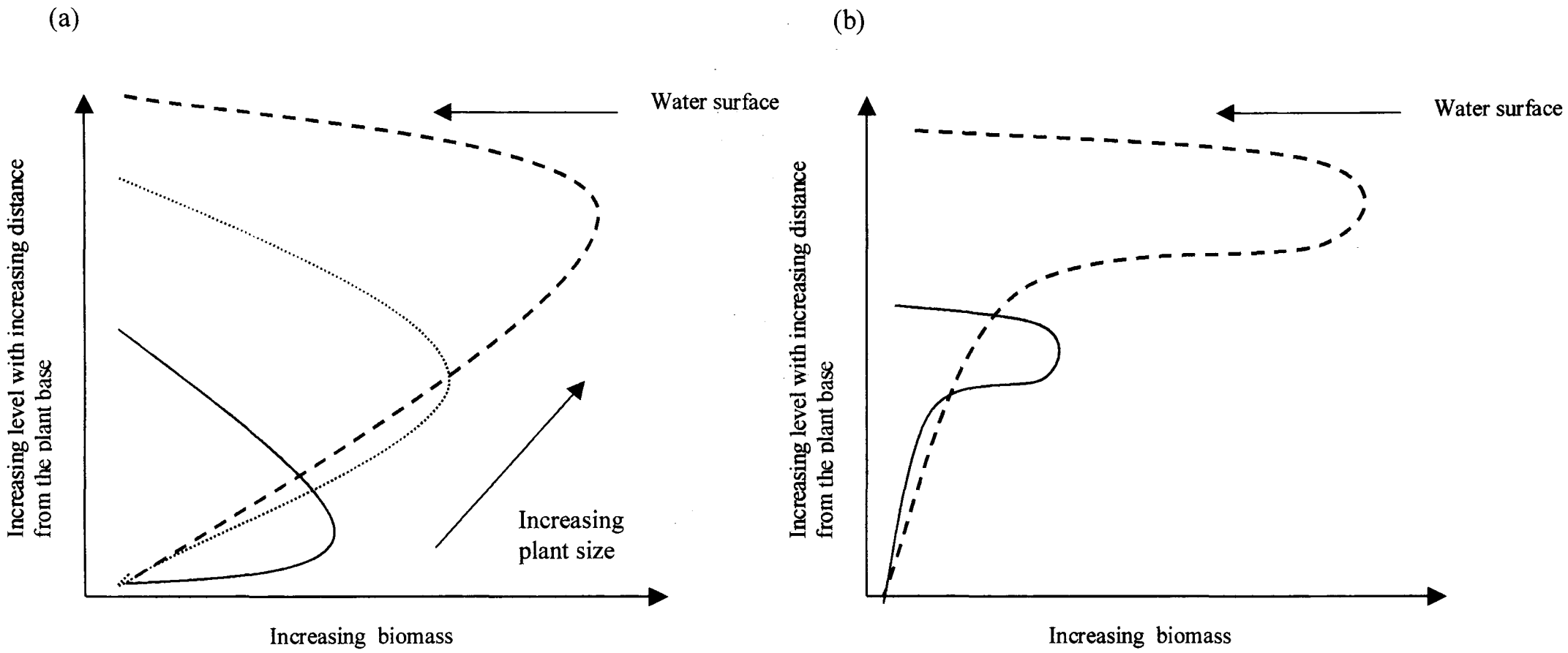


Fig. 3.3 Theoretical changes in plant densities with increasing plant size. (a) A relatively inefficient architecture in which, particularly with young plants, biomass is concentrated at the base of the plant, as plants branch form close to their bases. (b) An efficient biomass distribution in which long stems are produced and branching occurs close to the water surface.

macrophyte community. They suggested that the high BD of invasive alien species such as *Elodea* spp. and *L. major* might, in part, contribute to their success in displacing native flora. However, Duarte and Kalff (1990) commented that species with similar growth forms (e.g. *Elodea*, *Egeria* and *Hydrilla*) also have similar BD's. Therefore, for displacements between species with similar growth forms, such as those examined here, it seems unlikely that differences in total BD alone play a decisive role. During the above studies, biomass density was measured as an average for the whole water column and the potentially influential distribution of that biomass within the vertical profile was not taken into consideration. In particular, workers took no account of the ability to develop a canopy rapidly and efficiently, concentrating biomass towards the water surface, which may convey important competitive advantages particularly under light limiting conditions.

Simpson (1990) commented that the simple overgrowth of *E. canadensis* by *E. nuttallii* resulted in reduced light availability to the former. While this was attributed largely to more rapid stem elongation in *E. nuttallii*, it may also be achieved by differences in the architectural development of the two species. The actual form that a plant develops will depend upon both the position and time of branch production. Kunii (1984) described the growth of *E. nuttallii* as starting with the elongation of a single, long stem at the beginning of the growing season, with prolific branching taking place only when the stem approaches the water surface. Pokorny *et al.* (1984) describes the biomass of *E. canadensis* as being also concentrated at the water surface and similar observations have also been made for *L. major* (Schwarz and Howard-Williams, 1993). Despite the relative simplicity of this plant group where branch diameter is constant and branching position often consistent, the descriptions of macrophytes have been almost solely qualitative, with few attempts to quantify observed branching patterns. In describing maximum and seasonal changes in biomass, overall measures given in the literature provide little indication of the underlying architecture.

In order to recognise and describe branching patterns, it is necessary to define a workable construction unit or structure. A number of different unit types have been described that are both descriptive and functional (see Bell and Bryan, 1991). The

metamer is a repeatable constructional unit consisting of a node and a section of internode proximal to that node. It is descriptive and does not imply any physiological or functional connections, and is therefore one of the easiest to use for simple architectural descriptions. Metamers can be used to describe the architecture of a plant in modular terms, that is the build up of repeatable units. For descriptions of *Elodea* spp., these structural units are easily visualised, as the leaves occur in pseudo-whorls of three, spaced between internodes. For *L. major,* leaves are arranged spirally although can sometimes appear in pseudowhorls, and internodes are often extremely short. Metamers may be useful in the construction of a mathematical model, as they present an easily measurable structure. They can be grouped together into branching and inter-branch zones or grouped further still into branching orders. P. Gould and J. W. Eaton (unpublished data) have developed an architectural model for *E. canadensis* to describe its branching pattern. The plant is described in both orders and levels, enabling each branch to be uniquely labelled (Fig. 3.4). Ordering describes the lateral branching pattern, 0 order being the first or main axis, 1st order branches arise from the 0 order branch, 2^{nd} order branches arise from the 1^{st} order branches, and 3^{rd} order branches arising from the 2^{nd} order branches and so on. This architectural description has some similarities with geographical descriptions of rivers. Levels describe the points of insertion of a branch. As branches of *E. canadensis* occur every 7 nodes, an idealised plant can be divided into vertical levels, with each level consisting of 7 nodes and a branch position (Fig. 3.4). Each plant can then be described in terms of levels and orders. Practically however, variation in inter-branch distances within a single plant will result in changes in the height of levels, confounding overall measures of vertical biomass distribution. Kunii (1984) described the vertical profile structure of *E. nuttallii* by sectioning each plant into 10 cm vertical zones. Similarly, Schwarz and Howard-Williams (1993) divided the vertical biomass of *L. major* into 50 cm zones. This method was also used for the calculation of vertical profiles in the present study.

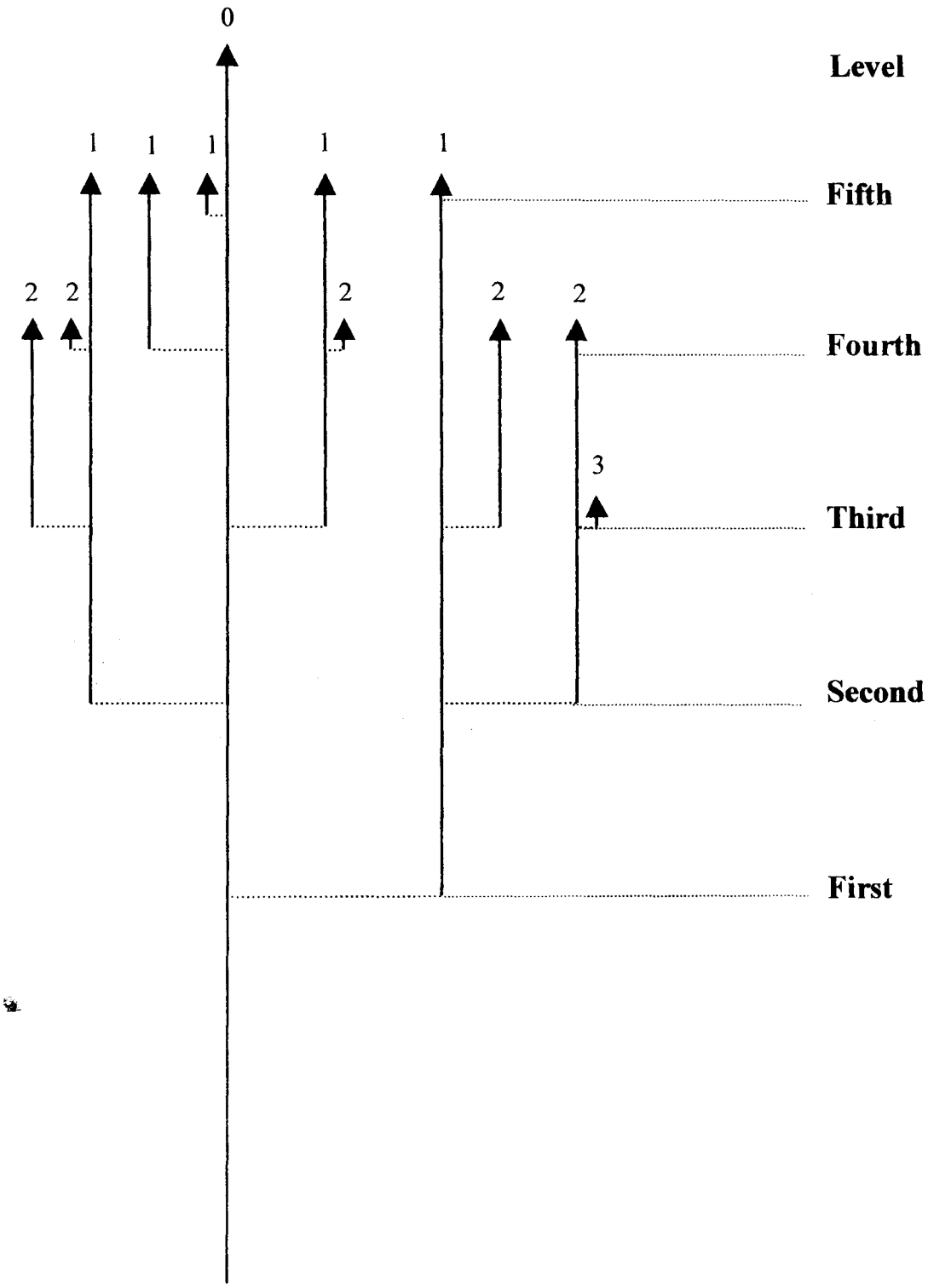


Fig. 3.4 Systematic representation of branching system with branching interval and time delay constant. Numbers indicate order number of each branch (i.e. 1, 2 or 3).

Plant forms of *Elodea* and *Lagarosiphon* are very variable and any structural unit used both for architectural descriptions and modelling must reflect the dynamic nature of the growth. The fate of a meristem is either to grow into a shoot, to abort or to become dormant and following dormancy, revert to either of the first two possibilities. As most plants grow, deviations occur away from the "standard model". One of the most common deviations is reiteration, that is the development of a dormant bud. This can occur in response to damage, favourable conditions or simply a variation from normal behaviour. One possible way in which reiteration may occur in *Elodea* and *Lagarosiphon* species is in the production of secondary branches (Fig. 3.1). These buds do not always develop and the mechansim of control of their development is unknown. They may become active in response to damage of the main axis on which the reiteration is located, or possibly as a result of light or space limitations. The development of these secondary shoots could have a significant impact upon the overall architecture of the plant, as their growth in abundance may result in the development of a denser plant. In addition, for the purposes of modelling it is necessary to understand whether the development of these secondary shoots contributes significantly to the overall biomass and architecture of a plant.

The aim of this chapter is to develop a clear understanding of the growth characteristics and architecture of the three species. This is necessary both for morphological comparisons between the species, and for the development of a mathematical model of their growth. Basic characteristics of internodes were studied such as mean internode length and the length of the internode elongation region. Branching was analysed for both laboratory grown and field collected material as it is important to ascertain the branching patterns of the species when grown under similar controlled environmental conditions for comparative purposes. Features such as the branching order complexity of the three species, i.e. lengths of 1^{st}, 2^{nd} etc order branching, the vertical distribution of the biomass and the contribution of secondary branching to the overall biomass were studied. In addition a short study was also performed to determine the minium viable shoot length. The growth patterns were related to the theoretical and observed plant architectures in laboratory and field collected material.

3.2 Methods

3.2.1 Internode elongation

To measure internode elongation, 6 shoots approximately 200 mm in length were selected at random for each species collected from sites Ec WB and En L and, *L. major* material cultured in mass cultures outdoors at the University of Liverpool. For *E. nuttallii* and *E. canadensis,* the leaves on each shoot were removed and the distances between successive nodes down the stem were measured using a ruler under a dissecting microscope. For measurement of internode length of *L. major* where leaves are arranged in a spiral, the numbers of leaves in successive 1 cm lengths from the apical tip were counted and a mean internode length per 1 cm length of stem calculated.

Numbers of internodes between successive branches were counted in 25 shoots of *E. canadensis*, *E. nuttallii* and *L. major* collected from the field (Table 3.1). Mean internode length was calculated from the measured distance (cm) and number of internodes between successive branches.

3.2.2. Branching

3.2.2.1 General

Branching of material collected from the field and also plants grown under laboratory conditions was assessed in terms of:

1. Total length of plant
2. Total number of apical shoot tips
3. Total number and length of 1^{st}, 2^{nd}, 3^{rd} etc. order branches
4. Vertical distribution of biomass per 10 cm zone
5. Number of broken apical tips per total number of apices
6. Secondary branching numbers

For the analysis of branching patterns, each plant was drawn to scale and then divided into branching orders and zones. As length and biomass have been found to be

linearly related (see Section 2.3.3), measurements of length within each zone or order can be used to approximate biomass. In addition, measurements of secondary branching (Fig. 3.1) were used to assess both the contribution of this branching to the overall biomass of each plant, and growth, in response to damage or breakage of the shoot on which they were situated.

Table 3.1

Sources and numbers of replicates of plants collected from field sites for branching analysis during this study, June-October 1998.

Species	Canal site	Grid ref.	No. Reps
E. canadensis	Nottingham Canal, Cossal	SK 478 429	5
E. canadensis	Shropshire Union Canal, Montgomery Branch, Walls Bridge	SJ 262 206	20
E. nuttallii	Lancaster Canal	SD 481 618	4
E. nuttallii	Ripon Canal	SE 433 687	5
E. nuttallii	Shropshire Union Canal, Montgomery Branch., Maesbury Marsh	SJ 304 247	8
E. nuttallii	Shropshire Union Canal, Montgomery Branch, Burgedin Locks	SJ 252 145	12
E. nuttallii	Rochdale Canal	SD 898 119	3
E. nuttallii	Huddersfield Broad Canal	SE 152 183	4
L. major	Leeds and Liverpool Canal, Stanley Dock	SJ 343 922	23

3.2.2.2 Branching of whole plants collected from the field

Branching was studied on whole plants collected from various canal field sites. Sites of collection and numbers of replicates per site are listed in Table 3.1. Of the field sites examined, relatively few contained either *E. canadensis* or *L. major*, consequently fewer replicates are available for these two species than for *E. nuttallii*. All data was collected from June to October 1998. Attempts were made to obtain

whole plants whenever possible, although the brittle nature of the plants made this difficult.

3.2.2.3 Seasonal changes in branching of E. canadensis

Unpublished data on *E. canadensis* collected by J. W. Eaton during 1975 and 1976 at two-week intervals at five sites along the Leeds and Liverpool Canal was examined. In the original data set, drawings were made of short apical sections of plant shoot, recording distances between branching points and lengths of branches of each section. This data is reworked here to show seasonal changes in terms of total plant length, percentage of first degree branching and numbers of apices per plant.

3.2.2.4 Branching in laboratory grown material

Laboratory grown material was used to study branching under controlled light and temperature conditions for comparison between species. Total plant length, numbers of apical tips, inter-branch distances and branching lengths were recorded from species growing in monoculture treatments of Competition Experiments 1 and 2 (Chapter 7). Total length and length of first order branches were also measured for 12 replicates of each species grown in nursery cultures for two weeks, as described in Chapter 2, Section 2.2.3.

3.2.3 The growth of different starting lengths

72 shoots each of *E. nuttallii* and *E. canadensis*, and 45 shoots of *L. major* were selected. For *Elodea spp.* 12 replicates of each of the following apical lengths were cut: 5 mm, 20 mm, 40 mm, 60 mm, 120 mm and 240 mm. For *L. major* 9 replicates of the following lengths were selected: 5 mm, 20 mm, 40 mm, 60 mm and 120 mm. All shoots were planted in 250 ml plastic beakers. Replicates were then placed in plastic buckets with 10 l of tap water, for *Elodea spp.* 4 replicates per bucket, and for *L. major*, 3 replicates per bucket. The buckets were placed randomly in temperature-controlled water tanks for a period of 14 days at 15 °C, with incident

PAR of approximately 100 μmol m^{-2} sec^{-1} at the water surface. After 14 days, the total length and total numbers of lateral branches were measured.

3.3 Results

3.3.1 Internode elongation

Fig 3.5 shows that the internode length of *E. canadensis* and *E. nuttallii* increased rapidly within the first 10 to 12 cm and appeared to level off thereafter. *L. major*, however, showed a gradual increase up to 15 cm from the apical tip (Fig. 3.6).

Internode lengths of the three species were also measured on 25 single length shoots of material collected in the field (see Table 3.2). Internode lengths were observed to increase with increasing distance from the apical tip towards the base of the plant with internode lengths varying from in excess of 3 cm to less than 1 cm for *Elodea* spp. (examples shown in Fig. 3.8). Internode lengths for *L. major* were considerably shorter than those for the *Elodea* spp. Internode numbers between successive branches did not show any consistent pattern with increasing distance from the apical tip.

3.3.3 Branching

3.3.3.1 Field material

Summaries of the growth parameters for each species are given in Table 3.2 for field collected material. Fig. 3.7 shows an example of a scaled drawing made of field collected plant material for architectural analysis. Branching was found to occur both within and outside the elongation zone. If it is assumed that the main axis and side branches grow at the same rate, differences in length between the main axis and a branch can be used as a measure of the delay between the production of a branching point and growth of the side shoot. Measurements suggest that this delay can be short, the minimum difference in length for *E. canadensis*, *E. nuttallii* and *L. major* being 3mm, 2 mm and 1mm respectively. Examination of the scaled

Table 3.2
Summary of architectural data for the three species from field collected data. TL = Total length (cm), DB = Secondary branching, TL of n^{th} order branches = Total length (cm) of plant before production of n^{th} order branches.

Species	Mean internode length (mm)	Length of elongation region (cm)	No. Internodes between branches	TL for 1st order branches	TL for 2nd order branches	Length (cm) apice^{-1}	No. 2nd DB cm^{-1}	No. broken apices per total No. apices
E. canadensis	13.3	10	7	15-50	100	18.10[a]	0.012[ca]	0.12
E. nuttallii	16.0	10	7	50-100	100-200	17.67[ab]	0.0039[a]	0.14
L. major	1.33	10-15	11	80	200-300	20.17[b]	0.0025[c]	0.16

[a] = Significant differences between *E. canadensis* and *E. nuttallii* (For significance levels see text)

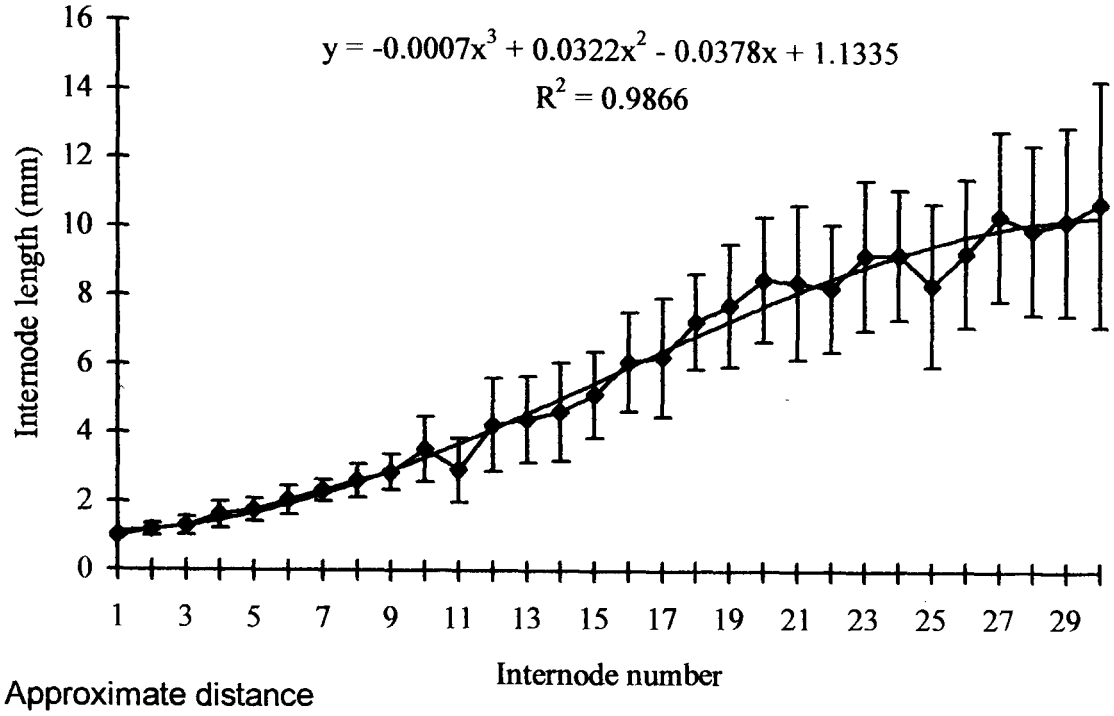
[b] = Significant differences between *E. nuttallii* and *L. major*

[c] = Significant differences between *E. canadensis* and *L. major*

Table 3.3
Combined data from Competition Experiments 1 and 2 showing the number of broken apices per total number of apices for each species.

Species	No. broken apices per total no. apices
E. canadensis	0.210[ac]
E. nuttallii	0.068[a]
L. major	0.012[c]

(a)



(b)

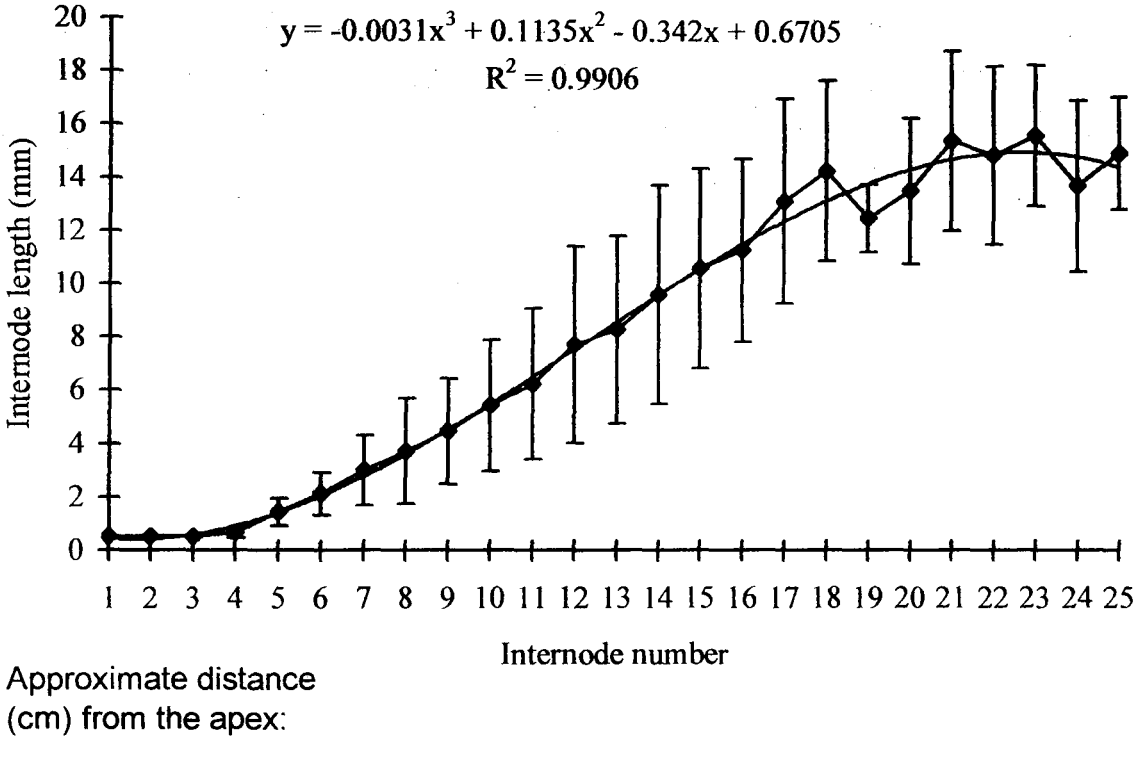


Fig. 3.5 The mean internode length (mm) (± 95 % confidence intervals) between successive nodes down shoots of (a) *E. nuttallii* and (b) *E. canadensis* starting from the tip.

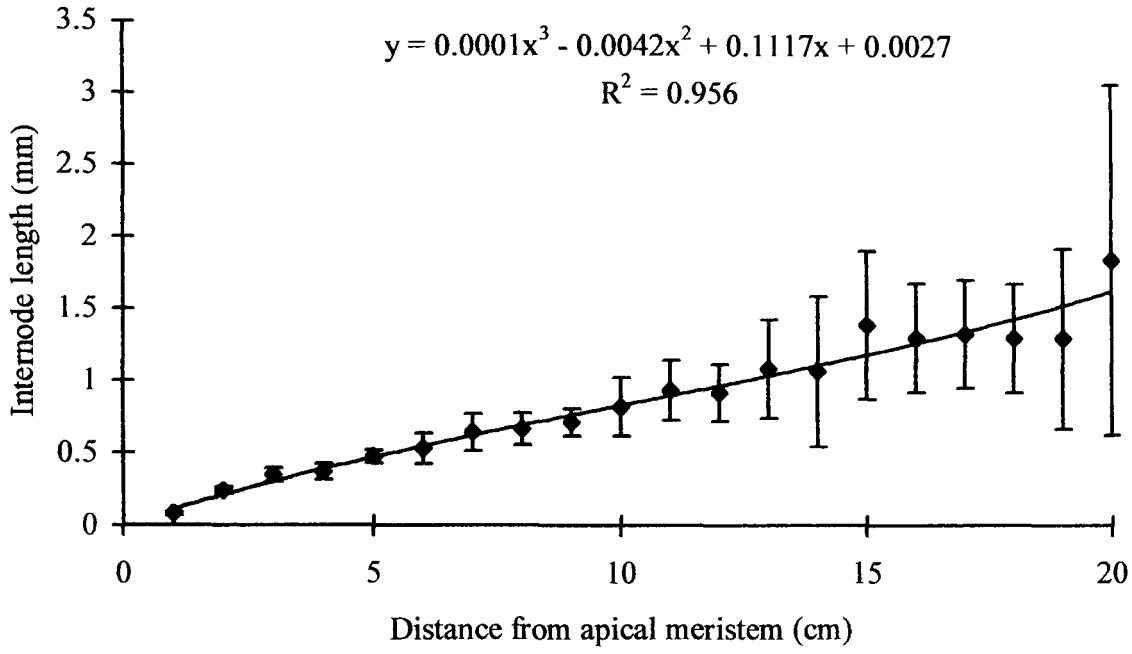


Fig. 3.6 Internode length (mm) (± 95 % confidence intervals) of *L. major* measured from counts of number of leaves per cm of shoot (n = 6).

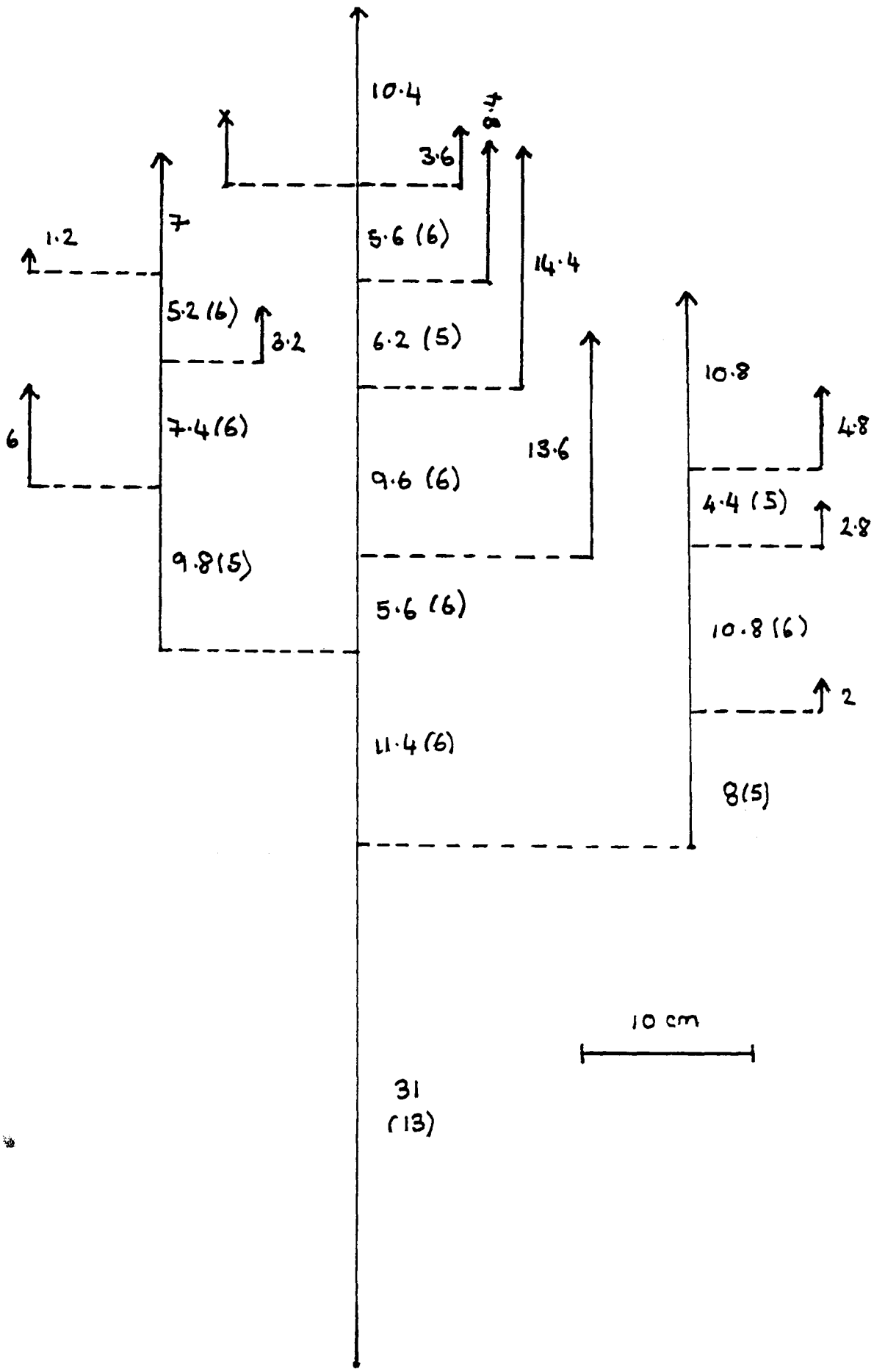


Fig. 3.7 An example of a scaled drawing of *E. nuttallii* collected from the Huddersfield Broad Canal showing the branching pattern. Values outside the brackets are distances between successive branches. Values in brackets are the number of internodes between branches. ↑ = growing apice, X = broken shoot.

drawings shows that the time delay between main shoot growth and growth of a side shoot decreases towards the apical tip (Fig. 3.9).

From examination of branching order of field material, *E. canadensis* was found to have first order branches for shoots greater than 10 cm in length (Fig. 3.10, a). Second order branching was only apparent in plants exceeding approximately 100 cm in total plant length. Very few third order branches were observed. Plants of *E. nuttallii* were generally much larger with more extensive branching systems than those of *E canadensis* (Fig. 3.10, b). First order branching was normally found for plants greater than 50 cm in total length, while second order branching was only observed for plants with a total length in excess of approximately 100 cm. Second and third order branches were more frequent for *E. nuttallii* plants than for *E. canadensis*. These differences in 2nd and 3rd order branching frequency may be a consequence of the larger plants of *E. nuttallii* found, an aspect discussed later. For *L. major*, the data collected from field samples was very variable and patterns in branching order were difficult to discern. One difficulty was caused by the frequent death of the main axis apical meristem. Often, a first order branch proximal to the dead tip would "take over" as the main axis, making interpretation of the branching pattern difficult as this could be classified as an increase in branching order. Where this had obviously occurred, the broken main axis and the first order branch "taking over" were here treated as a single branch. First order branching did not generally occur for plants with a total plant length of less than approximately 80 cm (Fig. 3.11). Second order branching was only apparent for plants with a total length exceeding 200 cm.

Measurements of vertical biomass distribution revealed distinct zonation, particular for *E. nuttallii* and *L. major*. *L. major* often had a lengthy section of unbranched main stem upwards from the base of the plant, with first and second order branches only towards the top of the plant and therefore closest to the water surface (Fig. 3.14, a). Although the biomass distribution of larger plants of *E. nuttallii* was similar to plants of *L. major*, growth of side axis did occur close to the plant bases (Fig. 3.13, a). Thus, smaller plants did not exhibit such clear vertical biomass distribution as larger plants. This zonation was not observed for *E. canadensis* (Fig, 3.12, a). Graphs of maximum plant length against zone containing maximum biomass

were expected to reveal a roughly linear trend if the pattern of biomass distribution with respect to the whole plant length did not vary, that is, if the maximum biomass was always towards the top of the plant. As expected from the examination of vertical biomass profiles, *E. nuttallii* and *L. major* did reveal relatively linear responses (Fig. 3.13, b and Fig. 3.14, b) although variation in biomass distribution for the smaller plants particularly of *E. nuttallii,* was evident. This response was much more difficult to interpret for *E. canadensis* (Fig, 3.12, b), although these plants did show some similarities with the smaller plants of *E. nuttallii.* This again reflects the lack of zonation of the biomass for the *E. canadensis* plants examined. As expected, a linear relationship was observed between the number of apices and total length of a plant for all three species (Fig. 3.15). Calculations of number of apices per unit length (Table 3.2) suggest that this relationship is similar for all three species, although *E. canadensis* had significantly less ($p = 0.05$) numbers of apices per unit length than *L. major*, but significantly more ($p = 0.05$) than *E. nuttallii*.

From data collected from June 1975 to June 1976, seasonal variation in branching of *E. canadensis* was apparent. Increased branching as a percentage of the total plant length was observed during the summer months (Fig. 3.16). During the winter shoots were found to be relatively simple in structure with few first order branches. The data also show that first order branching was only found for shoot lengths in excess of 10 cm (Fig. 3.17).

In the field data examined, *E. canadensis* had significantly greater ($p = 0.001$) numbers of secondary branches than either *E. nuttallii* or *L. major*. However, generally, second degree branching was not common for any of the species. While 46% and 48% of *E. canadensis* and *L. major* plants respectively had secondary shoots, in none of the plants did it occur more than 4 times. Only 27% of *E. nuttallii* plants were observed to have any secondary shoots, and none with more than six occurrences. Occasional secondary shoot growth was observed, located from the axis of scale leaves at the first node of broken stem shoots, suggesting that growth of these shoots may be in response to damage of the original shoot. Frequently, however, secondary shoot production had no apparent cause or stimulus. The number

of broken apices per total number of apices was similar for all three species in field collected material (Table 3.2).

In summary

1. *Branching occurred within the elongation zone and is possible within 5 mm of the apex for all three species.*
2. *Generally, first and second order branching was common for all three species, while branching in excess of third order was rare.*
3. *Plants of both E. nuttallii and L. major collected from the field had larger more extensive branching systems than those of E. canadensis.*
4. *E. nuttallii and L. major produced dense canopies, with the plant biomass concentrated close to the top of the plant. This was not shown for E. canadensis.*
5. *Branching of E. canadensis was found to increase during the summer period, with first order branches having a greater contribution to the total biomass during this period.*
6. *Growth of secondary shoots in field material was relatively rare and did not contribute substantially to the overall biomass of the plant.*

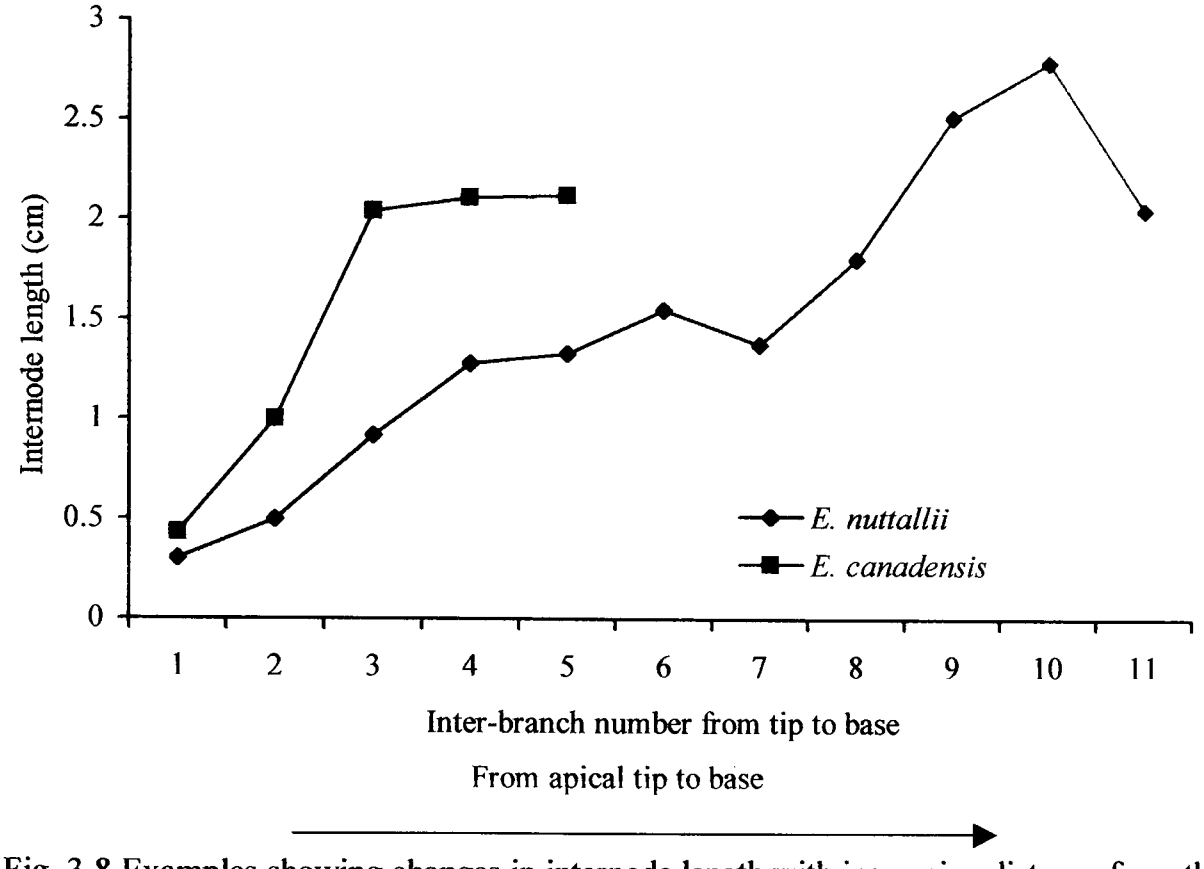


Fig. 3.8 Examples showing changes in internode length with increasing distance from the apical tip.

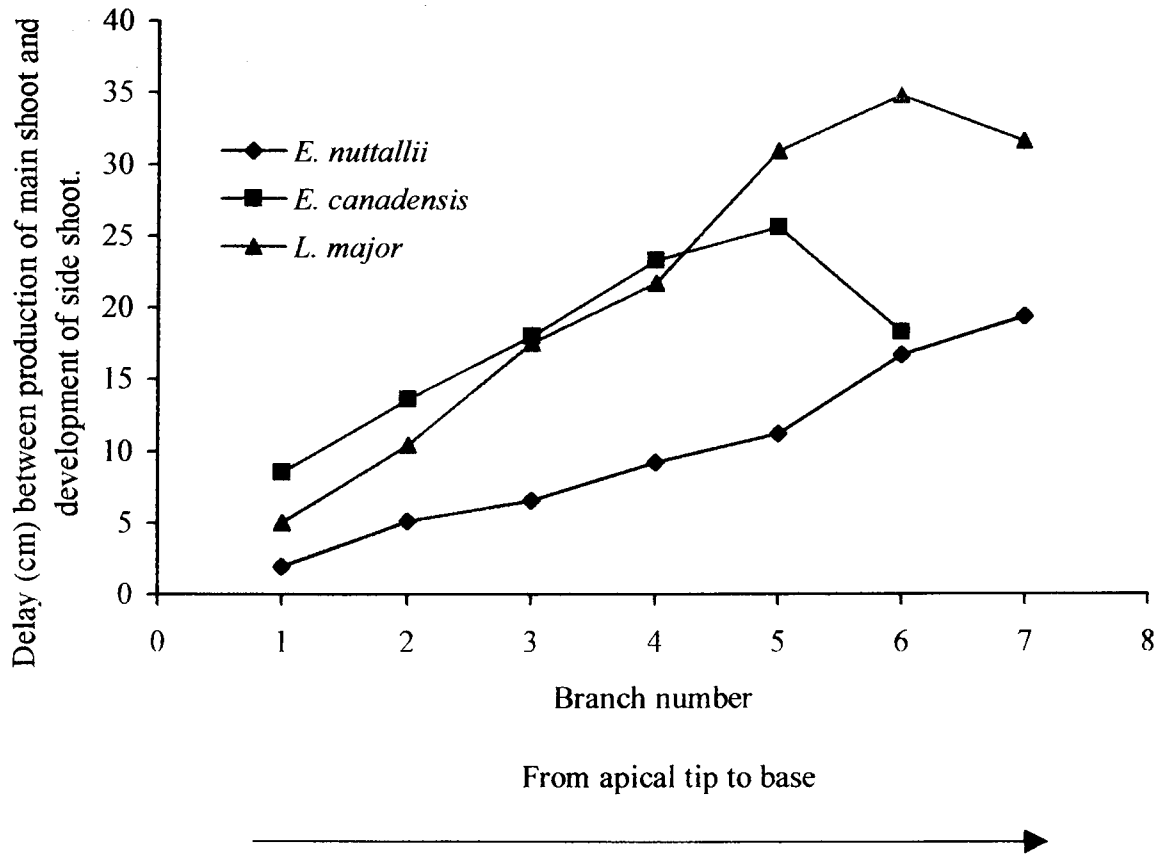
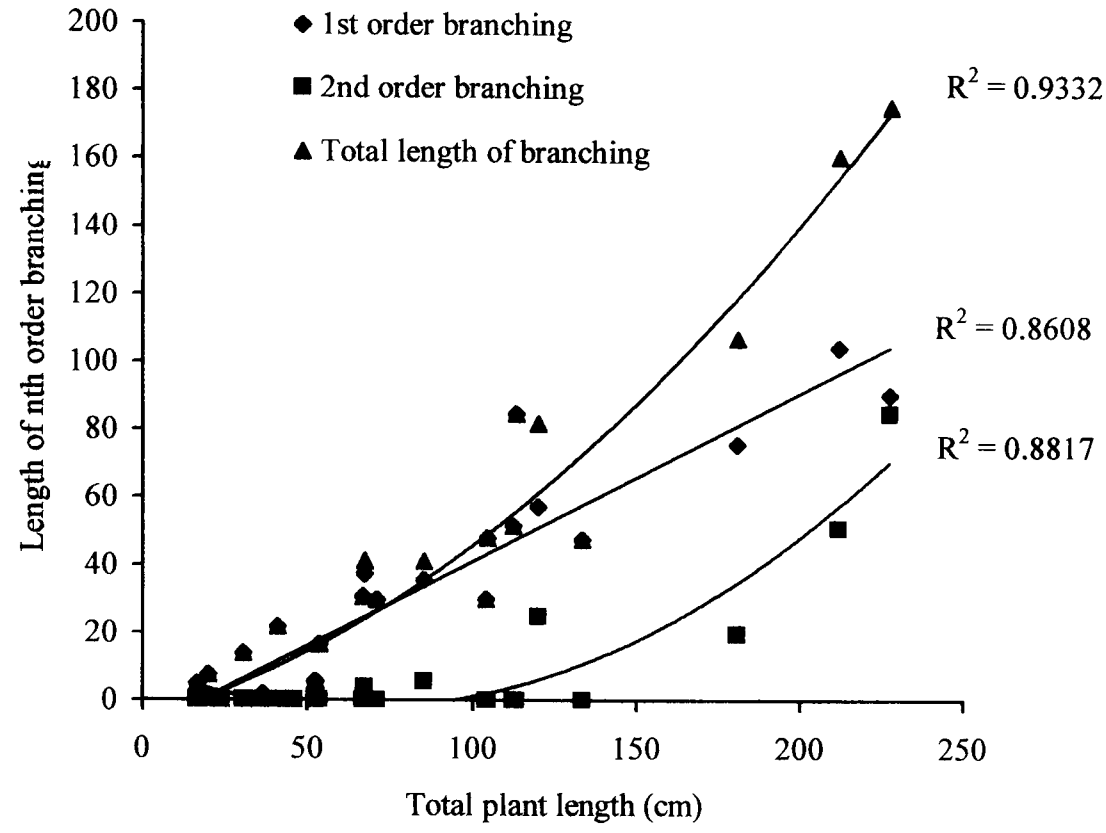


Fig. 3.9 Examples showing difference between main axis length and side shoot length as a measure of the delay between the production and growth of a side shoot with increasing distance down the stem.

(a)



(b)

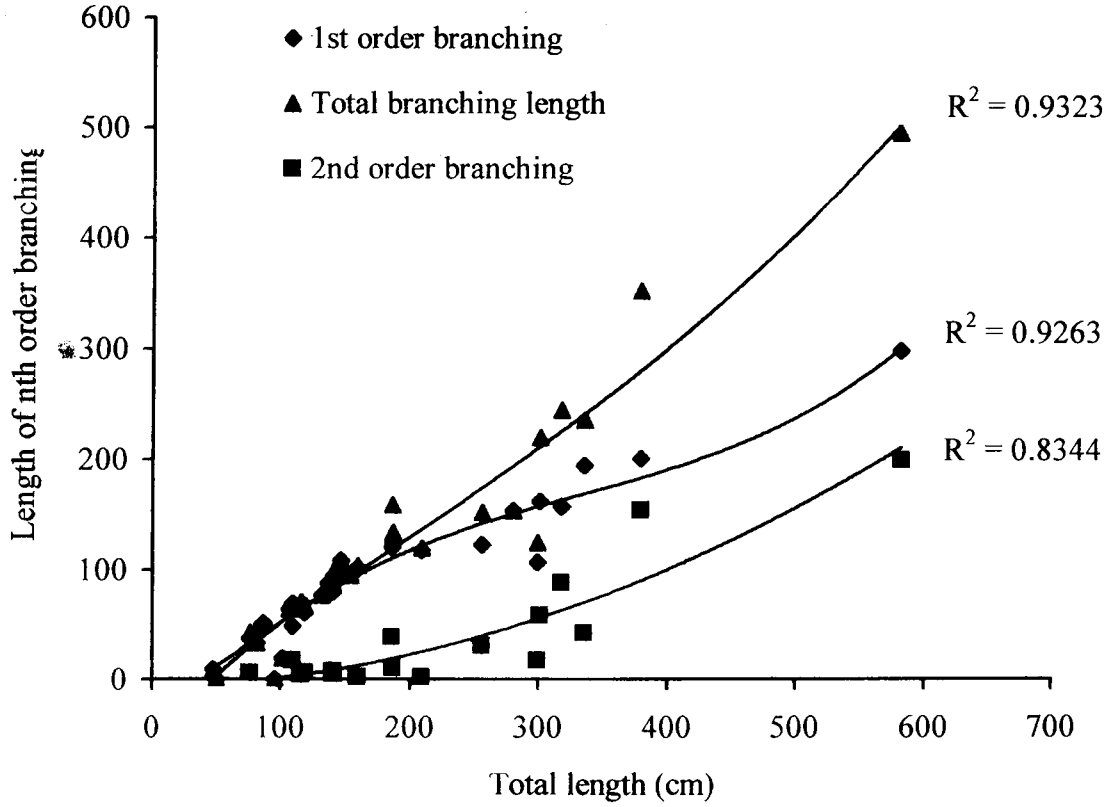


Fig. 3.10 Total branching lengths and lengths of first and second order branching of (a) *E. canadensis* and (b) *E. nuttallii* collected from the field.

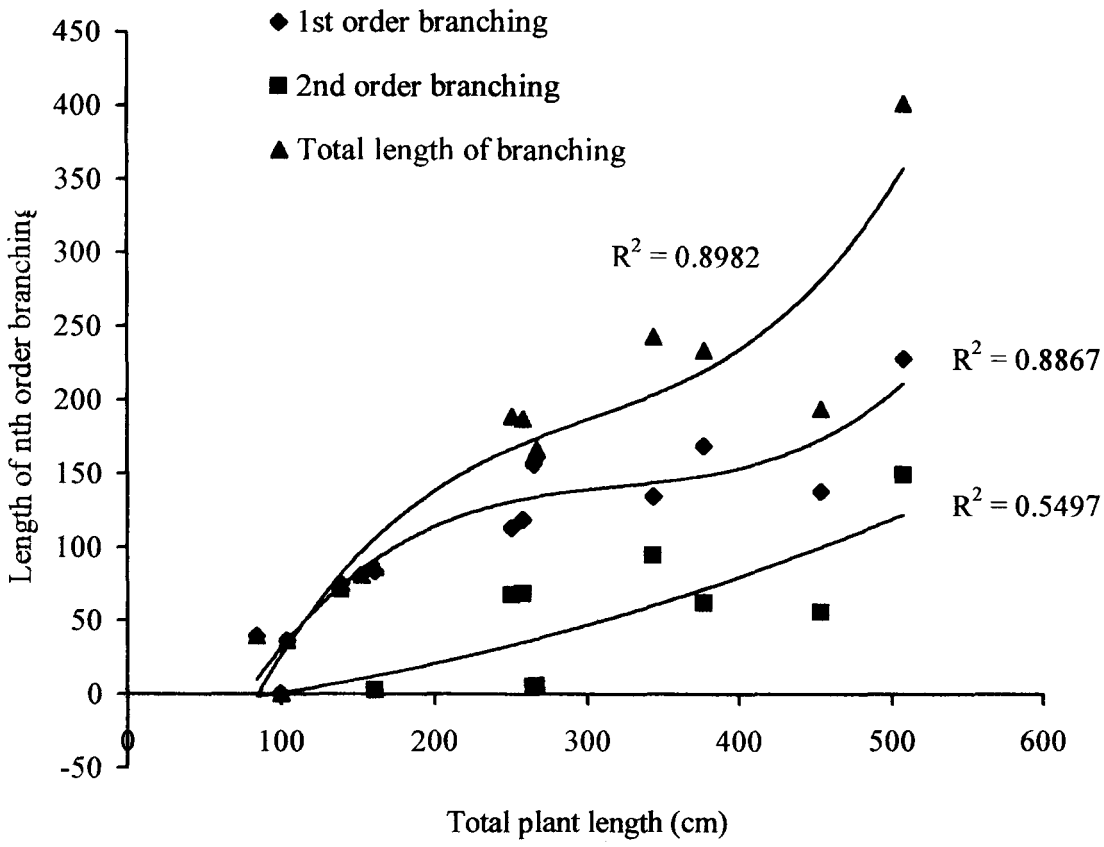
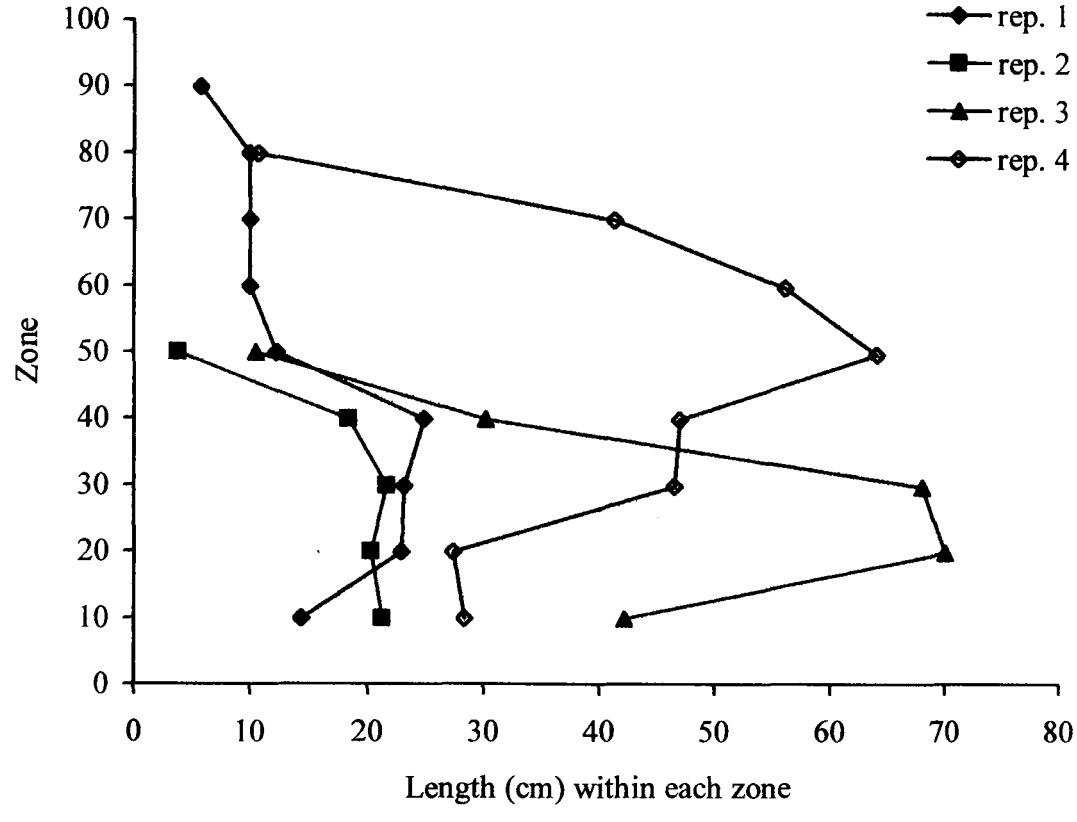


Fig. 3.11 Total branching lengths and lengths of first and second order branching of *L. major* collected from the field.

(a)



(b)

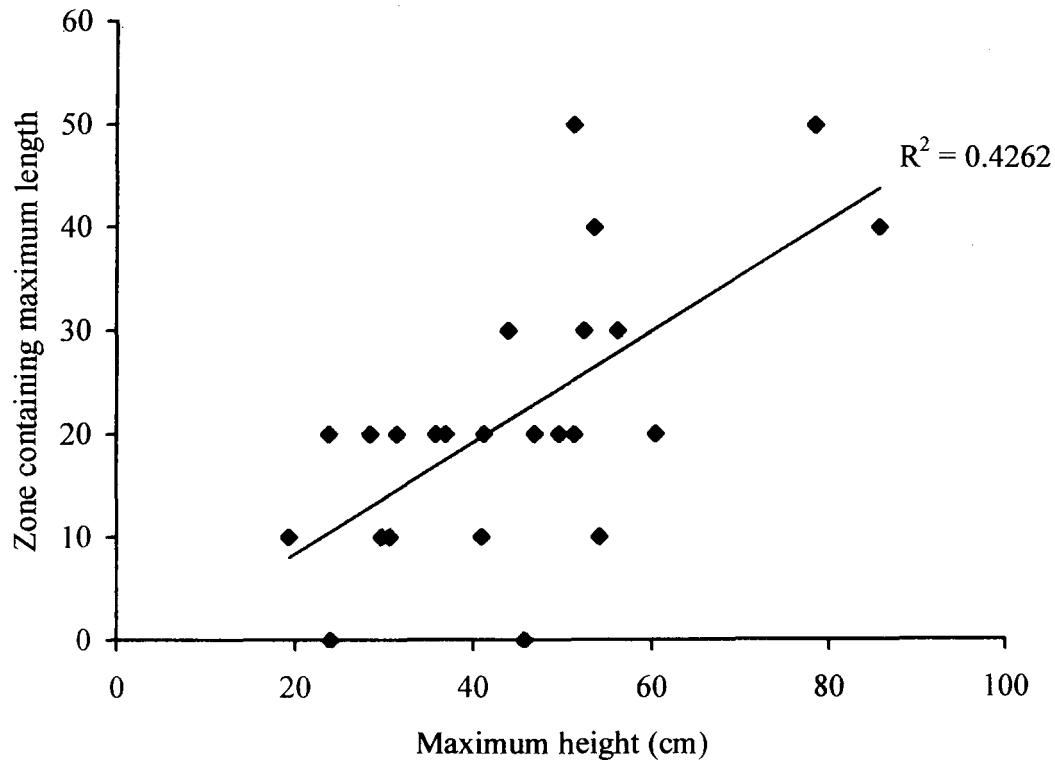
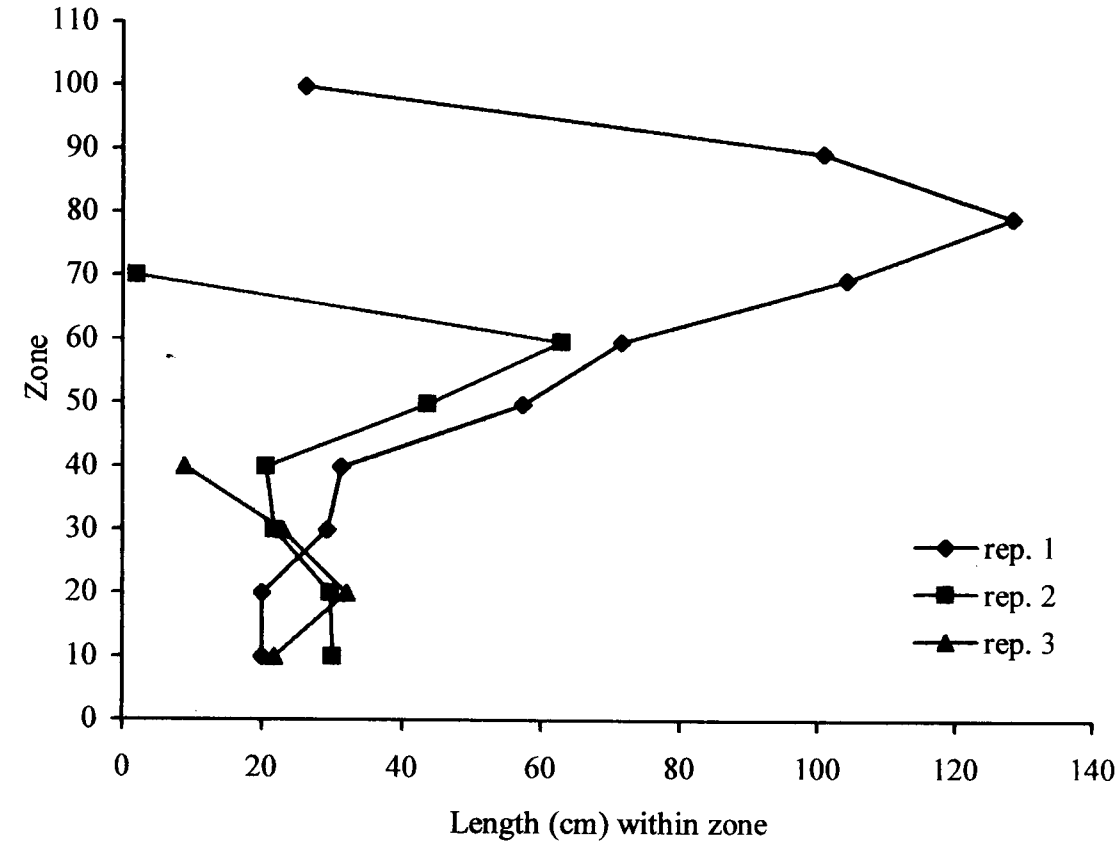


Fig. 3.12 (a) Examples of change in distribution of biomass of *E. canadensis* collected from the field with depth. (b) Graph showing maximum plant height (cm) against zone containing maximum plant length of *E. canadensis* from samples collected in the field.

(a)



(b)

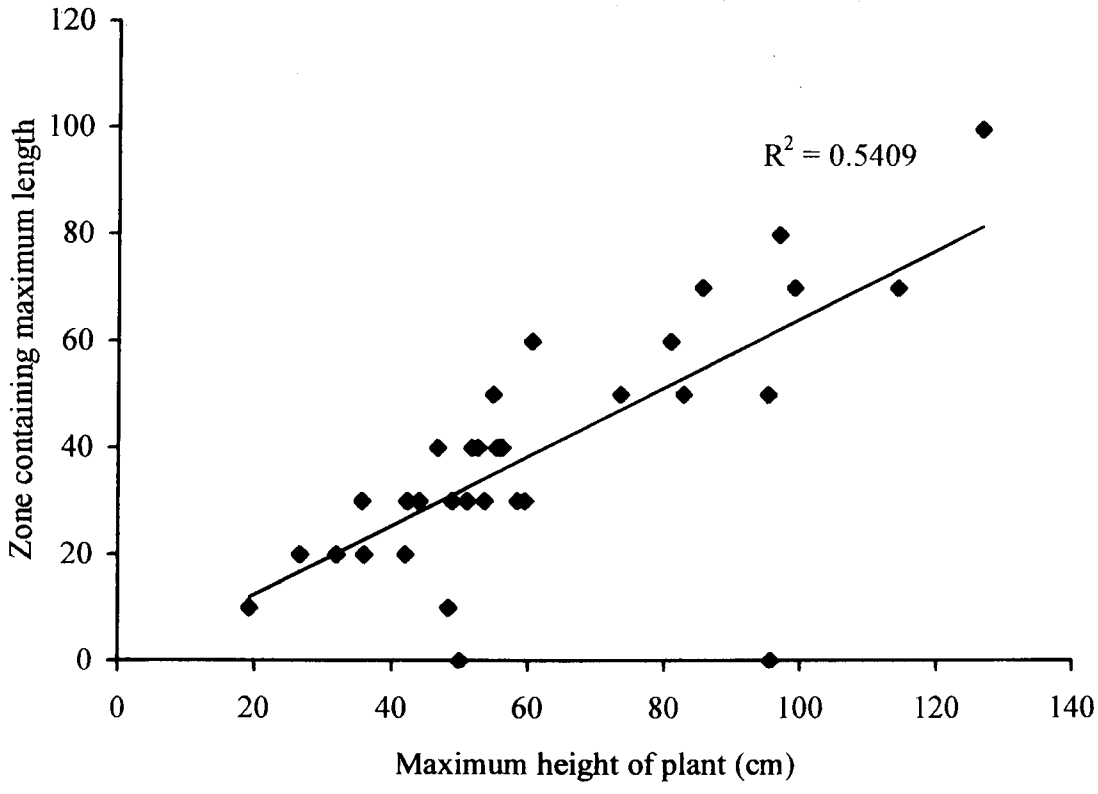
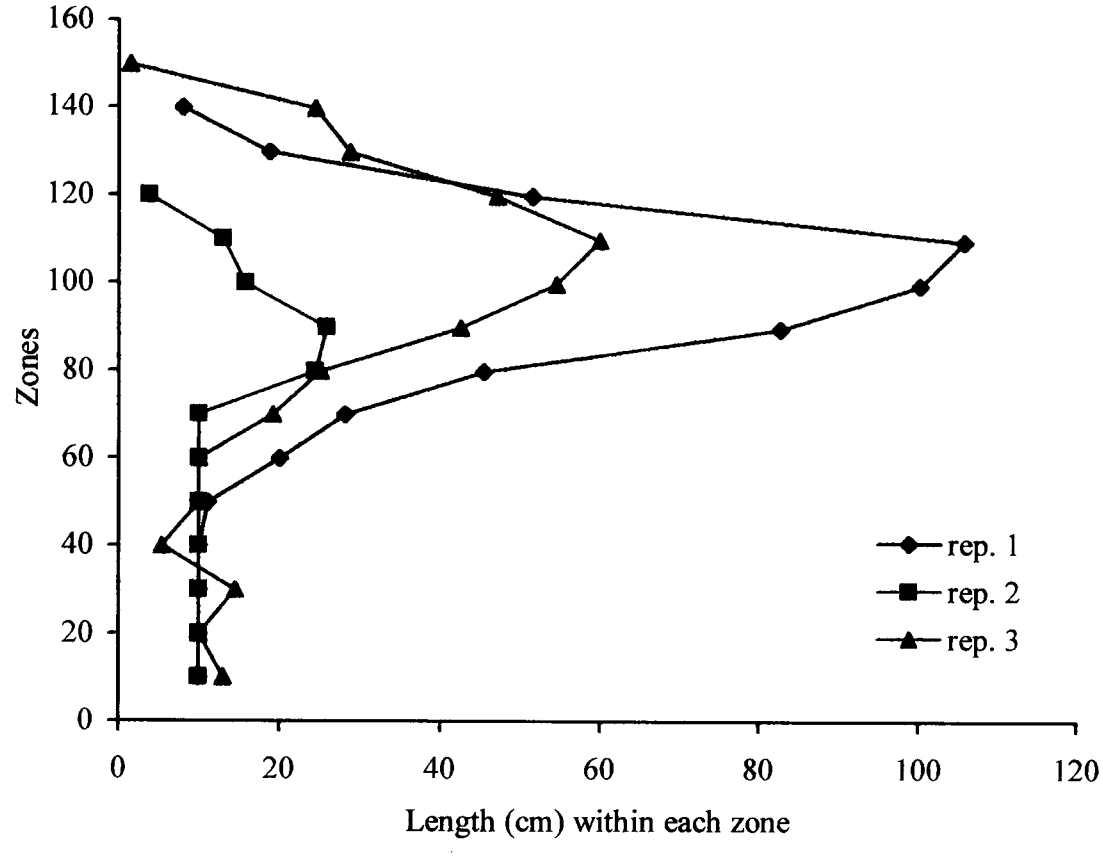


Fig. 3.13 (a) Examples of change in distribution of biomass of *E. nuttallii* collected from the field with depth. (b) Graph showing maximum plant height (cm) against zone containing maximum plant length of *E. nuttallii* from samples collected in the field.

(a)



(b)

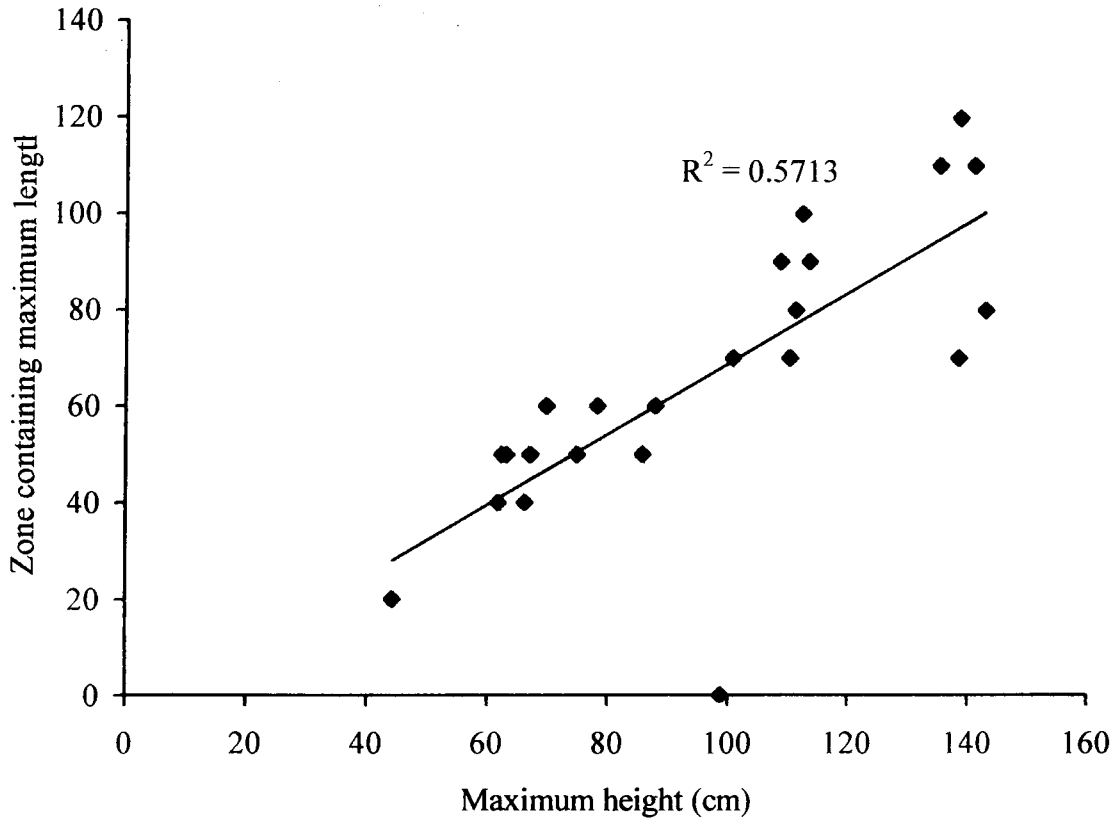


Fig. 3.14 (a) Examples of change in distribution of biomass of *L. major* collected from the field with depth. (b) Graph showing maximum plant height (cm) against zone containing maximum plant length of *L. major* from samples collected in the field.

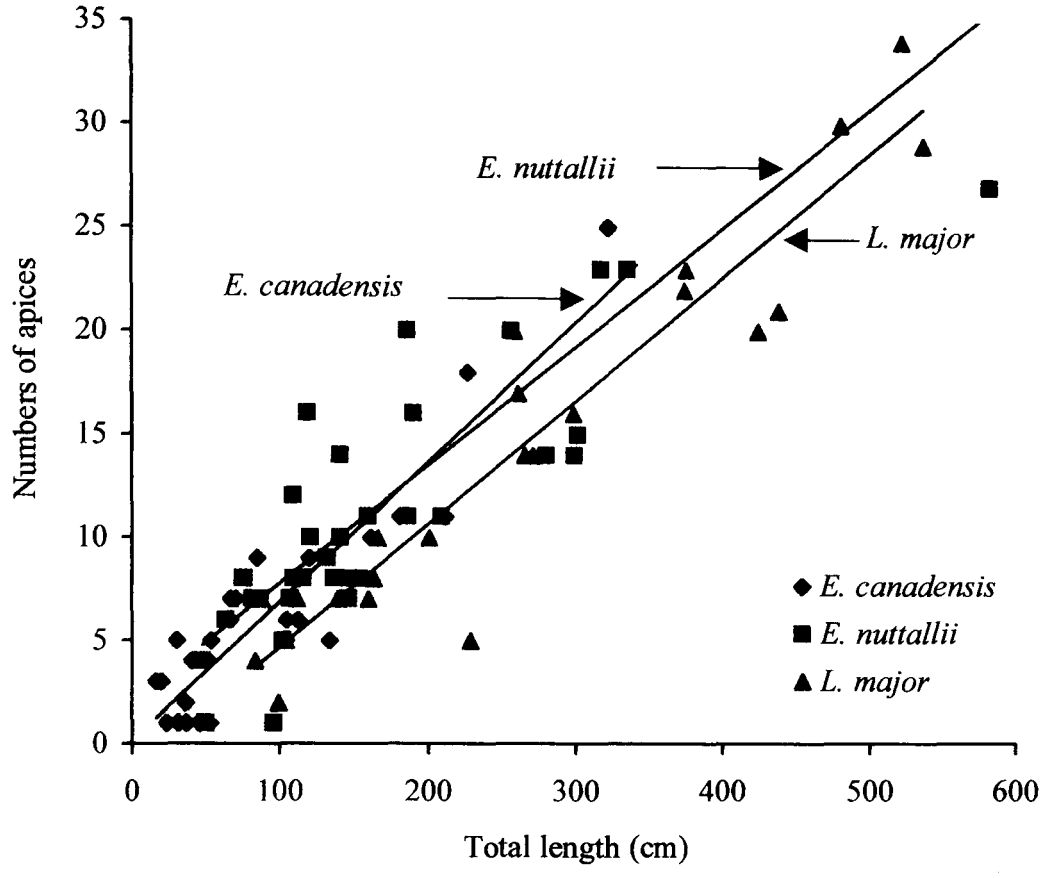


Fig. 3.15 Relationship between number of apices and total plant length (cm) of *E. canadensis* ($r^2 = 0.85$), *E. nuttallii* ($r^2 = 0.75$), and *L. major* ($r^2 = 0.90$) collected in the field.

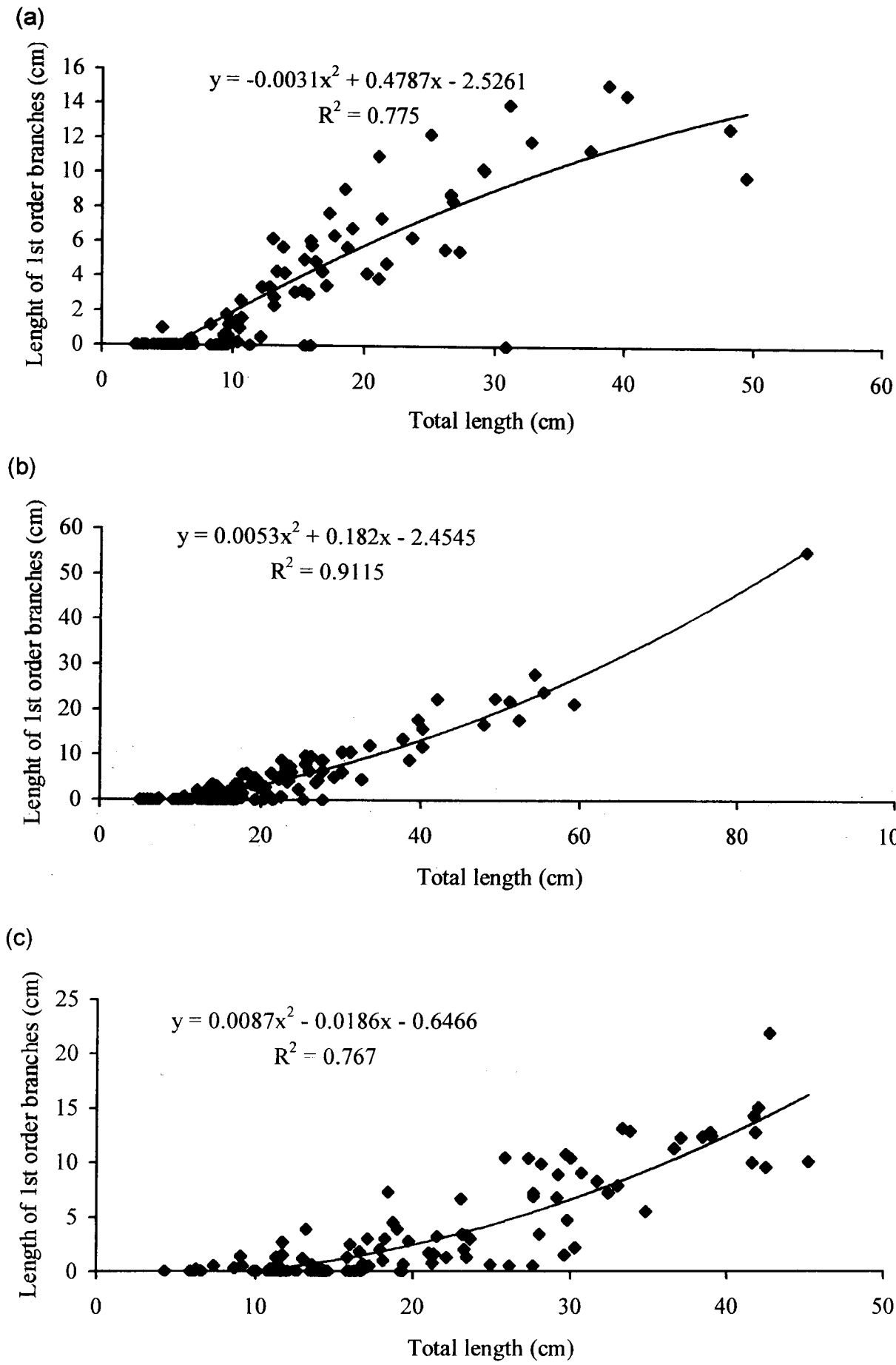
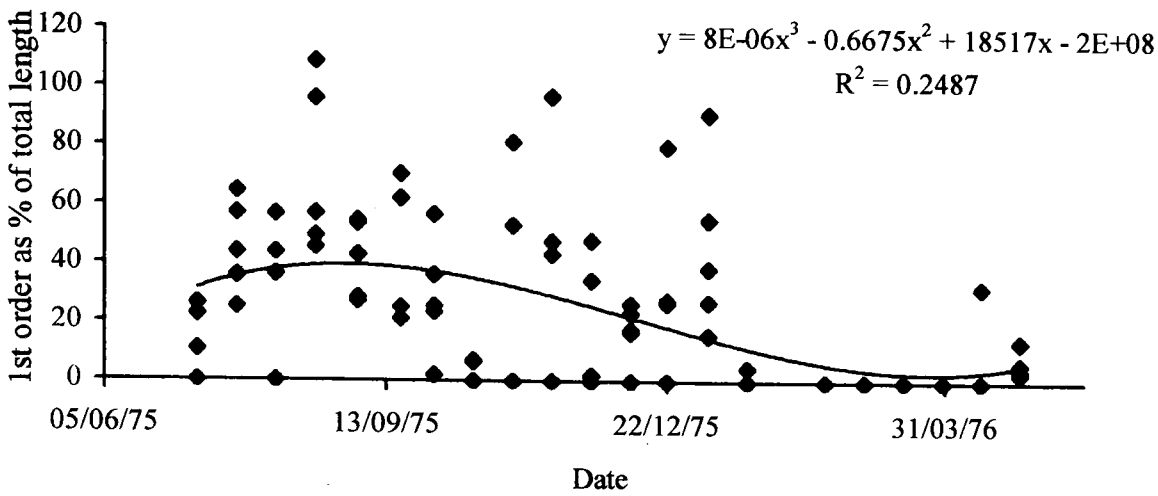
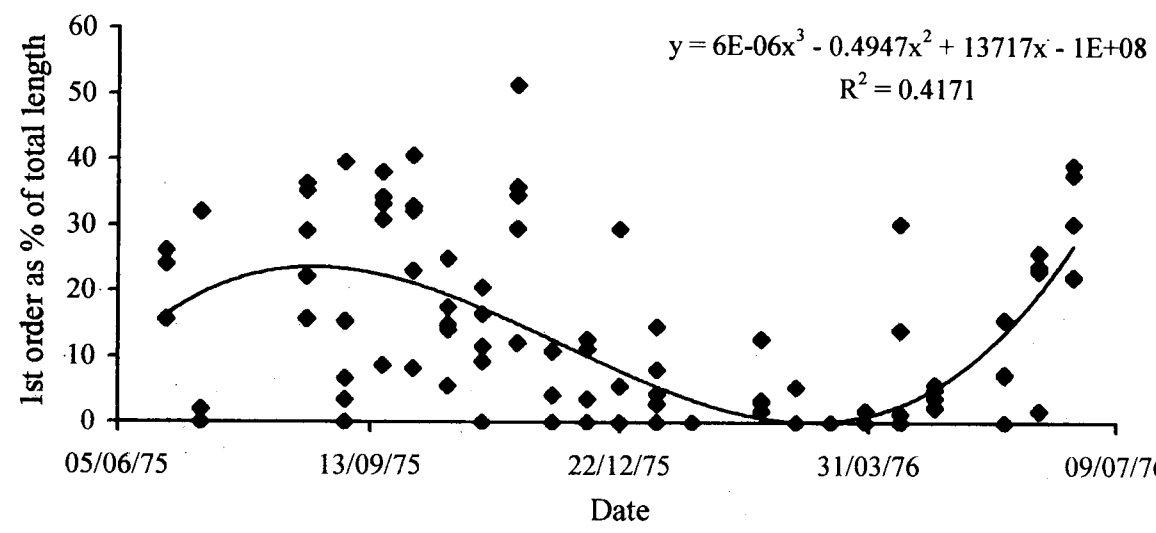


Fig. 3.16 Compilation of data from June 1975 to June 1976 showing relationship between total length and numbers of apices of *E. canadensis* collected from three sites along the Leeds and Liverpool Canal, (a) New Springs, (b) Haskayne and (c) Martland Mill.

(a)



(b)



(c)

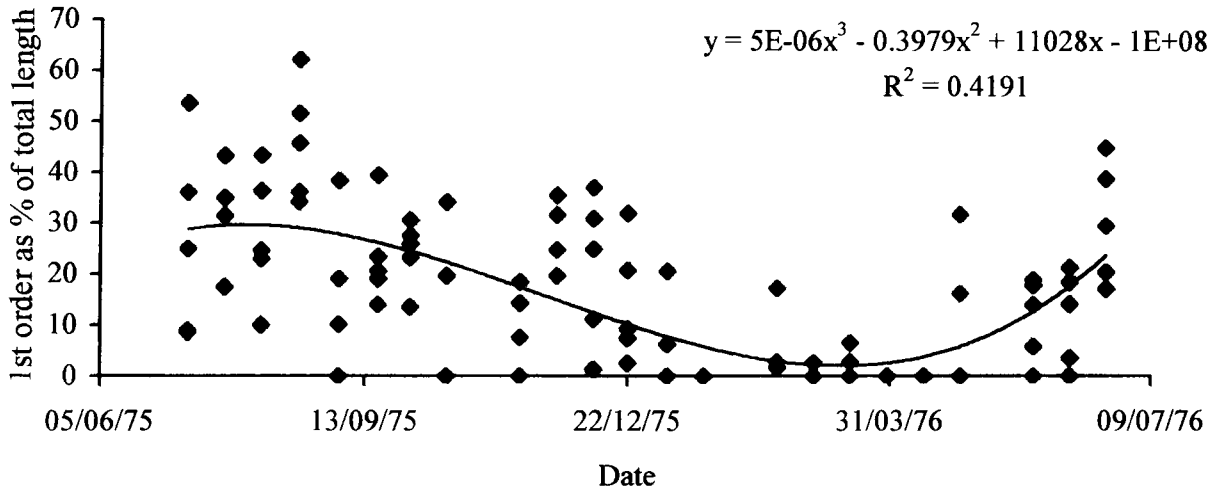


Fig. 3.17 Data from June 1975 to June 1976 showing changes in total length of 1st order branches as a percentage of the total length of each *E. canadensis* shoot. Plant material was collected from three sites along the Leeds and Liverpool Canal, (a) New Springs, (b) Haskayne and (c) Martland Mill.

3.3.3.2 *Laboratory grown material*

Data on architectural traits were collected from the monocultures used in competition studies described in Chapter 7 from Competition Study 1, Parts 1 (*E. nuttallii* vs *L. major*) and 2 (*E. nuttallii* vs *E. canadensis*). Graphs of dry weight against apex number reveal significant differences in both the slope and elevation of the best fit lines between *E. nuttallii* and *L. major*, with *L. major* having significantly less apices per unit dry weight (Fig. 3.18). On the other hand, a similar comparison between *E. nuttallii* and *E. canadensis* revealed similarities between these two species, although differences in the elevation of lines was found with significantly less apices per unit dry weight for *E. canadensis* in comparison with *E. nuttallii* (Fig. 3.18). On conversion of dry weight to total length, very different patterns were observed reflecting the differences in dry weight per unit length of the species (Fig. 3.19). Most noticeably, the total length of *E. nuttallii* was much greater than that of either *E. canadensis* or *L. major* despite the use of similar starting units (10 cm lengths with only a single intact apical tip). Best-fit lines fitted to data of *E. canadensis* and *L. major* suggest that, per unit length, these species have greater numbers of apical tips than *E. nuttallii*.

Examination of ordered branches, again shows substantial first order branching and relatively little second or third order branching in *E. nuttallii* (Fig. 3.20). Samples of *L. major* were also found to have significant first order branching and virtually no second order branching. Numbers of secondary shoots produced generally increased for *E. nuttallii* with increasing total plant length, although no discernible patterns in secondary shoot production were observed for either *E. canadensis* or *L. major* (Fig 3.21). *L. major* rarely produced secondary shoots.

Examination of plants from Competition Experiment 2 revealed a slightly different pattern in apical numbers per unit plant length. In this study, *E. nuttallii* was found to produce greater numbers of apical tips per unit length than either *E. canadensis* or *L. major* (Fig. 3.22, a). This also corresponds with an increase in secondary shoot numbers (Fig. 3.22, b). In this study, *E. nuttallii* produced large numbers of secondary shoots. Plants of *L. major* were observed to have few

secondary shoots (Fig. 3.22, b). It is significant that this experiment had over twice the duration of the first study, consequently higher experimental densities, more consistent with those found in the field during the summer period, were achieved.

A comparison between total length and production of first order branches following two weeks growth under nursery cultures revealed significant differences between the species (Fig. 3.23). In this period, *E. nuttallii* produced significantly greater ($p = 0.001$) total lengths of shoot and, numbers and length of first order branches.

Numbers of broken shoots during the competition experiments give a good indication of the potential for fragmentation of these three species as treatment prior to examination of the material is known (unlike the field material examined). As can be seen in Table 3.3, numbers of broken shoots are significantly ($p= 0.001$) greater for *E. canadensis* compared with either *L. major or E. nuttallii*. Experimental material of *L. major* rarely showed any damage.

In summary

1. *Per unit dry weight, the two Elodea spp. produced similar numbers of apices, but L. major produced far fewer per unit dry weight.*
2. *Per unit length, both E. canadensis and L. major produced greater numbers of apices compared with E. nuttallii.*
3. *E. nuttallii produced greater numbers of second order branches than L. major.*
4. *Secondary branching occurred more frequently in laboratory cultures compared with field material.*
5. *Secondary branching was common for Elodea spp., but rarely occurred in L. major.*
6. *Breakages were most frequent for E. canadensis, and least frequent for L. major.*
7. *Growth rates per unit length of E. nuttallii were higher than those of E. canadensis or L. major.*

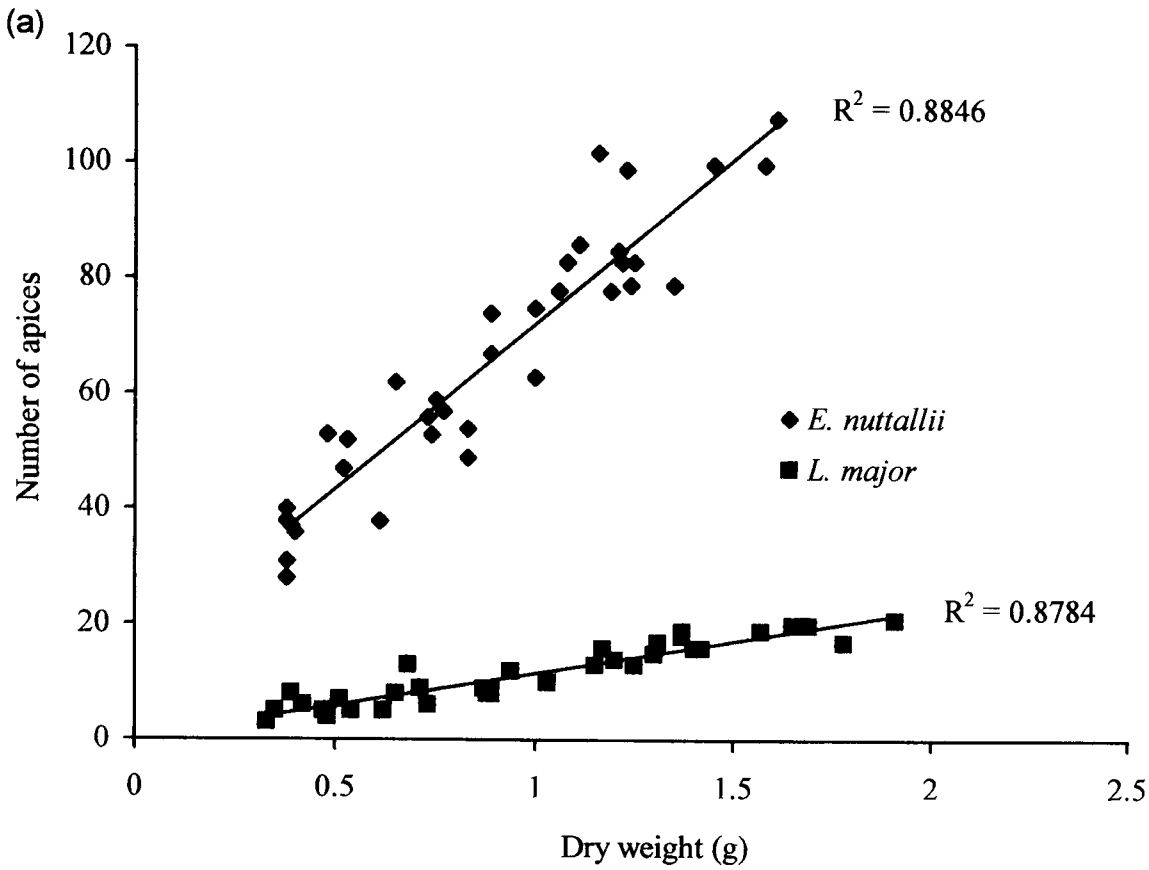
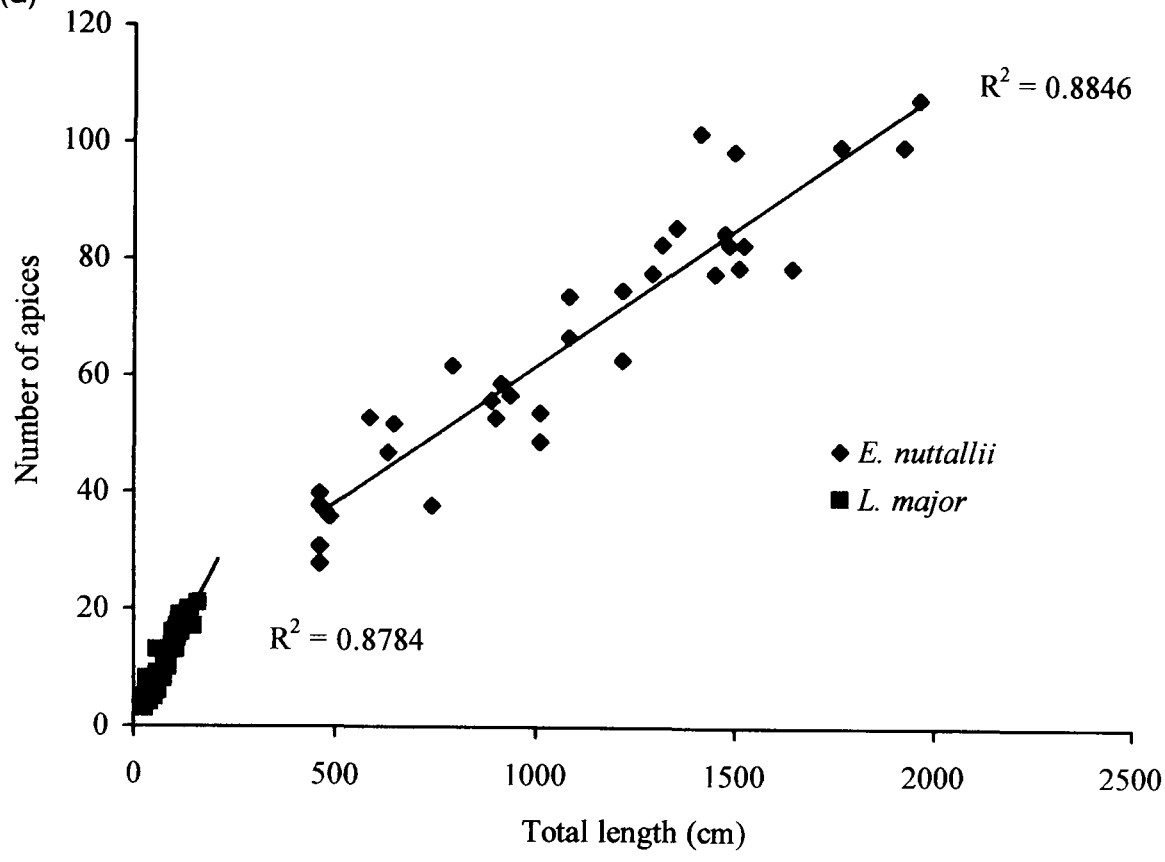


Fig. 3.18 Graphs showing numbers of apices per unit weight for (a) Competition Experiment Part 1, *E. nuttallii* vs. *L. major* and (b) Competition Experiment Part 2, *E. nuttallii* vs *E. canadensis.*

(a)



(b)

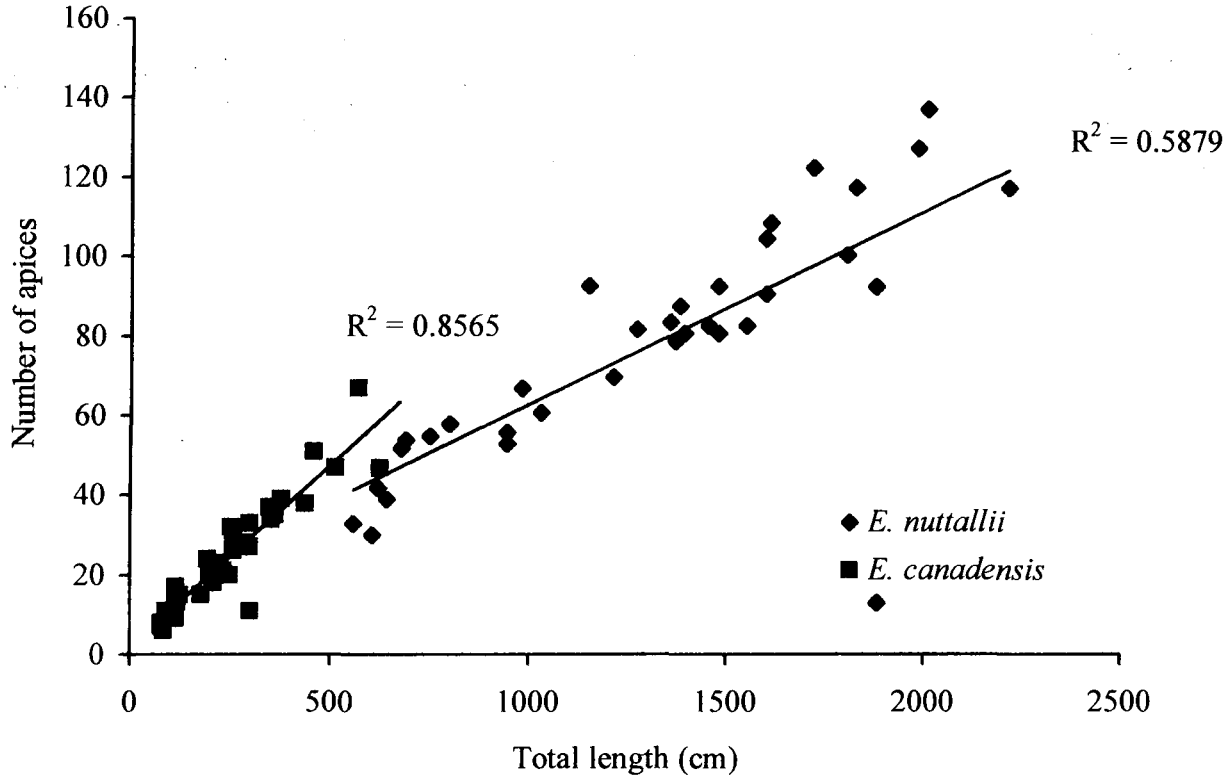
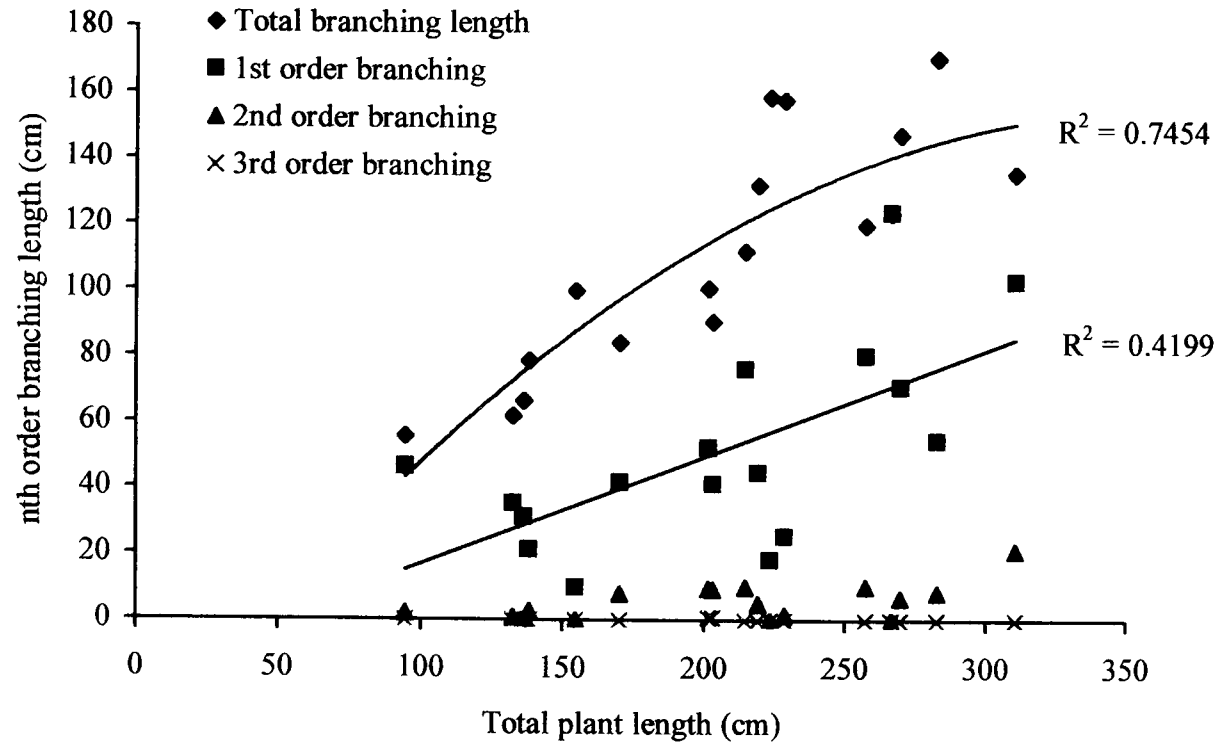


Fig. 3.19 Graphs showing numbers of apices per unit length, calculated from known conversion factors (Chapter 2 & Birch, 1990) for (a) Competition Experiment Part 1, *E. nuttallii* vs. *L. major* and (b) Competition Experiment Part 2, *E. nuttallii* vs *E. canadensis*.

(a)



(b)

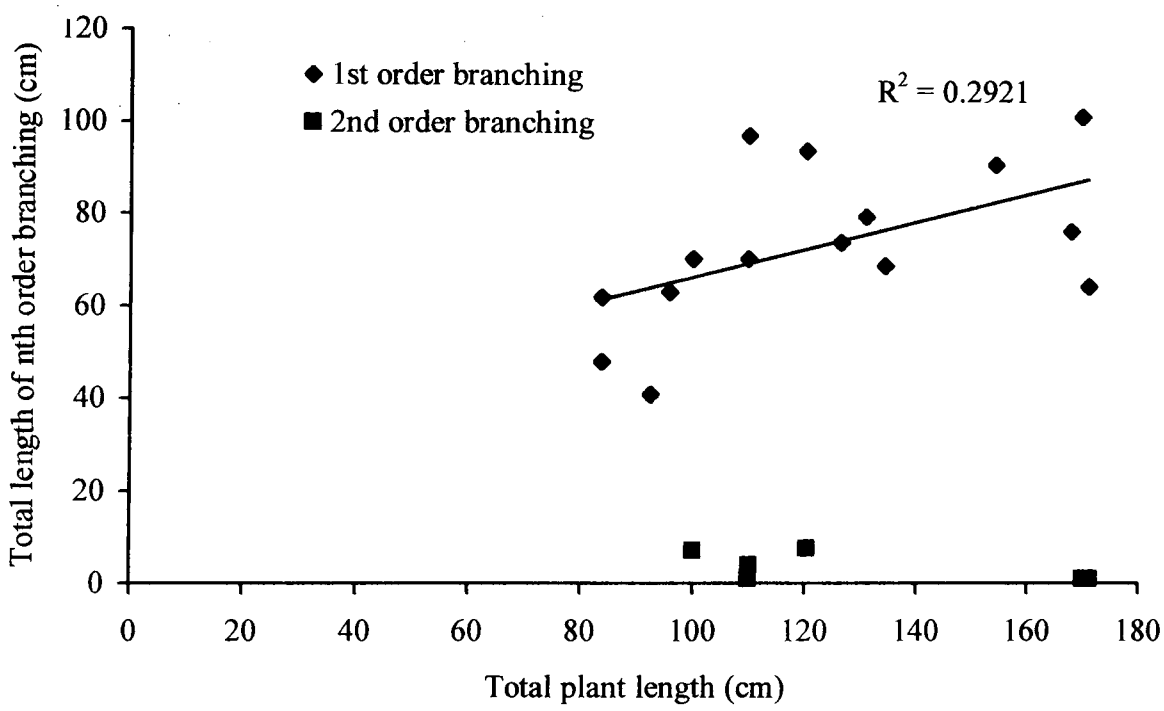


Fig. 3.20 Branch ordering of (a) *E. nuttallii* (n = 17) and (b) *L. major* (n=15) collected from Competition Experiment 1, Parts 1 and 2. As *L. major* had few 2nd order branches, total branching length corresponds approximately with 1st order branching points.

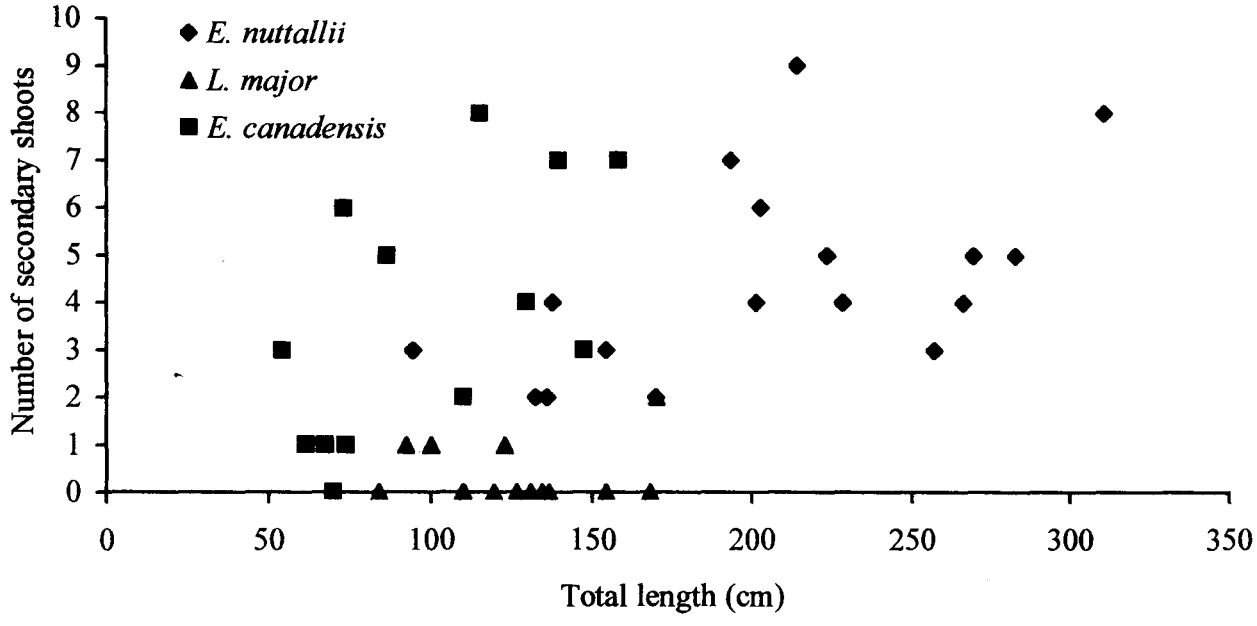
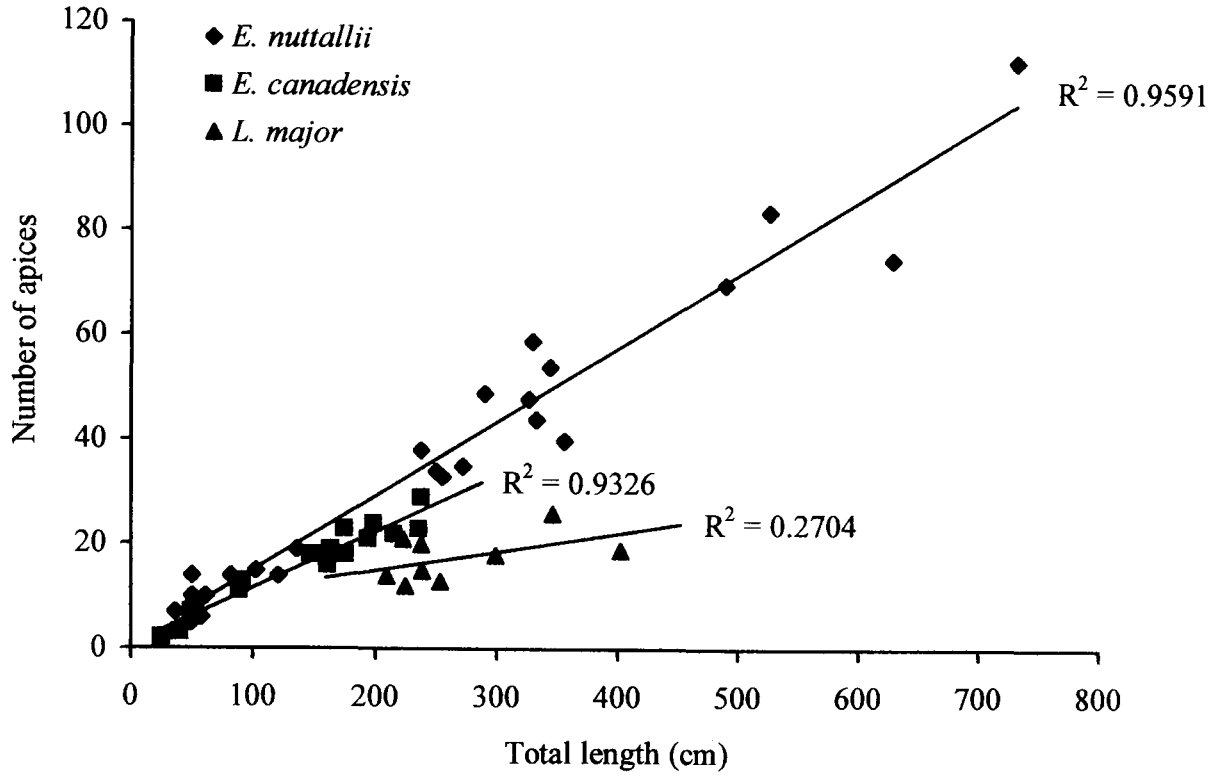


Fig. 3.21 Number of secondary shoot divisions agains total plant length. Data collated from Competition Experiment 1.

(a)



(b)

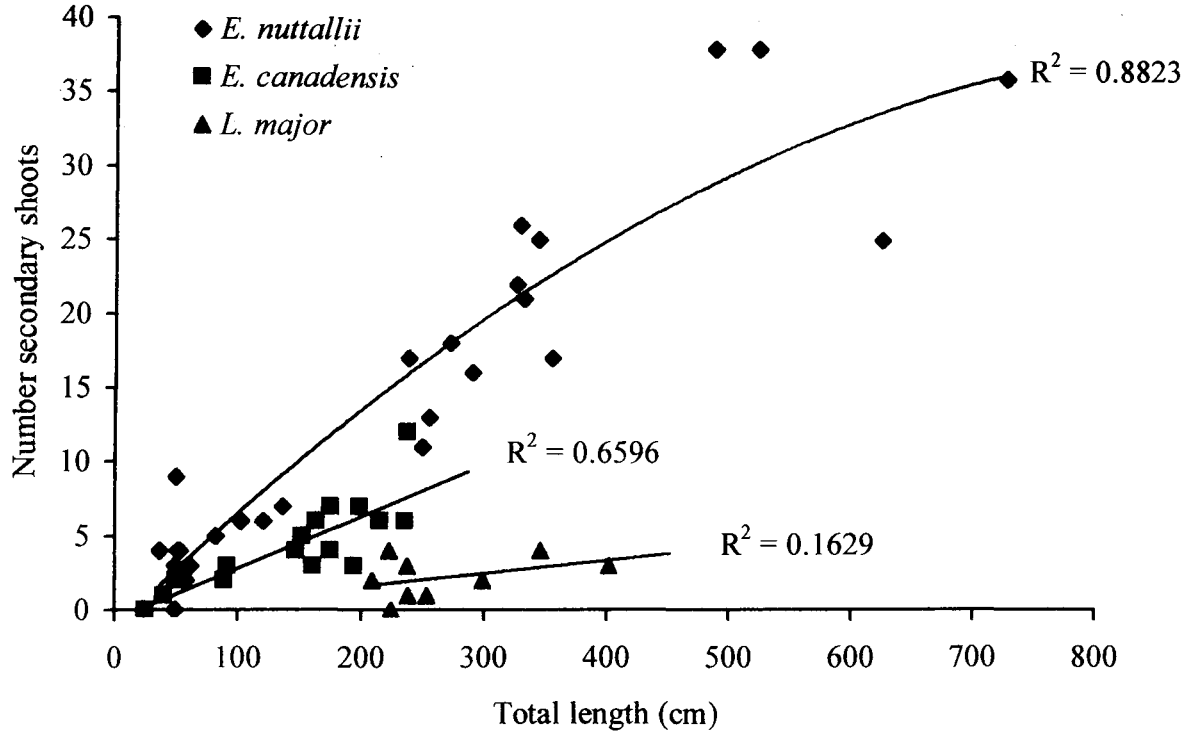


Fig. 3.22 Plant architecture data collated from Competition Experiment 2, (a) numbers of apical tips against total plant length (cm) and, (b) Number of secondary divisions against total plant length (cm).

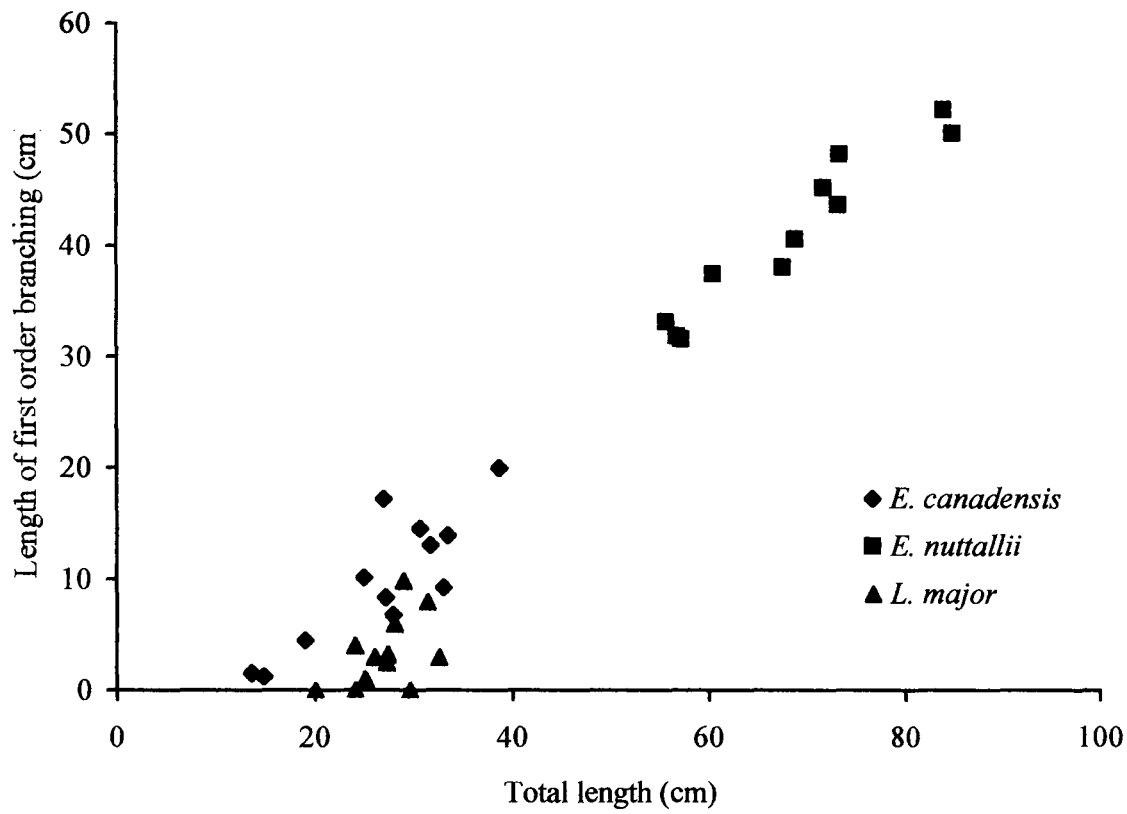


Fig. 3.23 First degree branching of *Elodea* spp. and *L. major* against total length of shoots grown for two weeks under laboratory conditions.

3.3.4 The growth of different starting lengths

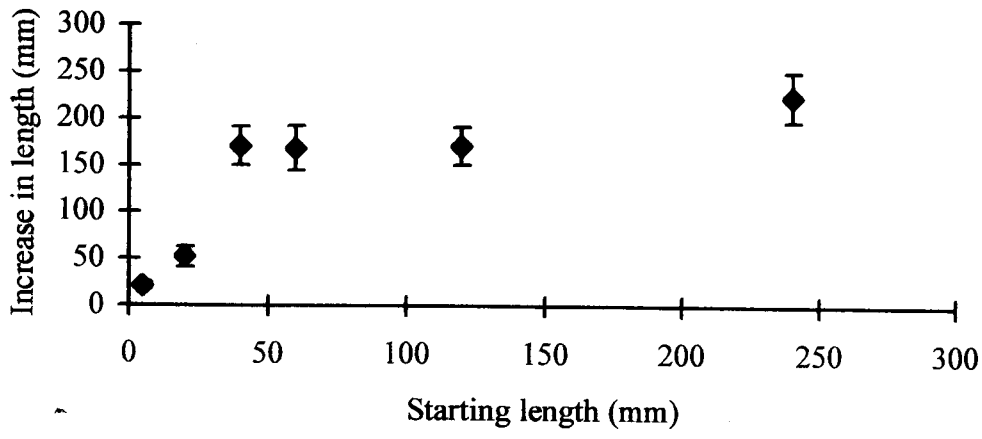
All initial shoot lengths of *E. nuttallii* and *L. major* used in this study were capable of growth. Increases in length (Fig. 3.24) were observed for starting shoots up to approximately 60 mm in length. The greatest growth rates for these species were observed for the smallest starting shoot length (5mm). Thereafter RGR rates were observed to decrease with increasing initial length (Fig. 3.25). For *E. canadensis* a different pattern was observed. Shoots of 20 mm and 40 mm starting lengths exhibited only slight growth and 10 out of 12 of the replicates with the smallest initial starting length (5 mm) died. Maximum RGR of *E. canadensis* were observed with initial starting lengths of 60 mm. A decrease in RGR was observed for replicates with starting lengths greater than this. During this study, *E. canadensis* appeared to be particularly prone to algal infestation (mainly epiphytic diatoms). This was most noticeable at the higher initial starting lengths. Significant algal growth was not observed in cultures of either *E. nuttallii* or *L. major*. It is therefore possibly that the reduced growth rates observed with the longer starting lengths of *E. canadensis* (120 mm and 240 mm) may be due to reduced light penetration through epiphytic algae growth on leaves, and increases in stress conditions (high pH and oxygen, and low CO_2 caused by the algal growth.

Branching was observed to occur for *E. nuttallii* with starting lengths equal to or greater than 20 mm (Fig. 3.26). *E. nuttallii* exhibited extremely rapid and prolific branching for starting units of length 40 mm and above, this is also reflected in length increases (Fig. 3.24 a) for these starting units.

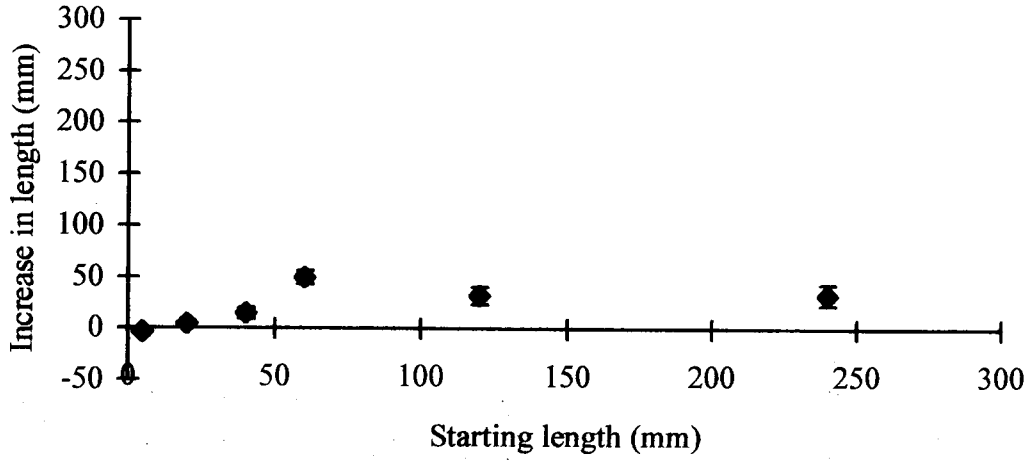
In summary

1. *Survival of shoots was greatest for E. nuttallii and L. major with almost 100 percent survival of even the shortest starting lengths (5 mm).*
2. *E. canadensis exhibited the highest mortality rate for shoots particularly for starting lengths of less than 60 mm.*
3. *Increases in length were greatest for starting lengths of 60 mm for E. canadensis and L. major, and 40 mm for E. nuttallii.*
4. *Relative growth rates (per unit dry weight) decreased with increasing starting lengths for E. nuttallii and L. major, but increased to a maximum for starting lengths of 60 mm, before decreasing for E. canadensis.*
5. *E. nuttallii had both the greatest growth rate and the greatest branching rate of the three species.*

(a)



(b)



(c)

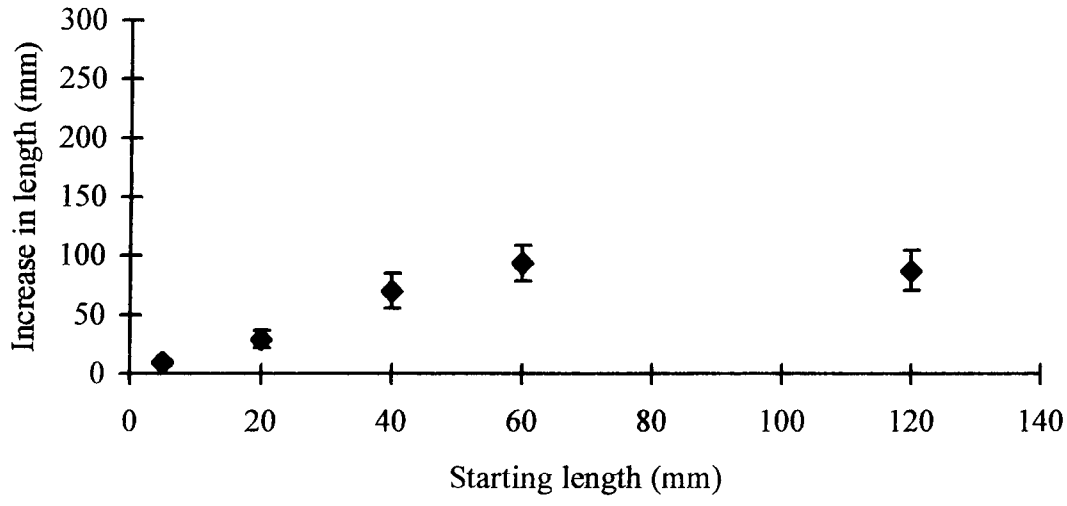
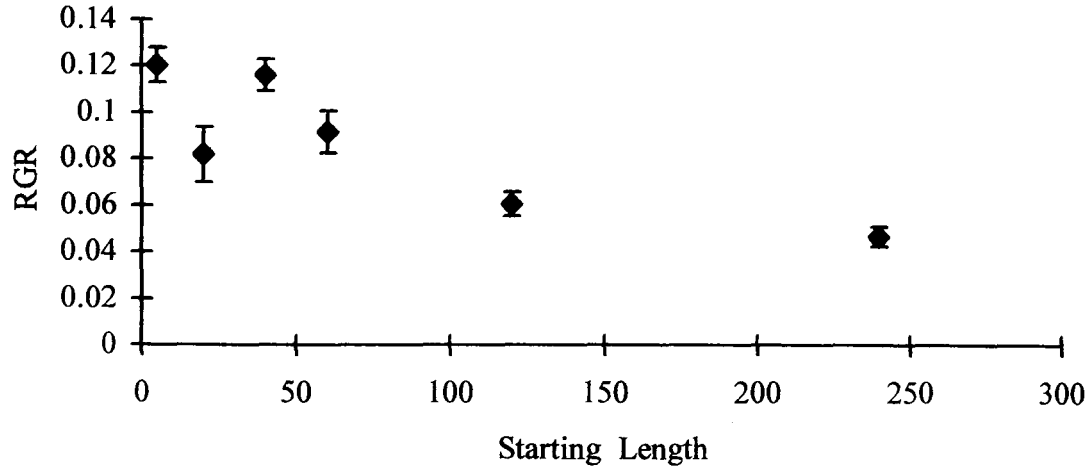
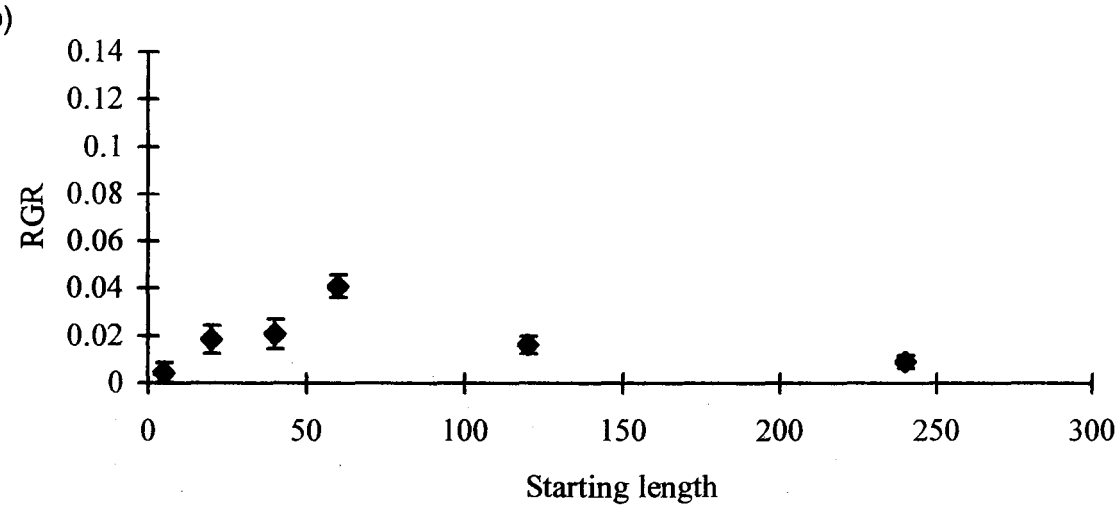


Fig. 3.24 The mean increase in length (mm) of a) *E. nuttallii*, b) *E. canadensis* and c) *L. major* of different starting lengths after two weeks in laboratory culture. (Mean + SE, n = 9-12)

(a)



(b)



(c)

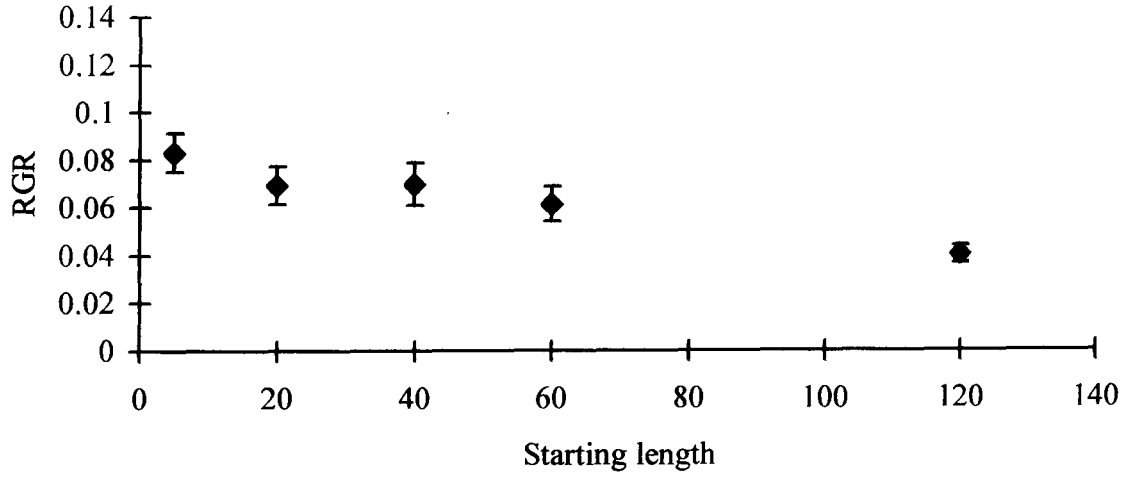


Fig. 3.25 The relative growth rate (mm mm^{-1} day^{-1}) of (a) *E. nuttallii*, (b) *E. canadensis* and (c) *L. major* of different starting lengths after two weeks in laboratory culture. (Mean + SE, n = 9-12)

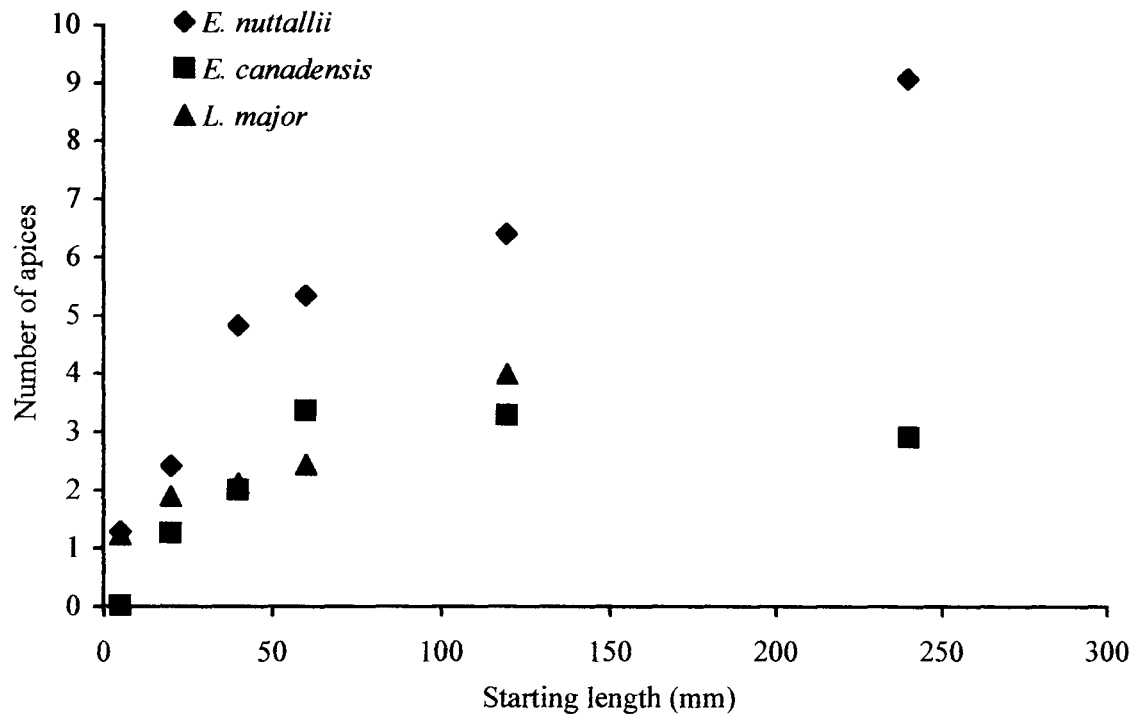


Fig. 3.26 The number of apices per plant of different starting lengths after two weeks in laboratory culture. Single, unbranched shoots having 1 apice and 0 apices indicating death of the starting unit.(Mean + SE, n=9-12)

3.3 Discussion

Internode lengths measured here for *E. canadensis* and *E. nuttallii* are similar to those reported by Simpson (1988). Internode lengths for *L. major* were considerably shorter being approximately 1.3 mm in length. Although the main zone of internode elongation was in the first 10 cm down from the apex for both *Elodea* spp., small further increases in internode length did occur below this point. The internode elongation region of *L. major* was less clearly defined although appeared to be within 15 cm of the shoot apex. As internode numbers between successive branches were not observed to vary down the stems, it must be presumed that changes in inter-branch distances arise solely from variation in internode lengths. Thus, shorter internodes will result in a denser, more compact growth form and vice versa. In morphological studies on *E. canadensis* and *E. nuttallii* at three different light intensities, Simpson (1988) observed that internode elongation showed no response to light intensity except for *E. canadensis* on nutrient poor sediment where a slight increase in internode length was observed at the lowest light intensity. However, it is likely that light quality plays an important role in determining internode length as shown for shoot elongation in *H. verticillata* (Van *et al.*, 1977). As discussed previously shoot elongation in this species was promoted green light and inhibited by red. It is probably that the light environment, both quantity and quality, is important in determining internode length and may also be important in controlling the development of other morphological features such as branching frequency and development of secondary shoots. Further studies, particularly on the effects of light quality on growth form, would provide valuable insight into control of growth form of these three species.

None of the field plants examined had branching of order greater than 3. However, the development of many secondary shoots, particularly under laboratory conditions, greatly increased the maximum branch order number (see Fig. 3.27). Due to the complex nature of plants collected in Competition Study 2 and the difficulties in determining the "main axis", ordering of these plants was not attempted. However, it was clear that in many cases secondary shoot production was in excess of 5, and in

Fig. 3.27 Drawing of an *E. nuttallii* shoot showing two secondary branches growing from close to the base of a lateral branch.

one case 8 secondary shoots were produced in successive branches on a plant of *E. nuttallii*. Visually, this appeared as if the eight branches and original side axis originated from almost the same point. If interpreted in terms of branch ordering, order numbers in excess of 9 are possible. As previously stated, interpretation of branching pattern of these species was often complicated by the lack of an obvious main axis. The plants appeared to loose their monopodial appearance and take on a more sympodial growth form. While none of the field examples examined in the present study exhibited this degree of secondary shoot production, under very high densities greater secondary shoot production has been observed in the field (personal observations of the present author). Secondary branch production in *E. nuttallii* plants may be a response to reduced space. Final biomass densities measured for Competition Study 2 were much greater than those measured for Competition Study 1 (See Chapter 7, Tables 7.4 and 7.5). In the former study, secondary shoot production was greater for plants of similar total length than in the latter. For example, a *E. nuttallii* plant of a total length of 150 cm had approximately 4 secondary shoots for Competition Study 1 and 10 for Competition Study 2. This response was not observed for either *E. canadensis* or *L. major* as similar numbers of secondary shoots were produced in both competition studies for these species. The ability of *E. nuttallii* to produce secondary branches may allow this species to take advantage of a localised increases in limiting resources and will allow the development of an extremely dense canopy close to the water surface.

Differences in total plant length against length of 1st and 2nd order branching reflect the observed vertical profiles of the species. In field-collected material, both *E. nuttallii* and *L. major* grew tall single stems before the significant production of 1st order branches. However, 1st order branches were observed in even quite short plants of *E. canadensis*. A greater initial height before the development of 1st order shoots does not necessarily imply a more efficient growth form as 1st order shoots can still develop from the base of a shoot rather than close to the plant apex where light intensities will be greatest. However, particularly for *L. major*, branching points were observed to remain dormant and this is reflected in the efficient canopy formation observed for this species in vertical biomass profiles. However although *E. nuttallii* grew rapidly, when 1st order branches were produced they were observed frequently

to develop close to the base of the plant, therefore not taking advantage of the higher light intensities towards the shoot apex. Thus, it appears that the main difference in growth between the *Elodea* spp. was in the growth rate and not the architectural form.

Field material of *E. canadensis* was collected towards the middle of the growing season, July and August. It was therefore expected that sufficient time had elapsed from the start of the growing season to allow for establishment and growth of a substantial canopy, yet before the advent of significant breakage and rotting of the lower shoot sections that is characteristic during the autumn period. However, these plants were not as large, measured in terms of length, as those of *E. nuttallii* and *L. major*. Field observations, particularly in the disused Nottingham Canal, also confirm that although *E. canadensis* had achieved a relatively high biomass at this site, plants were not forming dense canopies at the water surface but still growing up towards the surface quite late in the season. Similar observations were also made on the Montgomery section of the Shropshire Union Canal. Thus, in competition with *E. nuttallii* and *L. major*, plants of which were found to have developed a substantial zonation in biomass, *E. canadensis* would be at a distinct disadvantage. Rapid growth and canopy formation by competing species, particularly *E. nuttallii*, would further reduce the slow growth of *E. canadensis* through increased shading of its shoots while they were still lower down in the water column. This is supported by the observations of Kundel (1990) and Simpson (1988) who both suggest that the rapid growth rate of *E. nuttallii* may result in it over-growing *E. canadensis* and effectively shading out the latter species.

It is difficult to compare the growth forms observed in field material with those of laboratory cultured material as the latter were grown in shallow buckets where light intensity does not vary to the same extent with depth as in the field. In addition, there is not sufficient space for the development of a distinct vertical profile. However, this approach does have the advantage of allowing direct comparisons of growth patterns between the three species under controlled conditions. During a two-week growth period, both the total length of *E. nuttallii* and the lengths of first order branching was significantly greater than either *E. canadensis* or *L. major* (Fig. 3.23).

In addition, *E. nuttallii* plants analysed from Competition Study 1 showed significantly greater total length and numbers of apices than either *E. canadensis* or *L. major*. This feature, in combination with rapid canopy production and differences in over-wintering may result in this species being effectively able to pre-emptively shade competing species.

Measurements of the survival and growth of different initial lengths of the three species also emphasised differences between the three species. While short shoot sections (5 mm in length, with an intact apical tip) of *E. nuttallii* and *L. major* survived the culture period, nearly all of the shortest starting lengths (5 mm) of *E. canadensis* died. In fact, survival of shoots of *E. canadensis* did not significantly improve until starting lengths exceeded 40mm. This suggests that spread of *E. nuttallii* and *L. major* may be enhanced by the ability of even the smallest fragment to survive. However, while small fragments can survive, it was apparent that attached apices are to some extent supplied with resources from older parts of the shoot as greater increases in length are observed for longer shoots, up to approximately 60 mm in starting length. These results suggest that growing apices are to some extent supported by resources from plant parts within approximately 60 mm of the growing tip. It should however be noted that, although slight, differences in light intensities received by the apical tips due to differences in height of the shoot are likely to confound these results and a further study in which differences in height between the different shoot lengths were compensated for by elevating the shoots would clarify this point.

Analysis of plant growth and architecture indicate that in competition between *E. canadensis* and *E. nuttallii*, the latter's ability to grow rapidly, forming tall plants which produce significant numbers of branches with efficient high level canopy production may convey a competitive advantage. Both *E. nuttallii* and *L. major* produced canopy structures with the majority of their biomass concentrated towards the water surface, although the development of the *L. major* canopy may take a longer period of time due to its slower growth rate. Additionally, survival of shoot fragments extremely small in length may promote the spread of both *E. nuttallii* and *L. major*. However, while fragmentation readily occurs in *E. nuttallii*, *L. major* is a

sturdier, stronger plant and breakages are generally not as frequent under controlled laboratory conditions as either *Elodea* spp. Thus, this may partially explain the relatively slow spread of *L. major* in the UK, despite its introduction over twenty years prior to that of *E. nuttallii*. The high proportion of broken tips noted in *L. major* material from field sites can not be accounted for as prior conditions at the site, both chemical and physical, are not known.

Chapter 4 LIFE HISTORY AND SEASONAL GROWTH

4.1 Introduction

Dense stands of macrophytes may resist invasion by other submerged species by monopolising local resources. During the main growing season well-established species already dominate local resources. As Sculthorpe (1985) suggests a newly invading species may therefore exert its influence early on in the season, before established species have attained their maximum growth. In the following chapter, the effects of temperature on photosynthesis and growth are investigated. In addition, tissue starch concentrations are measured since starch reserves may both aid the survival of plants under adverse conditions, and may also provide essential resources for re-growth during early spring.

During the autumn, apical shoots of *Elodea nuttallii* often become detached and sink on to the sediment, forming dense mats of short, green-leaved stems (Kunii 1982, 1984). As the temperature increases in the following spring re-growth occurs from these shoots (Cook and Urmi-Konig, 1985). They grow rapidly upwards, branching profusely to form a dense canopy at the water surface. In northern temperate regions, the greatest plant biomass is achieved between June and August (Engle, 1988; Kunii, 1984). During the latter part of the summer and autumn, basal leaves die back, the stems begin to disintegrate and the plants become largely detached from the substrate, shedding apical shoots which continue the vegetative cycle. *Elodea canadensis* differs from *E. nuttallii* in its ability to produce distinct over-wintering buds or turions (Fig. 4.1). Turions are axillary, terminal buds with dense clusters of tightly packed leaves (Sculthorpe, 1985). These develop on shoots of *E. canadensis* in late autumn. They remain dormant on the bottom sediment during the winter before developing new growth in the following spring (Catling and Wojtas, 1986; Nichols and Shaw, 1986). While dormancy is used to describe the state in which the apices over-winter, it would be more accurate to describe this as enforced dormancy. Enforced dormancy is dormancy that is imposed by external environmental conditions, e.g. the absence of normal requirements for growth. It can be broken as soon as the growth requirements are met. For example, growth of *E. canadensis* recommences as soon as temperature and light intensity increase (Cook and Urmi-Konig, 1985).

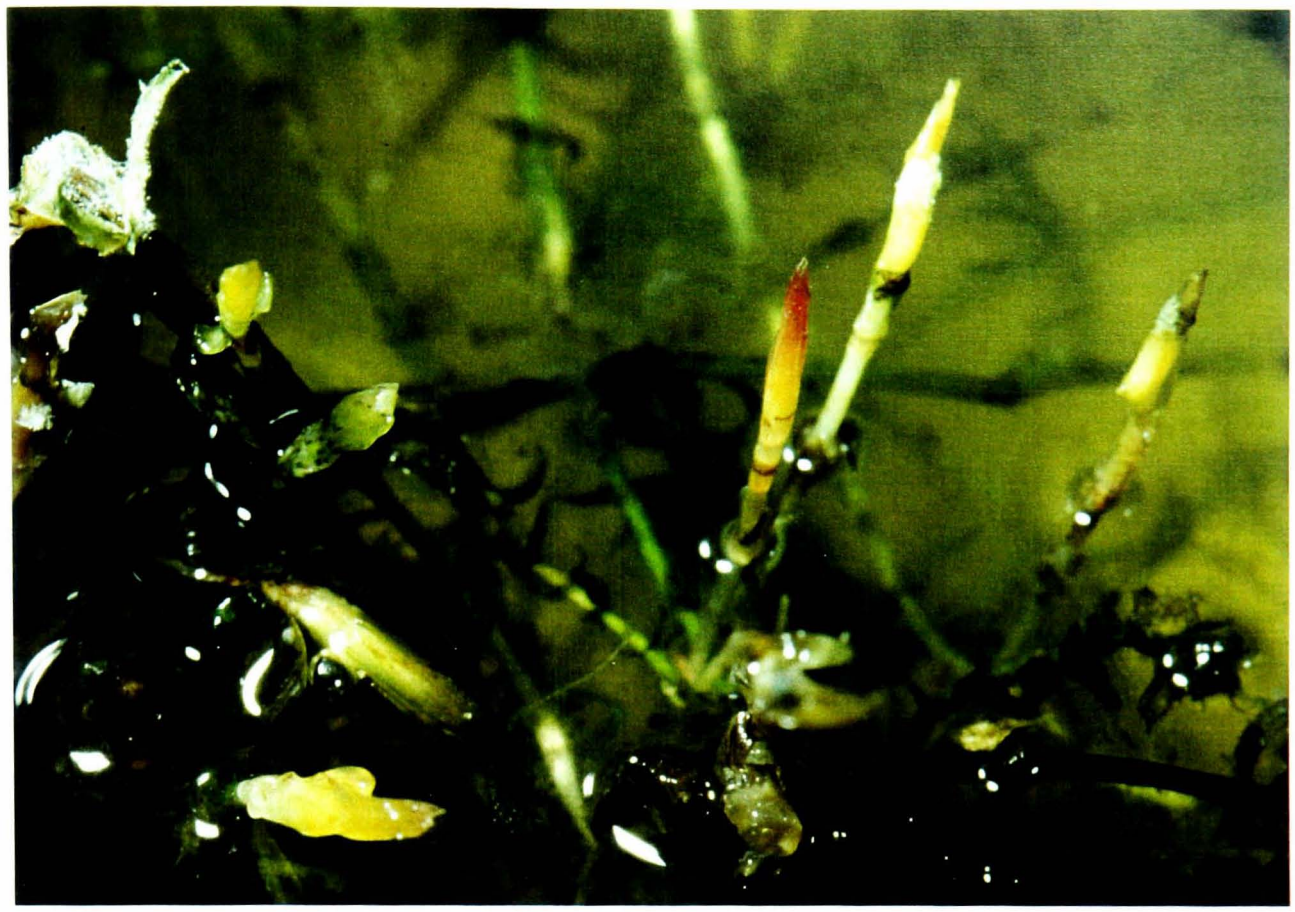
Fig. 4.1 (a)

Photograph showing over-wintering turions of *E. canadensis*

Fig. 4.1 (b)

SEM of stem cross section of *E. canadensis* turion showing large numbers of starch grains within cells.

a)



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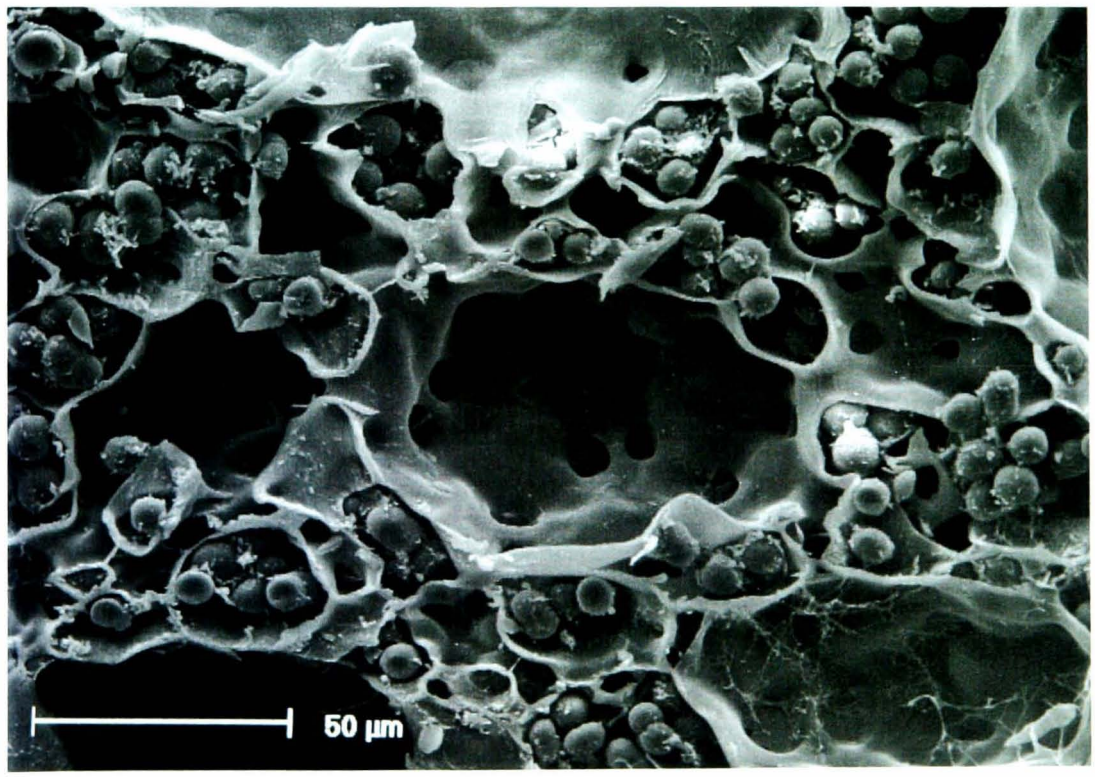


Less information is available on the over-wintering strategy of *Lagarosiphon major*. Personal observations suggest that at least in the British Isles, little die back occurs and growth may continue throughout the winter period. Schwarz and Howard-Williams (1993) found no distinct seasonal pattern in stand biomass of *L. major* growing in Lake Taupo, New Zealand. Even during the winter period a biomass of almost 300 g dry weight m^{-2} was recorded. Machena *et al.* (1990) reported that *Lagarosiphon ilicifolius,* a morphologically similar species, is perennial and grows all the year around, although the main growing season in Lake Kariba, Zimbabwe, is during the summer, October to February. *L. major* has no specialised over-wintering buds or turions.

Seasonal growth patterns of many temperate submerged macrophytes typically follow a similar pattern, with maximum growth achieved during spring and summer, and a virtual cessation of growth in the winter. A species that can grow throughout the winter period, even minimally, or grow rapidly at the start of the growing season at lower temperatures than competing species, may gain a competitive advantage. These features provide the potential for the early occupation of space and dominance of limiting resources within the water body. For example, the ability of *Eleocharis acicularis* to out-compete *E. coloradoensis* may well be related to its establishment and growth early in the season, despite its smaller, lower stature (Ashton and Bissell, 1987). Ashton and Bissell attribute this to tolerance of low light and temperature conditions by *E. acicularis*. Kunii & Maeda (1982) reported that growth of *E. nuttallii* started earlier in the growing season than was the case for all other species present except *Potamogeton crispus*. Competitive displacement may consequently take place early in the season, the subsequent field distribution observed during the summer period being the outcome, and not itself competitive displacement in action.

Implicit in the ability to grow early in the growing season and/or throughout the winter is survival and growth at low temperature. Both *E. canadensis* and *E. nuttallii* are reported to exhibit minimal growth at 4 to 5 °C (Madsen and Brix, 1997; Kunii, 1981). For *E. canadensis*, Madsen and Brix (1997) attributed the suppression of photosynthesis and growth at 5 °C to possible loss of physiological integrity, as found with chill-sensitive plants (Lyons 1973; Larcher 1995). They also commented

that reduced growth rates may be idicative of changes in physiology as plants go into an inactive stage for winter survival. Studies on *E. nuttallii* indicated that while minimal growth does occur at low temperature (5 °C), substantial growth of roots and shoots does not occur until the water temperature reaches between 8.2 and 12 °C (Kunii, 1982, 1984). This corresponds with field observations on *Elodea* spp. of substantial growth during March and April, when water temperatures are often in this range.

While temperature will have pronounced physiological effects upon the initiation of growth, other factors may be important in determining initial shoot survival and growth. Some aquatic species possess organs such as tubers (e.g. *Potamogeton spp.)* or turions (e.g. *Elodea canadensis*) that are rich in starch (Janauer, 1981) (Fig. 4.1). While many macrophyte species do not possess specific storage organs, old attached stems such as those from last year's growth may supply resources for the growth of new shoots. Rorslett *et al.* (1986) state that old stems of *E. canadensis* provide a source of nitrogen and phosphorus for new shoots, it is therefore likely that starch resources in older stems are also utilised in this manner. Ozbay (1998) observed higher relative growth rates for *E. nuttallii* stems with a 5 cm section of old stem attached than without. Mobilisation of reserves from this old tissue could aid the growth of new shoots at the beginning of the growing season, allowing plants to grow up to the euphotic zone of the water column where strongly positive net photosynthesis could then be achieved. Plants with high internal starch reserves may also be better able to tolerate stress conditions, such as the low temperature and light conditions that characterise the early seasonal environment. These species would thus have a distinct competitive advantage at the beginning of the growing season.

The aim of the work described in this chapter was to investigate whether temperature responses and use of storage products influence, or may even be central to, the outcome of competition between *E. canadensis*, *E. nuttallii* and *L. major* in the field. Field and laboratory studies were made to characterise the response of growth, physiology and, possession and utilisation of storage products to temperature under both controlled laboratory conditions and in the field.

4.2 Methods

4.2.1 Winter growth in the field

Winter growth of the three species was measured in the field. Twelve 10 cm shoots of each species were collected from laboratory cultures maintained at 15 °C and ~80 μmol m^{-2} s^{-1}. Shoots were then planted singly in 200 ml plastic cups filled with canal sediment and placed in 10 l buckets filled with tap water previously aerated for 24 h. Shoots were grown for two weeks in nursery cultures to allow establishment. Following nursery culturing, shoot length (cm) was measured and each potted shoot transferred to a shallow white 5 litre bucket, four shoots per bucket. These were then placed at a depth of 0.5 m in the Leeds and Liverpool Canal at Melling (SD 384 004), a section of waterway with little boat traffic or side vegetation. Plant lengths were measured in the field after 5 and 10 weeks and water temperatures taken on the day of plant measurements. From measurements of shoot length (cm) relative growth rates per unit length were calculated. Loss of replicates prevented further measurements being taken after 10 weeks.

4.2.2 Photosynthetic rates of winter material

Photosynthetic and respiratory rates were measured on plant material of *E. canadensis*, *E. nuttallii* and *L. major* collected from the field sites Ec N, En M and Lm L respectively. Prior to measurements, all material was kept under controlled laboratory conditions, initially at 5 °C (similar to that encountered in the field) and gradually over 48 hours brought up to 15 °C. All measurements were made according to the proceedures described in Section 2.5 within five days of collection at 15 °C at a light intensity of 290 μmol m^{-2} s^{-1}.

4.2.3 Starch, nitrogen and phosphorus content in field material

The method used for starch analysis was adapted from McCready *et al.* (1950). It is based upon the reaction of anthrone with carbohydrate to form a blue-coloured complex, as first reported by Dreywood (1946). A simplified approach was developed and used here in which simple sugars were removed using ethanol and the residue fraction with the insoluble starch analysed following a simplification of the

method of McCready *et al.* (1950), which involves solubilising the starch in perchloric acid. Preliminary analysis revealed no significant differences between values obtained with this simplified approach and the more lengthy procedure followed by McCready *et al.* (1950) (See appendix III for method development and results).

Plant samples of *E. canadensis*, *E. nuttallii* and *L. major* were collected from field sites listed in Table 4.1. Different sites were used as a consequence of the death all the vegetation in the Leeds and Liverpool canal following the salt intrusion mentioned previously. Consequently, samples taken after January 1997 were collected from the Montgomery section of the Shropshire Union Canal for *E. nuttallii* and *E. canadensis*. No comparable field site was known at this time for the collection of *L. major* material for analysis. Fresh weights were estimated after spinning samples in a spin drier for 30 seconds. Samples were then dried to a constant weight at 40°C and re-weighed. Starch, nitrogen and phosphorus analyses were performed on whole plant samples unless otherwise specified. The nitrogen and phosphorus content of plant material was analysed following the methods described in Allen (1989). Total nitrogen analysis was measured using the kjeldahl procedure followed by the colorimetric method for determination of ammonium nitrogen using the indophenol-blue reaction (as described in Table 2.2). For phosphorus tissue content determination, phosphorus was first extracted in sodium hydroxide then analysed as for total phosphorus (See Table 2.2 for Methods).

Table 4.1

Source sites for collection of field material for analysis of starch content. For explanation of site codes see Chapter 2, Table 2.1.

Species	Sites
E. canadensis	Ec N, Ec WB
E. nuttallii	En M, En BL
L. major	Lm SD

4.2.4 Temperature Studies

Two temperature studies were conducted under controlled laboratory conditions. For the first, experiment plant material was not acclimatised before use

and no supplementary nutrients were. A brief description of this first study is included below for comparison with later results.

4.2.4.1 Temperature study 1

Plant material of *E. canadensis*, *E. nuttallii* and *L. major* was collected from sites Ec N, En N and Lm L. Eighteen 8 - 10 cm shoots of each species were selected, cleaned carefully to remove epiphytes and fresh weight measurements of individual shoots taken. Shoots were then planted in 250 ml plastic cups filled with canal sediment and placed in 3 l jars, filled with 2.5 l of tap water previously aerated for 24 h. Jars were placed randomly in three thermostatically controlled water tanks with temperatures adjusted to 10, 15 and 20 °C. Jars were aerated throughout the experiment.

To ensure growth conditions were similar for all species and treatments, with the exception of temperature various chemical parameters were monitored. pH readings were taken 2 cm below the water every two to three days. Conductivity measurements were taken in conjunction with the pH readings. Alkalinity measurements were made every 14 days. From the measurements of temperature, pH, conductivity and alkalinity, total carbon and the carbon fractions CO_2*, HCO_3^- and CO_2^3 calculated. After 35 days plants were harvested. Measurements of shoot fresh weight, root fresh weight, total length of shoots and roots, and total number of apical tips were then made.

4.2.4.2 Temperature Study 2

Following the first experiment (Temperature Study 1), it was felt that the rapid changes in temperature from field conditions to experimental cultures involving a rapid temperature change of 10 °C, may have contributed to the death of a number of replicates during the experiment. In addition, differing degrees of acclimation may have been responsible for the variability in growth of replicates within the same treatment. As a consequence, for a second experiment an acclimation regime was introduced which it was hoped would reduce the stress experienced by shoots, through a more gradual controlled rise in temperature.

Supplementary nutrients were also added to an adequate supply of nutrients and to avoid confounding the effects of temperature with nutrient limitations.

Plant material for this second study was collected from sites: Ec N, En N and Lm L for *E. canadensis*, *E. nuttallii* and *L. major* respectively during early spring, when water temperature was approximately 7 °C. The collected plant material was first cleaned carefully to remove algae before being placed in a bucket filled with tap water in a temperature controlled growth room at 5 °C for 24 hours, after which time the plants were transferred to a 10 °C water tank for a further 24 hours. Plant material to be used in 15 °C and 20 °C treatments was then transferred to a 15 °C water tank. After 24 hours, plants to be grown at 20 °C were transferred to a 20 °C water tank. From the acclimatised plant material, 10 shoots, each with a length of 10 cm and intact apical tips and no side shoots were selected as starting units. Species were grown for a period of two weeks at the specified temperature (10, 15 or 20 °C) in nursery cultures to allow establishment of the shoots. At the start of the experiment initial fresh weight and dry weight measurements were estimated from sub-samples of each species at each temperature (n = 4). Death of a number of replicates during nursery culturing prevented higher replication. The six remaining shoots were placed singly in 3 litre glass jars, filled with 2.5 litres of tap water. Jars were then randomly assigned positions in the three thermostatically controlled water tanks. Jars were continuously aerated throughout the experiment. Nitrogen and phosphorus concentrations were measured after 7 and 14 days. 18 replicates were selected at random and SRP, nitrate and ammonium concentrations analysed according to methods described in Table 2.2. On both occasions 50 μg P l^{-1} were added to all replicates to maintain phosphorus concentrations between 50 and 100 μg l^{-1}. Continuous monitoring (pH, conductivity and alkalinity) was as described for Temperature Study 1.

After 22 days growth at the specified temperature, photosynthetic and respiratory rates were measured as oxygen evolution or uptake using a Clark type O_2 electrode as described in Chapter 2, Section 2.5. A slide-projector with tungsten bulb provided incident light of 100 μmol m^{-2} s^{-1} PAR, a light level equivalent to that received by the plants during the experiment. Temperature was controlled using a

water jacket fed from a thermostatically regulated water bath. For physiological work, temperatures of 10, 15 and 20 °C were used in accordance with the temperature to which the plants had been grown in during the experiment. After the physiological measurements, all plants were harvested. Fresh weight was estimated by removing excess water from the shoots by padding dry with paper towels. Plants were then separated into shoot and root components, number of apices were counted, and each component dried to a constant weight at 40 °C.

4.2.5 Cold and freezing injury

The effects of low temperatures on cell integrity were measured through solute leakage, detected as the consequent increase in medium conductivity. Preliminary investigations (Appendix V) found no increase in solute levels following treatment at 4.5 °C or 13 °C showing that no freezing damage occurred. Consequently, the temperature regimes chosen for the present study were below this. Fresh plant material of *E. nuttallii* and *L. major* was collected from cultures (En L and Lm L). Fresh plant material of *E. canadensis* was collected from Ec No. Plant material was cleaned and 0.7 g fresh weight of *E. canadensis* and *L. major*, and 0.5 g fresh weight of *E. nuttallii* weighed out. Plant material was then placed in plastic 25 cm^3 containers and 20 cm^3 of tap water added, with three replicates per treatment. An initial tap water conductivity measurement of 235 µS was recorded. Controls containing only 20 cm^3 of tap water were also prepared. Treatments and controls were then assigned to different temperature regimes, i.e. 3, 1, -10 and –18 °C for 12 hours. Following the treatments, containers were transferred to a 15 °C growth room and conductivity measurements repeated once the medium temperature had reached 15 °C.

4.3 Results

4.3.1 Growth and photosynthesis of winter material

No replicates showed positive growth in the field during the first month of measurements (Fig. 4.2). The water temperature at the time of measurements was 4.5 °C. The high variability and negative growth rates observed were likely to be a consequence of damage occurring while taking measurements, as plants were found to be extremely brittle and fragmented easily. However, significant growth of both *E. nuttallii* and *L. major* was observed for the second month of measurements, during which time the water temperature rose from 4.5 °C to 11 °C. The relative growth rate of *E. nuttallii* for this second period was significantly higher ($p = 0.05$) than that of either *L. major* or *E. canadensis*. Recorded relative growth rates of *E. canadensis* were still negative, indeed little growth of this species was observed throughout the experiment.

Physiological measurements on winter material were made following a short period of acclimation. *E. nuttallii* had significantly higher ($p = 0.005$) rates of photosynthesis than either *E. canadensis* or *L. major* (Fig. 4.3). No significant differences were observed between the latter two species. Respiration rates of *L. major* were significantly higher ($p = 0.05$) than those of *E. canadensis*.

4.3.2 Measurements of starch, N and P content from field samples

Measurements of starch content made on material collected from the field revealed a high degree of variability (Table 4.2). Generally, starch concentrations in the *Elodea* spp. were higher than those in *L. major*. The data do not suggest any seasonal patterns in starch, nitrogen or phosphorus content. Nitrogen concentrations were similar between species. Concentrations of phosphorus in *E. canadensis* were found to be higher than in either *E. nuttallii* or *L. major*. Phosphorus concentrations were similar in *E. nuttallii* and *L. major*. Starch levels measured in turions of E. canadensis collected from Ec N [211.86 (± 7.20) mg g^{-1} DW ($n = 4$)] were significantly higher ($p = 0.01$) than levels measured in other material of *E. canadensis* collected.

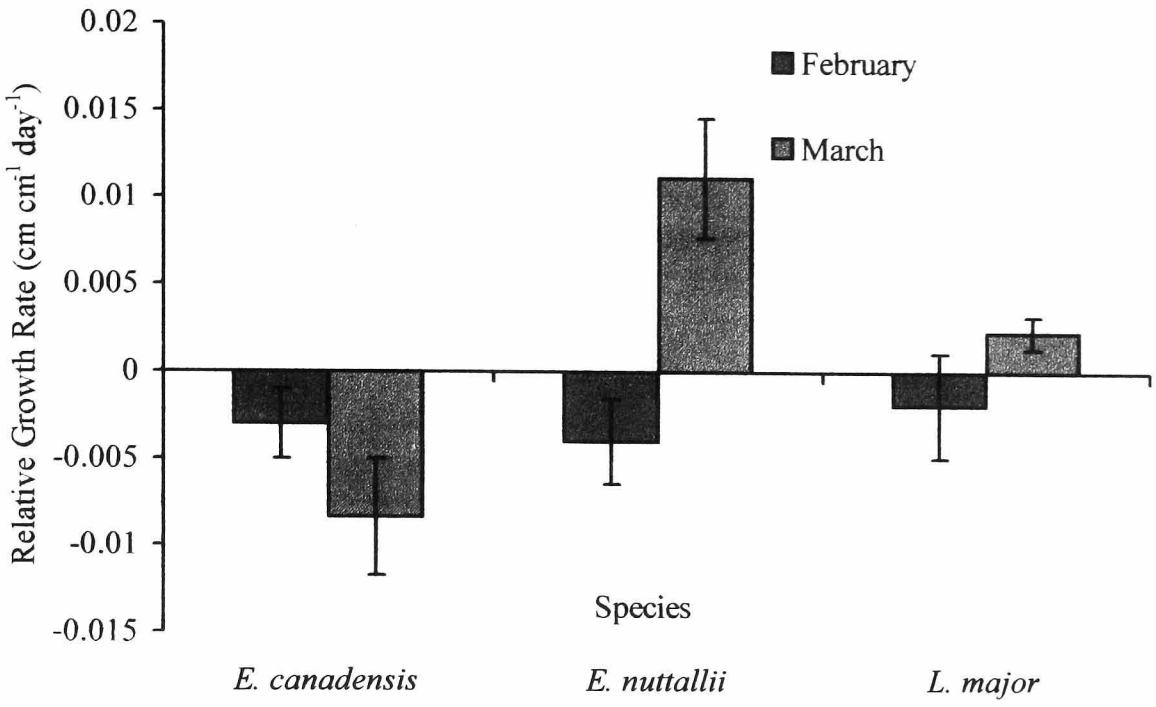


Fig. 4.2 In situ measurements of relative growth rates (cm cm^{-1} day^{-1}) of *E. canadensis*, *E. nuttallii* and *L. major* for Febuary and March 1997 in the Leeds and Liverpool Canal, Melling. (Mean $\pm$ SE, n = 9 -12)

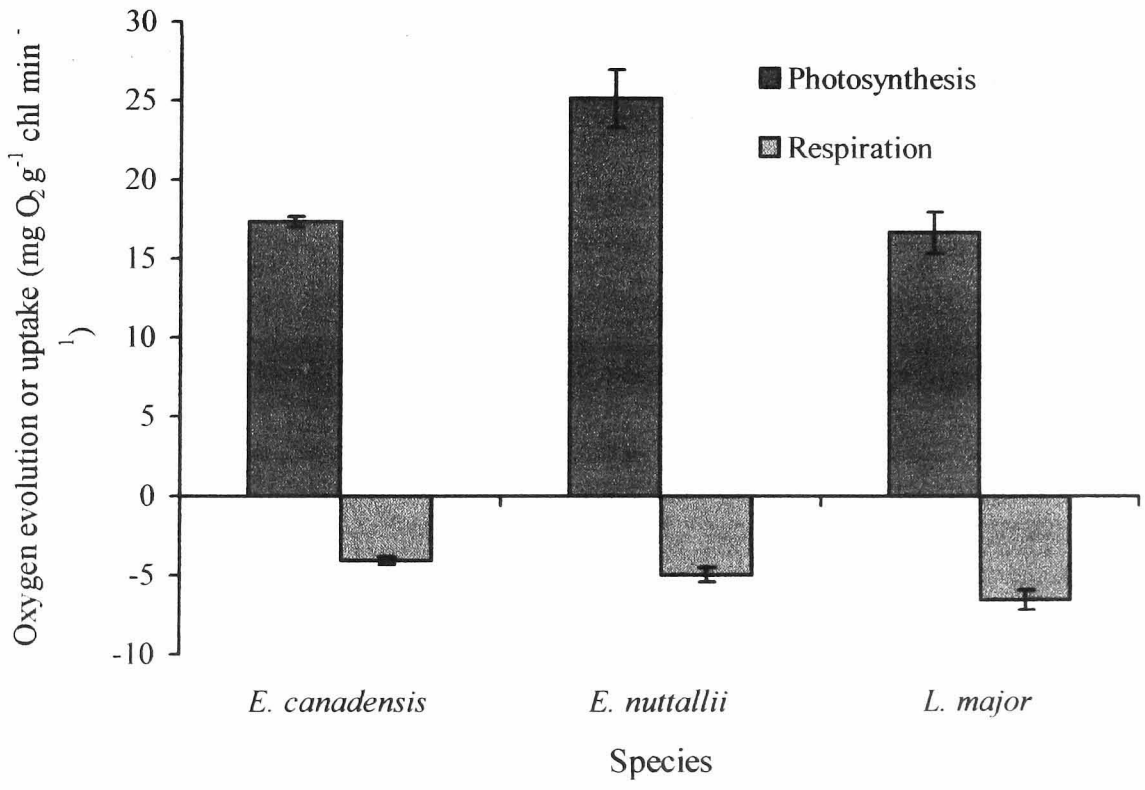


Fig. 4.3 Photosynthetic and respiratory rates of plant material collected from the field in February 1997. (Mean $\pm$ SE, n = 6)

Table 4.2
Changes in Fresh : dry weight ratio (FW:DW), starch (mg g^{-1} dry weight) , N (mg g^{-1} dry weight) and P (mg g^{-1} dry weight) content of material collected from the field.

Species		Date										
		9/9/96	5/11/96	12/12/96	14/01/97	24/01/97	19/2/97	23/5/97	2/6/97	3/7/97	29/9/97	14/3/98
E. canadensis	FW:DW	-	10.0	10.75	-	9.79	10.50	10.10	8.33	-	11.76	12.32
	Starch	63.99	47.80	118.73	-	27.09	61.68	40.86	81.72	26.22	19.72	28.52
	N	14.41	15.04	15.47	-	15.79	16.58	18.35	21.62	20.93	20.25	19.77
	P	7.91	6.75	8.31	-	8.62	8.09	2.56	6.63	5.99	5.88	5.56
E. nuttallii	FW:DW	-	10.92	10.12	8.94	-	8.47	11.41	8.30	-	7.55	13.16
	Starch	33.92	40.09	30.07	41.38	-	121.81	30.07	75.55	93.08	66.30	47.80
	N	14.27	20.82	17.97	19.03	-	18.96	17.89	18.93	15.4	9.55	17.09
	P	1.9	3.05	2.95	1.94	-	1.33	3.75	5.86	3.91	2.33	5.71
L. major	FW:DW	-	7.24	6.73	10.00	-	-	10.02	-	-	-	-
	Starch	71.70	35.46	28.52	21.48	-	-	-	-	-	-	-
	N	12.29	11.47	14.73	11.93	-	-	16.21	-	-	-	-
	P	2.08	1.7	1.93	2.39	-	-	2.21	-	-	-	-

4.3.3 Temperature Study 1

Significant differences between the mean pH of treatments (10, 15 or 20°C) were observed at the beginning of the experiment. However, the actual difference between treatment means was less than 0.2 pH. In all cases an increase in pH was observed over the experimental period (Fig. 4.4). By the final measurement, day 35, the treatment at 15 °C had a significantly higher pH ($p = 0.05$) than treatments at 10 or 20 °C. No significant differences in pH were observed between species. The initial mean Total CO_2 was 1.076 ± 0.01 mmol l^{-1}, with calculated proportions of free CO_2, bicarbonate and carbonate as 7.2 %, 92.3 % and 0.5 % respectively. By the final harvest the mean Total CO_2 had decreased to 1.03 ± 0.02 mmol l^{-1}. A breakdown of Total CO_2 into the various carbon fractions revealed a decrease in free CO^2 to 3 % and a proportional increase in bicarbonate (96.2 %) and carbonate (0.8 %). No significant differences were observed between culture treatments or species.

A high mortality rate was exhibited by a number of *E. nuttallii* replicates over the experimental period (Table 4.3). Only one replicate from the other two species showed a reduction in weight (*L. major* replicate at 10 °C). In addition to a reduction in weight, *E. nuttallii* plants appeared chlorotic and damaged, the original shoots planted had in many cases disintegrated, leaving only a new short pale shoot close to the base of the stem. It is suggested that death may result from damage during handling of the plants before planting. Consequently, for calculation of growth parameters, dead replicates were removed from subsequent calculations.

Table 4.3

Percentage survival of shoots following temperature treatments.

Species	Temperature		
	10 °C	15 °C	20 °C
E. canadensis	100	100	100
E. nuttallii	83.3	50	66.7
L. major	83.3	100	100

Relative growth rates of *E. canadensis* and *L. major* were significantly greater ($p = 0.05$) for plants grown in the 20 °C temperature regime in comparison

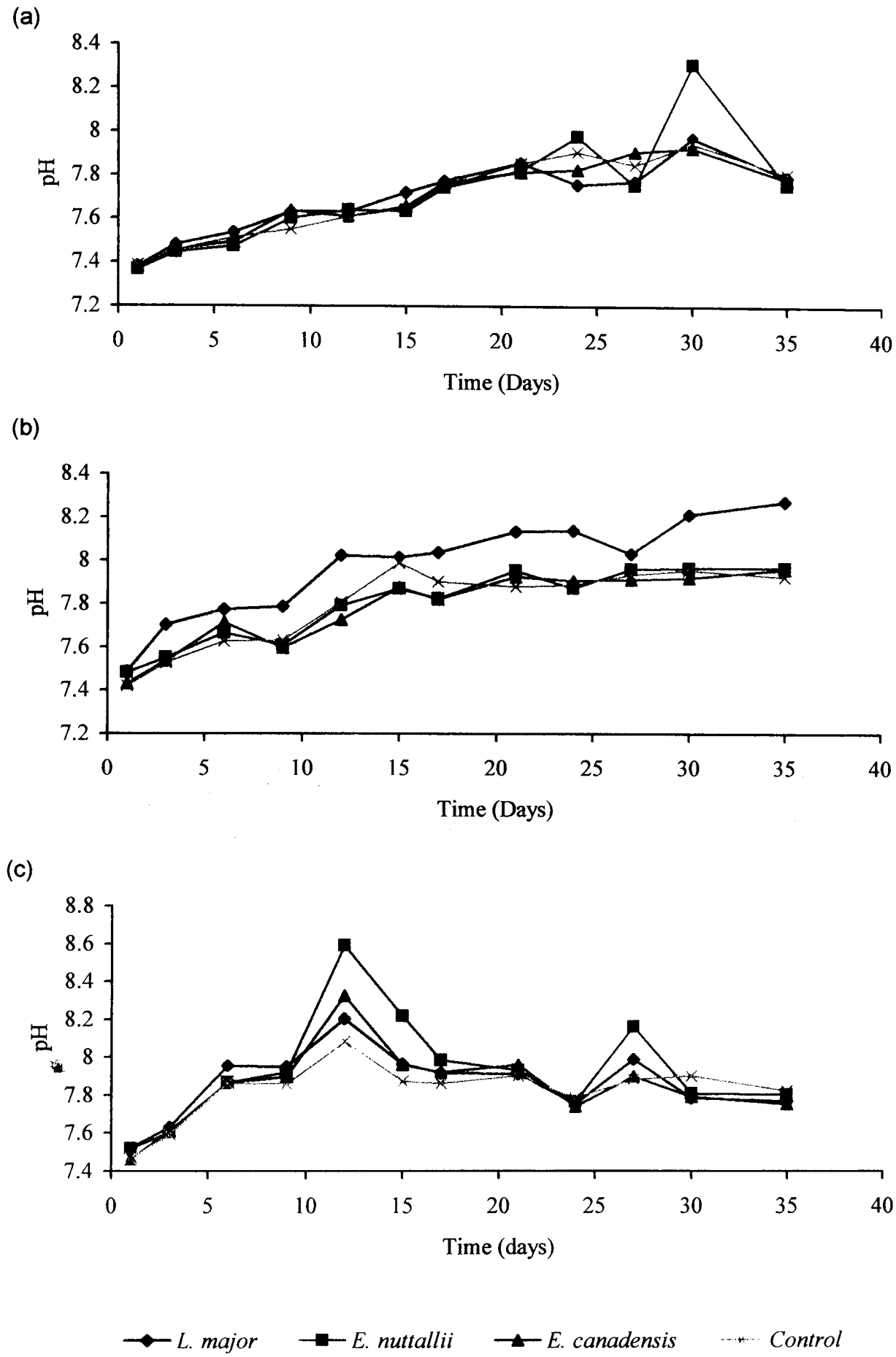


Fig. 4.4 Change in pH of different treatments over time at (a) 10 °C, (b) 15 °C and (c) 20 °C.

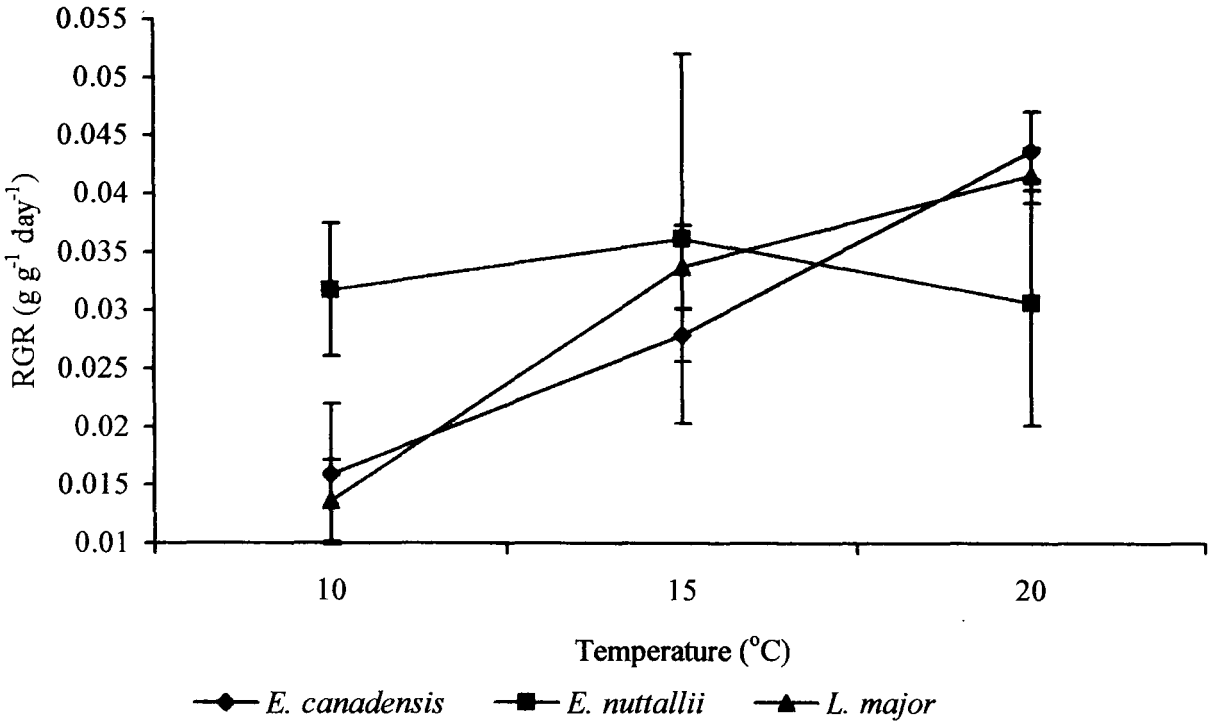


Fig. 4.5 Temperature Study 1, relative growth rate (as fresh weight) of three species as a function of temperature. Only replicates with positive growth rate were included in this analysis. (Mean ± SE, n = 3-6)

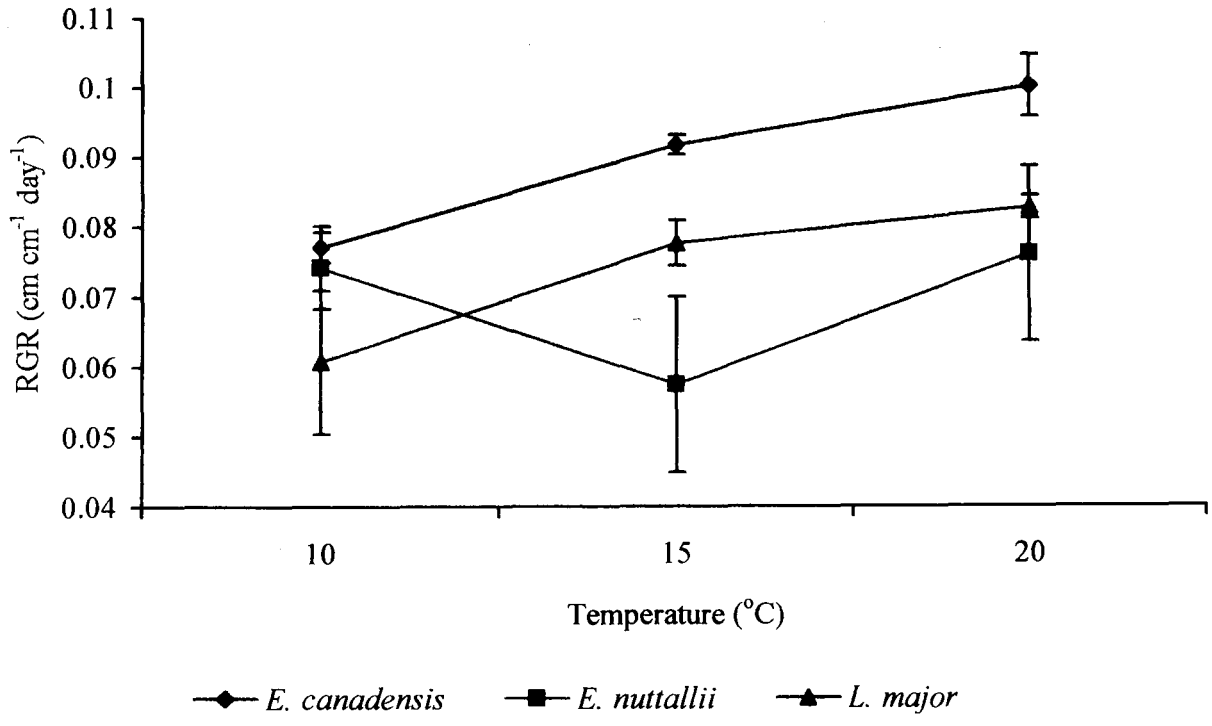


Fig. 4.6 Temperature Study 1, relative growth rate measured as increase in length of the three species. (Mean + SE, n = 6)

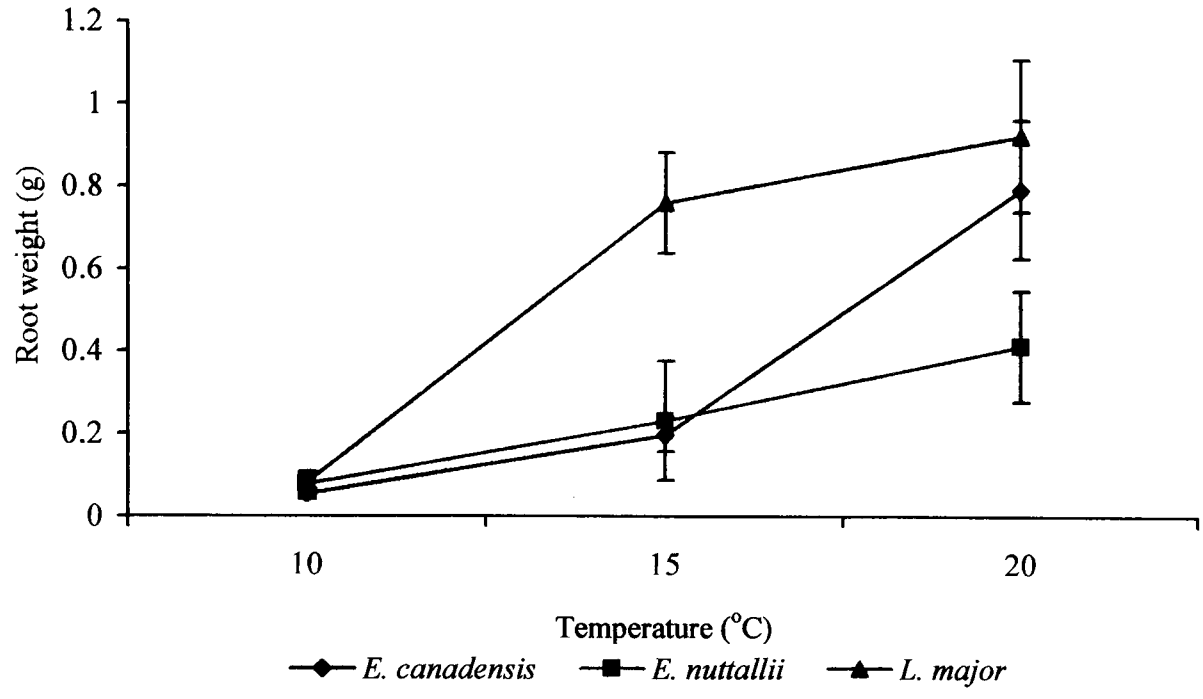


Fig. 4.7 Temperature Study 1, root weight (g) of three species as a function of temperature. (Mean ± SE, n = 3 - 6)

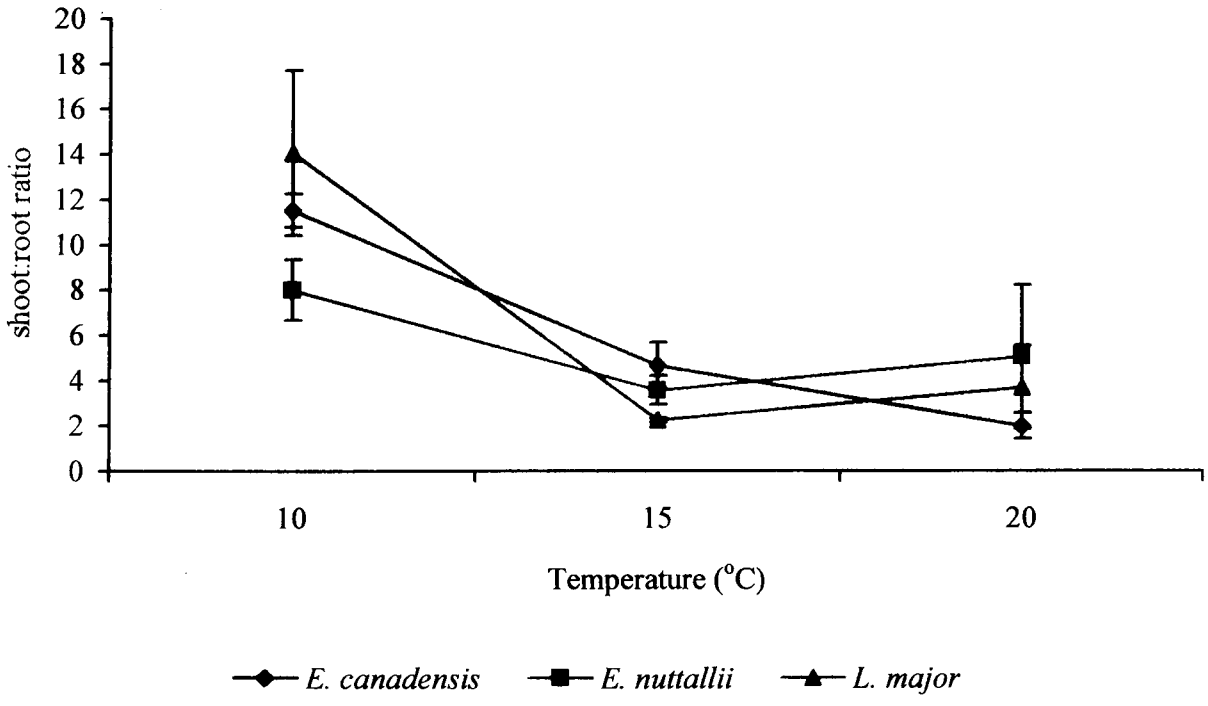


Fig. 4.8 Temperature study 1, shoot : root weight ratio of three species as a function of temperature. (Mean ± SE, n = 3 - 6)

with those grown at 10 °C. Q_{10} (10 °C – 20 °C) values of 2.76 and 3.07 were found for *E. canadensis* and *L. major* respectively (Fig. 4.5). Although an increase in growth rate with temperature was observed for *E. nuttallii*, due to the high variability of the results, the difference was not significant (Q_{10} (10 °C – 20 °C) = 1.96). A similar pattern in relative growth rates was observed when calculated per unit length (Fig. 4.6). Root fresh weight increased with increasing temperature (Fig. 4.7). Root fresh weight of *E. canadensis* grown at 20 °C was significantly greater than that of plants grown at 10 °C and 15 °C. Root fresh weight of *L. major* grown at 15 and 20 °C was significantly greater ($p = 0.05$) than that grown at 10 °C (Fig. 4.7). No significant differences were observed between root fresh weight of *E. nuttallii* grown at 10, 15 or 20 °C. The shoot to root ratio was observed to increase with increasing temperature for all three species (Fig. 4.8).

4.3.4 Temperature Study 2

The relative growth rates of both *Elodea canadensis* and *E. nuttallii* increased with increasing temperature (Fig. 4.9). For both species, significant increases ($p = 0.05$) in rates were observed between 10 and 20 °C. Q_{10} (10 °C – 20 °C) values were 2.04 and 2.60 for *E. canadensis* and *E. nuttallii* respectively. The relative growth rate of *L. major* responded only slightly to temperature, with no significant differences being observed between any of the treatments for this species (Q_{10} (10 °C – 20 °C) = 1.15) (Fig. 4.9). No significant differences were observed in fresh to dry weight ratios for any of the treatments, although both *E. nuttallii* and *L. major* showed a slight decrease in the ratio with increasing temperature (Fig. 4.10). Numbers of apices g^{-1} dry weight for *E. nuttallii* plants grown at 20°C were significantly greater ($p = 0.05$) than those grown at 10 °C (Fig. 4.11). No significant differences were observed for either *E. canadensis* or *L. major*. Root weight was also observed to increase with increasing temperature (Fig. 4.12). Photosynthetic and respiratory rates were measured at the growth temperature (Fig. 4.13). No significant differences in photosynthetic rates with increasing temperature were observed for either *E. canadensis* or *E. nuttallii* (Q_{10} (10 °C – 20 °C) values of 1.44 and 0.98 respectively). *L. major* exhibited significantly greater ($p = 0.05$) photosynthetic rates at 15 and 20 °C than at 10°C, with a Q_{10} value of 2.4. No significant differences were observed

between pH of treatments, mean pH being pH 8.37. Temperature had no apparent effect upon the respiration rates of the species.

Table 4.4

Starch content (mg g^{-1} dry weight) of plant material. (Mean ± SE, n = 4)

Species	Temperature	
	10 °C	20 °C
E. canadensis	54.71 (8.40)	29.23 (2.57)
E. nuttallii	105.14 (25.55)	13.89 (2.68)
L. major	66.92 (11.23)	29.02 (3.61)

Starch analysis revealed significantly higher ($p = 0.001$) concentrations of starch in plants of all three species grown at 10 °C compared with plants grown at 20 °C (Table 4.4). Although significant differences were not observed between the species, possibly due to the high variability of the *E. nuttallii* data, *E. nuttallii* had greater levels of starch than either *E. canadensis* or *L. major* grown at 10 °C, but less than the other two species at 20 °C. Concentrations found in *E. canadensis* and *L. major* were similar. Overall, *E. nuttallii* showed a significantly greater reduction in starch ($p = 0.05$) with increasing temperature than either *E. canadensis* or *L. major*.

4.3.5 Cold and Freezing injury

No significant changes in conductivity were recorded following the cold treatments (i.e. 3 °C and 1 °C), but freezing treatments (i.e. –10 °C and –18 °C) were followed by significant increases ($p = 0.001$) in conductivity (Fig. 4.14) showing significant cell solute leakage due to freezing damage into the surrounding medium.

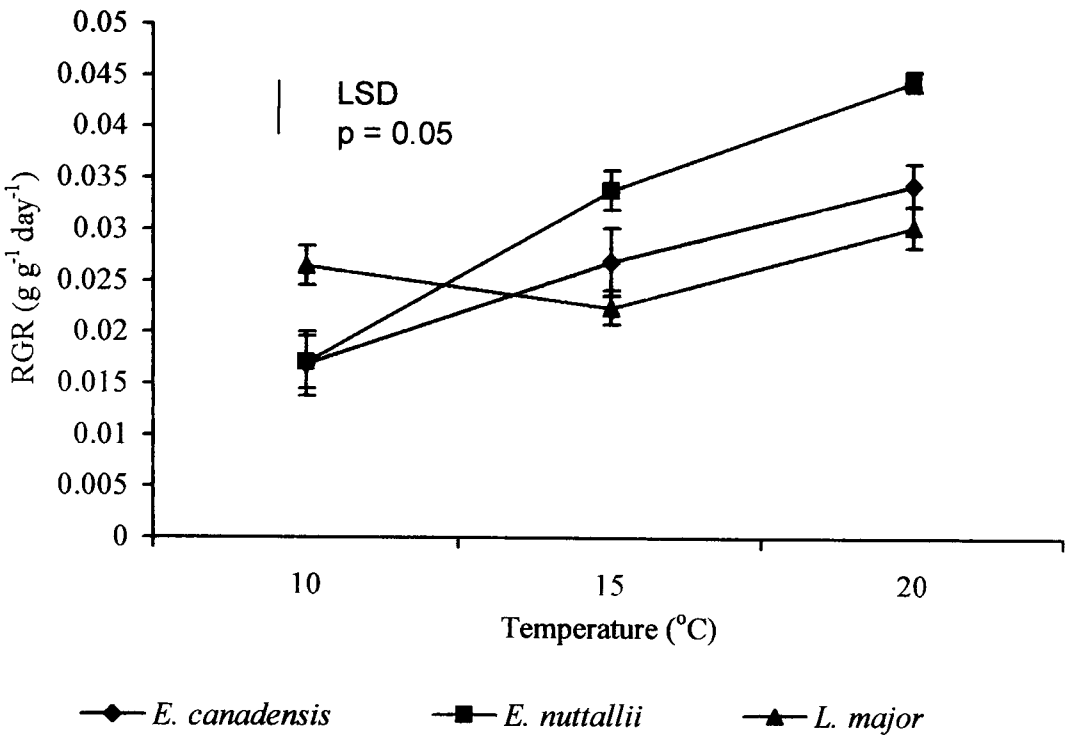


Fig.4.9 Temperature Study 2, relative growth rates of *E. canadensis*, *E. nuttallii* and *L. major* as a function of temperature. (Mean ± SE, n = 6)

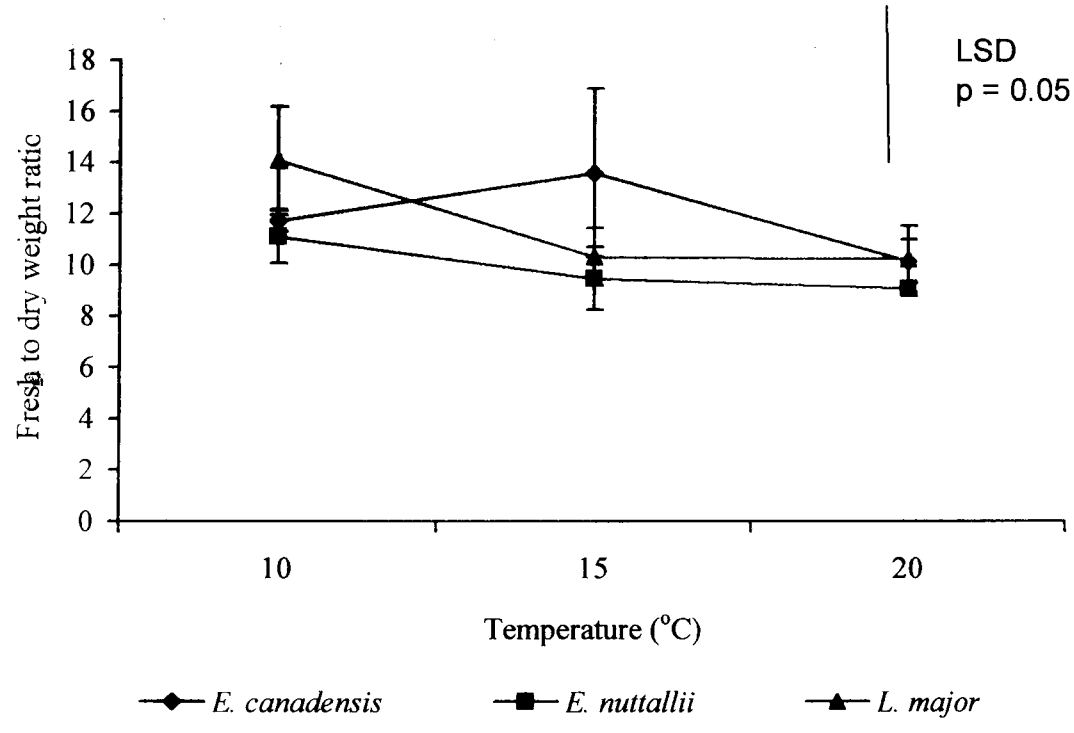


Fig. 4.10 Temperature Study 2, fresh weight to dry weight ratios of *E. canadensis*, *E. nuttallii* and *L. major* as a function of temperature. (Mean ± SE, n = 6)

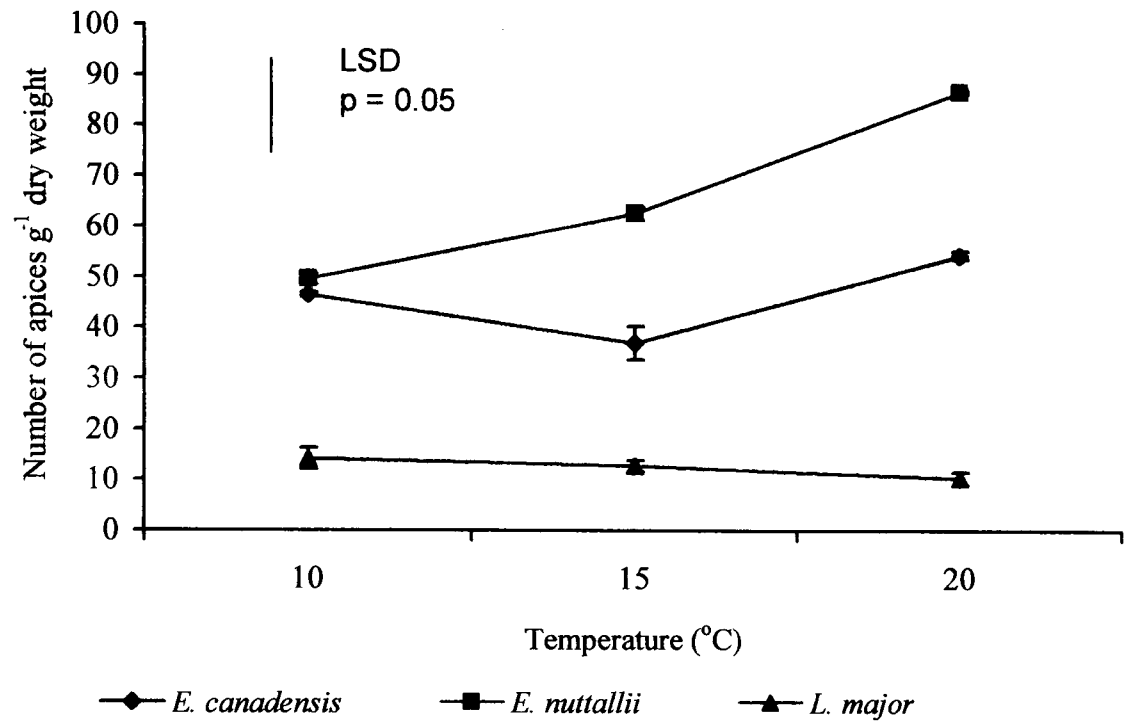


Fig. 4.11Temperature Study 2, number of apices g^{-1} dry weight for *E. canadensis*, *E. nuttallii* and *L. major* as a function of temperature. (Mean ± SE, n = 6)

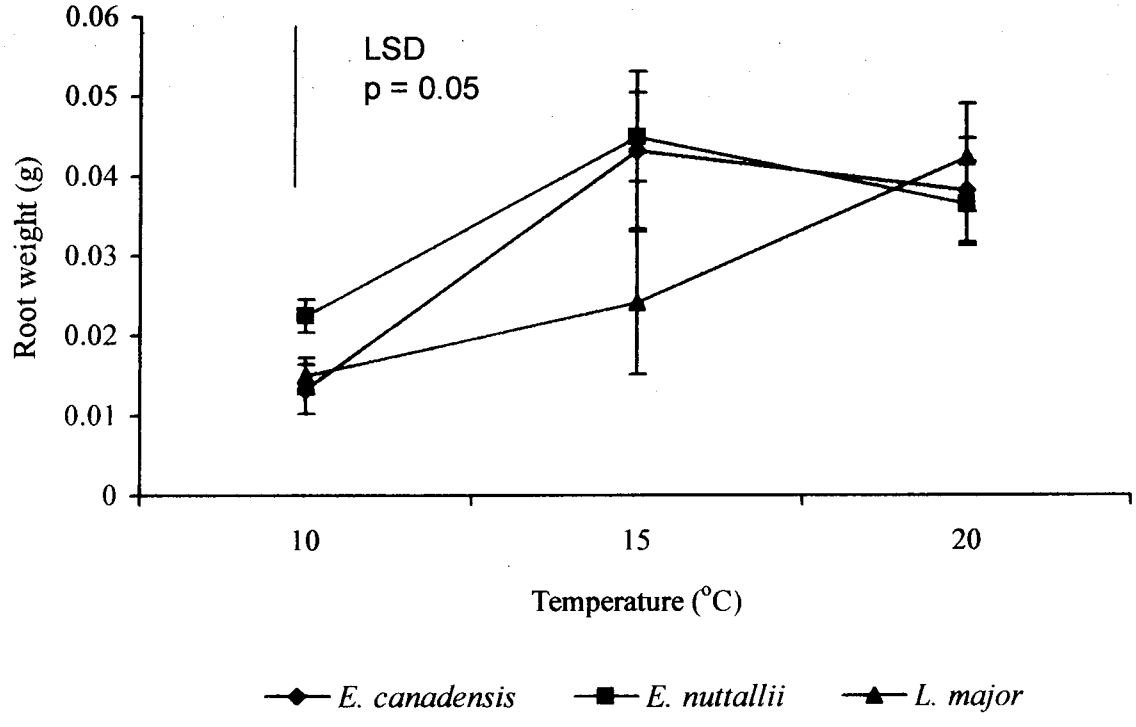


Fig. 4.12 Temperature Study 2, root weight (g) of *E. canadensis*, *E. nuttallii* and *L. major* as a function of temperature. (Mean ± SE, n = 6)

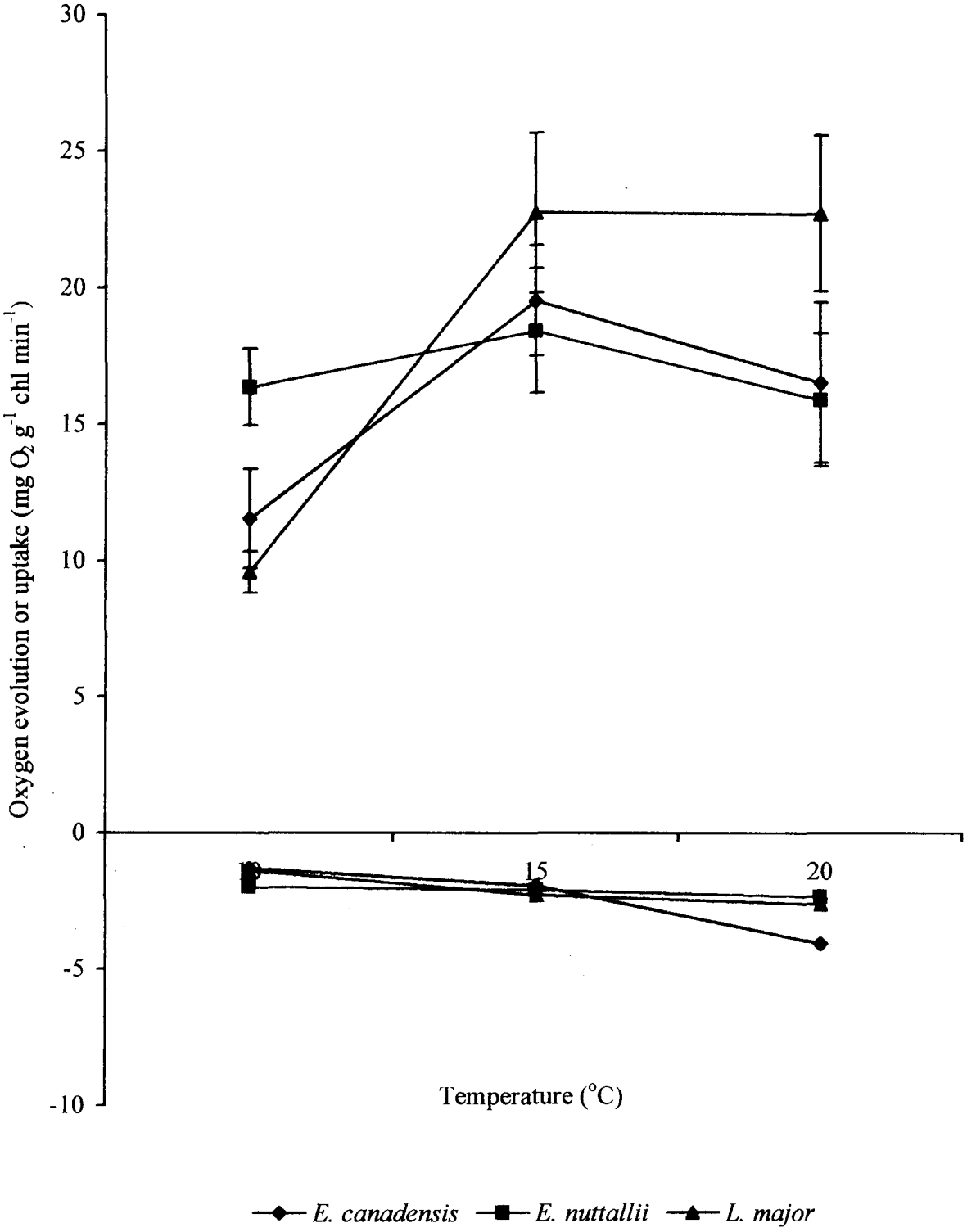


Fig. 4.13 Photosynthetic and respiratory rates of *E. canadensis, E. nuttallii* and *L. major* as a function of temperature. (Mean $\pm$ SE, n = 5). Significant differences (p = 0.05) between photosynthetic rates of *L. major* at 10 °C, and 15 and 20 °C, no significant differences for *E. canadensis* or *E. nuttallii*.

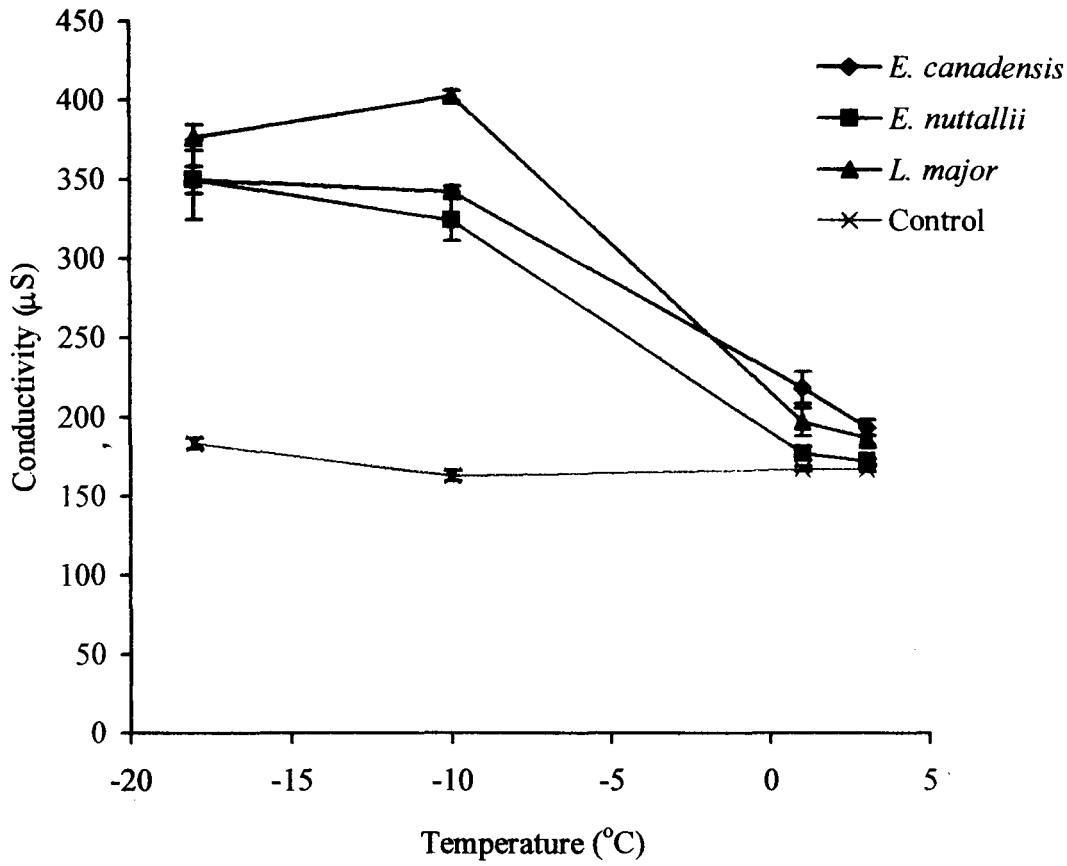


Fig. 4.14 Measurements of conductivity (μ S) in medium following temperature treatments. (Mean ± standard error)

4.4 Discussion

Under field conditions, when temperature rose to 11 °C, substantial growth of *E. nuttallii*, and to a lesser extent *L. major*, was observed. This parallels findings of other studies on *Elodea nuttallii*. (i.e. Kunii, 1981, 1984) where only very minimal growth at 5 °C occurred, active growth not being observed until the temperature was approximately 10 °C. The lack of growth of *E. canadensis* in the field suggested that this species may require higher temperatures for significant growth to occur. However, in subsequent laboratory studies, active growth of all three species occurred at 10 °C and significant differences were not found in the growth rates of *Elodea* spp. at this temperature. Growth of *E. canadensis* at 10 °C under laboratory conditions consisted of the production of short, densely packed buds similar in morphology to over-wintering turions observed in the field. Growth of *E. canadensis* in both Temperature Studies 1 and 2 was positively correlated with increases in temperature, although RGR were consistently higher in the second study. A positive correlation between temperature and RGR for *E. nuttallii* was only observed during the second study. No significant effects were observed during the first study.

In comparing the two temperature studies significant differences ($p = 0.05$) in root growth were observed between the two temperature studies, with greater root growth apparent in the first study. This suggests that plants were nutrient limited in the first experiment, particularly at 20°C. While the role of roots in the accumulation of nutrients from the sediments is the subject of much dispute between authors (e.g. Carignan, 1980; Barko and Smart, 1981), it has been found in subsequent studies (see Chapter 6) that root growth does decrease with increasing nutrient concentrations in the surrounding water medium. Thus, it seems likely that roots do play a role in the accumulation of nutrients, although the extent to which is likely to depend upon the relative concentrations in the sediment and surrounding medium. The growth parameters measured, namely RGR, root growth and numbers of apices, suggest that once the temperature starts to increase above 10 °C, rapid growth of *E. nuttallii* occurs, as accumulated weight, increase in length and branching density. These features will aid the rapid occupation of space by *E. nuttallii* at the beginning of the growing season, allowing this species to dominate locally available resources. As observed in the field (e.g. Schwarz and Howard-Williams, 1993), growth of *L.*

major did not show any distinct seasonal patterns in Temperature Study 2. Similar relative growth rates were observed over the experimental temperature range studied. The discrepancy between the results of the first and second study suggest a possible further factor influencing the growth of these species, that of nutrient limitation. This lack of growth seasonality under the more eutrophic conditions of the second study may allow *L. major* to grow, probably slowly, throughout the winter period. Lack of significant die back over the winter will convey a distinct competitive advantage over neighbouring species. At the beginning of the main growing season, *L. major* will already occupy space and resources within the photic zone of the water body, thus gaining an advantage over competing species.

Physiological measurements made did not show a response to increased temperature as suggested by growth measurements. No distinct pattern was observed for either *Elodea* spp. although *L. major* did show some increase with increasing temperature between 10 and, 15 and 20 °C despite this species showing little growth response to temperature. Investigations into the interactive effects of light and temperature upon the growth of *E. canadensis* by Barko *et al.* (1982), indicate that at light levels similar to the relatively low ones used in the present study, the effects of temperature are not as pronounced as at higher light levels. This may, in part, be the reason for the lack of response noted here, although one would expect the growth results to show a similar response. However, studies also suggest a degree of acclimation to long term temperature experiments (Madsen and Brix, 1997). Previous studies with macrophytes have shown that the responses of growth to changes in water temperature correspond well with changes in photosynthetic rates with short term fluctuations in temperature (Santamaria and van Vierssen, 1997). However, physiological measurements on plant material grown over a prolonged period at different temperatures show less temperature dependency than would be predicted from short-term experiments (Madsen and Brix, 1996). Low temperature have been found to induce physiological changes, such as more efficient photo-protection mechanisms, lower optimal temperatures and lower Q_{10} values (Li & Morris, 1982; Davison, 1987; Falk *et al.*, 1990). Adaptations to long-term exposure to low temperature will be important in the overall survival and growth of species.

The results of the freezing experiment suggest that all three species studied are chill tolerant but not freeze tolerant. Freezing damage results in rupturing of cells as ice crystals form within the cell membranes (Lyons, 1973; Larcher, 1995). Chilling injury has been suggested as a reason for the low growth of *E. canadensis* at 5 °C by Madsen and Brix (1997). These results indicate that this is not the case, as cell damage was not apparent in 1 or 3 °C treatments. However, further studies are required to clarify the effects of freezing and chilling injury as differences between the species may become apparent during longer-term exposure to low temperatures. In addition, the method used here was relatively insensitive, only measuring large changes in conductivity. At low temperatures it is possibly that limited cell damage may occur to only some cells, in this case solute damage may not be detectable using the current equipment, but may nevertheless be sufficient to result in shoot death.

The availability of stored resources may be critical to the survival of a plant in times of stress, e.g. low light and/or low acquisition of CO_2. Such conditions are likely to occur early in the growth season. *E. nuttallii* and *E. canadensis* over-winter as short green compact stems lying on the substrata. Under these conditions both light and CO_2 are likely to be limiting. The water itself will attenuate light, while periphytic algae and sediment are likely to reduce both light and CO_2 availability. Estimations of carbohydrate requirement made by Best and Dassen (1987) for *E. nuttallii* indicate that carbohydrate reserves may be inadequate to meet the demands of the plant even when including the mobilisation of starch. They suggest alternative sources of carbon, namely the mobilisation of protein and degradation of glycuronates. However, it seems likely that the growth of new shoots is, at least initially, be supported by older stems. Starch concentrations found in the present study resemble those found by previous authors using similar techniques with values of <3 to ~60 mg g^{-1} dry weight quoted for *E. canadensis, E. nuttallii* and *C. demersum* (Best and Werf, 1986; Best, 1977; Best and Dassen, 1987). Levels of starch in turions of *E. canadensis* were considerably higher than those recorded in other shoot samples. During spring, growth of *E. canadensis* may depend upon the frequency of over-wintering turions as a starch source for the development of new shoots.

Starch concentrations in *L. major* were generally found to be lower than those in *Elodea* spp.. The ability of this species to survive during the winter is therefore unlikely to be due to high internal reserves and suggests some other mechanism. Observations on this species suggest that in periods of extreme stress, this species becomes inactive exhibiting little growth, but also showing no die back. This is supported by the low photosynthetic rate of winter field material of *L. major* measured. In view of the relatively low starch reserves present, *L. major* may be able to down regulate photosynthesis and respiration, and simply maintaining existing plant material.

During the present study whole plants were used during the starch analysis. However, the distribution of starch within the plant may also be important. If starch is concentrated mainly in the older stems, with few reserves in the younger stem shoots, in periods of stress starch reserves may not be readily mobilised. This may account for the die back of younger shoots of *Elodea* spp. observed during experimental studies. Observations of growth suggest that following the die back of the main growing shoot during nursery culturing, re-growth occurred from older shoot sections. In contrast, *L. major* showed little die back, even following damage to the growing tip. If starch reserves are more readily mobilised, or within the facinity of the apex, these shoots may be able to survive stress without dying back through the immediate mobilisation of starch. Thus, not only total starch concentrations but also starch distributions within plant shoots may be important in determining how a species responds to stress. This is an aspect that requires further study.

The brittle nature of plant material found in the winter study, particularly *E. nuttallii,* is likely to aid dispersal of the species. This corresponds with the fragmentation of *E. nuttallii* shoots in early autumn reported by Kunii (1984). The rapid spread of *E. canadensis* during the 19th century may also be partly attributed to the brittle nature of the shoots. However, *L. major* is a stronger, far less brittle species, so dispersal may be a much slower process for this species. This observation suggests a reason for the slow spread of *L. major* in this country, despite its relatively early introduction (1944).

4.5 Summary

1. Growth of *Elodea* spp. was positively correlated with temperature in the absence of nutrient limitations.
2. *E. nuttallii* may gain significant competitive advantages through rapid growth at the beginning of the growth season.
3. No evidence was found to suggest that *E. canadensis* and *E. nuttallii* differ in the temperature at which significant growth will occur at the beginning of the growing season.
4. Evidence suggests that *L. major* does not respond to changes in water temperature under non-limiting nutrient conditions, showing little die back over the winter period. This lack of seasonality may be a considerable advantage allowing the plant to continue growing throughout the winter.
5. Starch concentrations measured in field material were highest in the *Elodea* spp.. High storage reserves may allow this species to withstand periods of stress (i.e. low temperature, low light etc.) through the metabolism of internal reserves.
6. High concentrations of starch were observed in over-wintering turions of *E. canadensis*. These may be critical for successful re-growth in spring.

Chapter 5 COMPARATIVE PHYSIOLOGY OF THE THREE SPECIES

5.1 Introduction

Macrophytes can create dense monoculture stands. In stagnant or slow moving water bodies, water quality within these stands is often characterised by high O_2, and pH, and low CO_2* during periods of active photosynthesis. Oxygen concentrations can rise to well above saturation levels. *Hydrilla* mats produce levels in excess of 200 % saturation (Van *et al.*, 1976) and a pH maximum of 10.2 (Hough and Wetzel, 1976). *Elodea nuttallii* is reported to produce maximum values of approximately 180 % O_2 saturation and pH in excess of 9 (Jones *et al.* 1996). CO_2* levels within plant stands during periods of active photosynthesis may be reduced to concentrations below the reported CO_2* compensation points for many macrophyte species (e.g. Jones *et al.*, 1996). In stress conditions, such as those that can develop within a dense plant stand, photosynthetic rates are reduced, and in some cases shoots may exhibit net respiration. Implicit to the theory of resource-mediated interference between neighbouring stands driving a species displacement process, is a differential ability of the species involved to tolerate stress conditions. Competitive advantages will be gained by those species that are best able to photosynthesise and consequently grow under these stress conditions of high pH, low CO_2* and high O_2 concentrations.

In many fresh water systems, inorganic carbon supply is often the principal factor limiting the photosynthesis of macrophytes (Madsen and Maberly, 1991). Dissolved inorganic carbon (DIC) concentrations are extremely variable in freshwaters. The principal forms of DIC found in the fresh water body are CO_2* (dissolved CO_2 and H_2CO_3), HCO_3- (bicarbonate) and CO_3^{2-} (carbonate). Whilst CO_2* is potentially available as a carbon source for photosynthesis in all macrophytes, some species can additionally utilise bicarbonate, but none can utilise carbonate directly (Raven, 1970). Temperature, pH, ionic strength and the partial pressure of CO_2 determine the proportions of individual carbon forms (Stumm and Morgan, 1980). The concentration of H^+ ions, numerically described as pH (= -log[H+]), largely determines the equilibrium position of CO_2 reactions. When dissolved in water, some CO_2* reacts to form H_2CO_3 (carbonic acid). This dissociates to form HCO_3- (bicarbonate) and CO_3^{2-} (carbonate). Increases in pH of the water body, and therefore a reduction in concentrations of H^+ ions, will result in a progressive change in the CO_2

equilibrium. At pH values below pH 6.5, most dissolved carbon will be in the form of CO_2*. With increases in pH from pH 6.5 to 9, HCO_3^- is the dominant carbon fraction present, while CO_2* concentrations diminish to virtually zero at pH 9. Above pH 9, HCO_3^- becomes scarce and a large proportion of CO_2 is in the unavailable form of CO_3^{2-}.

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+ \leftrightarrows 2H^+ + CO_3^{2-}$$

The total inorganic carbon concentration [CT] also changes with pH, with an increase in pH resulting in a reduction in total carbon. Rapid photosynthesis both depletes carbon and shifts the equilibrium towards bicarbonate and carbonate. Extremes of pH may also have a direct effect on physiology, interfering with the co-transport of H^+ ions across the plasmalemma, thereby disrupting the active transport of essential nutrients into the cell (Raven, 1984).

In many eutrophic water bodies with typically high pH values in the range pH 7 to 8, available carbon may be a critical factor limiting photosynthesis (Van *et al.*, 1976; Sondergaard and Sand-Jensen, 1979; Madsen and Maberly, 1991; Rattray *et al.*, 1991a; Jones *et al.*, 1996). Concentrations of CO_2* recorded in the field during active photosynthesis (e.g. 3.6 μmol l^{-1} - Jones *et al.*, 1996) can fall close to the lower end of the range of CO_2* compensation points reported for many macrophyte species (Bain and Proctor, 1980; Allen and Spence, 1981; Maberly and Spence, 1983; Bowes and Salvucci, 1989). Exploitative strategies by macrophytes that avoid carbon limitation or ameliorate it through physiological and morphological adaptations are likely to convey a distinct competitive advantage. Many macrophyte species have a high surface area to biomass ratio (Specific Leaf Area). The highly dissected or thin leaves of many aquatic plants will both increase the area for interception of carbon resources and, to a lesser extent, shorten internal diffusion pathways (Maberly and Madsen, 1998). Some species exhibit mechanisms that may enhance uptake of carbon, such as crassulacean acid metabolism (CAM) or C_4-like fixation. Species exhibiting the CAM photosynthetic pathway temporally separate the processes of initial CO_2 fixation at night, and the subsequent process of decarboxylation of malic acid and fixation by ribulose-bis phosphate carboxylase-oxygenase (RUBISCO) during the day. Thus these species can take advantage of night time increases in CO_2* availability.

CAM features have been found for several *Isoetes spp.*, *Littorella uniflora* and *Crassula aquatica* (Keeley and Bowes, 1982; Boston and Adams, 1983; Keeley, 1990). In plants exhibiting C_4 fixation, CO_2* is fixed to form malate or aspartate by PEP carboxylase in mesophyll cells. This is then transported to the bundle sheath cells for fixation by RUBISCO. Thus, C_4 plants have a distinct leaf anatomy known as Kranz anatomy, with spatial separation of initial CO_2* fixation by PEP carboxylase and subsequent fixation by RUBISCO. This mechanism concentrates internal CO_2*, enhancing the carboxylation efficiency of RUBISCO and reducing photorespiratory stress. Some aquatic plants may use a C_4-like fixation strategy without the Kranz anatomy associated with C_4 fixation in terrestrial species (Bowes, 1987). This has been reported for *Hydrilla verticillata* (Holaday and Bowes, 1980) and *Scirpus subterminalis* (Beer and Wetzel, 1981). Accumulation of four-carbon acids malate and aspartate have been observed in a number of macrophytes including *E. canadensis*, *L. major* and *E. densa* (Bowes, 1985). However, subsequent studies have found no evidence for C_4-like photosynthesis in *E. canadensis* (Madsen *et al.*, 1996).

Under CO_2* limiting conditions, bicarbonate is used by many submerged macrophytes as an alternative carbon source. Species such as *Ceratophyllum demersum, Elodea canadensis, E. nuttallii, Myriophyllum spicatum, Lagarosiphon major*, *Potamogeton perfoliatus* and *Potamogeton pectinatus* and are all capable of utilising bicarbonate (Allen and Spence, 1981; Maberly and Spence, 1983; Jones *et al.*, 1993; Maberly and Madsen, 1998). In contrast other species, such as *Callitriche* spp. and *Potamogeton polygonifolius,* are restricted to CO_2* as a carbon source (Maberly and Madsen, 1998). In addition to increasing the availability of inorganic carbon, bicarbonate uptake may also reduce photorespiration through increasing internal CO_2* concentrations in the vicinity of RUBISCO activity (Maberly and Madsen, 1998). There are obvious competitive advantages, particularly under conditions where CO_2* is limited, in being able to use an alternative carbon source. However, bicarbonate is less readily utilised than CO_2* (Sand-Jensen and Gordon 1984, 1986). Uptake is dependent on an active process as plant membranes are relatively impermeable to HCO_3^- (Lucas, 1983). In some species, including *Elodea* spp., and *Potamogeton* spp., evidence suggests that HCO_3^- is taken up via an active polar cation transport process (Prins and Helder, 1985; Elzenga and Prins, 1987;

Jones *et al.*, 1993). Utilisation of HCO_3^- by photosynthesising leaves depends upon an efflux of H^+ at the abaxial leaf surface via a proton pump. This acidification results in the external conversion of HCO_3^- to CO_2* or H_2CO_3 and it is these carbonic species that probably cross the plasmalemma into the cell (Prins *et al.*, 1982). This process, termed the polar leaf mechanism, is relatively inefficient, with high-energy costs involved in normal functioning, calculated at about 60 mmol m^{-2} s^{-1} (Jones 1994). In comparison, uptake of CO_2* occurs by diffusion and consequently has no direct energy costs (Raven and Lucas, 1985). The costs of utilisation of bicarbonate are reflected in the relatively low growth rate of plants utilising bicarbonate as a carbon source, despite its apparent plentiful supply in many fresh waters (Jones, 1994). Under conditions of low CO_2* availability, such as during periods of active photosynthesis, the efficiency of the bicarbonate uptake process, in energetic terms, will be an important determinant of photosynthesis and subsequent growth. Species with efficient bicarbonate uptake mechanisms may therefore expect be competitively advantaged under these conditions.

Submerged macrophytes will be subjected to both rapid diurnal and long term seasonal changes in availability of DIC, CO_2* and bicarbonate within the field. Many macrophyte species exhibit extreme plasticity in response to changing availability of carbon (Maberly and Spence, 1983). Increased affinity for HCO_3^- has been observed for many macrophyte species when DIC and CO_2* levels are depleted (Sand-Jensen and Gordon, 1986; Madsen and Sand-Jensen, 1994) and decreased affinities occur under high CO_2* concentrations (Sand-Jensen and Gordon, 1986; Adamec, 1993; Madsen and Sand-Jensen, 1994; Madsen *et al.*, 1996). Suppression of HCO_3^- uptake is also found to occur in *E. canadensis* under low light and nutrient limitation (Sand-Jensen, 1989). In view of the relative energy costs in bicarbonate uptake, there are obvious advantages in being able to change affinity for HCO_3^- in response to the availability of the various inorganic carbonic species.

Differing light saturation and compensation points may also play important roles in determining species dominance (Brown *et al.*, 1974). This is exemplified in the subtropical River Waikato, New Zealand. Here a correlation has been noted between water turbidity and changes in species dominance of a submerged

macrophyte community (Brown *et al.* 1974). *E. canadensis* predominated at the headwater. As turbidity increased downstream, *E. canadensis* was replaced by *Lagarosiphon major,* while a further increase in turbidity resulted in the replacement of *L. major* by *Egeria densa.* A correlation was found between light compensation points and turbidity with the lowest light compensation values found for *E. densa* and the highest for *E. canadensis.* The lower compensation points of *L. major* relative to the *Elodea* species could have important implications for this present study. This could indicate a more efficient photosynthetic mechanism for *L. major*, particularly in conditions of low light and/or high disturbance. The lower light compensation points may also allow the species to invade thick plant stands of other species where low light conditions prevail and still accomplish net positive photosynthesis and growth.

The aim of the study reported in this chapter was to characterise and compare the photosynthetic and respiratory responses of the three species. It was hypothesised that the competitive success of species may be related to their ability to tolerate conditions of high pH and oxygen supersaturation, and low CO_2*, since at high pH the inorganic carbon will be mainly available as bicarbonate. For all three species comparative measurements were made of photosynthetic rates per unit area and per unit chlorophyll, and the effects of pH increase on photosynthetic and respiratory rates were measured. Comparative measurements of bicarbonate uptake for all three species were made on material grown under limiting and non-limiting CO_2* conditions to characterise the abilities of the three species to acclimate to differing CO_2* availability. A number of previous studies have characterised the responses of *E. canadensis* and *E. nuttallii* to light, temperature, oxygen concentrations, pH and CO_2 * concentration (e.g. Simpson, 1981; Birch, 1990; Jones, 1994; Madsen and Sand-Jensen, 1994). There have been very few studies conducted on the physiology of *L. major,* so light saturation and oxygen response curves are reported here for *L. major*.

5.2 Methods

5.2.1 Culturing of material for physiological determinations

Plants were collected from Ec WB, En BL and Lm LC (see Chapter 2, Table 2.1 for explanation) and cultured in three 50 l tanks. A 10 cm thick layer of canal mud was placed in the bottom of each container. Tap water was poured in carefully to prevent sediment disturbance. The tanks were then aerated for 24 hrs before 5 to 6 healthy shoots were planted. The water was changed approximately every two weeks to ensure an adequate supply of nutrients and to prevent excessive phytoplankton growth within the media. Photo irradiance (PAR 400 – 700 nm) of approximately 100 $\mu mol\ m^{-2}\ s^{-1}$ was supplied by fluorescent tubes and temperature was maintained at 15± 1°C in a temperature controlled growth room.

5.2.2 Determinations of photosynthetic and respiratory rates

Photosynthetic and respiratory rates were determined as previously described (Chapter 2, Section 2.5). Previous studies have reported that buffered solutions interfere with photosynthetic rates (Prins *et al.* 1982; Prins and Helder, 1985; Elzenga and Prins, 1987). Working on *E. nuttallii,* Jones (1994) found a decline in photosynthetic rates in both buffered and unbuffered solutions. However, as the author states, some caution is needed when interpreting these results as the physiological condition of material (e.g. its degree of acclimation to HCO_3^- use) when collected may have a significant effect upon its ability to utilise bicarbonate. Preliminary studies (see Appendix II) on *E. canadensis* suggest that rates of photosynthesis may be underestimated at high pH when buffers are present. Consequently, during the determination of pH response curves and bicarbonate metabolism in the main study, buffer was not used. In the absence of buffer, pH values inevitably drifted during the course of the measurements. To allow for this, the pH of the media was taken at the start and end of each measurement to provide the pH range over which measurements were taken. For other treatments not requiring high pH media or comparison with high pH treatments, buffer was used. No significant effects on physiology have been reported at low pH (pH 7) and the stabilisation of pH allows statistical analysis to be performed easily and comparisons made with previous

authors who did use buffer (i.e. Simpson, 1981; Birch, 1990; Jones, 1994) to be made.

5.2.3 Comparative rates per unit area and per unit chlorophyll

For comparative measurements of photosynthetic and respiratory rates per unit area and per unit chlorophyll, six 10 cm long shoots of each species were selected. The leaf area (LA) was approximated by removing a representative leaf from a whorl 3 cm below the apical tip, or in the case of *L. major*, immediately adjacent to the leaves to be used for physiological measurements. These were then photocopied, the paper weighed and converted to a surface area using a conversion factor previously calculated from known segments of paper. Physiological determinations were then made on the remaining two leaves of the whorl in the case of *Elodea spp.* or a neighbouring leaf in the case of *L. major*. Forsberg medium was maintained at pH 7 with the addition of 50 mmol Tris buffer. Comparative measurements of chlorophyll per unit area were also made on plant material collected from the field (n = 12). (Origins of material used for measurements on field material: Ec Wb, En M, Lm L)

5.2.4 Light

For determination of a light response curve for *L. major,* photosynthetic and respiratory rates were measured at seven light intensities (5, 30, 50, 100, 200, 300 and 1000 $\mu mol\ m^{-2}\ s^{-1}$). Light intensity was determined using a Macam 101Q light meter. Physiological determinations were made as previously described with eight replicates per treatment. Forsberg medium was maintained at pH 7 with the addition of 50 mmol Tris buffer.

5.2.5 Oxygen

For determination of an oxygen response curve for *L. major,* photosynthetic and respiratory rates were measured at 3, 7, 10, 15 and 19 $\pm$ 0.5 mg $O_2\ l^{-1}$. Oxygen concentrations within the measurement chamber were increased using O_2 or decreased using N_2 gases. Physiological determinations were made as described previously with eight replicates per treatment. Forsberg medium was maintained at pH 7 with the

addition of Tris buffer (50 mmol). For comparative purposes, photosynthetic and respiratory rates were also determined at 3 and 19 mg O_2 l^{-1} for all three species.

5.2.6 pH

pH response curves were determined for all three species. Photosynthetic and respiratory rates were measured as previously described. pH measurements in the Forsberg medium were taken at the beginning and end of each measurement. In the absence of Tris buffer, pH varied significantly over the duration of each measurement. Total CO_2 and CO_2* concentrations were calculated from known values of pH, alkalinity, conductivity and temperature.

5.2.7 Bicarbonate experiment

Plastic beakers (250ml) were filled with canal sediment. To each beaker two 10 cm long shoots were planted, 8 beakers per species. The beakers and plants were then transferred to 3 l glass jars, each containing 2.5 litres of tap water adjusted by either bubbling with air previously passed through soda lime, which reduced the CO_2* to about half ambient (see Fig. 5.12) or bubbling with untreated air. For the latter treatments, water pH was adjusted every 24 hours to approximately pH 7.5 by the addition of weak HCl. Plants were grown for a period of two weeks. pH measurements were taken daily before the addition of HCl to determine the maximum pH reached. Alkalinity measurements made every two to three days by removing 25 ml of the water media and titrating with 0.01 N HCl to pH 4.5. From measurements of alkalinity, pH, conductivity and temperature, the proportions of carbon fractions (DIC, CO_2*, bicarbonate and carbonate) were calculated following the method of Mackereth *et al.* (1989).

For estimation of bicarbonate uptake, photosynthetic and respiratory rates were measured as previously described at approximately pH 6.5 and pH 9 in the absence of buffer. For measurement of starting rates, leaves were removed from a sub-sample of plant material cultured under the same conditions. At the time of harvest, total length, number of apical tips and dry weight of plants were measured. Finally, from growth measurements and photosynthetic and respiratory rates, an

estimate of metabolic cost of bicarbonate utilisation was made following Jones (1994).

5.2.8 Statistical treatment

For comparisons between treatment and species, Anova and Tukey tests were normally used. However, for comparisons between response curves where no appropriate linear transformation could be found, as for physiological measurement made in response to pH, least weighted regression was used. With this technique, best-fit lines were fitted to the data and the lines were then compared visually by fitting critical intervals to the line and looking for overlap.

5.3 Results

5.3.1 Photosynthesis and respiration per unit area and per unit chlorophyll

For both plant material cultured in the laboratory and that collected from the field, total leaf area of *L. major* was significantly larger ($p < 0.001$) than of either of the *Elodea* spp. (Table 5.1). Significant differences ($p = 0.05$) in leaf area between *E. canadensis* and *E. nuttallii* were only observed for field material, no significant differences being found for laboratory cultured material. Chlorophyll concentrations for plant material of all three species collected from the field were similar to values recorded for laboratory-grown material. Field grown *E. nuttallii* had significantly greater chlorophyll concentration per unit chlorophyll than either *E. canadensis* ($p < 0.001$) or *L. major* ($p = 0.025$). Significant differences were also found between field grown *E. canadensis* and *L. major* ($p = 0.05$).

No significant differences were observed in photosynthetic and respiratory rates expressed per unit chlorophyll between species (Table 5.2). However, photosynthetic rates per unit chlorophyll of *E. canadensis* were generally lower than those of *E. nuttallii* and *L. major*, but similar between the latter two species. No significant differences were observed between respiration rates per unit chlorophyll for the three species. Significant differences between species were observed in photosynthetic and respiratory rates expressed per unit leaf area (Table 5.2), being significantly greater ($p = 0.05$) for *E. nuttallii* and *L. major* than for *E. canadensis.* No significant differences in unit area rates were observed between the former two species.

5.3.2 Response to light, oxygen and pH

Light saturation curves for *L. major* were determined under constant DIC and within oxygen concentrations of 10 ± 0.5 mg O_2 l^{-1} (Fig. 5.1). Photosynthetic light saturation levels were achieved at an approximate light intensity of 200 μmol m^{-2} s^{-1}. Positive net photosynthesis was achieved at the lowest light intensity used (~ 5 μmol

Table 5.1 A comparison of leaf area and leaf chlorophyll concentration per unit area from field and laboratory grown material. Error is expressed in brackets as 95% confidence limits.

Species	Laboratory material		Field material	
	Leaf area (cm^2)	µg chl (cm^2)	Leaf area (cm^2)	µg chl (cm^2)
E. canadensis	0.292 [a] (0.07)	18.72 (2.88)	0.318[ac] (0.066)	16.03[ac] (1.32)
E. nuttallii	0.228 [b] (0.080)	24.44 (5.14)	0.198[bc] (0.019)	22.38[bc] (1.75)
L. major	0.515 [ab] (0.132)	22.89 (2.63)	0.490[ab] (0.043)	19.39[ab] (1.55)

[a] Significant differences between *E. canadensis* and *L. major*

[b] Significant differences between *E. nuttallii* and *L. major*

[c] Significant differences between *E. canadensis* and *E. nuttallii*

Table 5.2 Comparative measurements of photosynthesis and respiration rates per unit chlorophyll and per unit area (n=6). Error expressed in brackets as 95% confidence limits.

Species	Per unit chlorophyll mg O_2 g^{-1} chl min^{-1}		Per unit leaf area µg O_2 cm^{-2} min^{-1}	
	Photosynthesis	Respiration	Photosynthesis	Respiration
E. canadensis	16.82 (3.50)	6.32 (1.81)	0.316 [ca] (0.08)	0.114 [ca] (0.026)
E. nuttallii	22.67 (3.05)	7.66 (2.36)	0.546[c] (0.13)	0.178 [c] (0.045)
L. major	21.58 (5.66)	6.98 (1.23)	0.503 [a] (0.16)	0.159 [a] (0.033)

[a] Significant differences between *E. canadensis* and *L. major* at p = 0.05

[c] Significant differences between *E. canadensis* and *E. nuttallii* at p =0.05

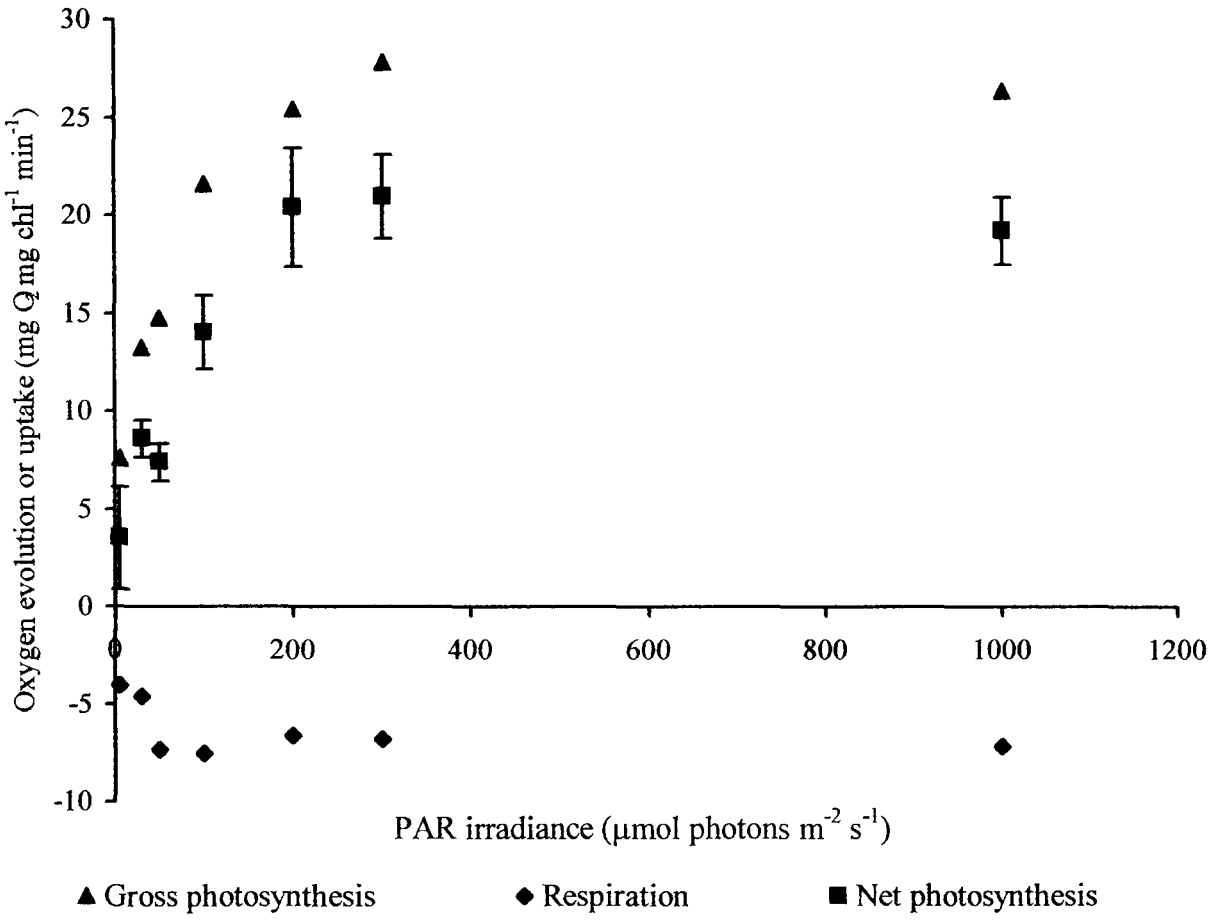


Fig. 5.1 Gross photosynthesis, net photosynthetisis and respiratory rates of *L. major* at different PAR irradiances. Error bars are 95 % confidence limits.

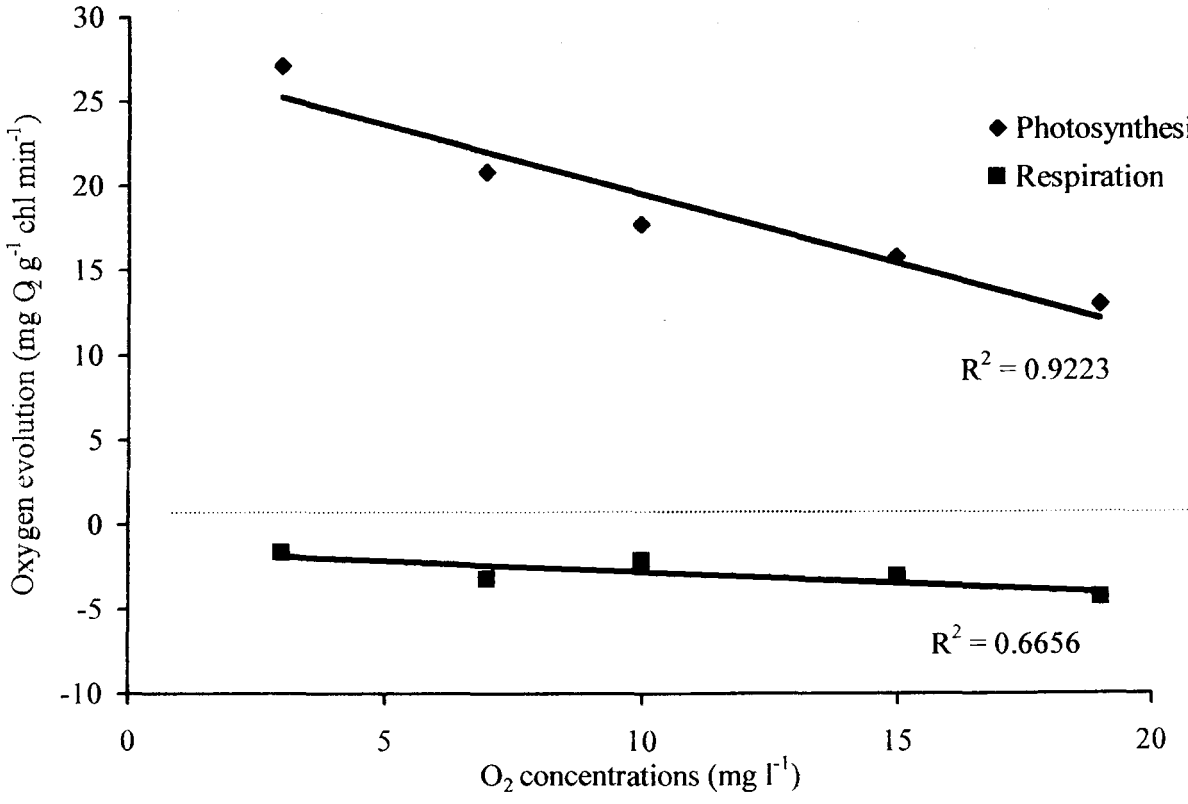


Fig. 5.2 The effects of increasing oxygen concentration on the net photosynthesis and respiration of *L. major* .

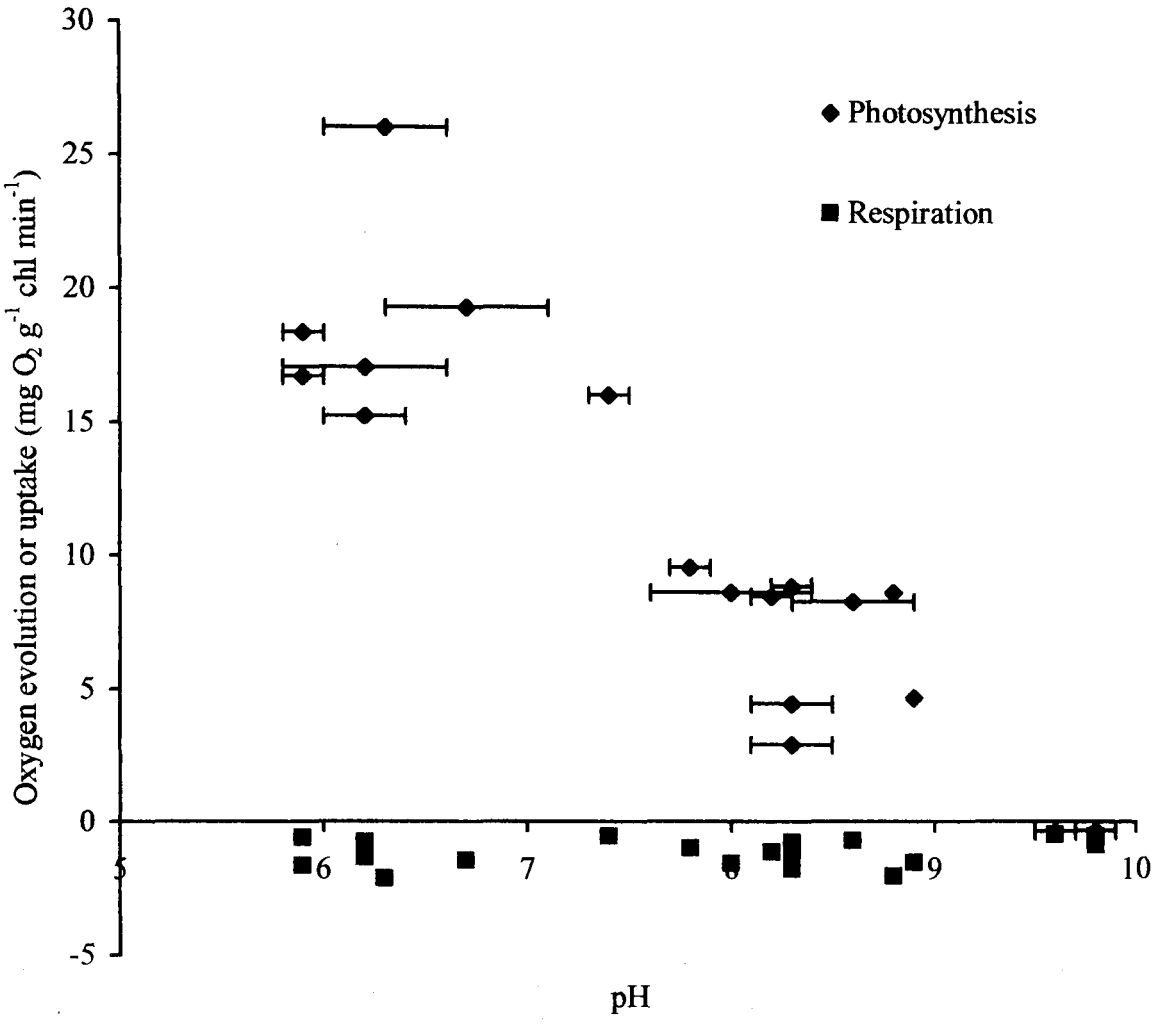


Fig. 5.3 Effect of pH in the incubation media on oxygen evolution and uptake of *E. canadensis* . Measurements were made in unbuffered solution with x error bars representing pH range over which studies were made. X error bars omitted from respiration data for clarity.

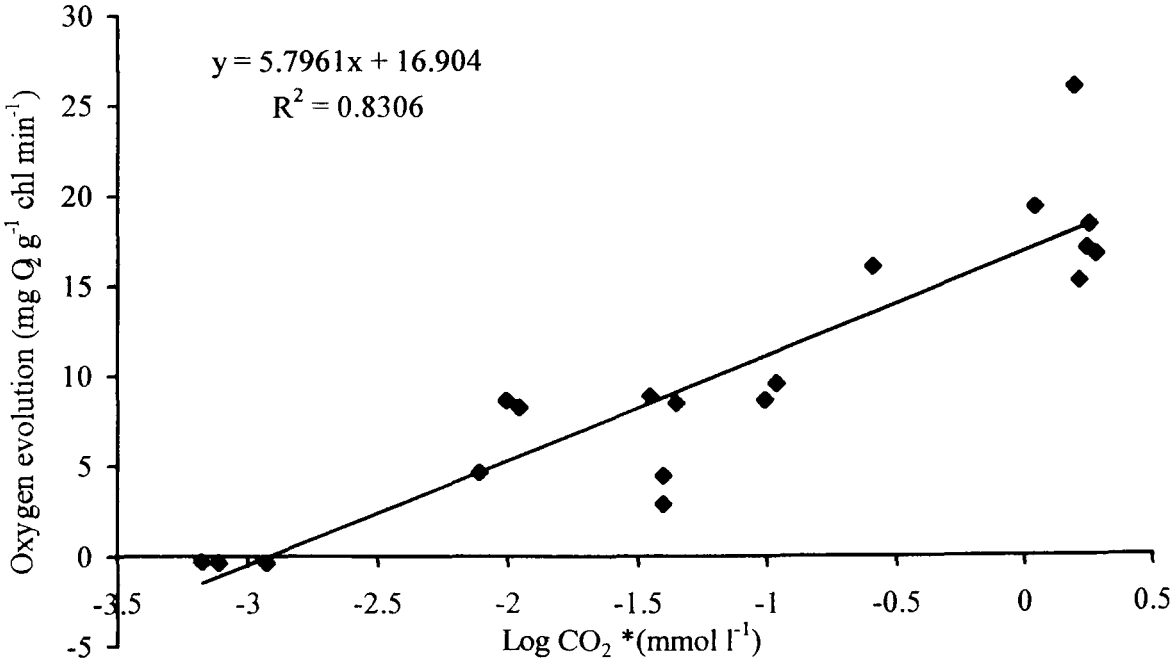


Fig. 5.4 Relationship between CO_2* in the incubation medium at different pH levels used and net photosynthesis of *E. canadensis* .

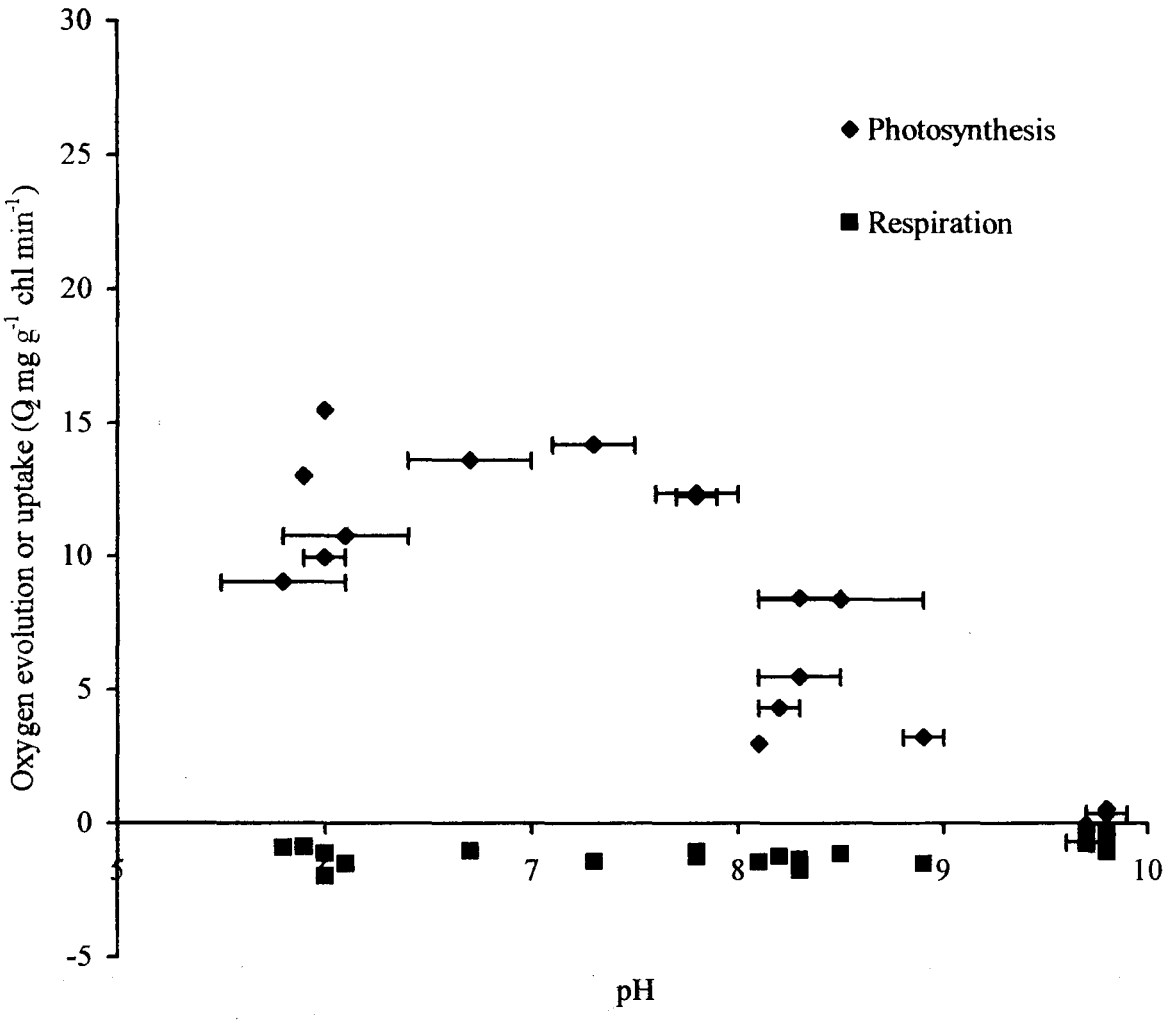


Fig. 5.5 Effect of pH in the incubation media on oxygen evolution and uptake of *E. nuttallii*. Measurements were made in unbuffered solution with x error bars representing pH range over which studies were made. X error bars omitted from respiration data for clarity.

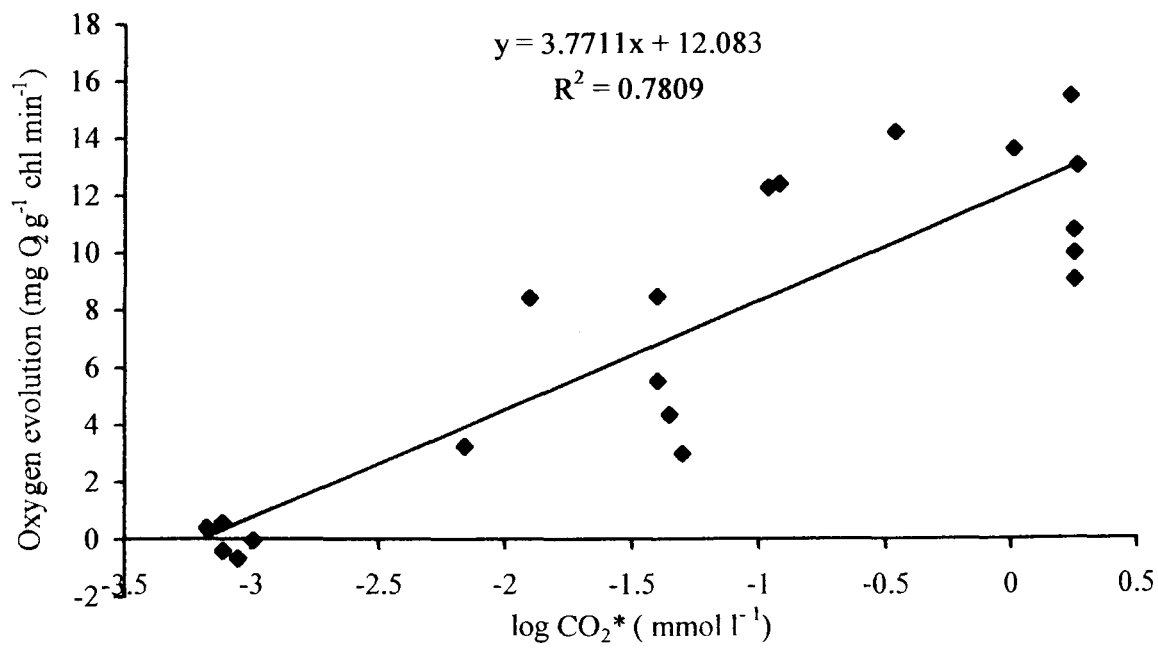


Fig.5.6 Relationship between CO_2* in the incubation medium at different pH levels used and net photosynthesis of *E. nuttallii*.

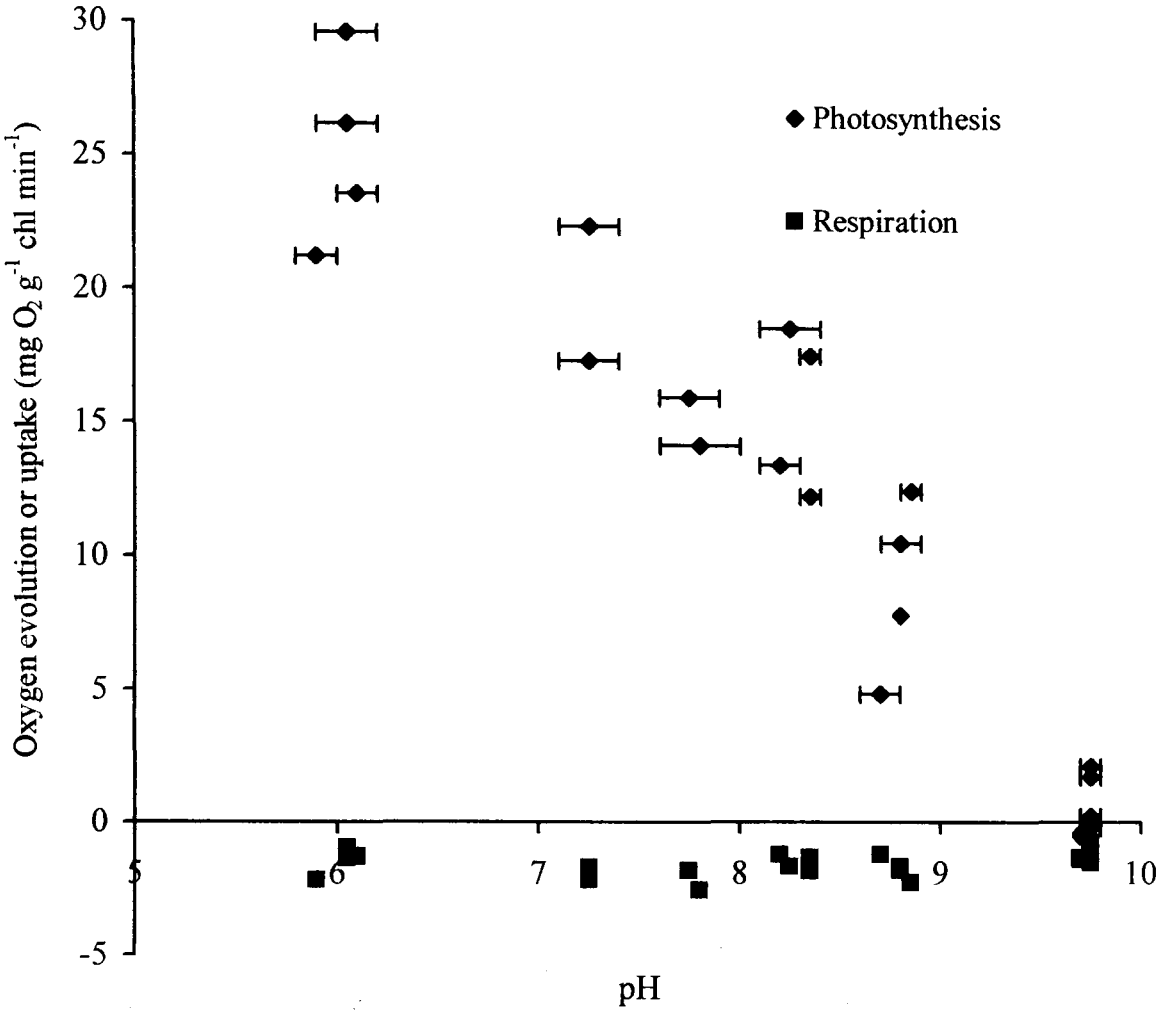


Fig.5.7 Effect of pH in the incubation media on oxygen evolution and uptake of *L. major*. Measurements were made in unbuffered solution with x error bars representing pH range over which studies were made. X error bars omitted from respiration data for clarity.

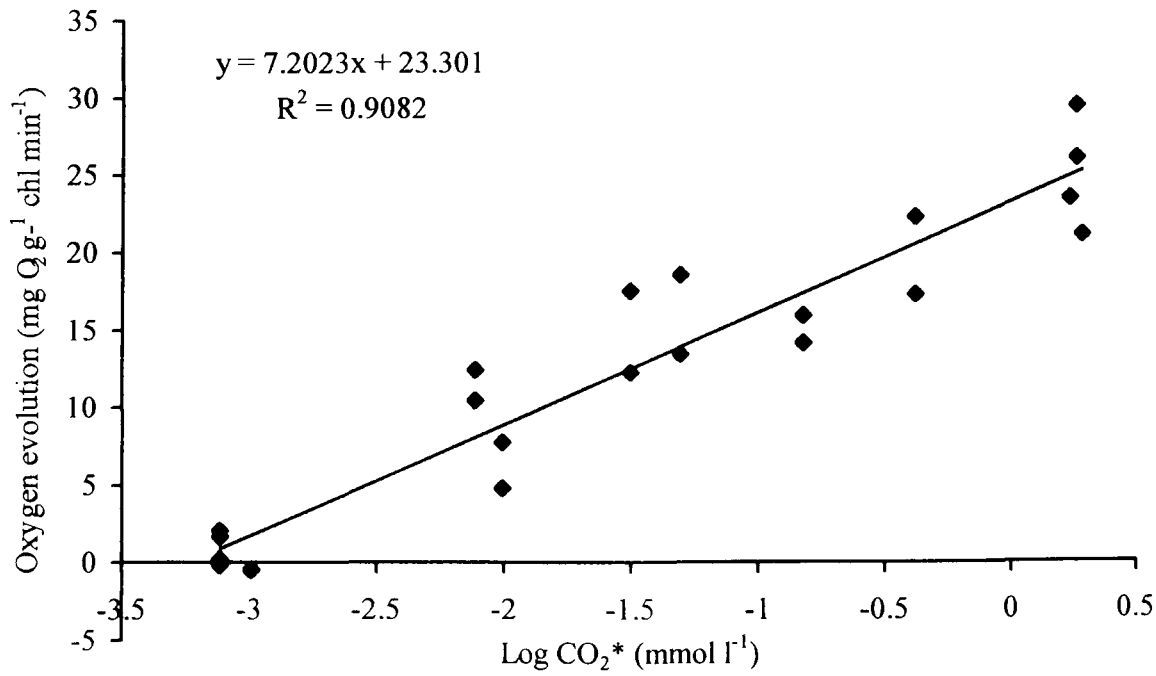


Fig. 5.8 Relationship between CO_2* in the incubation medium at different pH levels used and net photosynthesis of *L. major*.

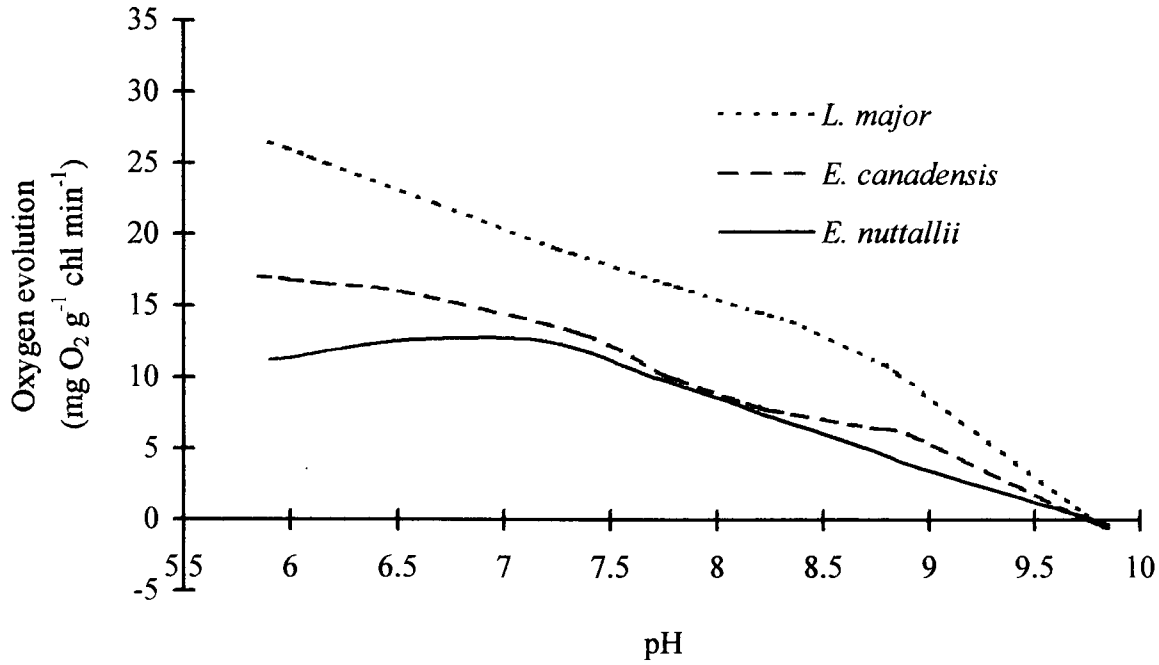


Fig. 5.9 Comparison of least weighted regression lines for photosynthetic rates in response to increasing pH of solution.

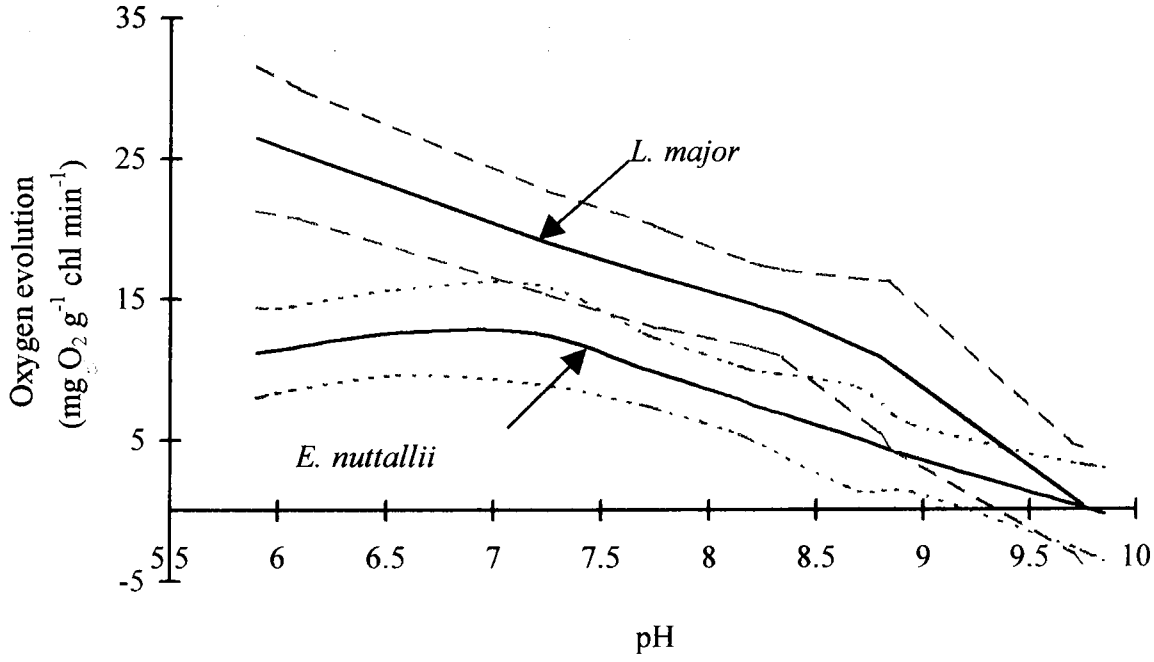


Fig. 5.10 Photosynthetic rates in response to increasing pH of solution. Critical intervals for best fit lines of *E. nuttallii* and *L. major*. Best fit lines and critical intervals for *E. canadensis* (not shown here for clarity) were intermediate between the two species shown.

m^{-2} s^{-1}). Respiration rates increased with light intensity up to approximately 100 $\mu molm^{-2}$ s^{-1} before levelling out. Increases in oxygen concentration under constant light and DIC resulted in a decrease in photosynthetic rates and an increase in respiratory rates of *L. major* (Fig. 5.2). Best-fit lines, fitted using linear regression, revealed a linear trend in both photosynthetic and respiration rates with decreasing photosynthetic rates and increasing respiration rates with increasing oxygen concentrations within the medium. Comparative measurements between the three species at 3 and 19 mg O_2 l^{-1} (Table 5.3) showed that *L. major* had significantly higher ($p = 0.05$) photosynthetic rates than *E. nuttallii* at both oxygen concentrations. Although photosynthetic rates of *L. major* were observed to be higher than those of *E. canadensis*, statistically significant differences were not found.

Table 5.3

Photosynthetic rates (mg O_2 g^{-1} chl min^{-1}) of the three species at two different oxygen concentrations. Error expressed in brackets as 95 % confidence intervals. (Significant differences at p = 0.05 between *E. nuttallii* and *L. major*.)

Species	Oxygen concentration	
	3 mg l^{-1}	19 mg l^{-1}
E. canadensis	19.7 (2.7)	16.7 (3.4)
E. nuttallii	19.2[b] (2.4)	15.4[b] (3.6)
L. major	24.0[b] (1.7)	21.9[b] (3.1)

[b] Significant differences between *E. nuttallii* and *L. major* ($p = 0.05$)

Under constant DIC (2.4 mmol l^{-1}), increase in pH of the Forsberg medium, and a consequent decrease in CO_2* concentrations, resulted in a marked decrease in photosynthetic rates and a slight increase in respiration rates of all three species (Figs. 5.3, 5.5 and 5.7). All three species achieved positive net photosynthesis over the whole range of pH values studied (pH 5 to pH 9.7.5). A comparison of best fit lines, fitted using locally weighted regression, and critical intervals for these lines (Figs. 5.9, 5.10) suggests that while the two *Elodea* spp. responded similarly to increasing pH, *L.*

major photosynthesised at higher rates over the pH range pH 5.5 to pH 9. Above pH 9 rates were similar between species. None of the species achieved net positive photosynthesis above pH 9.75. CO_2* concentrations were calculated from known values of total DIC, pH, temperature and conductivity. In order to compare the responses of the three species to decreasing CO_2* concentrations, best-fit lines were fitted to the log transformed CO_2* concentrations (Figs 5.4, 5.6 and 5.8). The linearized responses show, as expected, an increase in photosynthetic rates with increasing CO_2* concentrations in the Forsberg medium. Regression analysis revealed significant differences ($P < 0.001$) in the elevation of response lines between *L. major* and *Elodea* spp.. Significant differences ($P = 0.05$) were also found between the slopes of the response lines for *E. nuttallii* and *L. major*. No significant differences in the slopes of the response lines were observed between the two *Elodea* spp. Increases in pH were not found to have any effect upon respiration rates, as measured by oxygen uptake in the darkness.

5.3.3 Bicarbonate experiment

The two treatments applied, namely bubbling air through soda lime and untreated air with the daily addition of HCL, produced two CO_2* treatments, low and high (Fig. 5.12). CO_2* concentrations for all treatments were found to decrease over the 14 day duration of the experiment, final mean concentrations being 11.2 μmol l^{-1} and 1.6 μmol l^{-1} for high and low CO_2* treatments respectively. As measurements (alkalinity, pH and conductivity) were made prior to the addition of HCl, added to maintain a low pH (pH 7 to 8) for the high CO_2* treatment, the CO_2* concentrations shown in Figure 5.12 for the high CO_2* treatment represents minimum CO_2* concentrations. The final mean CO_2* concentration following the addition of HCl was 72.6 μmol l^{-1}. The mean CO_2* concentration calculated taking an average of the before and after HCL addition CO_2* concentrations was approximately 40 μmol l^{-1} CO_2*. Significant differences ($p = 0.05$) in CO_2* concentrations (high CO_2* treatment values taken as the minimum value reached) of the two treatment levels for each species were found by day 3. DIC concentrations were found to increase for all treatments, possibly as a result of release of CO_2 from sediment (Fig. 5.11). No significant differences were observed between relative growth rates and total length of

the species grown at the high CO_2* and low CO_2* treatments (Table 5.4). *E. nuttallii* had significantly greater numbers of apical shoot tips ($p = 0.05$) compared with *E. canadensis* and *L. major*, but no significant differences in this feature were found between the latter two species.

Initial photosynthetic and respiratory measurements showed significant differences in photosynthetic rates of *E. nuttallii* and *L. major* at pH 6.5 (Table 5.3). At the start of the experiment all species exhibited similar uptake of bicarbonate, revealed as low net photosynthetic rates at pH 9. After 14 days growth under the two CO_2* treatments, physiological differences were observed, although high variability resulted in few significant differences between treatments and species. Photosynthetic rates at pH 6.5 increased for both *Elodea* spp following the low CO_2* treatment, while *L. major* exhibited a decrease compared with initial photosynthetic rates at pH 6.5. After the high CO_2* treatment, photosynthetic rates of *E. canadensis* and *L. major* decreased while photosynthetic rates of *E. nuttallii* increased compared with initial rates at pH 6.5. Photosynthetic rates of all species at pH 9 increased following the low CO_2* treatment, compared with initial rates at pH 9. Rates in *E. nuttallii* and *L. major* at pH 9 after the high CO_2* treatments were similar to initial rates, although *E. canadensis* showed a decrease compared with initial rates. In a comparison between physiological rates at high and low CO_2* treatments, all species exhibited higher photosynthetic rates at pH 9 following the low CO_2* treatment compared with the high CO_2* treatment, although significant differences ($p=0.05$) were only found for *E. canadensis*. The ratio of photosynthetic rates at pH 9 to pH 6.5 (9/6.5), used to eliminate overall differences in the physiological rates of the material, were extremely variable. Both *Elodea spp.* showed increases in the 9/6.5 ratio following the low CO_2* treatment, and decreases after the high CO_2* treatment. Results for *L. major*, however, differed. The ratio of (9/6.5) increased following both high and low CO_2* treatments, compared with initial rates. This is possibly a consequence of the difficulty in maintaining a low pH for the high CO_2* treatment. The initial higher starting biomass of *L. major* resulted in rapid changes in water quality, such that the pH of some replicates of *L. major* reached pH 9 on a few occasions.

Following the method of Jones (1994), it was possible to calculate approximate costs of bicarbonate utilisation for each species. Jones (1994) estimated maximum photosynthesis without photorespiration (P_{max} Free) as 50% greater than that in water at equilibrium with air (P_{max}) for *E. nuttallii*. The estimate was made from an oxygen response curve determined for the species. Simpson (1981) reports a similar response curve for *E. canadensis*. The comparison between photosynthetic rates of the three species at 3 mg l^{-1} and 19 mg l^{-1} made in the present study suggest a similar response for all three species as photosynthetic rates were similarly depressed at the higher oxygen concentration. On the basis of these results it was decided to use a similar estimate to that used by Jones (1994) of a 50 % increase in maximum photosynthesis in the absence of photorespiration.

For clarification of the calculation method, values for *E. nuttalii* are used as an example. The value for P_{max} Free [40.86 mg O_2 g^{-1} chl min^{-1}] was estimated as 50 % greater than P_{max} [27.24 mg O_2 g^{-1} chl min^{-1}] from photosynthetic rates after the high CO_2* treatment, at pH 6.5, assuming that only CO_2* was being utilised as a carbon source (Table 5.4). P_{air}, photosynthesis at the mean CO_2* concentration measured for the high CO_2* treatment (40 umol l^{-1}) under ambient O_2 concentrations (~10 mg l^{-1}), was estimated from Fig. 5.6 as 6.88 mg O_2 g^{-1} chl min^{-1}. The overall cost in terms of reduction of photosynthesis is therefore:

$$P_{max}\text{Free} - P_{air} = 33.98 \text{ mg } O_2 \text{ g}^{-1} \text{ chl min}^{-1}$$

Taking into account the effect of night-time respiration (darkness was 8 hours out of every 24):

$$\frac{33.98 * 16}{24} = 22.65 \text{ mg } O_2 \text{ g-1 chl min - 1}$$

Assuming a 1:1 ratio of CO_2* fixed to O_2 released and using a value of 1 mole of CO_2* fixed to carbohydrate is equal to 25 moles of photons (estimate from Cyanobacteria, Raven & Lucas, 1985) values for number of photons required per unit chlorophyll per minute can be made.

$$17.70\ \mu mol\ photons\ g^{-1}\ chl\ min^{-1}$$

This value can then be converted to incident light using the known chlorophyll concentrations in leaves per unit leaf area (22.38 $\mu g\ cm^{-2}$) (Table 5.1).

$$66.02\ \mu mol\ photons\ m^{-2}\ s^{-1}$$

Under this incident light intensity, it is estimated that bicarbonate utilisation would not be beneficial as overall costs would exceed the benefits of utilising this carbon source.

Values for the three species are:

E. canadensis	30.75 $\mu mol\ photons\ m^{-2}\ s^{-1}$
E. nuttallii	66.15 $\mu mol\ photons\ m^{-2}\ s^{-1}$
L. major	25.76 $\mu mol\ photons\ m^{-2}\ s^{-1}$

A simple working cost can also be calculated by :

$$\text{Working cost} = \frac{P_{max}\ O_2 - P_{air}}{P_{air}} * 10$$

The working costs calculated for each species were:

E. canadensis	25%
E. nuttallii	50%
L. major	12%

Using these values it is possible to predict that use of bicarbonate will be efficient once photosynthesis is reduced below 75, 50 and 88 % of its maximum value by carbon limitation for *E. canadensis*, *E. nuttallii* and *L. major* respectively.

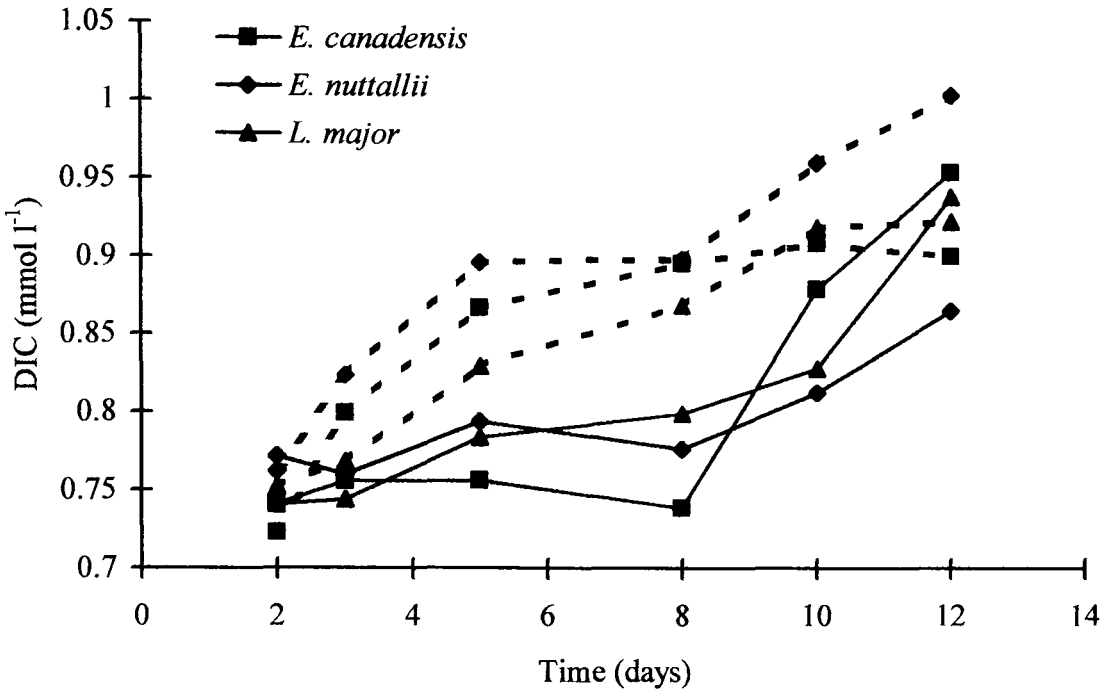


Fig. 5.11 Total inorganic carbon concentrations in the growth media, solid lines represent high CO_2* treatments, and dashed lines represent low CO_2* treatments

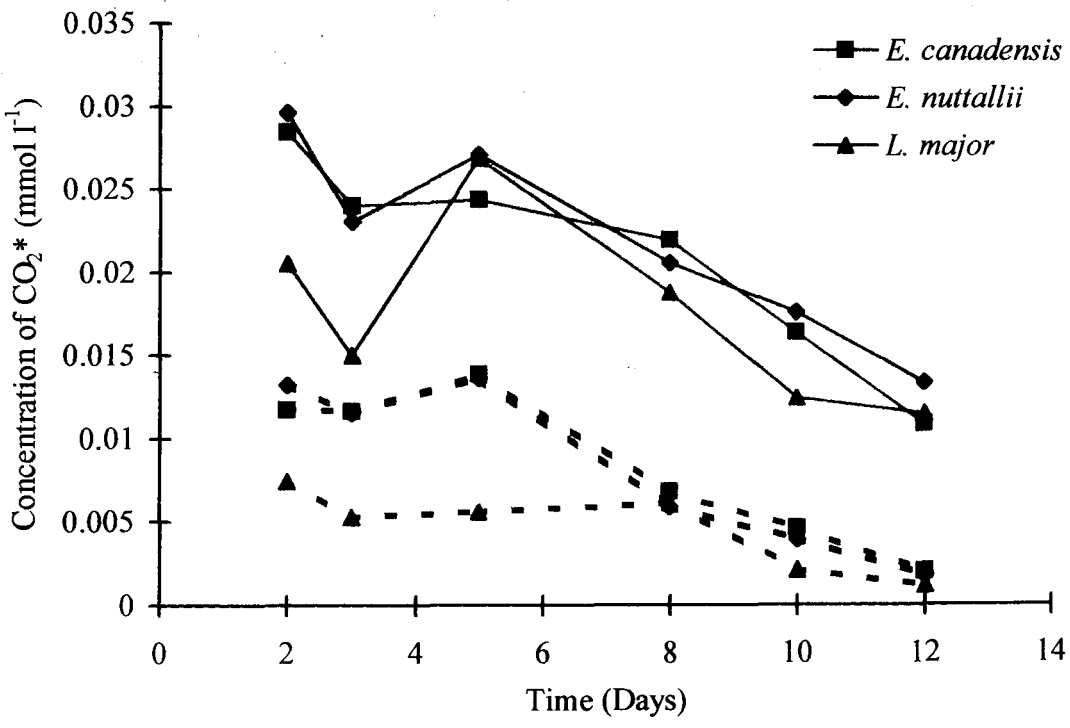


Fig. 5.12 Minimum CO_2* concentrations in the growth medium measured before the addition of HCL for the high CO_2* treatment. Solid lines represent high CO_2* treatments, and dashed lines represent low CO_2* treatments.

Table 5.4

Photosynthetic and respiratory activity of plant material at the start of the experiment and after 14 days grown at low CO_2* and high CO_2*. Values in brackets are 95 % confidence limits. * Significant differences at p = 0.05 between treatments. P = Photosynthesis, R = Respiration

species	Start					Low CO_2 *					High CO_2*				
	P (pH 6.5)	R (pH 6.5)	P (pH 9)	R (pH 9)	Ratio 9/6.5	P (pH 6.5)	R (pH 6.5)	P (pH 9)	R (pH 9)	Ratio 9/6.5	P (pH 6.5)	R (pH 6.5)	P (pH 9)	R (pH 9)	Ratio 9/6.5
E. canadensis	24.23	2.69	6.03	2.35	0.24	27.78	1.77	7.64 *	1.82	0.30	20.60	1.80	2.25 *	1.82	0.12
	(4.78)	(0.33)	(4.70)	(0.22)	(0.20)	(4.35)	(0.41)	(2.66)	(0.33)	(0.12)	(5.39)	(0.46)	(0.68)	(0.23)	(0.06)
E. nuttallii	19.46 [b]	3.11	5.78	1.96	0.35	27.63	2.52	10.24	2.50	0.42	27.24	2.49	6.64	2.82	0.25
	(5.40)	(0.70)	(1.90)	(0.39)	(0.13)	(5.77)	(0.58)	(2.19)	(0.36)	(0.15)	(6.65)	(0.41)	(2.14)	(0.58)	(0.07)
L. major	31.07 [b]	3.30	6.32	2.98	0.21	24.51	2.07	10.28	2.16	0.43	19.03	1.76	6.19	1.88	0.33
	(2.43)	(0.34)	(1.09)	(0.54)	(0.05)	(4.02)	(0.69)	(1.79)	(0.50)	(0.08)	(4.01)	(0.32)	(1.62)	(0.35)	(0.10)

[b] = **Significant differences between *E. nuttallii* and *L. major* (p = 0.05)**

Table 5.5

Growth measurements of plant material at the start of the experiment and after 14 days grown at low CO_2* and high CO_2* Values in brackets are 95 % confidence limits. * Signficant differences at p = 0.05 between *E. nuttallii* , and *E. canadensis* and *L. major.* Drywt = dry weight, RGR = relative growth rate

species	Start			Low CO_2 *			High CO_2*		
	Length cm	Apices Number	Dry wt g	Length cm	RGR $g\ g^{-1}\ day^{-1}$	Apices Number	Length cm	RGR $g\ g^{-1}\ day^{-1}$	Apices Number
E. canadensis	10	1	0.0223 (0.0053)	21.95 (4.12)	0.0599 (0.008)	2.5* (0.64)	19.1 (2.96)	0.0550 (0.012)	2.25* (0.49)
E. nuttallii	10	1	0.0188 (0.0042)	22.89 (1.72)	0.0526 (0.012)	4.25* (0.49)	21.96 (4.5)	0.0534 (0.014)	4.37* (1.12)
L. major	10	1	0.0663 (0.011)	16.75 (3.81)	0.0634 (0.009)	1.87* (0.44)	15.31 (3.84)	0.0541 (0.010)	2* (0.74)

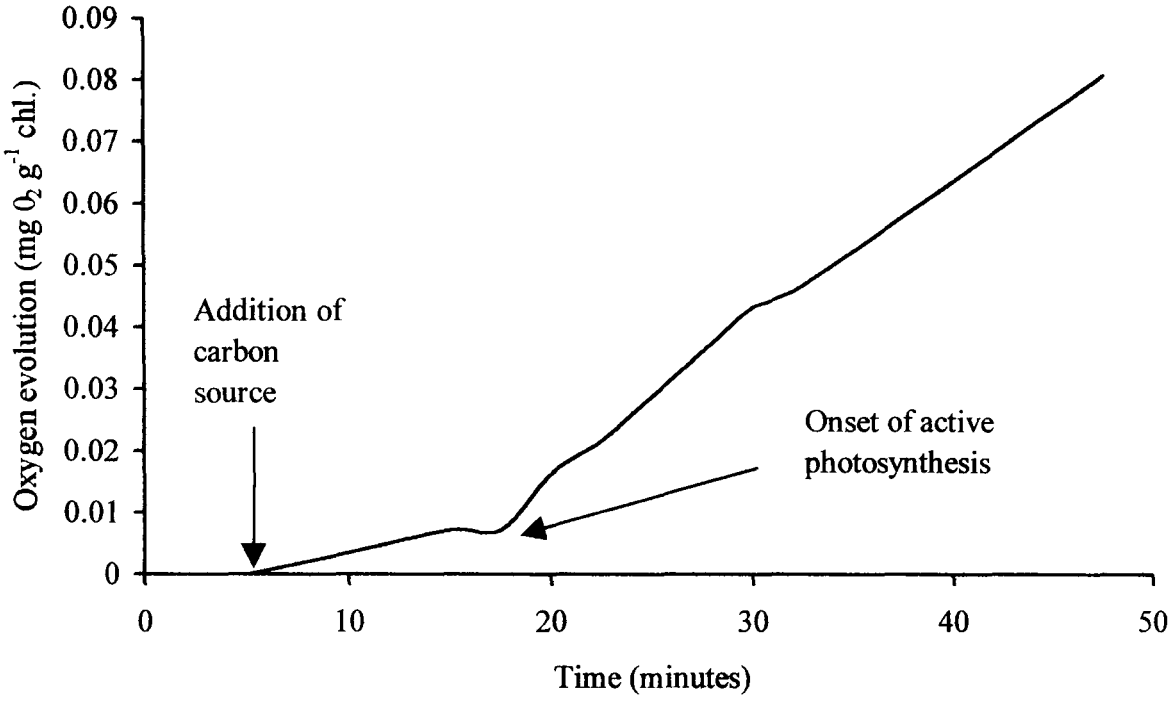


Fig. 5.13 Example showing delay between activation of photosynthesis with the addition of the carbon source, and active photosynthesis of *L. major*. pH drift for this study was found to be 0.1 of a pH unit, with an initial pH of 9.8, and a final pH of 9.7.

5.4 Discussion

Chlorophyll concentrations found in the present study for laboratory-grown and field material are greater than the range quoted by Nielsen and Sand-Jensen (1989) for 14 aquatic macrophytes (3.3 – 13.4 μg chl cm^{-2}) but more comparable with values (~ 21 μg chl cm^{-2}) quoted by other authors for *E. canadensis* and *E. nuttallii* (Simpson, 1981; Jones, 1994). Significantly higher photosynthetic rates per unit area were found for *E. nuttallii* and *L. major* than *E. canadensis*. This pattern was also observed for photosynthetic rates per unit chlorophyll made in the same study.

Light saturation curves for *L. major* follow the typical pattern found for many macrophyte species. In this study, photosynthesis was saturated at approximately 200 μmol photons m^{-2} s^{-1} (PAR)). Light saturation values for *L. major* quoted in the literature of 170 μmol photons m^{-2} s^{-1} (PAR) and 90 μmol photons m^{-2} s^{-1} (PAR) for oligotrophic and eutrophic lakes respectively (Rattray, 1989 reported in Schwarz and Howard-Williams, 1993) are similar. The light compensation point recorded in this study of below 5 μmol photons m^{-2} s^{-1} (PAR) is considerably lower than values previously quoted (15 μmol photons m^{-2} s^{-1} (PAR)) by Schwarz and Howard-Williams (1993). In work conducted on *Hydrilla verticillata* by Van *et al.* (1977), light compensation points were found to be correlated with the light intensity under which the species were grown. Thus, as light intensities used for culturing material in the present study were low (~ 70 μmol m^{-2} s^{-1}), the low light compensation point may well reflected this. The ability to adapt to changing light intensities with varying light compensation and saturation points is likely to be an important factor, particularly in light limiting environments.

While the general pattern in light response for *L. major* was found to be extremely similar to those of both *E. canadensis* and *E. nuttallii* in previous studies, overall maximum rates of photosynthesis found in the present study were lower. However, comparisons of physiological rates reported by different authors for different species should be made tentatively. Differences between photosynthetic and respiratory rates despite using the same measuring techniques are likely to result due to differences in growth conditions prior to measurements. Even in the present study, where physiological comparisons were made on material grown under controlled

environmental conditions, great variation in rates was found. High variability in physiological rates may be a manifestation of the physiological plasticity of individual leaves in adapting to environmental conditions in their immediate vicinity. Physiological plasticity is likely to be competitively advantageous allowing a plant to adapt to local environmental conditions, thus having the potential in a heterogenous environment to maximise photosynthesis and growth through the efficient use of available resource.

Increases in oxygen concentrations were found to decrease photosynthesis and increase respiration of *L. major* leaves. This response pattern is similar to that reported for *E. canadensis* and *E. nuttallii* in previous studies (Simpson, 1981; Jones, 1994). Comparisons between the three species made here also suggest that the effects of oxygen concentration on photosynthesis are similar. High oxygen concentrations, such as those studied here, result in photorespiratory stress as O_2 competes with CO_2* for RUBISCO sites. This was suggested as a reason for the observed depression in photosynthesis during late afternoon found during *in situ* field studies (Hough, 1974). However, while photorespiration may well contribute to this observed depression, Jones *et al.* (1996) suggest that depletion of carbon sources is likely to be the main cause.

In a comparison of pH response curves for the three species, the curve for *L. major* had the highest elevation over almost the entire pH range studied. Similarly, photosynthetic rates for this species at both 3 and 19 mg O_2 l^{-1} were high than those for either of the *Elodea* spp. The higher photosynthetic rates of *L. major* observed in these studies were contradicted in the results of the bicarbonate study. Although initial maximum photosynthetic rates of *L. major* were high, measured as photosynthetic rate at pH 6.5, following the acclimation treatments a reduction in maximum photosynthesis was observed. No apparent reason could be found for this reduced rate. In general, however, it appears that *L. major* has the potential for higher photosynthetic rates than either *Elodea* spp. This is also supported by the work of Jones (1994) who determined pH response curves for the three species, although in buffered systems. In this latter study, maximum photosynthetic rates of *L. major* were higher than those of either *Elodea* spp.. Response curves for the two *Elodea* spp. were largely similar between pH 6 and 10.

Measurements of growth following the bicarbonate treatments surprisingly revealed no significant differences either between species or between treatments. One would have expected growth rates to be reduced under the low CO_2* conditions, reflecting the lower photosynthetic rates when mainly utilising bicarbonate as a photosynthetic carbon source. As illustrated in the calculated costs of bicarbonate utilisation, energy costs of using bicarbonate are great compared to those of using CO_2* as a carbon source. This suggests that the experimental period was not sufficient to allow the lower photosynthetic rates to be manifested in lower species growth rates. Measurements of photosynthetic and respiratory rates showed that all three species responded to low CO_2* with increased affinity for bicarbonate reflected in a higher 9/6.5 ratio. However, following the high CO_2* treatment the affinity of shoots of *E. canadensis* for bicarbonate was reduced compared with both initial rates and rates following the low CO_2* treatment. This suggests that *E. canadensis* may rapidly lose its affinity for bicarbonate. Working on *E. canadensis*, Adamec (1993) found a very rapid decline in bicarbonate affinity (40 minutes) following exposure to high CO_2* concentrations. In longer-term experiments, Jones *et al.* (1993) found an increase in affinity for bicarbonate by *E. nuttallii* over 5 days (at 25 °C) and 8 days (at 15 °C). Sand-Jensen and Gordon (1986) reported an increase in bicarbonate uptake efficiency only after 56 days. However, as Jones *et al.* (1993) state, the plants used by Sand-Jensen and Gordon (1986) were already utilising a high proportion of bicarbonate before trials began, thus a further increase is likely to be a much longer process. Bicarbonate uptake rates for *E. nuttallii* and *L. major* following the high CO_2* treatment were similar to initial rates. It has frequently been observed that even at high CO_2* concentrations, affinity for bicarbonate may be reduced, but not eliminated altogether (Maberly *et al.*, 1996). The results from the present study suggest that at both low and high CO_2* concentrations, the affinity of *E. nuttallii* and *L. major* for bicarbonate is greater than that of *E. canadensis*. However, while the calculated costs of bicarbonate uptake for *L. major* were low, such that photosynthetic rates need only be reduced to 88 % below maximum for bicarbonate uptake to be metabolically advantageous, costs for *E. nuttallii* were substantially greater. Calculations of bicarbonate efficiency for *E. nuttallii* suggest that only above ~ 66 μmol photons m^{-2} s^{-1}, will bicarbonate usage become efficient. This is a higher value than for either *E. canadensis* or *L. major*. The value quoted here for *E. nuttallii* is very similar to that calculated for the same species (60 μmol photons m^{-2} s^{-1}) by

Jones (1994) further substantiating this result. Thus, under low light conditions when CO_2* is limiting such as within a dense macrophyte stand, both *L. major* and *E. canadensis* will have a competitive advantage over *E. nuttallii* as they will be able to maintain efficient use of bicarbonate. Thus, *L. major* can both photosynthesis at very low light intensities when CO_2* is plentiful but can also achieve net positive photosynthesis under low light conditions above ~30 μmol photons m^{-2} s^{-1} even when CO_2* becomes limiting. This may allow this species to grow within dense stands of another macrophyte species such as *E. nuttallii* during the initial period of invasion.

Notably, for treatments at high pH some leaves exhibited an initial lag phase during which time they were not achieving positive net photosynthesis (Fig. 5.13). This was apparent for all three species. After a short period of time, usually between 10 and 30 minutes, an increase in rate would be detected. This was observed both for measurements of pH response and of bicarbonate usage at pH 9. It is probable that during this initial lag phase the leaves are adjusting physiologically to the increased pH. This period may be required to develop the pH gradient across the leaf surface necessary for the external conversion of HCO_3- to CO_2*, as described for the polar leaf mechanism. Overall, this would suggest that most leaves required a period of adaptation. This raises the possibility that previous authors may not have allowed sufficient time for leaves to initialise bicarbonate utilisation and may, in part, suggest why such a difference in times required for adaptation to changing CO_2* and HCO_3^- concentrations are observed in the literature (i.e. Sand-Jensen and Gordon, 1986; Jones *et al.*, 1993). If this rapid adaptation occurs within the field, it will allow plants to adapt over a 24 hour light/dark cycle, during which there are diurnal pH and corresponding CO_2* and bicarbonate changes. The time taken for acclimation to changing CO_2* and bicarbonate conditions is of obvious ecological significance. Significant competitive advantages are likely to be gained by species that can rapidly adapt to changing water quality. Species that can respond to diurnal variations in CO_2* and HCO_3^- concentrations are likely to make more efficient use of available carbon, and will consequently gain a competitive advantage.

As previously stated there is some evidence in the literature (e.g. De Groote and Kennedy, 1977; Browse *et al.*, 1977) to suggest that some macrophytes may use a C_4 type fixation strategy without the associated Kranz anatomy. Although

subsequent studies on *E. canadensis* (e.g. Madsen *et al.*, 1996) report only very minimal concentrations of PEP carboxylase activity even under CO_2* limiting conditions, evidence is still lacking. Further studies, particularly on the metabolic pathways of *E. nuttallii* and *L. major,* are needed to provide further insight into the competitive abilities of these species.

5.5 Summary

1. Measured photosynthesis and respiration rates were very variable. This is likely to reflect the plasticity of the plants in their response to prior environmental conditions.
2. Light and oxygen response curves determined for *L. major* exhibited similar patterns to those of *E. canadensis* and *E. nuttallii* reported in previous studies.
3. *L. major* is capable of very high photosynthetic rates, with highest photosynthetic rates observed for this species.
4. All three species exhibited a plastic response to changing free carbon dioxide and bicarbonate concentrations. Photosynthetic rates at high pH (between 9 and 10) were observed to be higher following the low CO_2* treatment, suggesting increased bicarbonate affinity following acclimation to low CO_2* concentrations.
5. Results suggest that *E. canadensis* may lose its affinity for bicarbonate uptake more readily than either *E. nuttallii* or *L. major*.
6. *E. nuttallii* and *L. major* may gain a competitive advantage over *E. canadensis* by more rapidly invoking bicarbonate utilisation. The response times reported here (15 to 20 minutes) may be advantageous in conditions when there is a rapid onset of CO_2* depletion each day.
7. Calculated costs of bicarbonate utilisation suggest that *L. major* is an energetically efficient bicarbonate user. This may convey competitive advantages to this species under CO_2* limiting conditions.
8. Overall, no distinct differences in photosynthetic rates were observed. Even for plant material cultured under controlled laboratory conditions, great variation was observed and appears to be an innate characteristic of the three species.

Chapter 6 THE RESPONSE OF THE THREE SPECIES AND THEIR ASSOCIATED EPIPHYTIC FLORA TO NITROGEN AND PHOSPHORUS FERTILISATION.

6.1 Introduction

Increases in inorganic nutrient loading, particularly nitrogen and phosphorus, are indicative of eutrophication (Wetzel, 1988). High concentrations of nutrients have been shown to encourage prolific algal growth, both periphytic and filamentous algae (Mulligan and Baranowski, 1969; Phillips *et al.*, 1978). An increase in algal growth is often associated with a corresponding decrease in macrophyte growth (Phillips *et al.*, 1978; Wetzel, 1988), and in extreme cases macrophytes may eventually be eliminated (Moss, 1991). Changes in species composition, both flora and fauna, of fresh water systems with increasing nutrient loadings has been the subject of many studies (e.g. Mulligan and Baranowski, 1969; Mulligan *et al.*, 1976; Phillips *et al.*, 1978; Balls *et al.*, 1989). The decline in macrophyte growth has largely been attributed to competition for light, since prolific growth of epiphytic and filamentous algae may shade macrophytes and consequently severely restrict their growth (Phillips *et al.*, 1978) though the basis of competition has rarely, if ever, been experimentally proven, Further studies have, however, shown that epiphytes can successfully compete with their macrophyte hosts in nutrient acquisition from the surrounding water (Howard-Williams, 1981; Carignan and Kalff, 1982; Pelton *et al.*, 1996).

Despite field observations on macrophyte decline, studies in which high nutrient loading programmes are used have often failed to eliminate macrophytes (Howard-Williams, 1981; Moss *et al.*, 1985; Moss, 1991). The ability of macrophytes to buffer changes in nutrient concentrations, taking up excess nutrients that would otherwise be available for algal growth, may play an important role in preventing the switch from macrophyte to phytoplankton dominated system. Removal of excess nutrients by the macrophyte, thus reducing nutrient concentrations in the water body, will limit the growth of algae while allowing the macrophyte to continue growing through the mobilisation of accumulated resources and uptake of nutrients from interstitial waters. The nitrogen and phosphorus tissue content of many macrophytes are reported to be proportional to and dependent on the concentrations in the water

body (Gerloff and Krombholz, 1966; Wetzel, 1988; Portielje and Roijackers, 1995) and are often at "luxury" levels, indicating acquisition of nutrients without immediate use.

Other characteristics that may confer competitive advantages upon the macrophyte component are a high relative growth rate and a large surface area to volume ratio. These maximise the surface area available for absorption of nutrients and carbon sources and the interception of light. A high relative growth rate, coupled with the ability to take up "excess" nutrients rapidly, will allow the macrophyte to keep ahead of epiphytic algal colonisation by growing more rapidly than the epiphytic algae can, in sufficient numbers, colonise the newly produced shoots. However, slow growing plants with a low ability to take up excess nutrients, may be overtaken and deleteriously affected by algal cover, resulting in an eventual decline in their growth rate.

It is suggested that indirect competition, in which algae play an intermediate role, may determine the competitive success of a newly introduced invasive species. If an introduced species is better able to compete with the algae than an existing species, through the rapid uptake and accumulation of available nutrients, and a high relative growth rate, it is likely to displace it. This may account for the competitive success of both *Elodea nuttallii* in displacing *Elodea canadensis* and in *Lagarosiphon major* in displacing *Elodea spp.*. Experiments have shown that *E. nuttallii* and *E. canadensis* can survive in extremely eutrophic water bodies (Mulligan *et al.*, 1976; Spence, 1964; Ozimek *et al.* 1993; Portielje and Roijackers, 1995). However, field observations of species distribution on the Alsace flood plans, northern France, suggest that *E. nuttallii* has successfully displaced *E. canadensis* specifically from eutrophic water-bodies (Dendene *et al.*, 1993; Rolland and Tremolieres, 1995; Thiebaut *et al.*, 1997).

Within the UK, *E. canadensis* still appears to be common in Scotland, although it has been displaced from many lowland sites in England (See Chapter 1). It is unknown whether this distribution is the result of geographical isolation or niche separation of the two species based on water trophic status. Many oligotrophic sites in the UK tend to be isolated upland waters, reducing the likelihood of the

introduction of an alien species. Rolland and Tremolieres (1995) suggest that ammonium toxicity may play a vital role in the distribution of *Elodea* spp.. Their studies have shown that *E. canadensis* has greater susceptibility to ammonium toxicity than *E. nuttallii*. However, concentrations found in most inland freshwater bodies within the UK are lower than concentrations reported for toxic effects to be observed. Concentrations of ammonia recorded for British canals between 1978 and 1979 were rarely greater than 500 mg m^{-3} NH_4-N (Murphy, 1980), yet Ozimek *et al.* (1993) only found a reduction in growth of *E. canadensis* at ammonium concentrations exceeding 4 g m^{-3}. Dendene *et al.* (1993) observed a reduction in photosynthetic activity of *E. canadensis* at ammonium concentrations of 2.5 g m^{-3} and above. Similarly, Rolland and Tremolieres (1995) observed a significant reduction in growth of *E. canadensis* at 5 g $N\text{-}NH_4^+$ m^{-3} and a significant reduction in photosynthesis at ammonium concentrations of 2.5 and 5 g $N\text{-}NH_4^+$ m^{-3}. While these findings suggest interesting differences between these species, it seems unlikely that this is an important aspect in the displacement process in this country. *E. nuttallii* has been found to have a consistently higher growth rate than *E. canadensis* when grown under a range of nutrient conditions (Ozimek *et al.*, 1993), and a greater leaf area ratio (LAR) (See Chapter 3). Little information is however available upon the growth of *L. major* in relation to nutrient status. In an isolated publication, Rattray *et al.* (1991b) observed greater growth of *L. major* when grown on eutrophic sediments compared with oligotrophic lake sediments. Observations during previous studies in the present work indicate that *L. major* may have a high capacity to remove nutrients from the surrounding medium.

In this chapter the results of two experiments are reported. First, a time series experiment was performed to establish nutrient removal rates from solution in the presence and absence of *E. canadensis*, *E. nuttallii* and *L. major*. This study was done to determine whether nutrient decreases in the surrounding medium could be used as an indirect measure of plant uptake rates. Secondly, a growth experiment was conducted to quantify the effects of increasing nutrient loadings on the root and shoot biomass of the three aquatic macrophytes. In this study, the ability of these species to accumulate nitrogen and phosphorus in shoot tissue was evaluated, and the development of epiphytic algal communities on the surfaces of these species assessed.

6.2 Materials and Methods

E. canadensis was collected from Ec WB, *E. nuttallii* from En M and *L. major* material from stock cultures grown outside at Liverpool University. For the nutrient growth study, canal water was used for the growth media. This was collected from near Lydiate, Lancashire (Grid reference SD 373 059) on the Leeds and Liverpool Canal in 25 l carboys. On return to the laboratory the water was filtered twice through 25 TI 35, 40 * 40 μm mesh plankton netting to remove algae and suspended solids, before being stored until use at 10 °C in the dark to prevent algae growth. Total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate and ammonium concentrations were measured at the start of the experiment (for methods see Chapter 2, Table 2.2). Some chemical characteristics of the canal water are given in Table 6.2. Measurements of nitrogen and phosphorus content of plant dry tissue were made following the method described in Chapter 4, Section 4.2.3. For estimation of starting material, a sub-sample (n = 6 for the timed study, and n = 8 for the growth study) of each species were removed and cleaned. Measurements of fresh weight, numbers of apical meristems, shoot dry weights, root dry weights, N and P concentrations were made.

6.2.1 Time study

12 healthy 10 cm shoots of each species were selected. Each was planted in a 250 ml plastic cup filled with canal sediment and subsequently placed in 10 litre buckets filled with tap water. These were then grown as nursery cultures for two weeks prior to the start of the experiment to allow establishment of the shoots.

At the start of the experiment six plants of each species were transferred in their cups to 3 l glass jars. For controls six cups were selected at random and these plants removed, leaving only sediment. These were then also placed in 3 l jars. All jars were filled with 2.5 litres of tap water previously aerated for 24 hours. At time 0 nitrogen and phosphorus were added (50 mg m^{-3} as potassium dihydrogen orthophosphate and 1 g m^{-3} as ammonium nitrate). Levels of SRP, nitrate and

Table 6.1

Chemical characteristics of canal water medium. Error is expressed in brackets as 95 % confidence limits.

pH	7.7
Conductivity ($\mu S\ cm^{-1}$)	$4.9 * 10^2$
Oxygen ($g\ m^{-3}$)	11
Alkalinity (mequiv)	2.32
Nutrients :	
TP ($mg\ m^{-3}$)	197.51 (5.98)
SRP ($mg\ m^{-3}$)	35.15 (1.81)
SUP ($mg\ m^{-3}$)	138.21 (3.36)
PP ($mg\ m^{-3}$)	24.14 (4.40)
NO_3 ($g\ m^{-3}$)	negligible
NH_4^+ ($g\ m^{-3}$)	212.81 (14.35)
Phytoplankton chlorophyll ($mg\ m^{-3}$)	$2.34 *10^3$ ($2.46 *10^4$)
Carbon fractions ($mmol\ l^{-1}$):	
Total inorganic C	2.42 (0.089)
Free CO_2	0.11 (0.0039)
Bicarbonate	2.31 (0.085)
Carbonate	0.004 ($1.58*10^4$)

ammonium were then measured at time 0 (immediately after the addition of nutrients), and after 2 hours, 12 hours, 24 hours (excluding nitrate), 48 hours, four day and eight day intervals. Measured experimental nutrient levels were much greater than anticipated due to the high levels of SRP and nitrate in the tap water at the time of the experiments (Appendix I, Table 1).

6.2.2 Nutrient growth study

100 healthy 10 cm shoots of *E. canadensis* and *E. nuttallii* , and 50 10 cm shoots of *L. major* were selected. To achieve similar starting biomasses two shoots of *E. canadensis* and *E. nuttallii* and one of *L. major* were planted per pot in canal sediment. Plants were grown in nursery cultures for two weeks as described for the time study (Section 6.2.1). For the growth media, 2.5 litres of canal water were added to each experimental jar. Thirty plants of each species were selected at random from the nursery cultures and placed singly in each experimental jar. Nutrients were added as appropriate to the levels specified in Table 6.2 with six replicates per treatment. The base level present in the canal water before the addition of supplementary nutrients set the lower limit for the nutrient range to be studied.

The nutrient ranges chosen for this study are based upon phosphorus loading values quoted by Vollenweider and Kerekes (1981) for oligotrophic to hyper eutrophic water bodies. The ratio of P to N used (7N : 1P, by molecular weight) was that described by Redfield (1963) for phytoplankton in which either N or P is limiting growth.

Differences in the trophic status of the water body may lead to differential limitations in free CO_2* between treatments. Consequently total CO_2, free CO_2, bicarbonate and carbonate were estimated following Mackereth *et al.* (1989) using pH, temperature and alkalinity. Measurements of pH and conductivity were made every 3 or 4 days and alkalinity measurements weekly.

Table 6.2

Nutrient concentration and total loadings for nutrient growth study

Treatment number	P mg m^{-3} $week^{-1}$	Total P loading (mg m^{-3})	N g m^{-3} $week^{-1}$	Total N loading (g m^{-3})
1 (Base level)	30	120	0.21	0.84
2	60	240	0.42	1.68
3	120	480	0.84	3.36
4	240	960	1.68	6.72
5	480	1920	3.36	13.44

6.2.3 Harvesting

At harvest the length, fresh weight, dry weight and number of apical mertistems were measured/counted. Epiphyte growth was assessed by carefully removing each plant from its container and shaking it in 500 ml of distilled water for two minutes. Zimba & Hopson (1997) found this to be an adequate time for the removal of 88 % of diatom growth, the component of the epiphyte community found to be most strongly attached to the plant surface. A known quantity of water was then removed from the container, filtered using preweighed filter papers and dried to a constant weight at 45 °C for analysis of epiphyte biomass (mg). Phytoplankton growth in each jar was assessed using the procedure explained in section 2.6 using 120 ml of the growth medium. Plants samples were dried at 45 °C to a constant weight and then ground (Glen Creston Mill; particle size 1 mm) for analysis of nitrogen and phosphorus concentration.

6.2.4 Calculation of nutrient standing stock and transfer rates

Total nutrient accumulation, termed standing-stock, and nutrient transfer rates to the plant were calculated following Howard-Williams & Allanson (1981). Standing stocks (N) were calculated as:

Equation 6.1

$$N = dw * C$$

Where dw is the total dry weight of the plant and C the tissue concentration of nitrogen or phosphorus (mg g^{-1} dry weight). Transfer rates were calculated as:

Equation 6.2

$$\text{Transfer rates} = \frac{\ln B_1 - \ln B_2}{(t_2 - t_1)} * \frac{C_2 - C_1}{2}$$

Where C_1 and C_2 are the initial and final tissue concentrations of phosphorus or nitrogen, in biomass B_1 at time t_1, and biomass B_2 at time t_2.

6.3 Results

6.3.1 Timed study

All plants gained biomass over the experimental period nearly with the exception of two replicates which were presumably damaged during planting. Overall, the RGR of *E. nuttallii* was three times greater than that of *E. canadensis*, and almost twice that of *L. major*. Both *Elodea spp.* had at harvest significantly greater numbers of apical tips than *L. major*.

Table 6.3

Growth characteristics of shoots following timed study.

Species	Initial wt (g)	RGR (g g^{-1} d^{-1})	No. Apices	Root wt (g)
E. nuttallii	0.054	0.086	11.17[b]	0.013[b]
E. canadensis	0.134	0.023	9.33[c]	0.012[c]
L. major	0.226	0.048	4.33[bc]	0.027[bc]
LSD		0.067	4.85	0.013

[b] Significant difference between *L. major* and *E. nuttallii.* (p = 0.05).

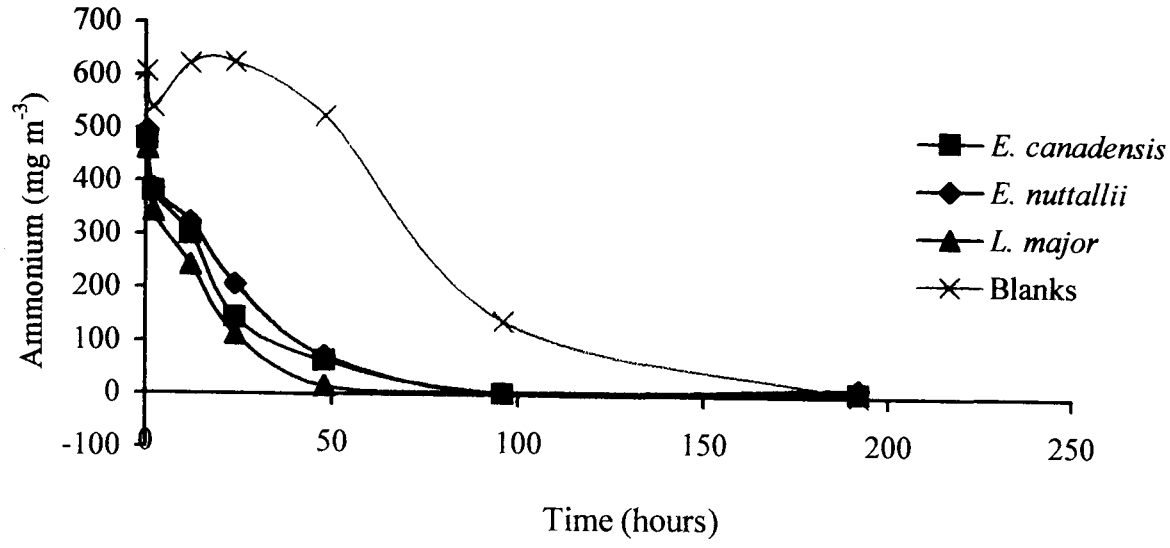
[c] Significant differences between *E. canadensis* and *L. major* (p = 0.05).

Table 6.4

Epiphyte and phytoplankton growth by the end of the nutrient uptake experiment. Epiphyte growth per unit area of macrophyte calculated from Leaf Area Ratios computed in Chapter 2, Section 2.3.2.3. (Mean + SE, n = 5-6)

	Species			
	E. nuttallii	*E. canadensis*	*L. major*	Control
Planktonic				
Chlorophyll (mg m^{-3})	8.80	5.18	9.30	7.96
	(2.34)	(1.12)	(1.70)	(1.90)
Epiphytes (mg g^{-1} dry weight)	231.62	397.23	146.38	-
	(61.59)	(97.66)	(23.54)	
Epiphytes (mg cm^{-2})	0.177	0.303	0.112	-
	(0.0.047)	(0.072)	(0.018)	

(a)



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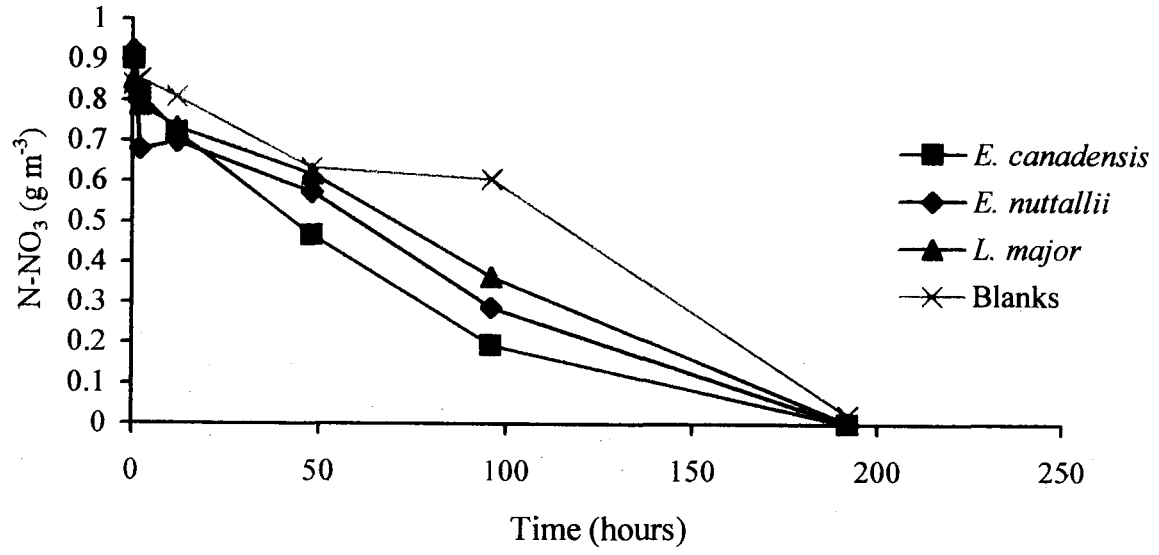

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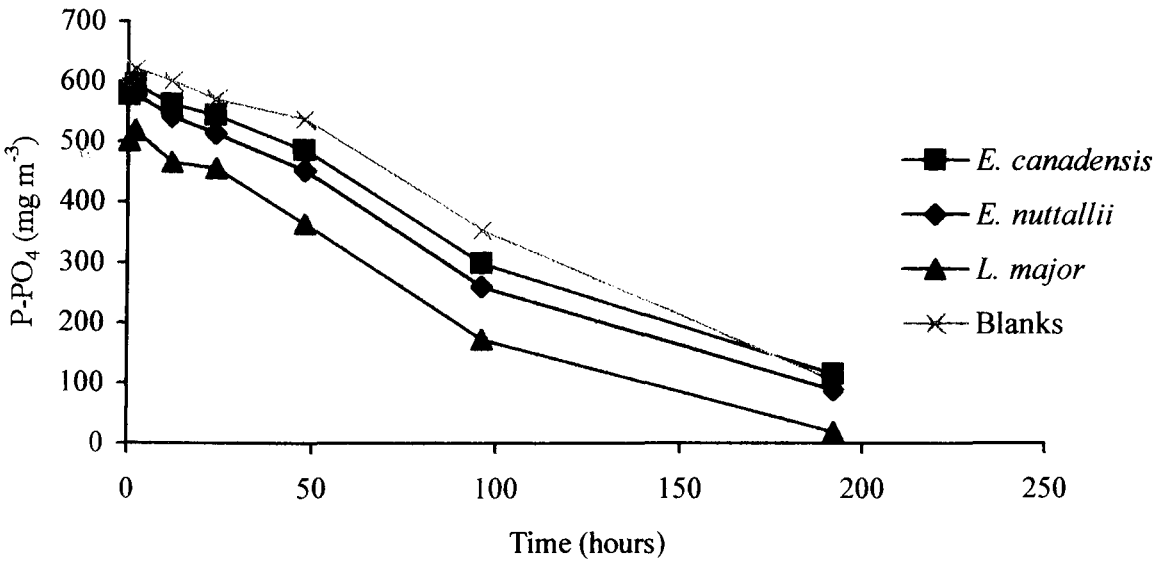


Fig. 6.1 Concentrations of (a) Ammonium (NH_4+), (b) Nitrate (NO_3-) and (c) Soluble reactive phosphorus (PO_4+) in the growth medium over time (hours).

Phytoplankton growth, measured as chlorophyll *a,* did not vary significantly between species or blanks (Table 6.4). Phytoplankton consisted almost totally of the unicellular green alga, *Chlamdomonas* spp.. While epiphytic growth was visibly greater on *E. canadensis* than on either *L. major* or *E. nuttallii*, significant differences were not obtained in analysis. Epiphytic algal communities present appeared to be similar between plant species. The communities were dominated by diatoms such as *Fragilaria spp.* and *Synedra spp.* and filamentous cyanobacteria, probably *Oscillatoria spp.*. Small numbers of green algae including *Chlamydomonas* and other unicellular coccoid green algae were also present. A qualitative assessment of the epiphytic communities present on the different species suggested that *E. canadensis* had a more established epiphytic algae community which included other diatoms such as *Gomphonema spp.*

Nutrient concentrations in the medium were measured on seven occasions over a period of eight days. Decreases in all nutrients studied (SRP, nitrate and ammonium) were observed both in the presence and absence of plants (Fig 6.1) In the absence of plants, SRP appeared to show a steady linear decrease, while ammonium showed a more rapid decrease between 48 and 96 hours. Nitrate levels did not show a significant decrease until after 96 hours.

Table 6.5

Initial and Final N and P concentrations in plant material, and transfer rates per day.

Species		Initial Concentration mg N or P g^{-1} dw	Final concentration mg N or P g^{-1} dw	Transfer rates mg N or P m^{-3} day^{-1}
E. canadensis	N	23.93	22.35	-0.018
	P	6.45	8.23	0.073
E. nuttallii	N	12.72	21.33	0.60
	P	3.64	6.52	0.32
L. major	N	20.66	19.96	-0.064
	P	4.12	5.49	0.088

Table 6.5 shows the initial, final and calculated transfer rates of nitrogen and phosphorus for the three species. Initial nitrogen concentrations within *E. nuttallii* were significantly lower ($p < 0.001$) than either *E. canadensis* or *L. major*. Initial concentration of phosphorus in *E. canadensis* were significantly greater than both *E. nuttallii* ($p < 0.001$) and *L. major* ($p = 0.005$). Final concentrations of both nitrogen ($p = 0.005$) and phosphorus ($p = 0.01$) within *E. nuttallii* plant biomass were significantly greater than initial concentrations. No significant differences between initial and final concentrations of nitrogen were found for either *E. canadensis* or *L. major*. By the time of harvesting, no significant differences in final concentrations of nitrogen or phosphorus between the three species were found. Transfer rates of *E. nuttallii* for both nitrogen and phosphorus were significantly greater than either *E. canadensis* ($p = 0.05$) or *L. major* ($p = 0.005$).

6.3.2 Growth Study

During the growth study, continuous monitoring revealed a trend towards increasing pH and a consequent decrease in total CO_2, free CO_2 and HCO_3^- (Fig. 6.2, a and b), and an increase in CO_3^{2} within the canal water medium for all treatments. No consistent differences in pH either between species or treatments were however observed. pH increased in all treatments to mean values >9 by day 21, although a great deal of variation was observed between replicates (min. pH 8.3, max. pH 10.6). By day 21, no significant differences in either total CO_2 or HCO_3^- concentrations between treatments or species were found. However, free CO_2 concentrations in treatments with *E. canadensis* were observed to be generally higher than those with *E. nuttallii*, and significantly higher ($p = 0.05$) than treatments with *L. major*.

Overall, the RGR of *E. nuttallii* was significantly greater ($p < 0.001$) than that of both *E. canadensis* and *L. major* (Table 6.6). No significant differences were found between the latter two species. RGR at the highest nutrient load (5) was significantly reduced ($p = 0.05$) compared with RGR for low nutrient loadings (1, 2 and 3) (Fig. 6.3). For all species a similar RGR response to increasing nutrient loadings was observed, that of a slight decrease particularly at the highest nutrient loading. A decrease in root growth and root to shoot ratio with increasing nutrient loadings was

observed (Fig. 6.4). The root to shoot ratio of *E. canadensis* was significantly greater ($p < 0.001$) than either *E. nuttallii* of *L. major*. An overall comparison between the highest and lowest nutrient loading regimes revealed a significant decrease ($p = 0.05$) in the root to shoot ratio, this was most pronounced for *E. nuttallii*.

Table 6.6

Overall growth characteristics of the three macrophyte species.

Species	RGR g g^{-1} d^{-1}	Root weight (g)	root/shoot ratio
E. nuttallii	0.086[ab]	0.081	0.107
E. canadensis	0.066[a]	0.106[c]	0.178[c]
L. major	0.063[b]	0.066[c]	0.096[c]
LSD (P = 0.05)	0.018	0.027	0.038

[a] Significant differences between *E. nuttallii* and *E. canadensis* ($p = 0.05$).

[b] Significant difference between *L. major* and *E. nuttallii.* ($p = 0.05$).

[c] Significant differences between *E. canadensis* and *L. major* ($p = 0.05$).

Within individual species an increase in epiphyte biomass both per unit dry weight and per unit area was observed with increasing nutrient loadings (Fig. 6.5, a and b). The data were however extremely variable and although trends could be discerned, statistical differences between treatments were not found. *E. canadensis* appeared to have greater epiphyte densities both per unit biomass and per unit area compared with *E. nuttallii* and *L. major*.

Nitrogen concentrations within the tissue did show a slight increase with increasing nutrient concentrations (Fig. 6.6). Highest concentrations were recorded in *E. canadensis,* which were significantly higher than either *E. nuttallii* ($p = 0.005$) or *L. major* ($p < 0.001$). Nitrogen tissue content was only weakly correlated with loading rates, and tissue concentrations under the low nutrient loading regimes (1 and 2) were significantly less ($p = 0.05$) than concentrations under the high loading regimes (4 and 5). Phosphorus concentrations did not exhibit an increase with increasing nutrient concentrations. Phosphorus tissue concentrations in *L. major* were significantly lower ($p < 0.001$) than in either *Elodea* spp.

Standing-stock of N and P in plant tissue did not increase with increasing nutrient loadings (Fig. 6.7). Overall, nitrogen standing-stocks in *E. canadensis* were significantly greater than either *E. nuttallii* ($p < 0.001$) or *L. major* ($p = 0.01$). Standing stocks of P were again significantly higher in *E. canadensis* than either *E. nuttallii* ($p = 0.05$) or *L. major* ($p < 0.001$). Significant differences ($p = 0.01$) in standing stock of P between *E. nuttallii* and *L. major* were also observed.

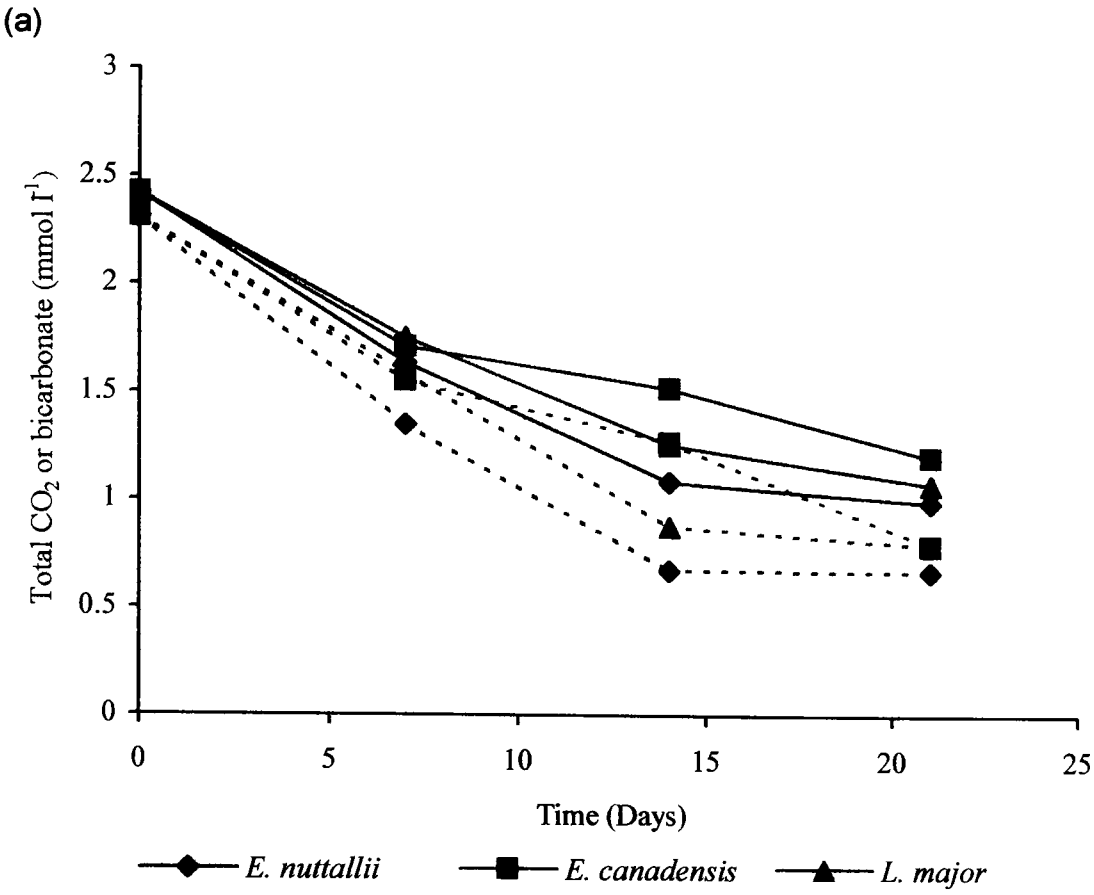


Fig. 6.2 Changes in (a) Total CO_2 (solid line) and bicarbonate (dashed line) and, (b) CO_2* concentrations in the growth media at the highest nutrient treatment.

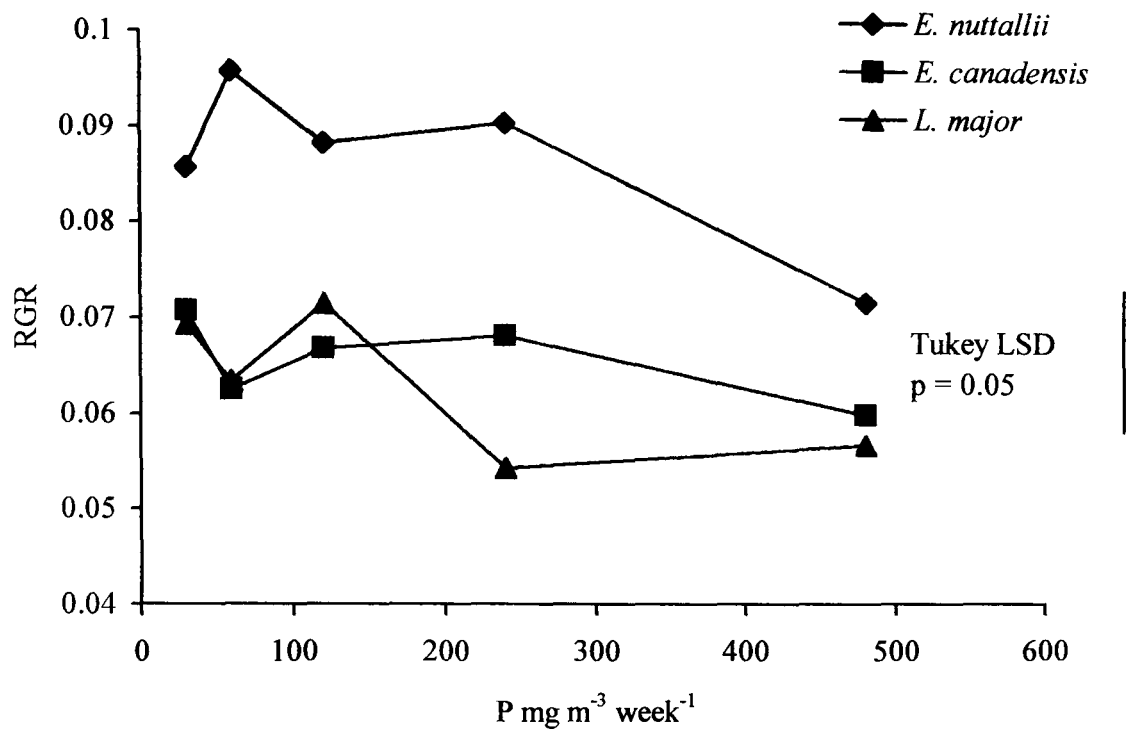


Fig. 6.3 Relative growth rate of the three species in response to increasing nutrient loadings. For simplicity only phosphorus loadings were used for graphical presentation; values for simultaneous loadings of nitrogen can be found in Table 6.2.

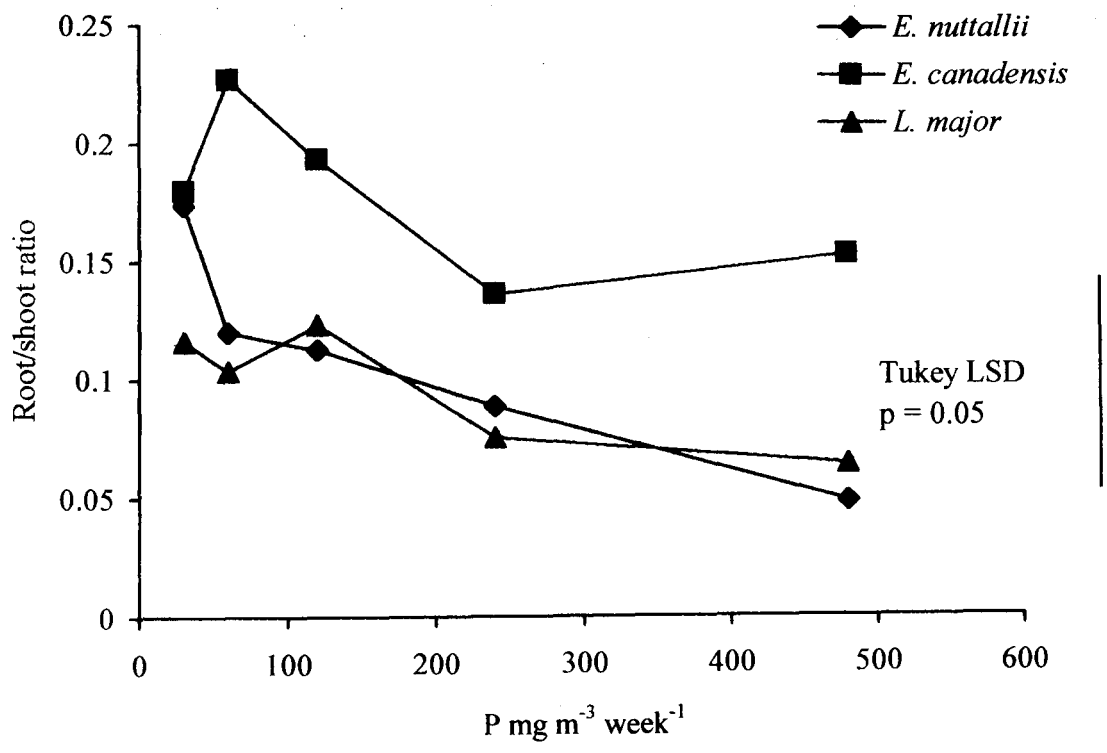
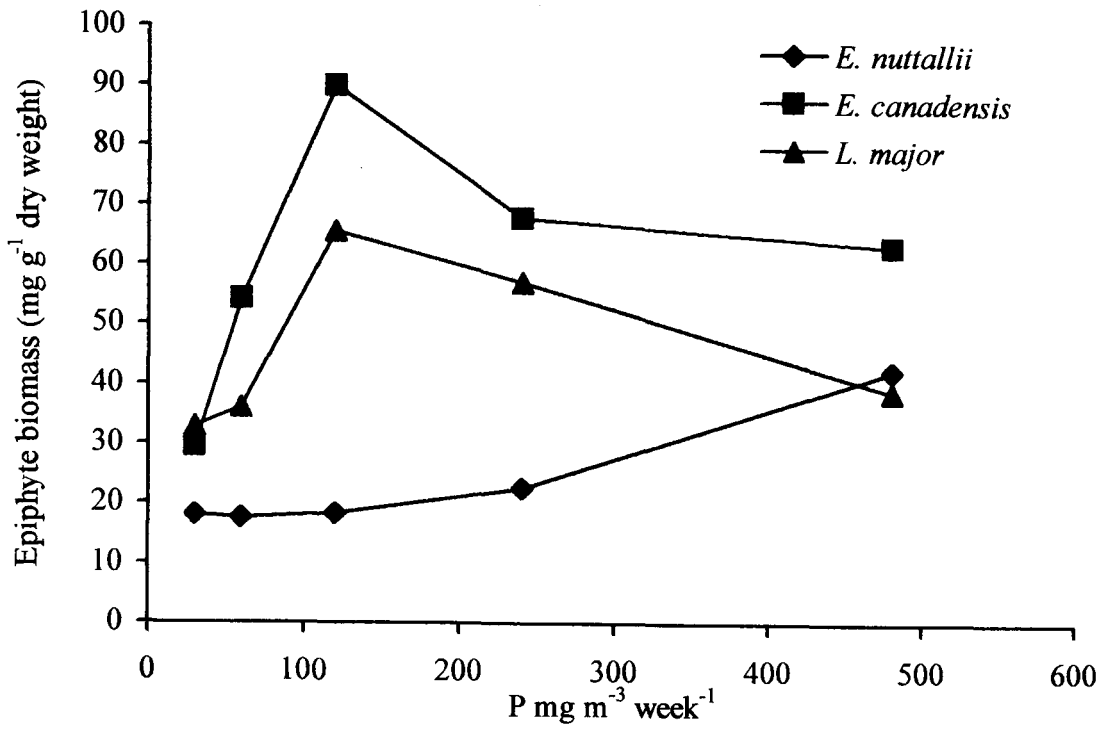


Fig. 6.4 Root:shoot ratios of the three species in response to increasing nutrient loadings. For simplicity only phosphorus loadings were used for graphical presentation; values for simultaneous loadings of nitrogen can be found in Table 6.2.

(a)



(b)

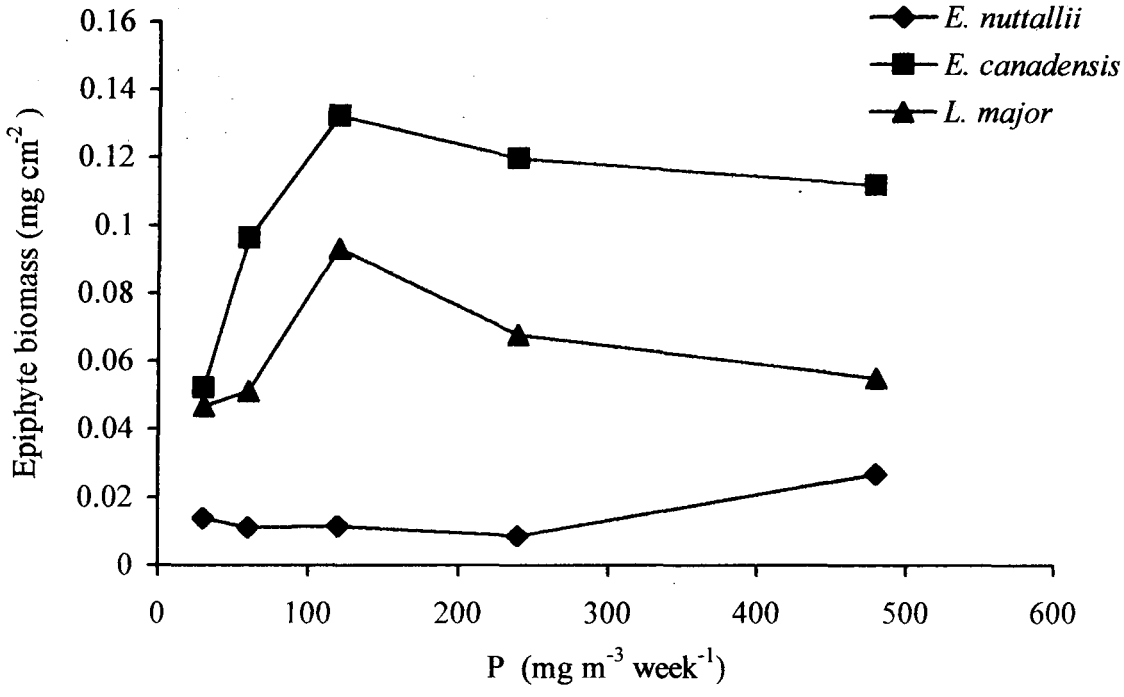
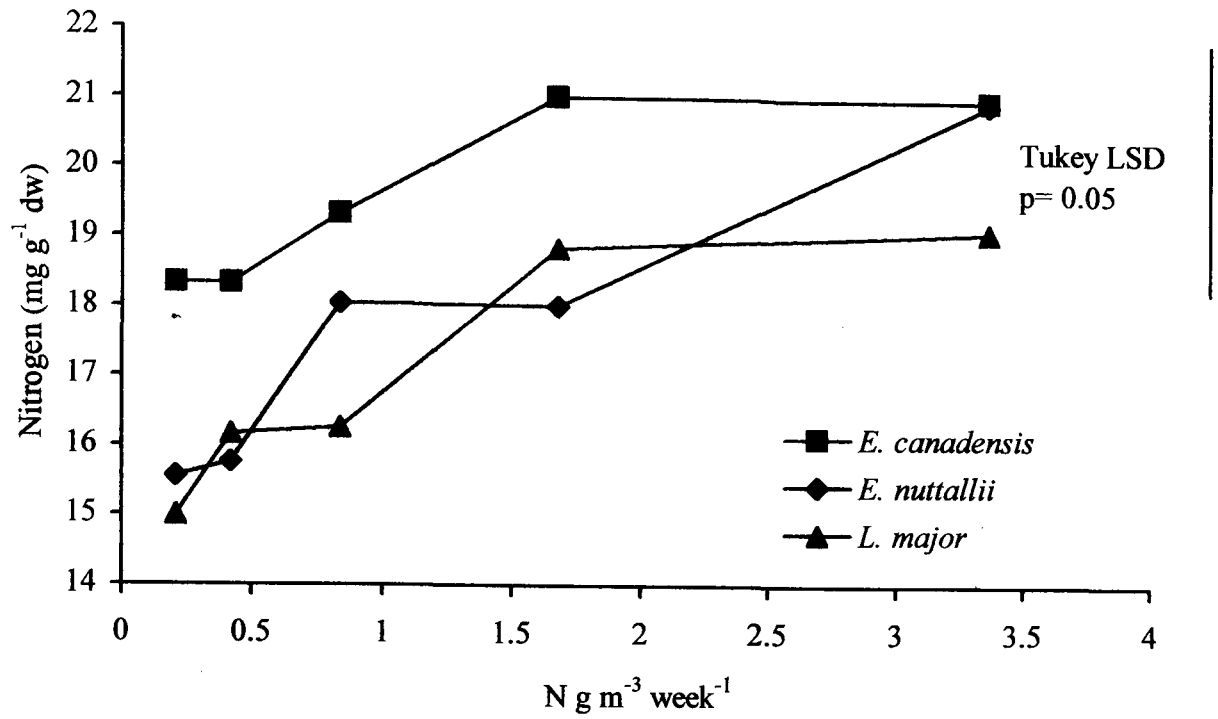


Fig 6.5 Epiphyte biomass at final harvest, (a) per unit dry plant biomass (mg g^{-1} dry weight) and, (b) per unit photosynthetic area (mg cm^{-2} plant surface area). Error bars are not included for simplicity as data were extremely variable.

(a)



(b)

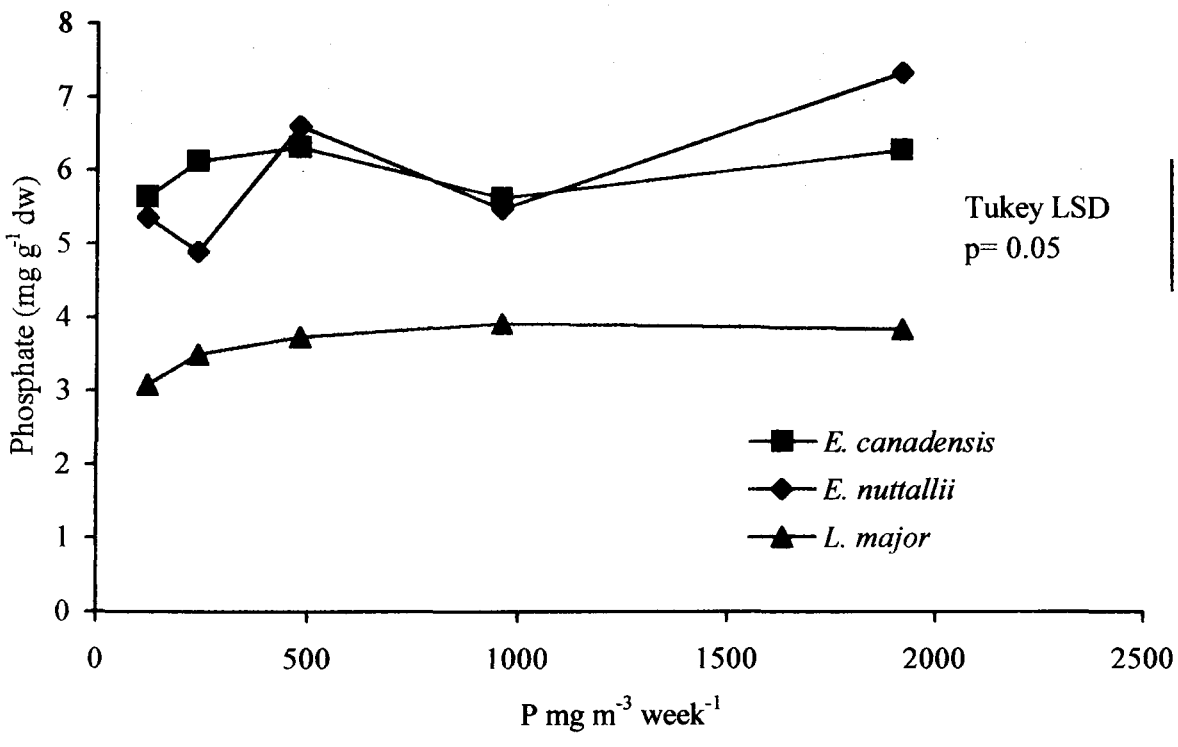
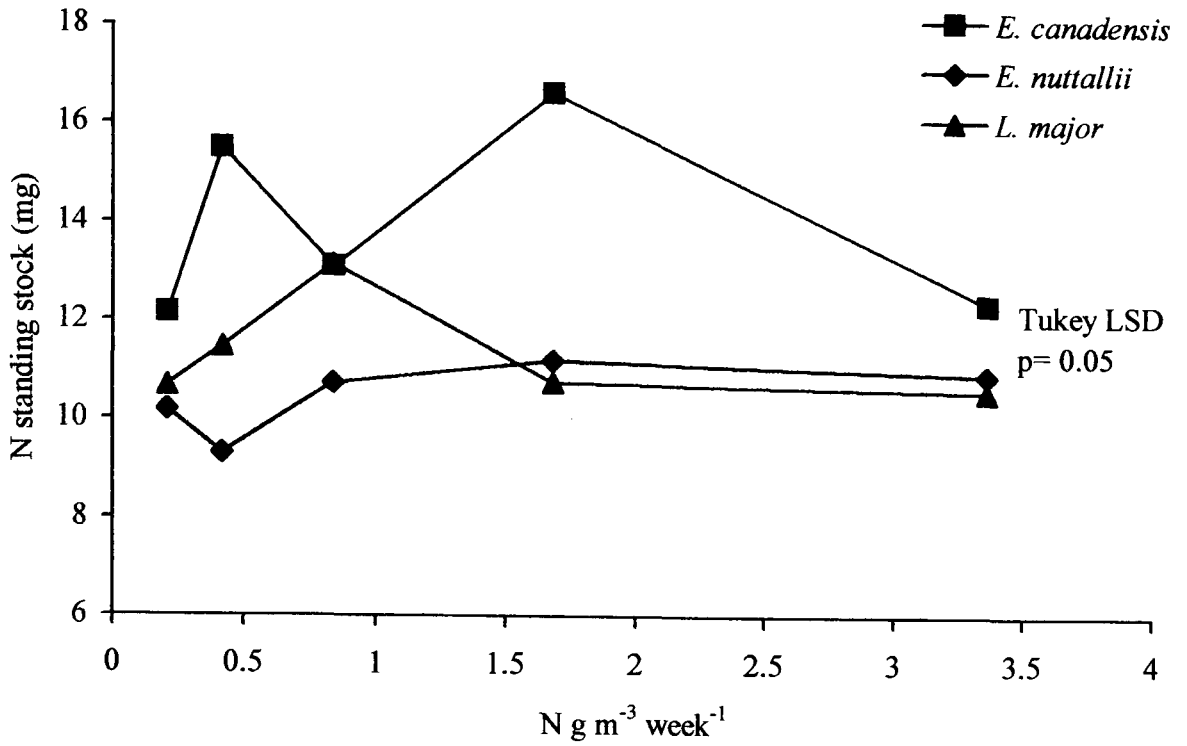


Fig. 6.6 Nitrogen (a) and phosphate (b) concentrations in dried material of the three species in relation to nutrient loading. (n = 6)

(a)



(b)

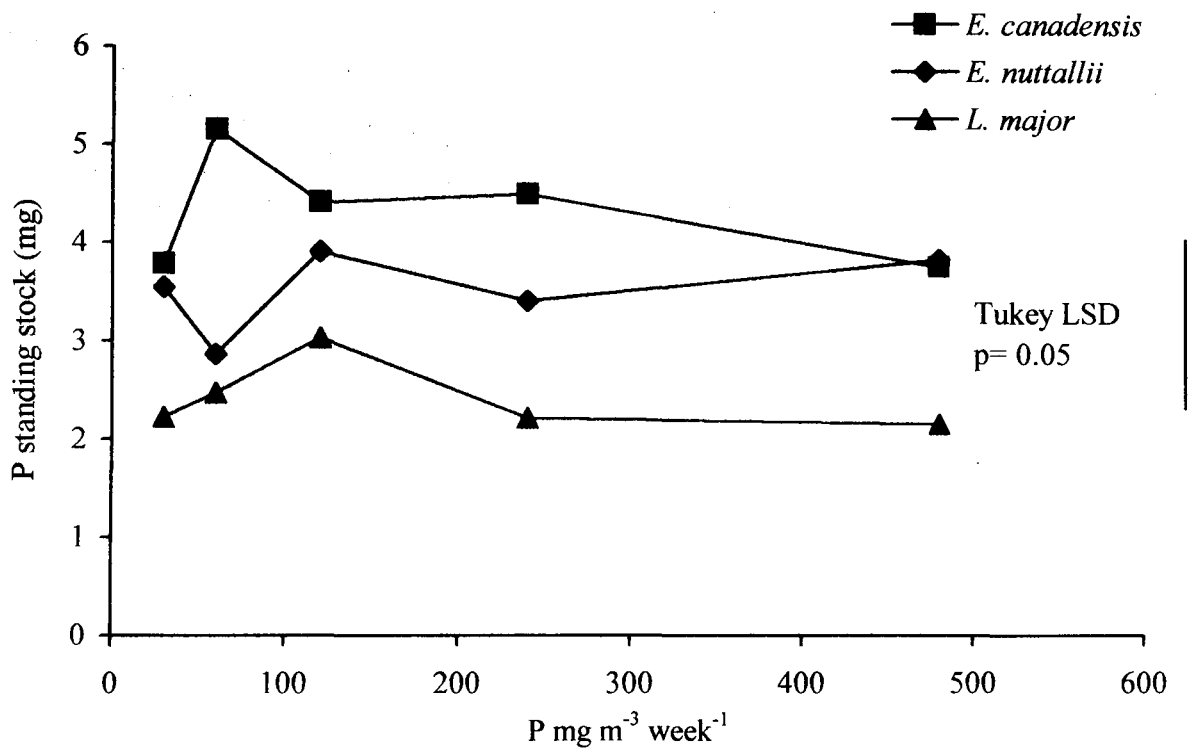


Fig. 6.7 Nitrogen (a) and phosphate (b) standing-stocks in dried material of the three species in relation to nutrient loading. (n = 6)

6.4 Discussion

The aim of this study was to investigate the nutrient uptake and growth of *E. canadensis, E. nuttallii* and *L. major* under increasing nutrient loadings. An initial timed study was carried out to investigate the potential for an indirect measure of nutrient uptake by the three species through the depletion of nutrients in the surrounding water body. Nutrient concentrations of ammonium, nitrate and SRP in the growth medium were observed to decrease during the timed study, both in the presence and absence of plants. This suggests that an indirect measure of nutrient uptake through depletion from the surrounding medium, as used by Ozimek *et al.* (1993), is not appropriate in the present study. It is likely that in the absence of plants, nutrients may have been adsorbed into the sediment. The redox potential in the surface sediment plays an important part in determining the exchange of nutrients between the water and the surface sediments. A high redox potential, as for well oxygenated systems, prevents release of phosphorus and ammonium from the surface sediment into the overlying water. In the present study, the addition of well aerated tap water and continuos aeration of the experimental systems resulted in characteristically rusty brown coloured sediment, indicative of oxygenated surface sediments due to the oxidation of the ferrous ion (Fe^{2+}) to ferric (Fe^{3+}). Under these conditions, inorganic phosphates may be adsorbed on ferric hydroxides and oxides or other minerals such as calcium, aluminium and clay (Jacobsen, 1977). Ammonium may be absorbed into clays and organic colloids or nitrified. The growth of phytoplankton in the growth medium may also have contributed to the observed nutrient depletions as relatively high concentrations of chlorophyll *a* were found at the end of the experiment. However, differences in ammonium concentrations were observed between replicates with and without plants. The rapid depletion of ammonium observed in the presence of plants prior to any noticeable effect on nitrate concentrations further confirms the preferential uptake of ammonium by macrophytes also reported by Ozimek *et al.* (1993). This preference is thought to be due to the ready transport of reduced nitrogen by the plant membrane transport systems located at the plasmalemmas of macrophytes.

Concentrations of nitrogen and phosphorus found in plant biomass were comparable with those of previous studies. Mean phosphorus concentrations varied between 0.31 and 0.82% DW, and nitrogen concentrations between 1.5 and 2.1% DW. Gerloff and Krombholz (1966) established minimum phosphorus concentrations of 0.13% DW for *E. nuttallii*, and concentrations in plant material in the present study were well above this value for all treatments. Rorslett *et al.* (1986) record values of 0.7% DW for *E. canadensis* in a Norwegian lake. Rattray *et al.* (1991b) recorded tissue concentrations of phosphorus in *L. major* of 0.1- 0.5 % DW. Nitrogen values found in the present study were lower than those found by Dendene *et al.* (1993) (3.1 - 5.1% DW) for *Elodea canadensis* and *Elodea nuttallii.* However results were comparable with those found by Best (1977) for *Elodea canadensis* (1.32% DW) and Rattray *et al.* (1991) for *L. major* (1.0 -2.5 % DW). Values found also compare favourably with nitrogen and phosphorus concentrations measured here for collected field material (Chapter 4, Table 4.1). Generally, concentrations of nitrogen and phosphorus within *L. major* tissue were significantly lower than either *Elodea* spp. for the nutrient study. Additionally, total standing-stock measurements were also observed to be lower suggesting that concentrations were not a result of nutrient limitation due to greater biomass densities of this species. In a previous comparison made between *E. nuttallii* and *E. canadensis*, Thiebaut *et al.* (1997) found that *E. nuttallii* had a greater accumulation capacity for phosphorus. This was not supported in findings of the present study which show that *E. canadensis* has a higher accumulation capacity. Eugelink (1998) states that while root uptake of phosphorus was similar between the species, leaf absorption capacity of *E. canadensis* was greater. Significant "luxury uptake" of nutrients was not observed in this study. An increase in nitrogen concentrations within plant tissue was observed overall, although this appeared to be particularly pronounced for *E. nuttallii*. This concurs with reports that nitrogen assimilation rates are proportional to nitrogen concentrations within the surrounding medium (Gumbricht, 1993). However, other authors have not found a correlation between nitrogen tissue content and the trophic status of the water or sediment (e.g. Rattray *et al.*, 1991b).

Nutrient loading over the range used did not have a significant effect upon the growth of any of the species, although *E. nuttallii* had a consistently higher growth

rate than either *E. canadensis*, or *L. major*. Many macrophyte species are capable of utilising sediment sources of both phosphorus and nitrogen (Welsh & Denny, 1979; Carignan, 1980; Barko and Smart, 1981, 1982; Chambers and Kalff, 1987; Barko *et al.* 1991; Rattray *et al.*, 1991b). Therefore the possible transfer of nutrients from the water to the sediment in the nutrient study similar to that observed during the timed study, is unlikely to have been an important factor controlling growth unless the species differ in their abilities to acquire and utilise sediment-bound nutrients. Studies suggest that the contribution of sediment and water to the plants nutrient budgets depend upon the relative proportions of nutrients within these two fractions (Denny, 1980; Carignan, 1982; Rattray *et al.*, 1991b). As macrophytes are capable of utilising sediment sources of nutrients, one might expect the root systems to have responded with increased growth under the lower nutrient loading regimes. Previous studies have shown a decrease in the proportion of roots to total biomass with increasing nutrient loadings (e.g. Mantai and Newton, 1982; Rattray *et al.*, 1991b). A decrease in root growth was observed at the higher nutrient levels, particularly in the case of *E. nuttallii* where a pronounced effect was observed. Main root production was directed towards the sediment. When the roots were in contact with the sediment, root hairs, which greatly increase the sediment surface area in which the roots were in contact, were produced. Increased production of roots under low nutrient conditions suggests that nutrients present in the sediment were contributing to the macrophytes nutrient budget. Differential nutrient uptake ability from the sediment, particularly under nutrient limiting conditions would be worthy of further study.

Carbon limitation has been shown to influence the efficiency with which *E. canadensis* uses nitrogen (Madsen *et al.*, 1998). Consequently, the nitrogen requirement at high and low CO_2 was found to be very similar as increased efficiency of nitrogen use by *E. canadensis* at high CO_2 balanced out the increased demand for nitrogen due to the higher growth rate of this species in this treatment. In relation to the present study, these results suggest that the low CO_2* conditions in the growth study will not have influenced the pattern of response in nitrogen tissue content or growth rates to increased nutrient fertilisation observed, although may have influenced the overall growth rates of the species. Madsen *et al.*, (1998) suggest that the low efficiency of nitrogen use at low CO_2* may be related to the ability of the

species to use bicarbonate as the bicarbonate system requires investments in an active uptake system, including nitrogen (Prins and Elzenga, 1989). The three species may respond differently at high and low CO_2* as previous results (Chapter 5) have shown that the species differ in the efficiency with which they utilise bicarbonate. Thus *E. nuttallii,* which exhibited the least efficient bicarbonate uptake in the present study, may have a higher requirement for nitrogen due to the need for greater investments in active uptake systems for bicarbonate. Further studies are needed, as it is not known whether there is a differential effect of CO_2* on the nitrogen requirement of the three species, or whether the efficiency of phosphorus uptake is effected by CO_2* availability.

The response of *E. nuttallii* to increasing nutrient loadings appears to be more plastic. This species did exhibit luxury uptake, particularly of nitrogen. Epiphyte growth appears to be reduced in the presence of *E. nuttallii* compared with *E. canadensis* and *L. major* both per unit biomass and per unit area. While, *E. nuttallii* does not have as great an ability per unit biomass as *E. canadensis* to accumulate nutrients in terms of either concentrations or standing-stock, its faster growth rate probably compensates and given time this species is probably very effective at removing nutrients. However, its main competitive advantage is probably in its ability to grow more rapidly than epiphytic algae can colonise the newly growing shoots.

Contrary to the original hypothesis, *L. major* did not show a greater capacity than the *Elodea* spp. to remove nutrients from the surrounding medium. However this species may gain a competitive advantage through internal recycling of nutrients. During autumn, die-back of many macrophytes and a subsequent release of nutrients results in a large flux of nutrients back into the nutrient pool. These released nutrients are consequently available to other macrophyte and microphyte species. Species, such as *L. major*, that retain a large proportion of their nutrients and do not exhibit substantial die-back, may both gain a head start through the mobilisation of internal nutrient resources and, may reduce subsequent growth of competing species due to nutrient limitations. In addition, *L. major*'s apparently low nutrient requirements may facilitate its growth in more oligotrophic waters and it may therefore become more of

potential problem in upland waters which, due to their often isolated situations, are slower to be colonised by alien species.

6.5 Summary

1. All three species showed preferential uptake of ammonium as a nitrogen source.
2. Increasing nitrogen and phosphorus loadings did not have a significant effect upon the growth rates of the three species.
3. Luxury uptake of nitrogen was observed, most noticably for the *Elodea* spp.
4. Root production was observed to be greatest in the lower nutrient treatments.
5. Epiphyte growth was observed to increase slightly with increasing nutrient loadings.
6. *E. canadensis* may be particularly susceptible to epiphyte shading due to its slow growth rate.
7. Although *E. nuttallii* did not have as great nutrient concentration per unit biomass or consequently nutrient standing stocks as *E. canadensis,* its faster growth rate may still result in this species being able to more efficiently remove nutrients from the surrounding water given sufficient time.
8. Lack of die back and a consequent release of nutrients back into the water column, thus reducing nutrient acquisition by competing species (whether algae or other macrophytes), may convey a competitive advantage on *L. major*.

Chapter 7 COMPETITION IN THE AQUATIC ENVIRONMENT

7.1 Introduction

What role competition plays in defining and regulating community structure is one of the most commonly asked questions in ecology. Most competition studies have been conducted with terrestrial species. There have been relatively few studies on aquatic species, e.g. Mc Creary *et al* 1983; Mc Creary and Carpenter 1983; Agami and Reddy 1989; Moen and Cohen, 1989; Kautsky 1991. Nevertheless, it seems likely that competition may play an important role in determining the structure and species composition of plant communities within the aquatic environment.

There have been many attempts to define competition (e.g. Milne and Milne, 1962; Harper, 1961). However, the term competition is used to describe such an array of different plant interactions that a concise definition is difficult if not impossible. Generally competition has been divided into two broad categories, direct and indirect interference. The first term, direct interference, includes allelopathy and physical contact, while the second term, indirect interference, is generally used to describe competition involving shared, limited resources. The latter term is also known as exploitative competition. However, there are also other forms of indirect competition such as "apparent" competition. Apparent competition is used to describe third party interference in which the investigated species differ in their ability to contend with a third species. In the context of this study, competition is used to mean resources mediated interference, that is, the sharing of limited resources between plants growing in the same habitat.

According to Tilman (1987) " ... competition comes solely from the process of acquisition and utilisation of limiting resources". Integral to this is the differing abilities of species to respond to the relative availability of limiting resources. In pair-wise competition experiments, the relative abundance of species has been shown to change in response to changes in the availability of limiting resources (Tilman 1987). Those species best able to capture and utilise limiting resources, while tolerating prevailing conditions, are likely to out-compete less competitive neighbouring species. While the ideas of resource mediated competition were developed with terrestrial species in mind, they can just as readily be applied to aquatic plants. In the aquatic

habitat, vigorous vegetative growth can give rise to largely homogeneous stands of submerged aquatic macrophytes. These stands can affect both the physical environment (e.g. light, temperature, hydrodynamics) and the chemical environment (e.g. oxygen, pH, inorganic and organic carbon, and nutrients). In stagnant or slow-moving waters, the effects of macrophyte stands on water quality are particularly pronounced. Waters surrounding macrophyte stands can frequently become supersaturated with oxygen, with pH rising to in excess of pH 9. Increases in pH result in decreasing availability of CO_2* (beyond pH 6.5) and bicarbonate (beyond pH 9), resources essential for photosynthesis and growth, while high O_2 has been shown to stimulate photorespiratory stress (Simpson *et al.* 1980). It is suggested that, especially in conditions of low bulk water flow, "envelopes" of high pH and O_2, and low CO_2 can extend well beyond the accepted boundary layer of leaves and ptentially could interfere with the growth of neighbouring plant stands. It is hypothesised that resource mediated competition between neighbouring macrophyte stands may be responsible for the displacement of one species by another. Competitive situations will favour those species that can most effectively suppress the photosynthesis and growth of neighbouring species, while tolerating conditions within the envelope.

7.1.1 Experimental design and interpretation of competition experiments

For most competition studies, experiments are simplified such that only two species are studied in pair-wise mixtures. Two principal experimental designs have been used: (a) replacement designs (de Wit, 1960), and (b) additive designs. The replacement design involves planting two species (i and j) in varying proportions at a constant total density (Harper, 1977) (Fig 7.1). In the additive design, the planting density of species i remains constant, while the planting density of species j varies. Consequently, in additive designs overall planting density of the mixture is greater than in the pure stand (Fig 7.1).

Experimental design and interpretation of results from competition studies has been, and remains, a controversial subject. According to Sackville Hamilton (1994), the two designs serve complementary purposes. He states that, "replacement series are appropriate for questions based on the similarity of competing taxa, such as biological resource complementarity, niche overlap (and) competitive exclusion or co-

existence ... and measures of competitive ability that quantify how limiting resources are partitioned between taxa." Additive designs assess "overall" competition without defining the nature of the interference. The latter has been commonly used in the study of crop:weed interactions, as interspecifc competition

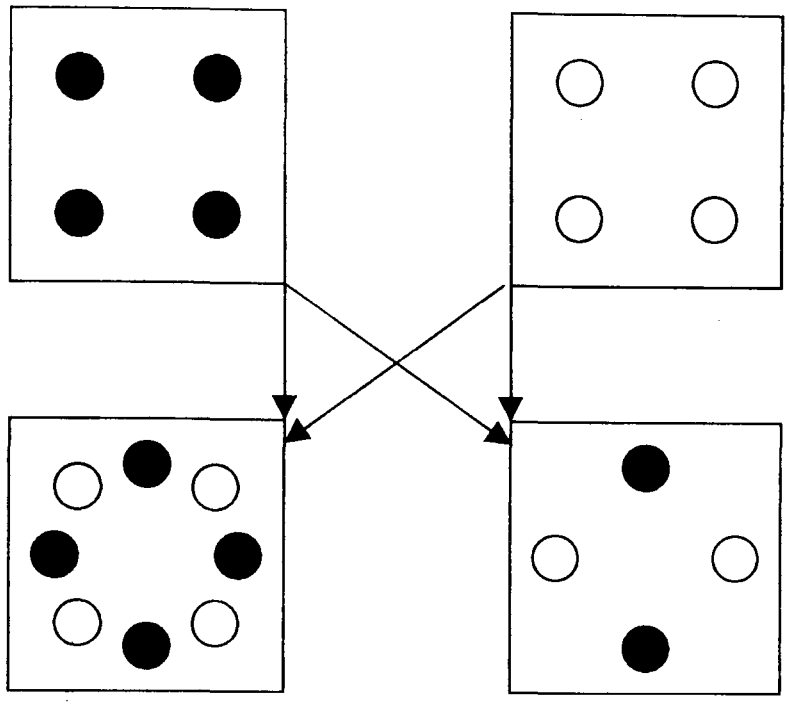


Fig. 7.1. Plant arrangement for pure stands of i (●) and j (○), and for the replacement and additive mixtures of i with j (from Snaydon 1991).

may be measured regardless of the intraspecific component. These arguments have been disputed by Snaydon (1991), who claims that the replacement design is basically flawed as it, "confounds the density of one component with that of the other, so confounding the effects of inter-component competition with the effects of intracomponent competition". Snaydon (1991, 1994) concludes that indices commonly used to analyse the replacement series are difficult or impossible to interpret. Similar criticisms have been expressed for the additive design. Rajmanek *et al.* (1989) criticised additive designs, stating that they, "result in simultaneous changes of proportion and total density and make interpretation of results difficult, seriously limiting their application". In view of the extensive criticisms of both experimental designs it is obviously necessary before attempting experiments to have a clear understanding of their relative advantages and limitations.

The replacement series has been almost universally used in the study of plant competition (Snaydon, 1991). The design of the replacement series can be represented on two Cartesian axes. The two monoculture densities of species 1 and 2 are situated on separate axis, X and Y respectively. A line joins the pure stand densities and is known as the replacement line. In the conventional analysis, a graphical representation of the results can be used, known as a replacement diagram. These are drawn either using the actual yields or the relative yields for each species in each mixture treatment. The relative yields of the two competing species are calculated separately as the yield of species *i* in a particular mixture (e.g. 50:50) divided by the yield of species *i* in monoculture at the same density (i.e. 50). These can then be incorporated into one diagram showing the respective changes in yield of each species with changing proportions. A number of indices have been developed for the analysis and interpretation of replacement series (e.g. Relative Crowding Co-efficient, Competitive Ratio, Relative Total Yield (RTY) and Co-efficient of Aggressivity). Connolly (1986) illustrated that the relative crowding coefficient, the coefficient of aggressivity, the competitive ratio and K_{12} all vary considerably depending upon the angle of the replacement line, while the RTY remains relatively stable. RYT is one of the simplest and most commonly used indices. It is calculated as the sum of the relative yields of each species in a particular mixture. However, RTY should only be used under the rather restricted assumption that the monoculture densities used are within the range that produces constant final yield (Silvertown & Lovett Doust, 1993), an assumption not always met. Jolliffe *et al.* (1984) and Spitters (1983) have proposed alternative methods for the analysis of de Wit style experimental designs. The latter may also be used for the interpretation of additive experiments. Roush *et al.* (1989) compared these two methods and the conventional replacement analysis using relative yields. They concluded that the conventional analysis was the least sensitive in describing the influences of either proportion or density on competitive interactions between the species. The method of Jolliffe *et al.* (1984) provided a detailed analysis on the effects of proportion on species interactions, while the model of Spitters (1993) gave a quantitative analysis of the effects of both proportion and density on species interactions.

In the method described by Jolliffe *et al* (1984) each species proportion in a mixture is compared with its projected and actual yield in monoculture at the same

density. This method avoids some of the problems of interpreting replacement series data using the conventional approach of de Wit (1960). Projected yields (Yp) in monoculture are calculated as the hypothetical yield of a single plant in the absence of intra- and inter- specific competition. Jolliffe *et al.* (1984) derived this approach from rectangular hyperbolic yield density responses (Roush *et al.*, 1989). The equations take the following form:

Equation 1

$$\frac{Ym}{Y\max} = \frac{N}{(Kn + N)}$$

In the linearized form:

Equation 2

$$\frac{1}{Ym} = \frac{1}{Y\max} + \frac{Kn}{Y\max}$$

where Ym is the actual vegetative yield per unit area, N the planting density, Ymax the theoretical maximum yield at infinite planting density, and Kn, the planting density at which 50% of constant final yield is achieved. For estimates of Ymax and Kn, 1/Ym is regressed against 1/Z. Equation 3 is the theoretical relationship between yield and density in the absence of intraspecific competition, named the projected yield (Yp):

Equation 3

$$Yp = \frac{Y\max}{Kn} * N$$

The difference between the projected yield and the actual (Ym) yields at any given density is a measure of the intraspecific competition (Eq. 4). Jolliffe *et al.* (1984) termed this the 'Species Monoculture Response'. Similarly, the difference between the yields in monoculture (Ym) and the yield in mixture (Yx) was termed the "Species Mixtures Response". The relative effect (Rm) of intraspecific competition on the yield of a species in monoculture is expressed as:

Equation 4

$$Rm = \frac{Yp - Ym}{Yp}$$

Similarly, the relative effects (Rx) of interspecific competition are defined as:

Equation 5

$$Rx = \frac{Ym - Yx}{Ym}$$

Spitters (1983) describes a multiple regression model known as the Reciprocal Yield Model (Roush *et al.*, 1989) with the following form:

Equation 6

$$\frac{1}{W_1} = b_{1.0} + b_{1.1}N_1 + b_{1..2}N_2$$

Where W_1 is the yield of species 1, N_1 is the planting density of species 1 and N_2 is the planting density of species 2. The partial regression co-efficient $b_{1.0}$ estimates the size of a single plant of species 1 in the absence of any competition, $b_{1.1}$ estimates intraspecific competition and $b_{1.2}$ estimates interspecific competition. The ratio of $b_{1..1}/b_{1..2}$ gives a measure of the relative effects of intra- vs inter-specific competition. A similar equation is also used to describe the competitive effects on species 2:

Equation 7

$$\frac{1}{W_2} = b_{2.0} + b_{2.2}N_2 + b_{2.1}N_1$$

Where W_2 is the yield of species 2, $b_{2.0}$ estimates the size of a single plant of species 2 in the absence of any competition, $b_{2.2}$ estimates the intraspecific competition and $b_{2.1}$ estimates interspecific competition of species 1 on species 2. However, this model can not be used for experiments with only a single replacement series as the two predictor values (N_1 and N_2) are negatively correlated. The resulting partial regression co-efficients (e.g. b_{11} and b_{12}) are therefore not unique (Bhattacharyya & Johnson, 1977).

In summary, the approach described by Joliffe *et al.* (1984) for the analysis of de Wit style competition studies provides a useful straightforward method for the interpretation of results. But, while sensitive to changes in proportions of species, it

does not provide a quantitative analysis on the effects of total density or the interactive influences of proportion and density (Roush *et al.*, 1989). The Reciprocal Yield Model of Spitters (1983) provides a more comprehensive description of species interactions although may be limited in its application to some replacement series data sets (i.e. single replacement series at one density).

Essentially, the choice of experimental design and method for interpretation depends upon the experimental objectives. However, design will also depend largely upon the limitations presented by both the species themselves (e.g. size) and the available facilities. In the next section some aspects of design practicalities particularly relevant to aquatic macrophytes are discussed.

7.1.2 Considerations for experimental design

Competition experiments between aquatic plants present a number of problems, particularly where species propagate wholly vegetatively. McCreary and Carpenter (1983) list a number of considerations and problems associated with the experimental approach to running competition experiments with submerged macrophytes.

a) Measurement of starting unit

Numbers of plants per unit area has frequently been used as a measure with starting density in competition studies of both terrestrial and aquatic plants. Where species propagate sexually, numbers of seeds or seedlings may be used as a measure. While number of plants per unit area has been used in studies on submerged macrophytes (i.e. Kautsky, 1991; Moen and Cohen, 1989), as many submersed macrophytes propagate vegetatively, it is often difficult to determine what constitutes a single plant. Instead, in order to standardise initial plant densities both within and between species, it may be necessary to make some measure of plant size. For ease of measurement, size is often expressed as dry weight. This may be appropriate where species are similar in morphology. However, where species differ, standardising one parameter, i.e. dry weight, may result in vastly differing quantities of another, i.e. number of apical meristems or shoot lengths. How size is measured, e.g. as shoot

length, fresh weight, dry weight, surface area or chlorophyll content, will largely depends upon what is considered important in determining the outcome of competition and hence a degree of pre-judgement is involved. Height or length may be important if the species compete for light or space. Likewise, surface area may be important if light or CO_2 are believed to be limiting factors, as a large surface area will increase photosynthetic surface area and facilitate gas exchange. Plant age may also be important, as physiological activity may be directly correlated with leaf age (See Chapter 5). Ideally more than one parameter should be measured.

b) Selection of overall starting densities

Once a measure of plant size has been made, initial starting densities must be chosen. In determining the amount of starting material, assumptions are made as to how and when competition occurs. For experimental design it is often stated that it may be appropriate to choose densities found in the field. However, these vary considerably both seasonally and in response to different environmental variables. In choosing high starting densities of plant material similar to densities found later in the growing season, it is assumed that competition occurs when plants are at their maximum biomass and not, for example, at the start of the growing season when plant biomasses are relatively low.

For the present study, dry weight was used as a measure of the initial planting density. Numbers of apical tips were also counted to give an indication of the branching frequency and consequent size of each plant. Relatively small starting units were used for both experiments, i.e. 10 cm shoot lengths with intact apical tips. This was for a number of both practical and experimental reasons. Practically, for replication purposes, small starting units are easier to select for uniformity, being less likely to have secondary branching. In addition, experiments described in Chapter 3 suggest that 10 cm shoot lengths are within the optimal length for survival of all three species when grown in monoculture. Experimentally, it was unknown beyond what density competition actually occurs. In addition to the above there are other factors that must be taken into account, such as disturbance to growth suffered by transplanted material. Nursery culturing reduces disturbance, allowing plants to establish before the start of the experiment. This method also reveals shoots damaged

during collection and preparation of plant material, so that these can be discarded to provide a more uniform starting innoculum.

7.2 Competition experiments

In the present study two competition experiments are described in which the three aquatic macrophytes, *E. canadensis, E. nuttallii* and *L. major* are grown together in pair-wise mixtures and monocultures. These aim to determine whether intra- or inter-specific competition is occurring and the relative strengths of any competition. Water quality parameters (pH, Total CO_2, CO_2^* and bicarbonate) within the media were monitored and dry weight (g) and numbers of apical tips were used to assess the overall effects on the growth of species.

7.2.1 Competition Experiment 1

The basic experimental design used in this study was that described by de Wit (1960). However, following the reinterpretation of the de Wit series as described by Jolliffe *et al.* (1984), the species were also grown at a range of monoculture densities equivalent to the various proportions in which they were used in the mixtures. Four densities were used and these are referred to as 1, 2, 3 and 4 beakers. Plants were grown in monoculture and in mixtures where the total overall density of both species in the mixture was 4 (i.e. 1:3, 2:2, and 3:1). There were five replicates of each treatment. Results were analysed following the method of Jolliffe *et al.* (1984), as this method was appropriate for a design run at one overall density using a range of monoculture densities. Due to size constraints, it was not feasible to perform all the planned plant combinations at the same time. Consequently, two pair-wise comparisons (Part 1: *L. major* vs *E. nuttallii,* Part 2: *E. nuttallii* vs *E. canadensis*) were run in succession. Within the available time constraints when healthy material was available in the field it was only possible to perform two of the three possible pair-wise comparisons. As the third comparison involving *E. canadensis* vs *L. major* has not been recorded in the field within the UK, it was decided not to perform this particular combination.

7.2.1.1 Preparation of plant material

E. nuttallii and *L. major* were obtained from stock cultures grown in experimental tanks at the University of Liverpool. *E. canadensis* was collected from

Ec N. At the beginning of the experiment, plant-starting units were selected for uniformity. For all three species, single 10 – 12 cm long shoots were selected. As *E. canadensis* and *E. nuttallii* are similar in morphology, a 10 cm shoot length of each species resulted in similar dry weight (see chapter 3, Table 2.3). *L. major* has approximately two to three times greater dry weight per unit length. In order to equalise the starting densities of the species, 2 shoots of *E. canadensis* and *E. nuttallii* and a single shoot of *L. major* were planted per beaker. Two hundred and twenty shoots of each *Elodea* spp. and one hundred and ten shoots of *L. major* were selected, cleaned carefully to remove any epiphytic growth and planted two per beaker for *Elodea* spp. and singly for *L. major* in 200ml plastics beakers containing standard amounts of canal sediment. Shoots were then grown for two weeks in nursery cultures prior to the start of each experiment. More shoots were nursery cultured than actually required for the experiment to allow for death of a few replicates due to damage during collection and preparation, and sub-sampling.

Table 7.1

Initial planting densities for 1, 2, 3 and 4 pots in monocultures and mixtures approximated from subsamples (n = 7) for Experiment 1, Parts 1 and 2.

	Initial calculated plant densities (g dry weight m^{-2})			
Species	1	2	3	4
E. nuttallii	2.38	4.77	7.15	9.53
L. major	5.09	10.19	15.28	20.37
E. nuttallii	2.64	5.28	7.92	10.56
E. canadensis	3.06	6.11	9.17	12.22

7.2.1.2 The experimental design

At the start of the experiment, 55 ten litre green plastic buckets were filled with tap water and placed overnight in the three large thermostatically controlled water tanks. From the nursery cultures, 80 beakers of each species were selected, plus a minimum of seven which were used to assess the number of apical tips and dry weight of the starting material (see Table 7.1). Plants were placed in buckets in accordance with the number of beakers required for each treatment, i.e. 1, 2, 3 or 4

per bucket. There were five replicate buckets per treatment. The treatments were then randomly positioned in the three temperature-controlled tanks.

7.2.1.3 Monitoring

Monitoring of treatments was as follows:

- pH was measured every 2-3 days.
- Conductivity measurements were initially taken every 2 - 3 days, but after two weeks measurements were taken weekly.
- Alkalinity was measured weekly on all replicates.

From alkalinity, pH and conductivity, measurements the quantities and proportions of Total CO_2, free CO_2, bicarbonate and carbonate were determined. Water levels were maintained in each bucket with the weekly addition of tap water.

7.2.1.4 Nutrient Concentrations

Supplementary nutrients were added in order to simulate nutrient levels found within British canals in which the experimental species occur or have done so in the past. This encompasses a range of nutrient concentrations described by Murphy (1980), generally in excess of 1.5 mg l^{-1} $N0_3$ - N and 20 µg l^{-1} PO_4 -P and thus characterised as eutrophic by Vollenweider (1968) and Moss (1998). Initial supplementary P and N were supplied at 50 µg l^{-1} PO_4 - P and 1 mg $N0_3$ - N l^{-1}. When added to the small amounts of N and P present, presumably both in the tap water and as a result of release from the canal sediment, initial mean concentrations of nutrients within the medium were: 67.91 µg l^{-1} PO_4 - P and 1.14 mg $N0_3$ - N l^{-1}. Nutrient concentrations were monitored as previously described on a weekly basis with analysis of treatments 2:2 mixtures, and 4:0 monocultures and 0:4 monocultures. Thus concentrations were assessed in both mixtures and monocultures at the highest plant density. Nutrients were maintained at 0.5 - 1 mg $N0_3$ - N l^{-1} and 25-50 µg l^{-1} PO_4 - P with the addition of concentrated solutions of potassium dihydrogen

orthophosphate and ammonium nitrate, giving final concentrations of 1 mg $N0_3$ - N l^{-1} and 50 μg l^{-1} PO_4 - P. Nitrate and orthophosphate were monitored on a weekly basis. Water samples were taken and analysed with the methods described in Chapter 2, Table 2.2.

7.2.1.5 Harvesting

All treatments were harvested after five weeks. Interpretation of data from replacement series can be made at three levels, namely the individual plant, the species and the total mixture. Due to difficulties in separating plants of the same species within each replicate bucket, these were grouped together. Each plant was removed carefully from the container and washed to remove soil and epiphytic algae from it before being separated into shoot and root components. The numbers of apical meristems were counted and shoots were then placed into pre-weighed paper bags and dried at 60 °C to a constant weight for dry weight analysis.

7.2.2 Competition Experiment 2

The aim of this second experiment was to examine the longer-term effects of growing species in monocultures and mixtures, thus allowing greater densities to be achieved. The results of this study were analysed using Spitter's (1983) Reciprocal Yield Model, although it is recognised that with this rather limited data set these results can only give an indication of the differing responses to intra- and inter-specific competition exhibited by the three experimental species. A second competition experiment was run in which plants were grown for ten weeks. A simpler experimental design was used in which species were grown at two densities, 2 and 4 plants in monoculture, and in 2:2 mixtures.

7.2.2.1 Collection and preparation of plant material

Elodea spp. plant material was collected from Ec AB and En BL. *L. major* plant material was taken from stock cultures grown outside at the University of Liverpool. Plant material was prepared and nursery cultured as for Competition

Experiment 1 with 55 beakers per species, two shoots of *Elodea* spp. per beaker and a single shoot of *L. major* per beaker.

7.2.2.2 Experimental design

At the start of the experiment 36 ten litre green plastic buckets were filled with tap water and placed overnight in the three large thermostatically controlled water tanks. The treatments were set up with 2 and 4 plants for monoculture densities and 2:2 combinations for mixtures placed randomly in 10 l buckets, with four replicates per treatment. From the nursery culture, 16 shoots of *Elodea* spp and 8 shoots of *L. major* were selected randomly for approximation of starting dry weights (Table 7.1). pH was measured every 2 to 3 days initially, and after 3 weeks, occasionally. Nutrient additions and harvesting were as for Competition experiment 1 (see section 7.1.2.4 and 7.1.2.5 for details). Plants were harvested after 12 weeks.

Table 7.2

Starting densities and numbers of apical tips for Competition Experiment 2, approximated from subsamples (*Elodea* spp. n=16, *L. major*, n=8) for monocultures (2 and 4 shoots) and mixtures (2:2 shoots) following nursery culturing.

	Initial calculated plant densities (g dry weight m^{-2})		Mean number of apices	
	2	4	2	4
E. canadensis	3.02	6.04	6.6	13.2
E. nuttallii	4.88	9.76	25.2	50.4
L. major	8.88	17.76	11.2	22.4

7.2.3 Results and discussion

7.2.3.1 Competition Experiment 1

The pH of the growth media rose rapidly for monoculture treatments of *E. nuttallii* and *L. major* (Fig 7.2), although the rise for *E. canadensis* was slower. Intraspecific differences in pH between treatments of different starting densities were not pronounced, although the slowest increase was found for the lowest initial starting densities. The maximum pH's achieved by all species grown both in monoculture and in mixtures were not significantly different. Differences between treatments were less than 0.1 pH unit. pH increases observed in monocultures were very similar to those observed in the highest density (4) monoculture treatments, although the mixture treatments with the greater proportion of *E. canadensis* present (1:3) exhibited the slowest increase in pH (Fig. 7.3). Measurements of diurnal pH change made in monocultures at the highest densities (See Appendix VII) show that pH decrease at night was only in the region of 0.4 pH units. As the pH did not appear to drop to a level below which CO_2* became available even following a night-time respiration period, bicarbonate was the principal source of carbon throughout this experiment. This is reflected in monitored concentrations of DIC, CO_2* and bicarbonate in cultures. Total CO_2 concentrations within the growth media decreased throughout the experiment (Fig 7.4). Of the various inorganic carbon fractions present, bicarbonate was the most abundant. CO_2* concentrations exhibited a rapid reduction during the first week of the experiment (Fig. 7.5). For all treatments with *E. nuttallii* and *L. major*, within 7 days CO_2* concentrations were less than 1 $\mu mol\ l^{-1}$. Treatments with lower initial starting densities of *E. canadensis* showed a less rapid decrease in CO_2* concentrations. Bicarbonate concentrations decreased in a very similar manner to total CO_2 concentrations (Fig. 7.6). An increase in carbonate was also observed (Fig. 7.7). Again, changes observed in mixtures were extremely similar to those observed in monocultures at the higher densities (3 and 4) (Fig. 7.8 and 7.9).

In monoculture treatments, relative growth rates of *E. nuttallii* were significantly higher ($p = 0.001$) than those of *E. canadensis* or *L. major* (Fig. 7.10). The relative growth rates of *L. major* appear to be higher than those of *E. canadensis* for lower density treatments (1,2 and 3), although direct comparison between these

species is difficult, as these species were not grown simultaneously in this experiment. Both *E. nuttallii* and *L. major* exhibited a slight decrease in RGR with increasing initial planting density, indicative of intraspecific competition at the highest monoculture planting density. However, the RGR of *E. canadensis* exhibited a slight increase with increasing planting density (Fig. 7.10). In a comparison between species grown in monocultures and in mixtures, for *E. nuttallii* and *L. major,* at lower planting densities, growth rates in mixtures (i.e. 1:3 and 3:1) were less than those in monocultures (1:0 and 0:1). However, this was only significant ($p = 0.05$) for comparisons between *E. nuttallii* treatments grown at 1:0 and 1:3 (Fig 7.11 and 7.12). *E. canadensis* exhibited slightly greater growth in mixtures than in monoculture at lower planting densities (1 and 2).

Table 7.3

Estimates of Ymax and Kn made using monoculture data

	Species	Ymax (g m^{-2})	Kn (g m^{-2})
Part 1:	*E. nuttallii*	76.34	15.86
	L. major	117.64	51.51
Part 2	*E. nuttallii*	60.24	9.39
	E. canadensis	217.39	148.17

In fitting the experimental data to the model described by Joliffe *et al.* (1989), estimates were made of Ymax and Kn (Table 7.3). Ymax is particularly low for *E. nuttallii* and *L. major*, when compared with estimates of average biomass quoted in the literature (i.e. 300 g m^{-2}) (Duarte and Roff, 1991[1]). It is however, difficult to compare the maximum biomass achieved in the field with that found in the laboratory, as this will largely depend upon the prevailing environmental conditions and limitations in resources. In conditions of high CO_2* availability, species will inevitable achieve higher biomass densities than under identical conditions except with low CO_2* availability.

[1] For this study, biomass densities were calculated per unit fresh weight. An approximation of 1:10 dry to fresh weight was used to convert this data to values per unit dry weight.

Examination of relative monoculture and mixture response diagrams revealed differences in the responses of species to intra- or inter- specific competition (Fig. 7.13). Intraspecific competition was observed to increase with increasing planting densities for both *E. nuttallii* (Parts 1 and 2) and *L. major*. Intraspecific competition was not observed for *E. canadensis*. For Competition Study Part 1 (*E. nuttallii* vs *L. major*) the relative effects of intra- and inter- specific competition on yield of *L. major* were similar. The data suggest that for *E. nuttallii*, higher densities of *L. major* (2 and 3) did result in reduced yield of *E. nuttallii* (i.e. interspecific competition), although the data is far from conclusive. In the second study (Part 2, *E. nuttallii vs E. canadensis*), the relative yield responses of the species when grown in mixtures did not reveal any significant trends. Growth of *E. nuttallii* in mixtures was extremely similar to that in monocultures suggesting that no interspecific competition was occurring. *E. canadensis* exhibited enhanced growth when grown in a low proportion in mixtures with *E. nuttallii* (1 *E. canadensis* : 3 *E. nuttallii*).

The results of competition study 1 tentatively suggest that for *E. nuttallii* and *L. major*, intra and inter- specific competition may be roughly equal. This is further confirmed by the extremely similar water quality conditions (pH, Total CO_2, CO_2*, bicarbonate and carbonate) that were created by all three species. Even at the lowest starting biomass, rapid changes in water quality were observed. In view of these results, the proposed mechanism of displacement though the differential ability of the species to both create and tolerate conditions of stress, is unlikely to be the means by which an introduced species displaces an established species. The similarities in conditions created would result in an established species not being able to distinguish between stress conditions created by a competing species and that created by itsself. If this were the case, one would expect the species to coexist and suggests that some other mechanism(s) is likely to be important in the displacement process. However, results of this study suggest that the initial planting densities may have been too low and the experiment not allowed sufficient time for detectable interspecific competitive effects to occur, as was evident for *E. canadensis*. Final biomass densities for treatments in Competition Experiment 1 are given in Table 7.4, these suggest that final densities are generally lower than those quoted for field densities of macrophyte stands (Duarte and Kalff, 1990; Duarte and Roff, 1991). This again supports the view

that densities may have been too low, or the experiment not allowed sufficient time for a substantial growth of biomass. In Competition Experiment 2, a longer experiment was performed to confirm (or otherwise) the results of the first experiment.

For all graphs showing monitoring data of Competition Experiment 1, the following key applies:

Planting density	Symbol
1	×
2	▲
3	■
4	◆

For details of actual planting density (g DW m^{-2}) of each species see Table 7.1

Part 1 : *E. nuttallii* vs *L. major*

Part 2: *E. nuttallii* vs *E. canadensis*

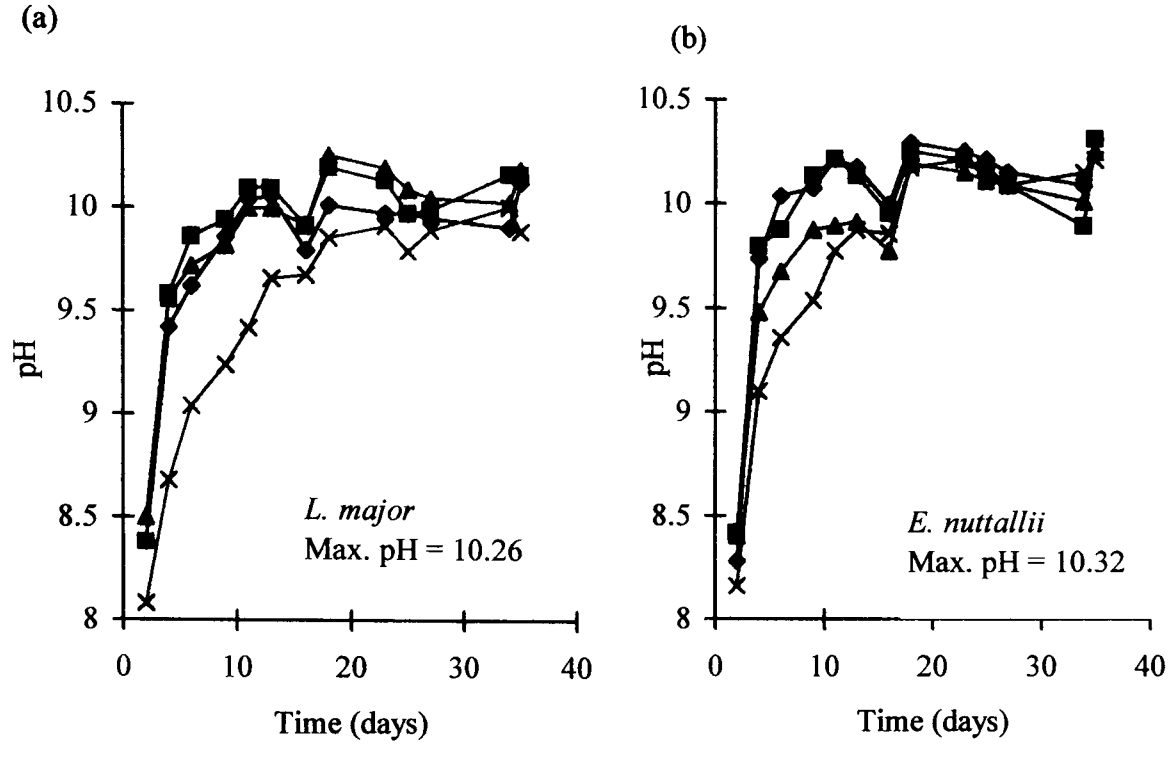
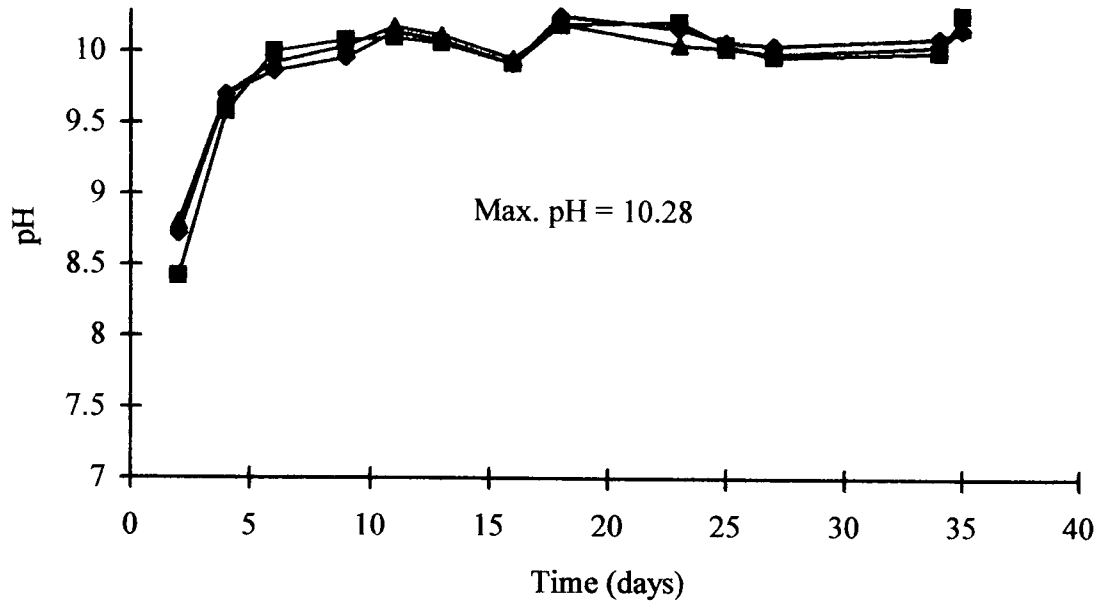


Fig. 7.2 pH of monocultures over time. (a) Part 1, *L. major* , (b) Part 1, *E. nuttallii* , (c) Part 2, *E. nuttallii* , (d) Part 2, *E. canadensis*. (n = 5)

(a)



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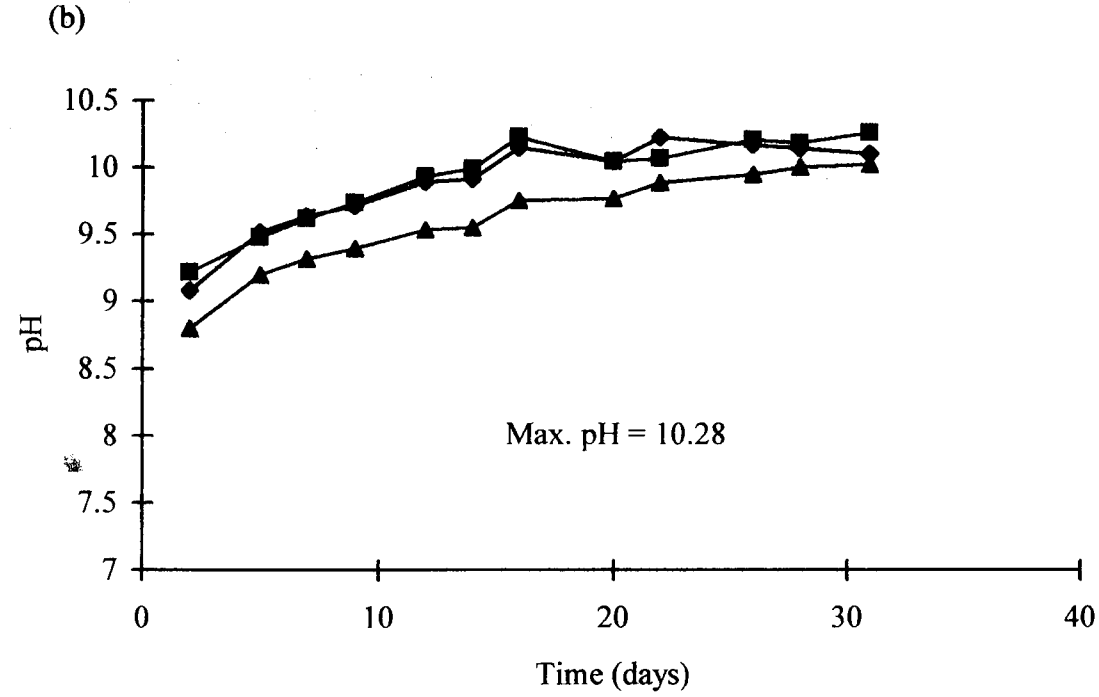


Fig. 7.3 pH of mixtures over time. (a) Part 1, *L. major : E. nuttallii,* (b) Part 2, *E. nuttallii : E. canadensis.* (n = 5)

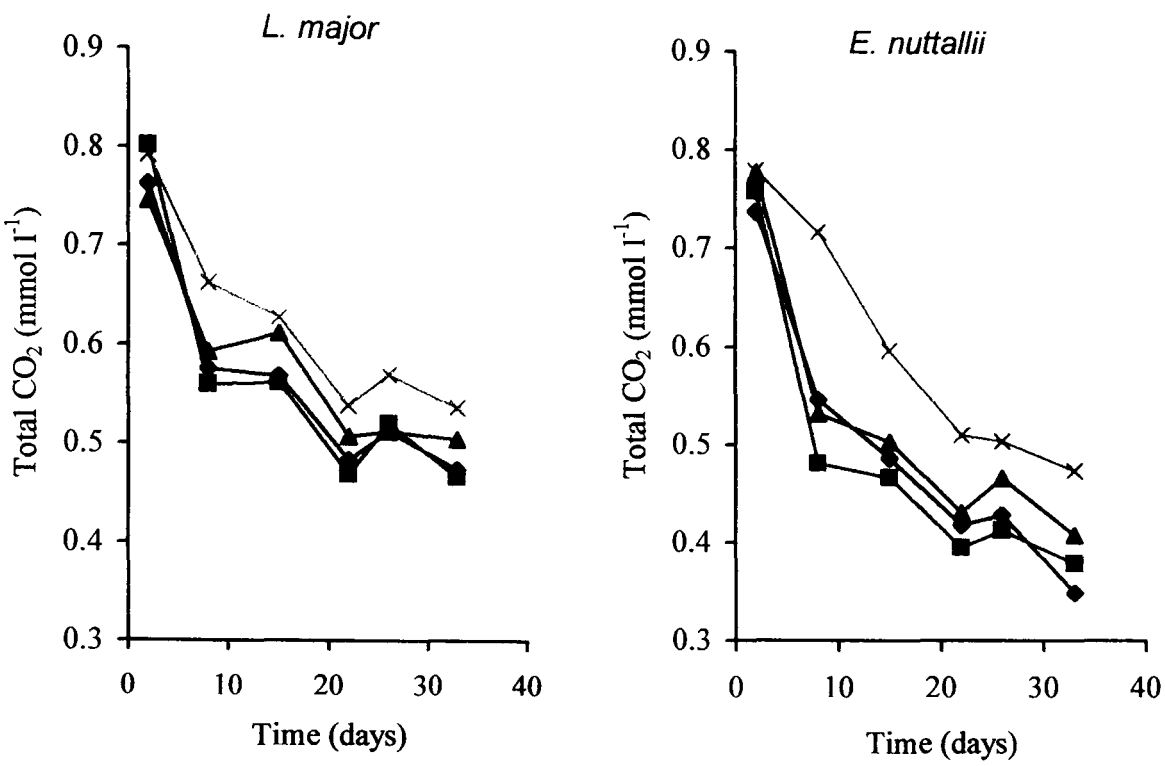
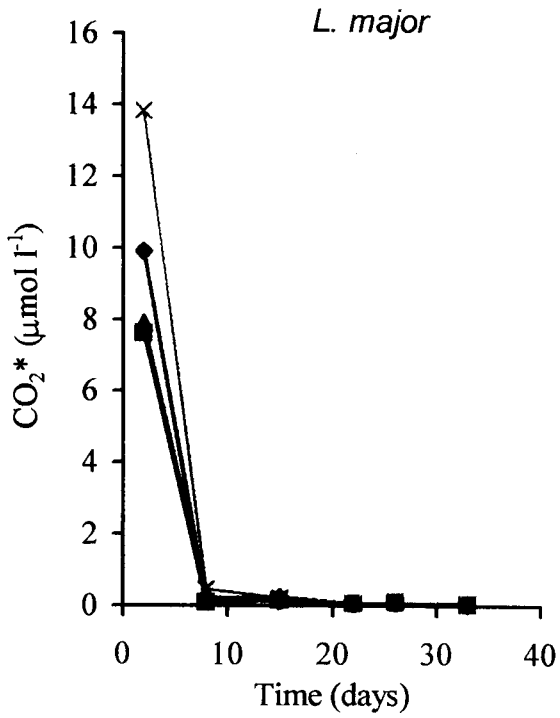
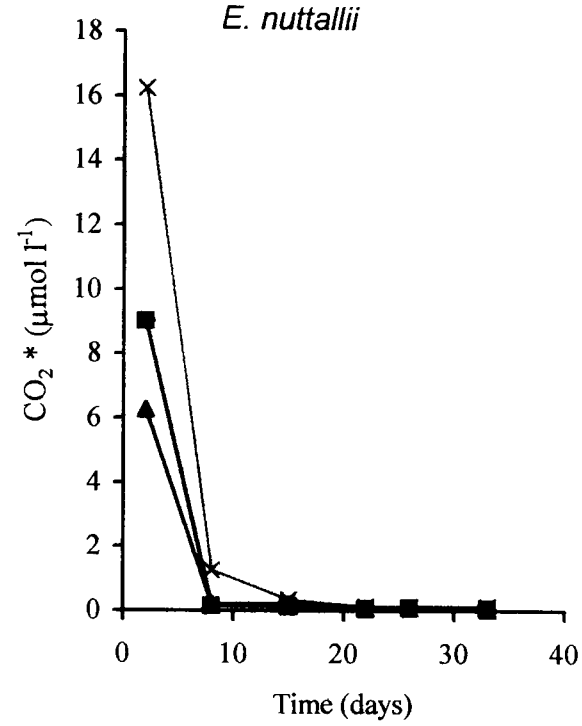


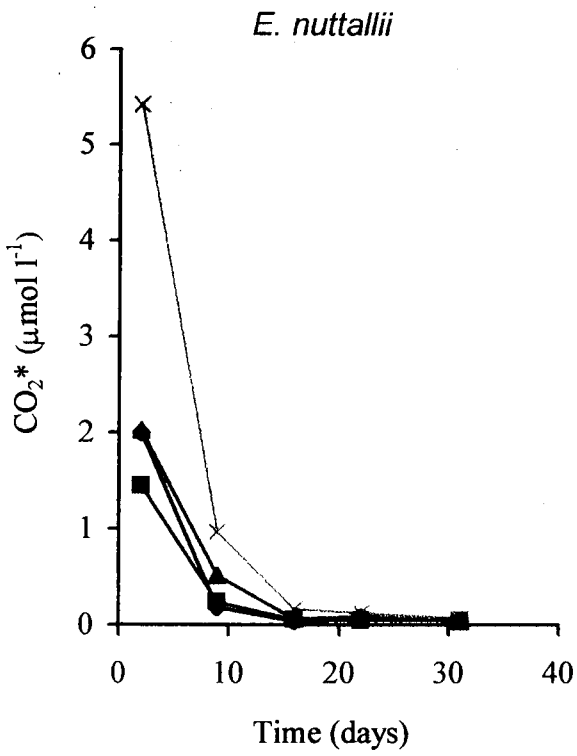
Fig. 7.4 Competition Experiment 1. Changes in Total CO_2 (mmol l^{-1}) of treatments with species grown in monocultures for Parts 1 and 2.

Part 1





Part 2



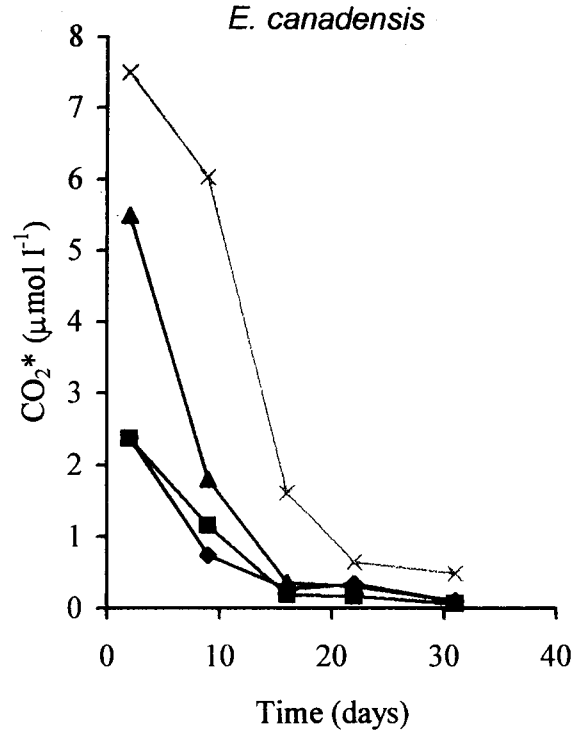


Fig. 7.5 Competition Experiment 2. Changes in CO_2* (µmol l^{-1}) of treatments with species grown in monocultures for Parts 1 and 2.

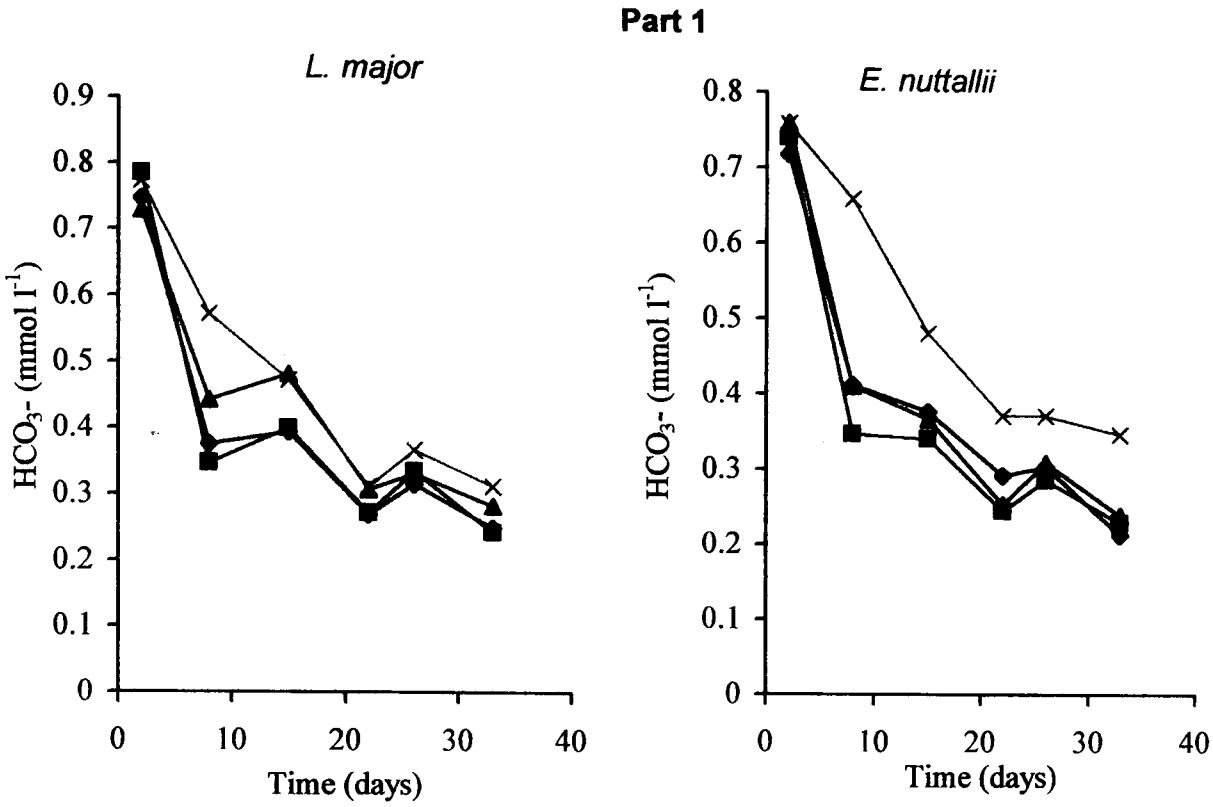
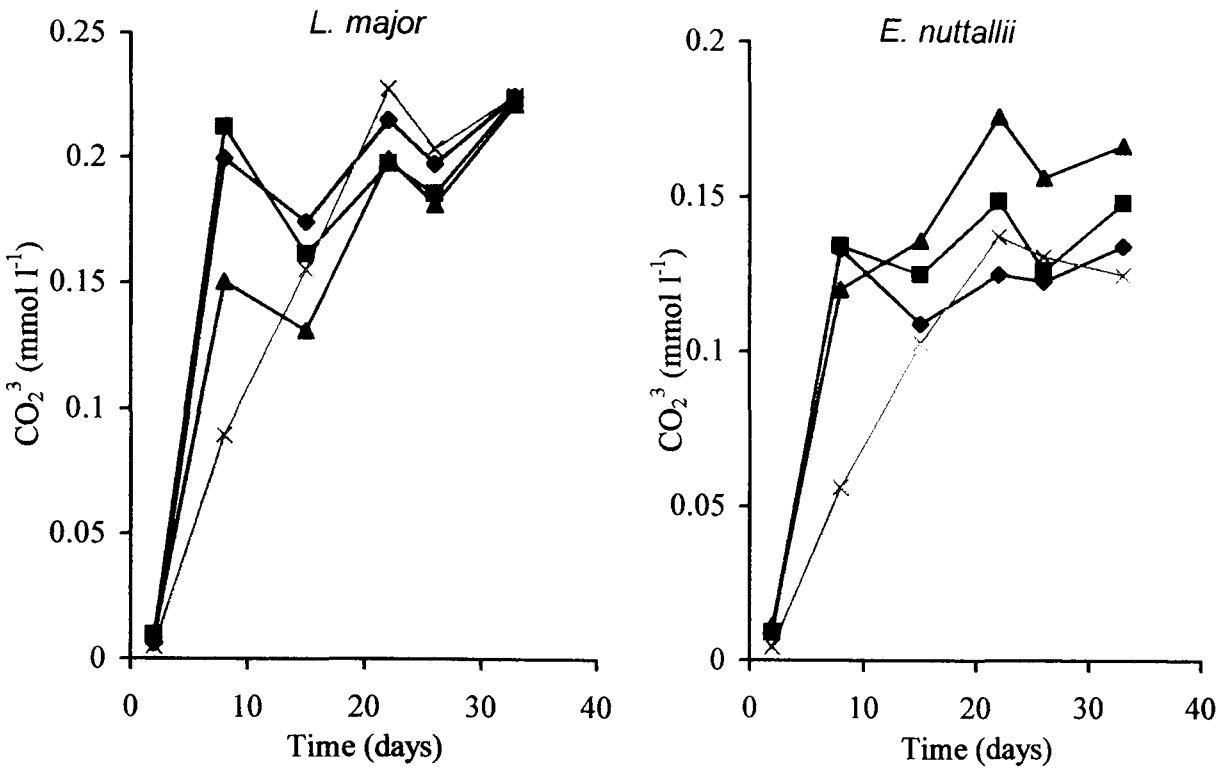


Fig. 7.6 Competition Experiment 2. Changes in HCO_3^- (mmol l^{-1}) of treatments with species grown in monocultures for Parts 1 and 2.



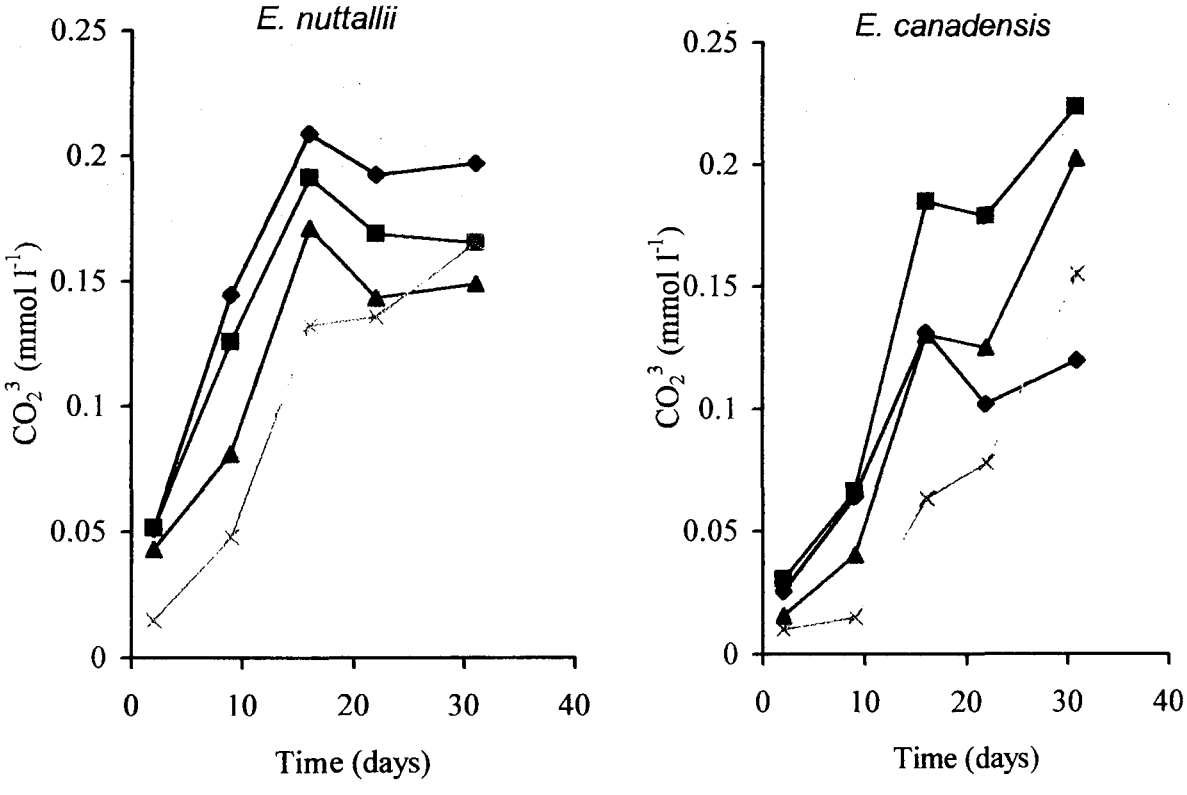
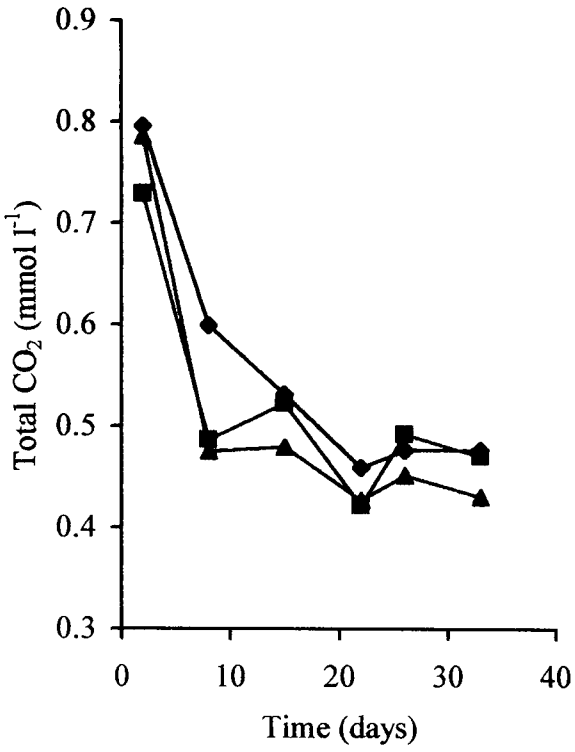
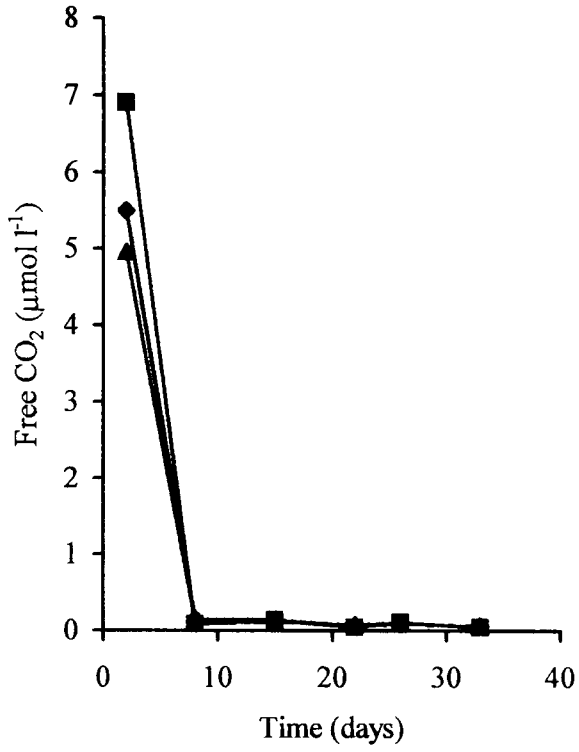
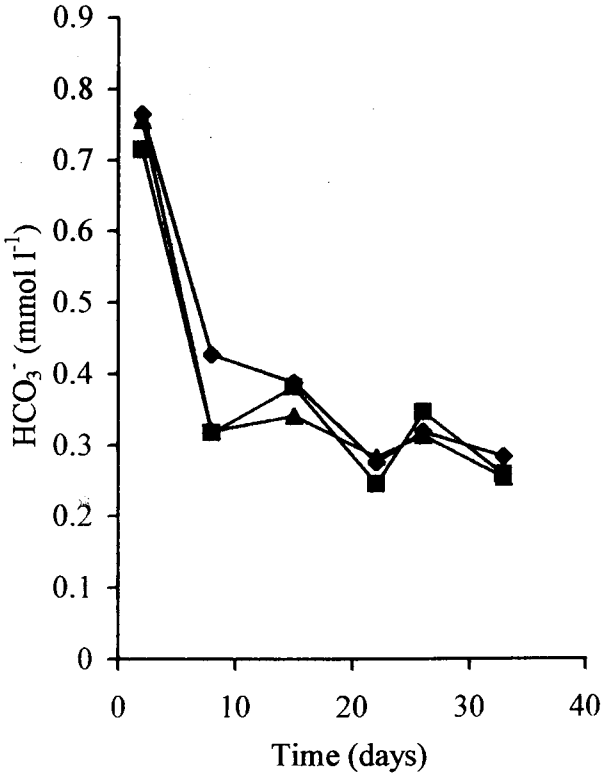


Fig. 7.7 Competition Experiment 1. Changes in CO_2^3 (mmol l^{-1}) of treatments with species grown in monocultures for Parts 1 and 2.







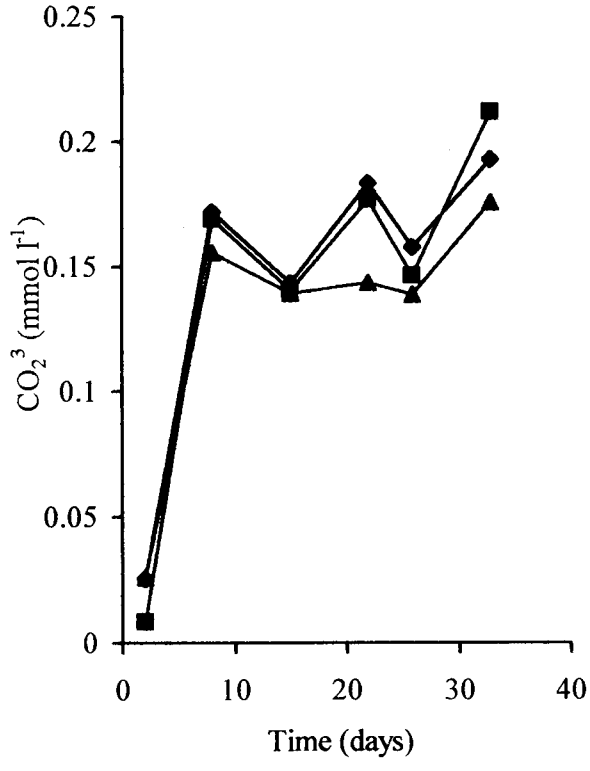


Fig. 7.8 Competition Experiment 1. Part1, *L. major* vs *E. nuttallii*. Changes in Total CO_2, CO_2 * , bicarbonate and carbonate in mixtures.

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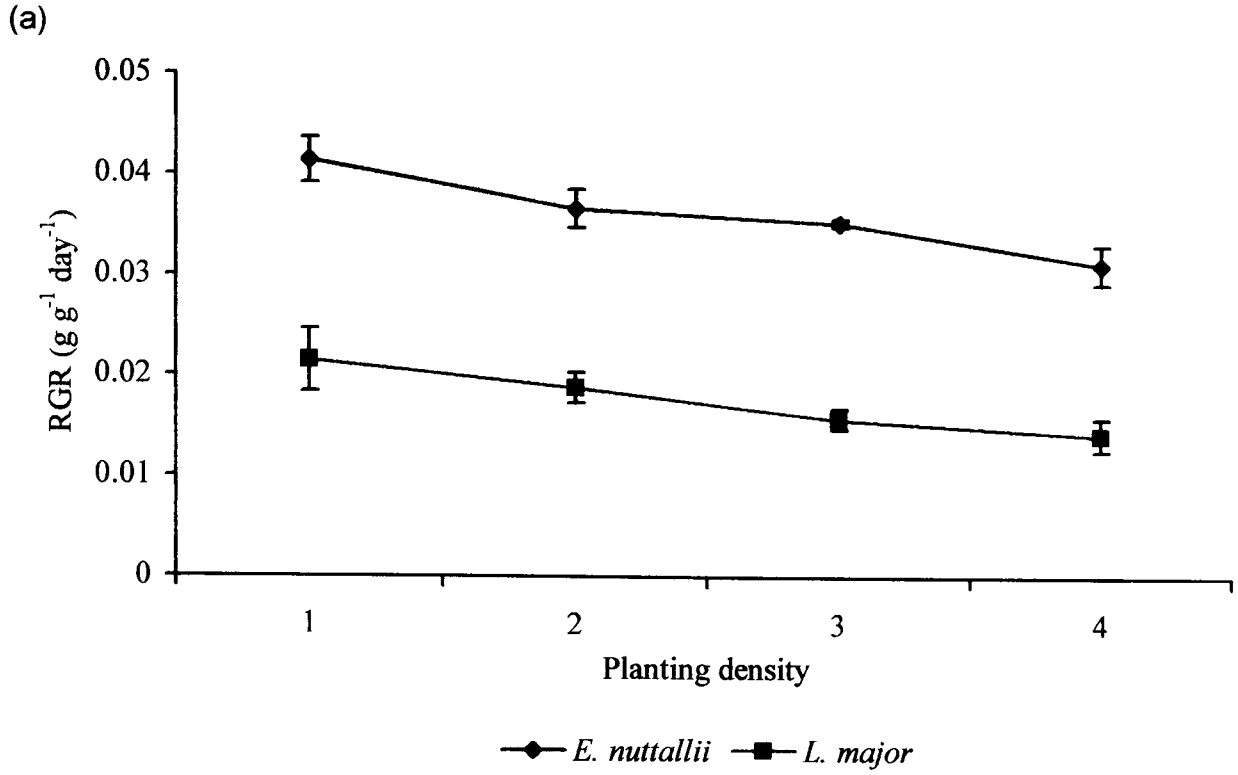


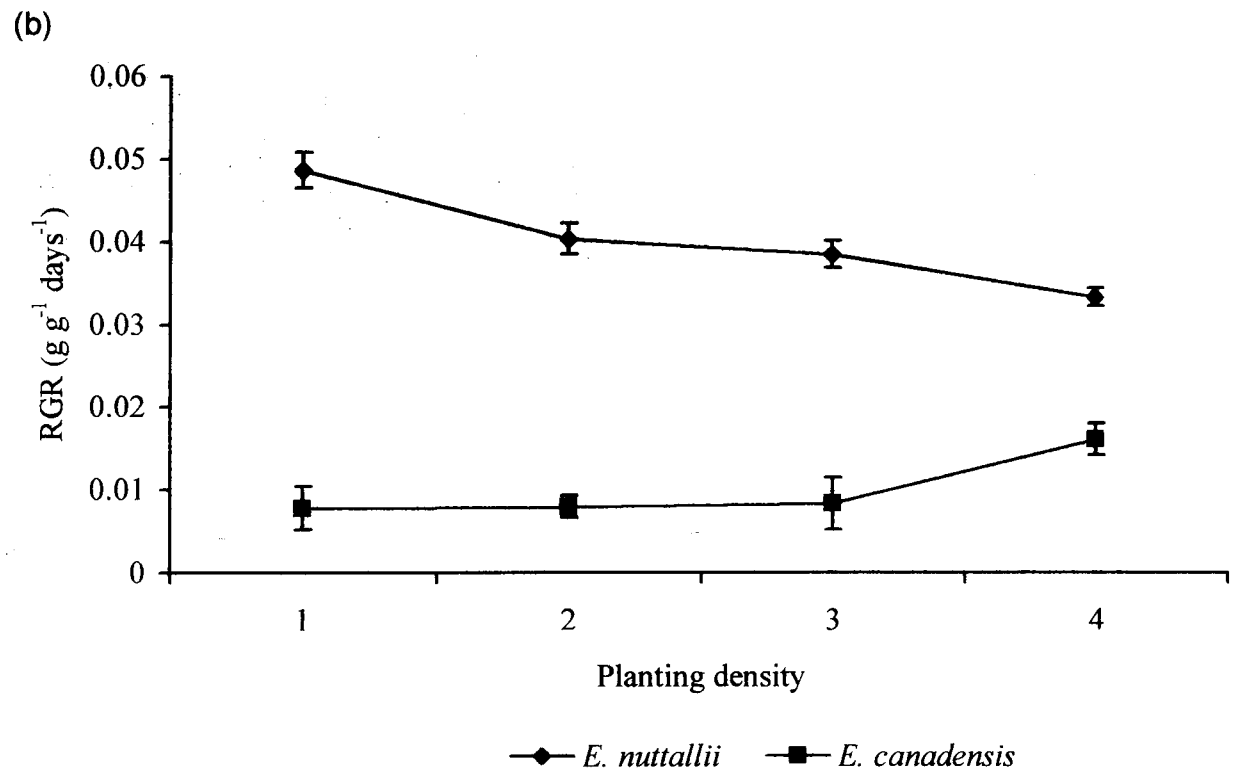
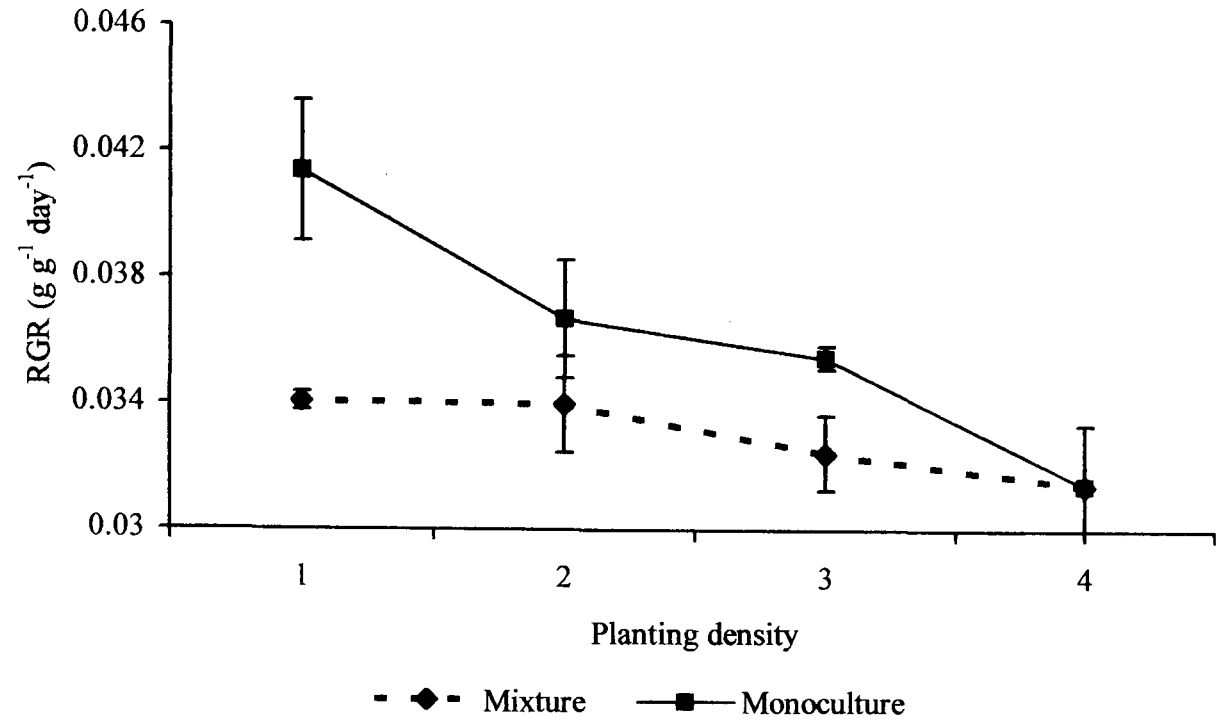


Fig. 7.10 Relative Growth Rates (RGR) of (a) *E. nuttallii* and *L. major* and, (b) *E. nuttallii* and *E. canadensis* grown in monoculture. (Mean $\pm$ SE, n = 5)

(a)



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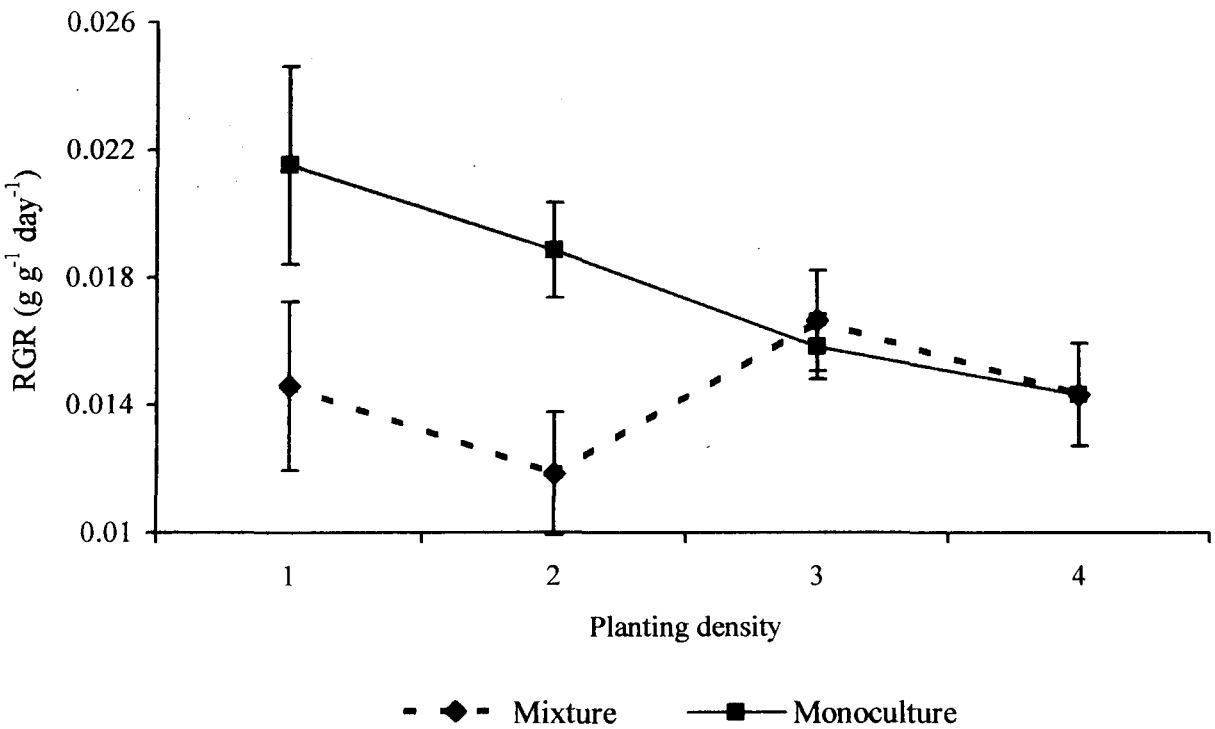


Fig. 7.11 Relative Growth Rates (RGR) of (a) *E. nuttallii*, and (b) *L. major* grown in moncultures (solid line) and mixtures (dashed lines). (Mean $\pm$ SE, n = 5)

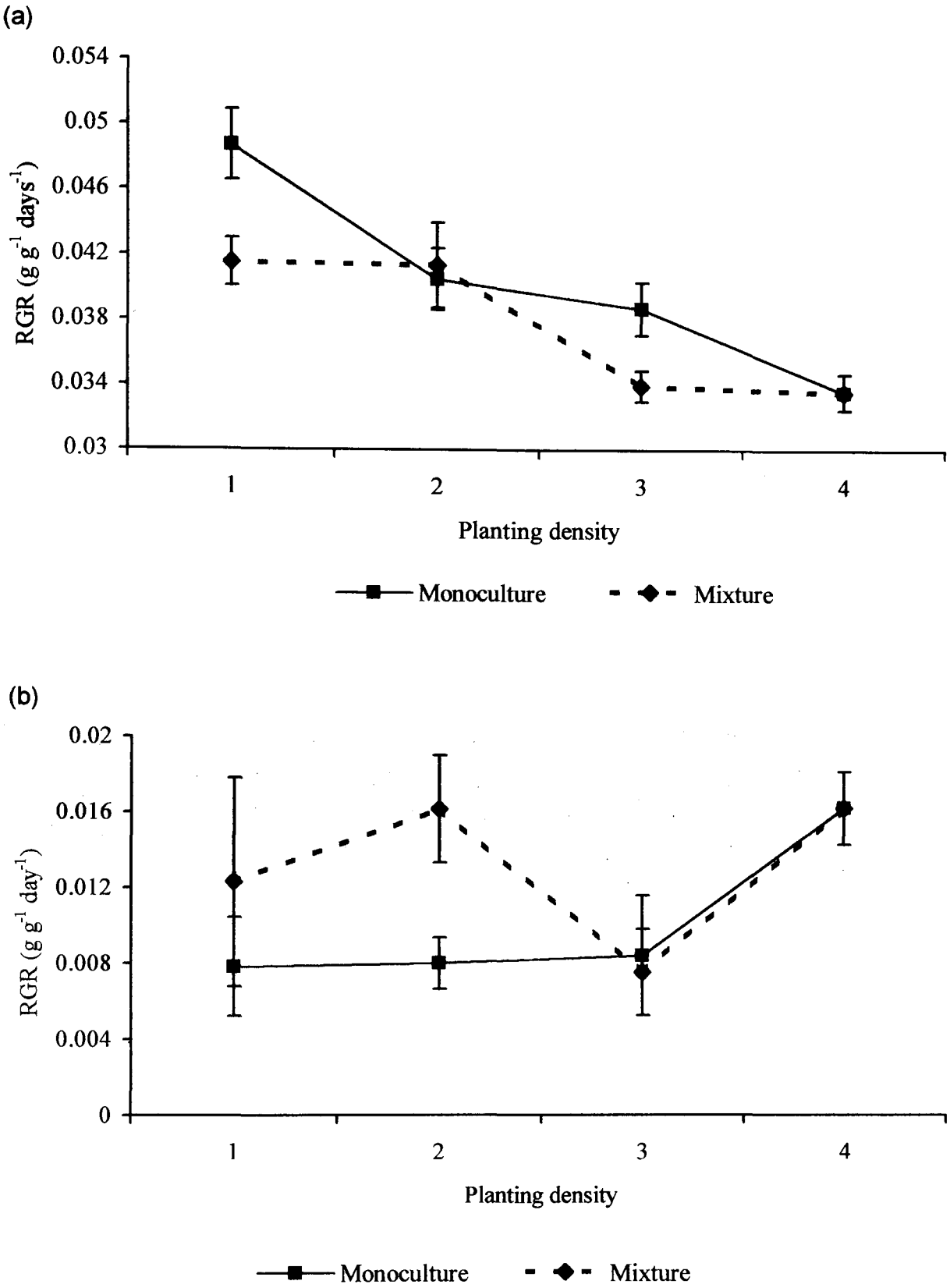
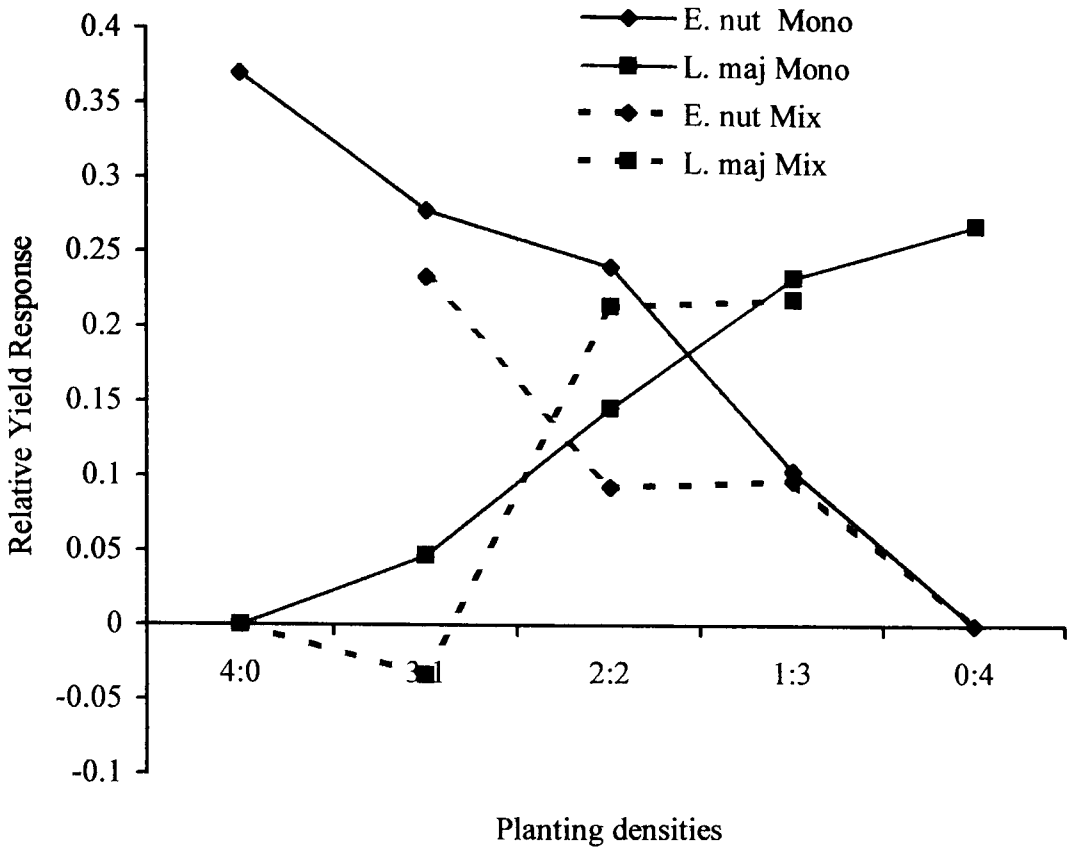


Fig. 7.12 Relative Growth Rates (RGR) of (a) *E. nuttallii*, and (b) *E. canadensis* grown in moncultures (solid line) and mixtures (dashed lines). (Mean $\pm$ SE, n = 5)



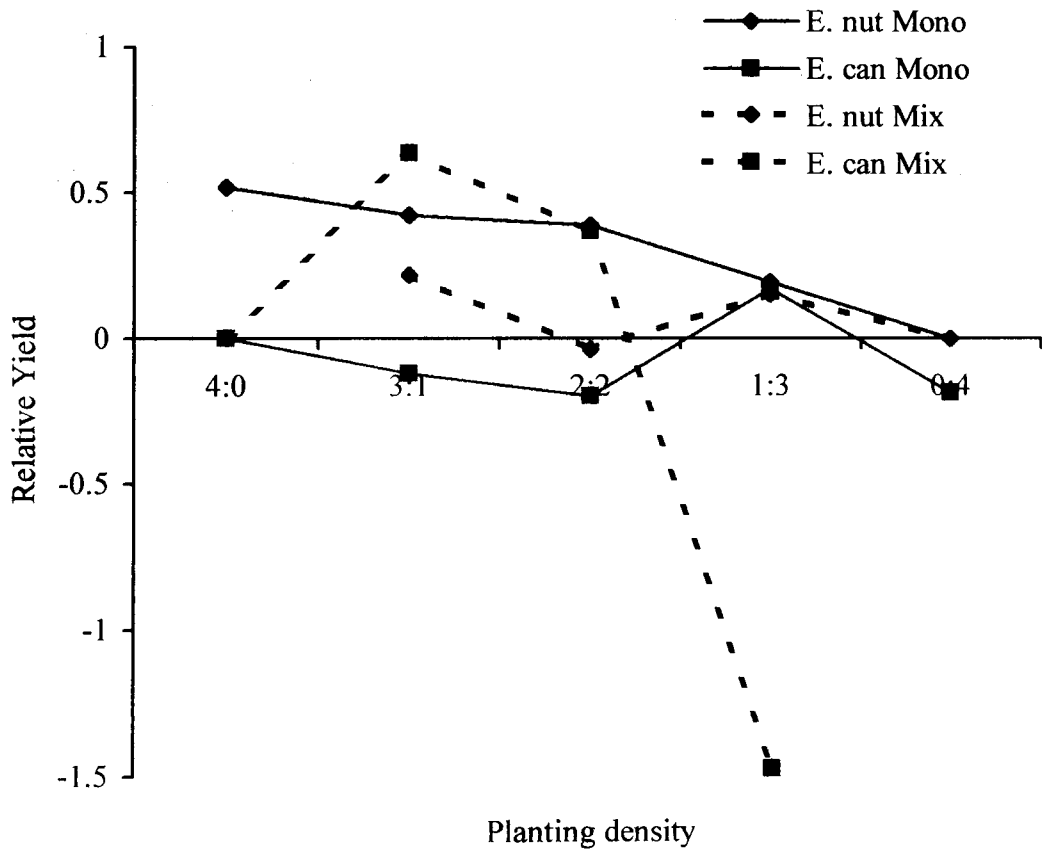


Fig. 7.13 Relative monoculture (solid lines) and relative mixture (dashed lines) responses for Competition Experiment, parts 1 and 2.

Table 7.4

Final biomass densities (BD) (g m^{-3}) of treatments for Competition Experiment 1.

Part 1 *E. nuttallii* vs *L. major*		Part 2 *E. nuttallii* vs *E. canadensis*	
Treatment	BD	Treatment	BD
En * Lm (3:1)	152.2	En * Ec (3:1)	141.4
En * Lm (2:2)	153.8	En * Ec (2:2)	138.6
En * Lm(1:3)	174.	En * Ec (1:3)	113.4
En (4:0)	142	En (4:0)	160.4
En (3:0)	121.8	En (3:0)	143.4
En (2:0)	85.4	En (2:0)	101.6
En (1:0)	50.4	En (1:0)	67.0
Lm (0:4)	166.4	Ec (0:4)	51.8
Lm (0:3)	131	Ec (0:3)	38.9
Lm (0:2)	97.4	Ec (0:2)	25.9
Lm (0:1)	54.4	Ec (0:1)	13.0

Table 7.5
Final biomass densities (BD) (g^{-1} m^{-3}) of Competition Experiment 2

Treatment	BD
En (2)	184.1
En (4)	338.3
Ec (2)	118.3
Ec (4)	188.8
Lm (2)	267.0
Lm (4)	340.1
En * Ec (2:2)	256.2
En * Lm (2:2)	340.0
Ec * Lm (2:2)	260.2

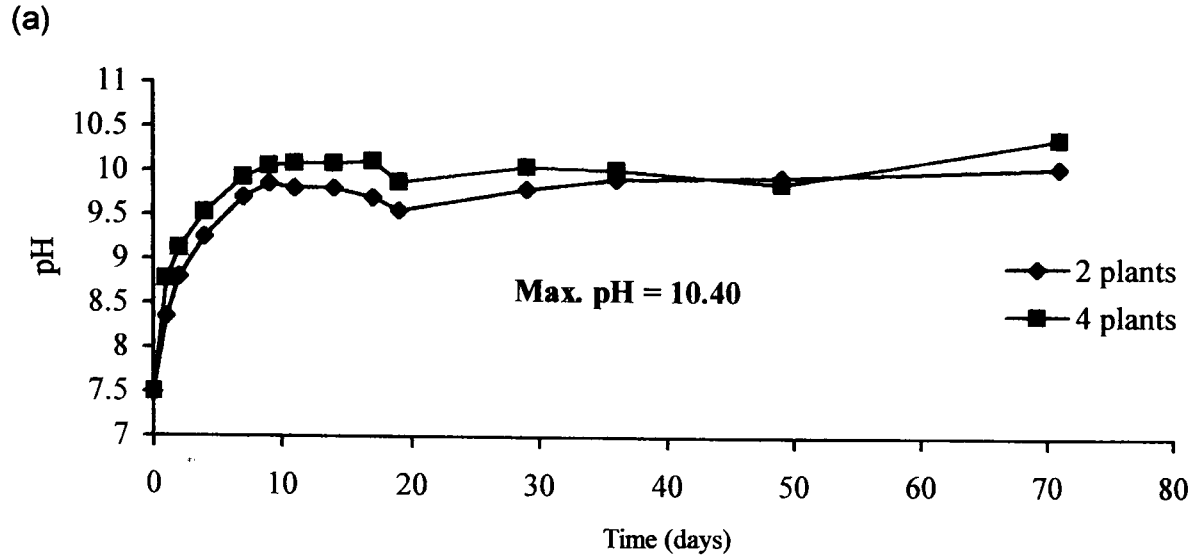
7.2.3.2 Competition Experiment 2

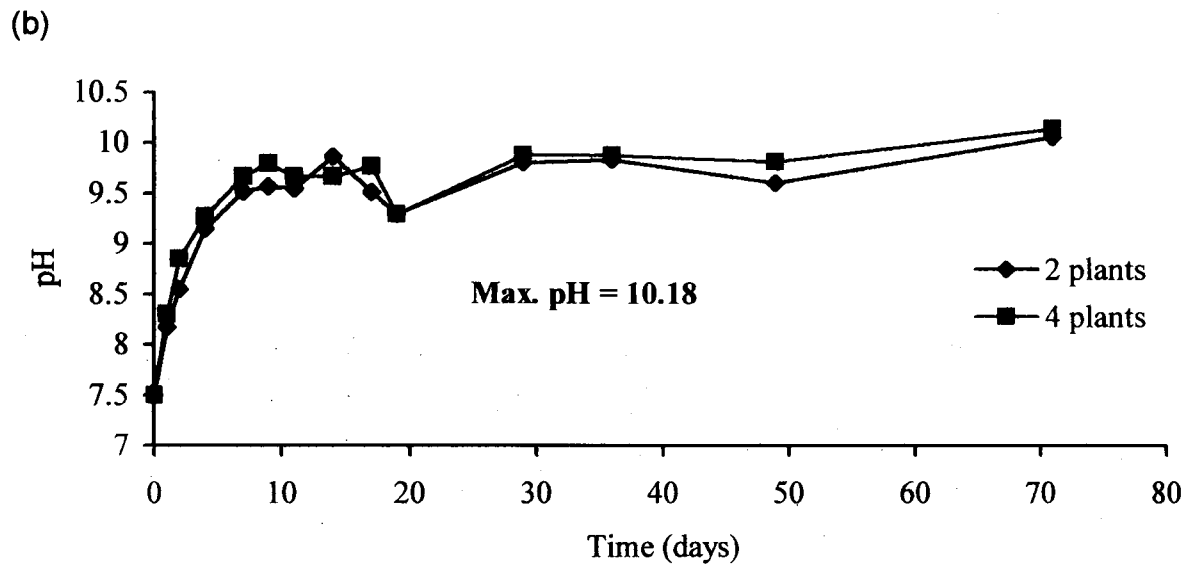
The rise in pH of the growth media was similar for all treatments (Fig. 7.14 and 7.15). Maximum pH values were similar to, although slightly lower than, those recorded for Competition Experiment 1. Measurements of final biomass densities made in this second competition study are closer to those quoted in the literature (i.e. Duarte and Kalff, 1990; Duarte and Roff, 1991) for *E. canadensis* and other macrophytes similar in morphology (Table 7.5). All species exhibited a reduction in growth rates with increasing density, either through addition of the same species (i.e. intraspecific competition) or through the addition of a second species (i.e. interspecific competition) (Fig. 7.16). Although the experimental design of Competition Experiment 2 was limited in order to study the three pair-wise comparisons simultaneously, the fitted Reciprocal Yield model did appear to interpret the results in accordance with visual estimates made of the raw data. For *E. nuttallii* in competition with either *E. canadensis* or *L. major,* intraspecific competition was greater than interspecific competition. The ratio of the partial coefficients suggests that 1 g DW m^{-2} of *E. nuttallii* and 3 g dry weight m^{-2} *E. canadensis* have an equivalent influence on the growth of *E. nuttallii*. For studies with *L. major,* 66 g DW m^{-2} of *L. major* had an equivalent effect to 1 g dry weight m^{-2} of *E. nuttallii* on the growth of *E. nuttallii.* While this value does appear to be great, the dry weight results (Fig. 7.16) do confirm that despite the much greater densities of *L. major* present in the treatments, this species had very little impact upon the growth of *E. nuttallii*. In fact, the growth of *E. nuttallii* in mixtures was not significantly different from that in monocultures. For *L. major*, interspecific competition was greater than intraspecific competition. Again partial coefficients suggest that 5.8 g DW m^{-2} of *L. major* had an equivalent effect to 1 g DW m^{-2} of *E. nuttallii* on the growth of *L. major.* Similarly, 4 g DW m^{-2} of *L. major* had an equivalent effect to 1 g DW m^{-2} of *E. canadensis*. Finally, in competition studies with *E. canadensis*, interspecfic competition was greater than intraspecific competition when this species was in competition *with E. nuttallii*, and intraspecific competition was greater than interspecific competiton when in competition with *L. major*. Thus, 1.5 g DW m^{-2} *E. canadensis* has an equivalent effect to 1 g DW m^{-2} of *E. nuttallii* on the growth of *E. canadensis*, and 1.34 g DW

m^{-2} *L. major* has an equivalent effect to 1 g DW m^{-2} of *E. canadensis* on the growth of *E. canadensis*.

The competition studies described in this chapter show that even the lowest initial planting densities resulted in a rapid changing in water quality. Yet, only slight effects were observed on the relative growth rates of *E. nuttallii* and *L. major* at the higher planting densities. In addition, the water quality conditions created by the species were extremely similar. These results suggest that as the species create very similar conditions, they would not be able to differentiate between intraspecific effects on water quality, and interspecific effects on water quality. Therefore, it seems unlikely that the differential ability to create stressful conditions will be an important driving force in the observed displacements between *E. canadensis*, *E. nuttallii* and *L. major*. In short, on the basis of the first experiment, intra- and inter- specific effects are similar.

The second competition study, however, suggest that competition is occur between the species. These results suggest that in competition between the three species, *E. nuttallii* would displace both *E. canadensis* and *L. major*, as the intraspecific effects *E. nuttallii* had on its self are greater than those effects caused by the competing species. Whereas, the interspecific effects caused by *E. nuttallii* had a greater effect on *E. canadensis* and *L. major* than these species had on themselves. In short, the interspecific effects were greater than intraspecific effects. In competition between *E. canadensis* and *L. major*, *L. major* would competitively displace *E. canadensis* as the interspecific effects *L. major* had on *E. canadensis* were greater than the intraspecific effects *E. canadensis* had on itself.





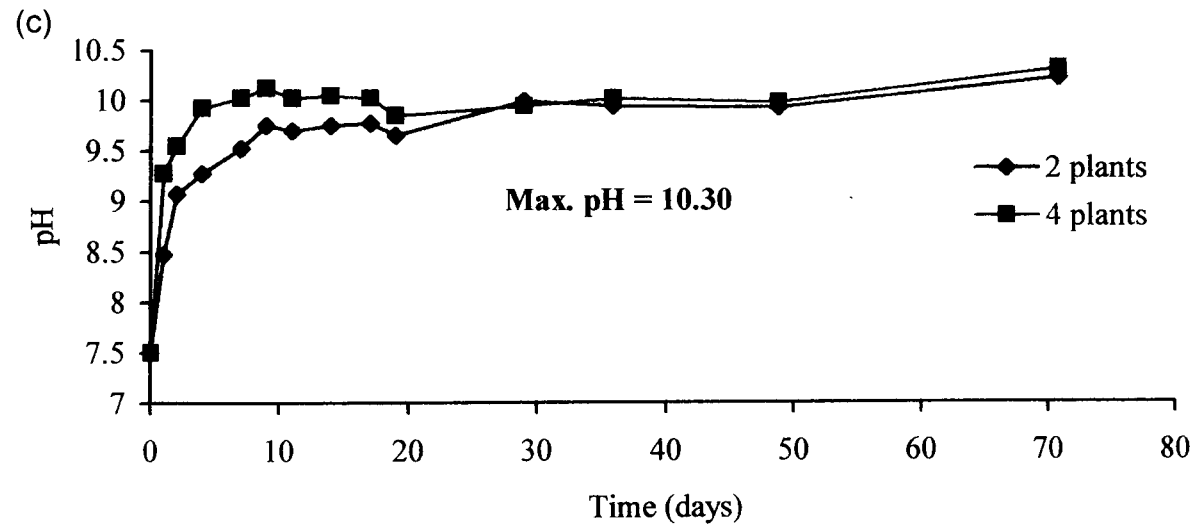


Fig. 7.14 Competition Experiment 2. pH of monocultures of (a) *E. canadensis*, (b) *E. nuttallii* and (c) *L. major*.

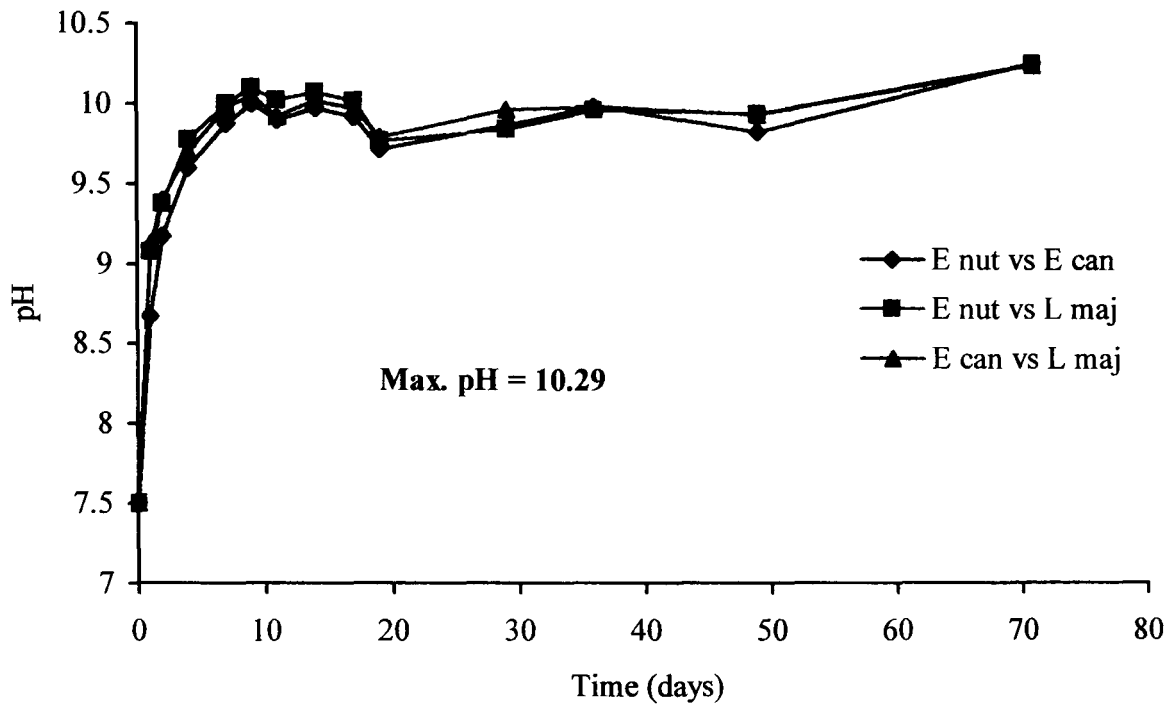


Fig. 7.15 Competition Experiment 2. pH of mixtures.

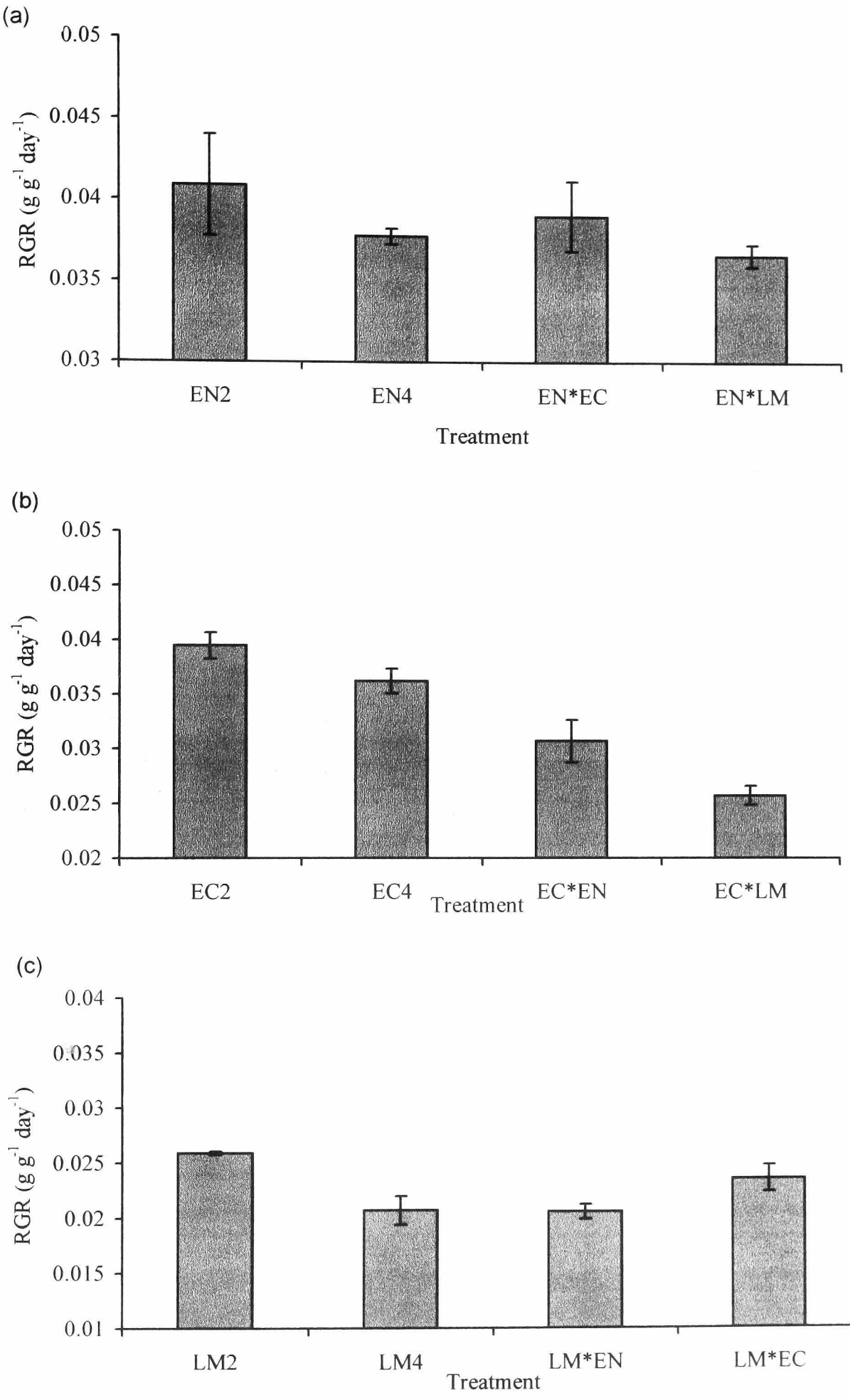


Fig. 7.16 Competition Experiment 2. Relative Growth Rates (g g^{-1} day^{-1}) of (a) *E. canadensis*, (b) *E. nuttallii*, and (c) *L. major* in the presence and absence of a competitor over an 85 day period. Error bar + standard error, n = 4

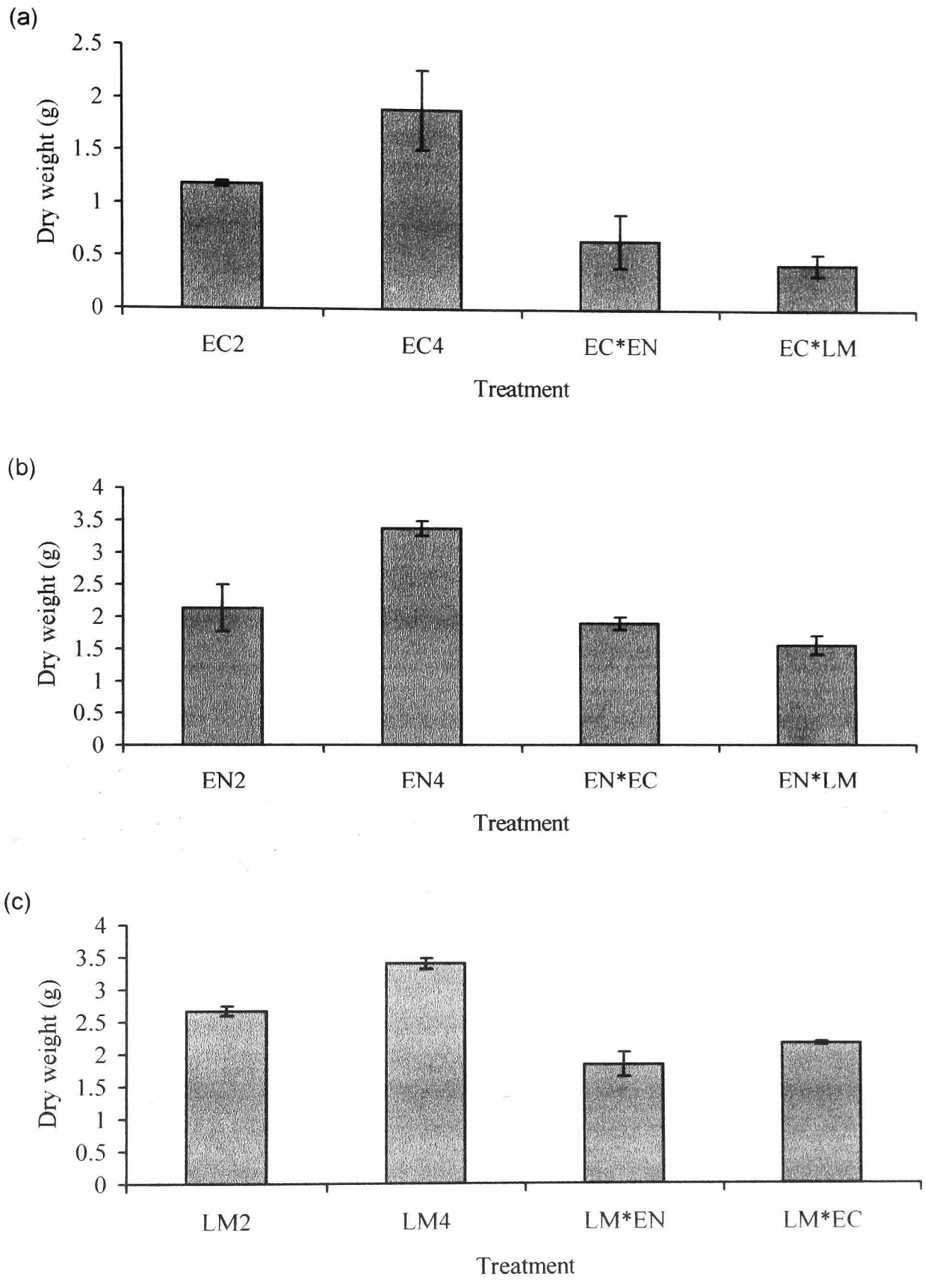


Fig. 7.17 Dry weight (g) of (a) *E. canadensis* , (b) *E. nuttallii* , and (c) *L. major* in the presence and absence of a competitor over an 85 day period. Error bar + standard error, n = 4

7.3 Summary

1. Even at the lowest planting densities used plants had a significant effect upon pH, Total CO_2, free CO_2, bicarbonate and carbonate.

2. Water quality conditions created by the three species were similar.

3. In both monocultures and mixtures *E. nuttallii* grew faster and achieved greater biomass than either *E. canadensis* or *L. major*.

4. Increasing monoculture planting densities resulted in increased intraspecific competition for *E. nuttallii* and *L. major* in Competition Experiment 1. For Competition Experiment 2, intraspecific competitive effects were observed for all three species.

5. Results suggest that under the experimental conditions described in Competition Experiment 2:

E. canadensis VS *E. nuttallii*	Intraspecific < Interspecific
E. canadensis VS *L. major*	Intraspecific > Interspecific
E. nuttallii VS *E. canadensis*	Intraspecific > Interspecific
E. nuttallii VS *L. major*	Intraspecific > Interspecific
L. major VS *E. nuttallii*	Intraspecific < Interspecific
L. major VS *E. canadensis*	Intraspecific < Interspecific

Chapter 8 MATHEMATICAL MODELLING: FUTURE CONSIDERATIONS

Mathematical modelling has a number of uses:

- It allows testing of interpretations arising from results of practical work
- It is possible to study theoretically combinations of variables that would not be possible to achieve practically.
- Variables, which due to practical reasons may not be studied in great detail, or only within confined limits, during practical work, may be studied in greater detail.

Originally it was intended that information and data collected during this study would be used in the construction of a mathematical model of the three species. Although preliminary work on the basic mathematics was completed, due to time constraints significant progress was not made with the actual model construction. However, a future model is intended that will be based upon both the work in the present study and information available in the literature. In the following summary important features for inclusion are given.

As the model is intended to be a visual model with which the user can see the growth of the plants over time, a first and important consideration is whether length can be used to represent dry weight biomass. Measurements made in Chapter 2 of length against dry weight for *E. canadensis* and *L. major*, and those of Birch (1990) on *E. nuttallii* show a linear trend between length and dry weight for all three species. As this is so, increases in dry weight biomass may be represented as length. The next consideration is what unit to select as the basic structural building block for a model. This can range from a single cell, to a whole plant. In Chapter 3, internode lengths and numbers between successive branches were studied. It is suggested from the present study that an internode and node unit (i.e. one metamer) may be an appropriate building block for a model. This unit has the advantages of being easy to count and measure; yet detailed enough to provide structural variation in response to changes in environmental conditions.

In the following section, a summary is given of known information concerning the basic structural unit or metamer and how this relates to branching frequency and distance:

- Internode number (~7 for *Elodea* spp., ~ 10 for *L. major*) is relatively consistent between branches.
- If it is assumed that internode numbers between successive branches remain constant, changes in distance between successive branches are therefore the result of changing internode length.
- The internode elongation region is ~10 cm long for *Elodea* spp. and ~15 cm long for *L. major*, although this varies depending upon the length of successive internodes.
- Branching in *L. major* is highly variable and patterns are difficult to discern.

Distance between branching points towards a main shoot apex shorten due to decreases in internode length. Increases in apparent branching density towards the apex, however, may also be due to a decrease in the delay between the production of a bud and initiation and growth of that bud. The following points may be noted for branching for all three species:

- Branching will take place within the elongation region. The minimum distance between the growth of a main shoot and the development of a side axis is less than 5 mm if it is assumed that the main branch and the side axis grow at the same rate.
- The time delay between production of the main shoot and development of a side shoot on the main shoot decreases towards the shoot apex.

Chapter Three also illustrates the importance of several architectural features of the plants, namely secondary branching and the ability to form a dense canopy. A significant secondary branching was only observed under laboratory conditions in the present study, it is impossible to predict how important it is under field conditions and further information is needed on this topic. In the formation of a dense canopy evidence in this study is not sufficient to suggest any significant differences in the

efficiency of canopy production as only relatively short plants of *E. canadensis* were found in the field. However, this in itself is an important factor as it is implicitly linked to an important difference between the species, their relative growth rates. In both the present study and in other comparative studies between the *Elodea* spp. (e.g. Simpson, 1990; Ozimek *et al.*, 1993), *E. nuttallii* exhibits a greater RGR than *E. canadensis*. A range of evidence suggests that *E. nuttallii* has an intrinsically higher growth rate than either *E. canadensis* or *L. major*.

In the next step, in model construction, it will have to be decided at what point the environment affects the development of a metamer. During this study all three species have been shown to be extremely plastic physiologically. Evidence in the literature also shows these plants to have a wide variety of morphologies, with variation in leaf size, shape and orientation and large differences in internode lengths. While localised differences in environmental parameters such as light intensity, temperature, carbon availablity, O_2 concentrations and water flow probably account for the variation found, it is difficult to determine at just what point in the development of a shoot the physiological and morphological features are determined. Morphology is probably determined during the maturation of a bud. This is most applicable for leaf shape, where leaves mature and show little increase in size below 1 cm beneath the apical tip. For internode length, it is more difficult to determine. Is internode length pre-programmed at the time of production or may it still be influenced by subsequent changes in environmental conditions? Although the latter is an interesting and likely possibility, this response would be extremely difficult to incorporate into a model. It may be necessary therefore to construct the model such that the morphology of a metamer is determined at the time of initiation. However, difficulties arise when incorporating physiological variation into a model. All three species have shown acclimation to different environmental conditions. In the present study, acclimation to changing carbon availability was shown to be an important feature differentiating the three species. It appears important to incorporate this physiological plasticity and acclimation into a model, particularly in response to changing carbon availability, but it is not immediately obvious how this can be done.

The present study, indicated not surprisingly, that the effect of temperature o the growth rate of the species is an important feature for inclusion in a model Particularly important will be the effect of temperature on relative growth rate.

In order to simplify the model, at least initially, the effects of trophic statu on growth and third party interactions with algae need not be incorporated Experimental evidence suggests that if there are affects, these are likely to b extremely subtle, and unlikely to be a main driving force in the displacement process

In the next phase of the model's development, a simple model will b constructed under constant summer conditions. At this stage features such a internode elongation and branching patterns will be incorporated. Once this i complete, seasonal and diurnal changes in environmental parameters will b considered. These will initially include light and temperature variation, latter o encompassing restrictions in available carbon and bicarbonate. Finally, acclimatio will be considered, particularly in response to carbon and bicarbonate availability.

Chapter 9 GENERAL DISCUSSION

The aim of this study was to determine what drives the observed species displacements from *Elodea canadensis* to *Elodea nuttallii* to *Lagarosiphon major*. It was hypothesised that the differential ability of species to generate stress conditions (i.e. high pH, low DIC and CO_2*, and high O_2) and to successfully survive those conditions is instrumental in the ability of one species to displace another.

Implicit to this hypothesis is the ability of the three species to exhibit differential abilities to create and tolerate stress conditions during periods of active photosynthesis. Tolerance of increases in O_2, pH, decreases in CO_2* and the differential ability to take up bicarbonate were all examined. Physiological measurements suggest that the three species exhibit extreme plasticity in physiological activity, with different species exhibiting the highest rates of photosynthesis under different environmental conditions. The response of *L. major* to oxygen concentrations was similar to that recorded for *E. canadensis* (Simpson, 1981) and *E. nuttallii* (Jones, 1994), but in the comparative studies presented here, photosynthetic rates of *L. major* were higher than those of the *Elodea* spp. The tolerance of all three species to pH was great, with positive photosynthesis recorded from pH 6 to pH 9.7, although, again the highest photosynthetic rates were recorded for *L. major*. This confirms that all three are capable of utilising bicarbonate as an alternative carbon source when CO_2* is depleted. Studies were made in which species were acclimated to high and low CO_2* conditions. A comparison of photosynthetic rates under CO_2* limiting and non-limiting conditions, i.e. at pH 6.5 and pH 9, suggests that *E. nuttallii* and *L. major* more readily adapted to changes in CO_2* and bicarbonate concentrations than does *E. canadensis*. Following the high CO_2* treatment, both *E. nuttallii* and *L. major* were still capable of utilising bicarbonate to a higher extent than *E. canadensis*. In view of reported diurnal variability in CO_2* availability, this may be an extremely important feature.

In a comparison of the stress conditions created by each species, little difference was observed in those parameters measured, namely pH, CO_2* and bicarbonate. All species generated high pH conditions, in excess of pH 10.2 on

equent occasions. When grown in monocultures at four different starting densities Competition Experiment 1), initial density-dependent differences in the generation f high pH and low CO_2* were observed. The lowest densities had the slowest rates f pH increase and the lowest rates of CO_2* decrease. *E. canadensis* was also bserved to generate these changes in water quality more slowly than either *E. uttallii* or *L. major*, possibly a reflection of this species slower growth rate during ıese experiments. Initial differences in stress generation between the species at the tart of the growing season may be important in the process of competitive isplacement. A slower growing species such as *E. canadensis* may encou ıter more ıpid depletions in available CO_2* when in the presence of a competing species that enerates stress conditions rapidly. However, differences in the generation of high H and low CO_2* were not normally prolonged beyond the second week of each xperiment. Physiological studies showed no positive net photosynthesis at pH alues greater than 9.75, it is therefore unlikely that under these conditions of high H and low CO_2* and bicarbonate, significant photosynthesis would be taking place. CO_2* concentrations were rapidly depleted to levels below CO_2* compensations oints reported for these species in the literature and thus, the main source of carbon vould be bicarbonate, although above pH 9.7, photosynthesis appeared to cease ltogether. Calculations of bicarbonate utilisation efficiency suggest that *L. major* is more efficient bicarbonate user than either *Elodea* spp. Thus, it would be expected ıat during conditions where CO_2* levels were depleted rapidly, *L. major* would ave a significant competitive advantage.

Growth of species in mixtures and monocultures at different densities suggest ıat under the experimental conditions even at the lowest planting densities, stress onditions of high pH, and restrictions in CO_2* and bicarbonate availability are ıpidly created. If the proposed hypothesis of stress generation/toleration has validity ı the displacement of one species by another, one would therefore expect to have ɔund some interspecific competitive effects in these experiments. Despite this, it vas apparent that little interspecific competition was taking place during the growth f the mixture cultures. It is possible that the advantages gained by *E. nuttallii* and *L. ıajor* in rapid acclimation to the changing CO_2* and bicarbonate environment may ave been lessened by the lack of a significant reduction in pH during the night time.)iurnal measurements revealed that during the experiments the pH did not drop

sufficiently for significant CO_2* to accumulate during the hours of darkness. Thus any potential advantages achieved by a rapid ability to invoke bicarbonate utilisation would not be realised during this study.

Other, non-physiological measurements, of features such as growth rates and life history traits were included in this study to assess their roles in determining the competitive ability of each species. One of the most significant features observed in the present study was the rapid growth of *E. nuttallii.* This species had a consistently higher growth rate than either *E. canadensis* or *L. major* for many of the experiments, e.g. nutrient studies and temperature studies at 15 and 20 °C. Rapid growth combined with a high potential for branch production, is likely to be an extremely important factor in competition between this species and *E. canadensis.* The latter species is generally much slower growing, with less potential for extensive canopy production. This is in accordance with observations made in the field that in sites where the species are found together, *E. canadensis* is frequently found growing beneath a dense canopy of *E. nuttallii.* This was noted during this study in the Lancaster Canal (SD 481 618). Light levels beneath a dense canopy are likely to severely restrict the growth of understorey species. While low light compensation points may facilitate survival, plants beneath a canopy can be in almost complete darkness. It seems likely that in this situation *E. canadensis* will be eventually eliminated from sites in which *E. nuttallii* is also present. Previous studies comparing the light compensation points of *E. canadensis* and *L. major* suggest that one reason for the success of *L. major* may be its low light compensation point, this allowing this species to survive and grow even under dense canopies of established species. This was also confirmed in the present study, where positive photosynthesis of *L. major* was observed at lower light intensities than those reported for either *E. canadensis* or *E. nuttallii.*

Previous authors have suggested that early seasonal growth may be instrumental in the displacement process. If one species either starts growth earlier in the season, before competing species, or simply grows more rapidly at the start of the season, this species will have a competitive advantage. The RGR of *L. major* at 10°C was significantly higher than that of either *Elodea* spp. for Temperature Study 2. Field observations of this species also suggest that it does not die back significantly

over the winter period. Throughout the course of this study, *L. major* was observed to survive extremely stressful conditions without showing any visible die back. This will confer two advantages. Firstly, *L. major* will have a height advantage over competing species when conditions improve, i.e. the water temperature increases, or CO_2* or bicarbonate concentrations increase giving this species an advantage. Secondly, lack of die-back means that nutrients such as nitrogen and phosphorus incorporated into the plant structure are not lost as the plant rots and hence are retained for future growth, rather than released back into the environment to become available to potentially competing species. While the growth rates of both *E. canadensis* and *E. nuttallii* were similar at 10°C, the RGR of *E. nuttallii* was much higher than that of *E. canadensis* at 15°C. Thus, these species probably both start growing at the start of the growth season as the temperature approaches 10°C. However, with further increases in temperature, *E. nuttallii* growth increases more rapidly than that of *E. canadensis,* thus this could result in the subsequently elimination of *E. canadensis* through the local dominance of resources such as light, CO_2*, space and nutrients by *E. nuttallii*.

Starch reserves may be critical for supporting new growth during spring before the new shoots are able to attain positive net photosynthesis and, survival during periods of high stress. Results of starch analysis showed that the *Elodea* spp. had slightly greater starch concentrations per unit dry weight overall than *L. major*. However, it is likely that not only the total starch concentration is important, but also the distribution of starch within the plant. High reserves in close proximity to the growing tip may allow a species to survive short-term stresses. Thus in future work it would be important to determine the localised distribution of starch and also the degree of physiological integration in terms of support provided to a growing tip by the rest of the plant.

Differences in the observed responses of species to the different temperature regimes under nutrient-depleted and none nutrient-depleted conditions led to the hypothesis that nutrient status may play a critical role in determining the competitive success of a species. Indeed, *E. canadensis* was observed to grow much more successfully than *E. nuttallii* under nutrient-depleted conditions in Temperature Study 1. It was hypothesised that a species with a high relative growth rate and high

nutrient uptake capacity may more successfully compete with algae, particularly under highly eutrophic conditions. Thus, in high nutrient conditions, a species that can successfully strip nutrients from the surrounding water body, thus reducing availability to competing species and microphytes, may be considered to have a competitive advantage over other species. Results showed that the growth of the three species was reduced only slightly with increasing nutrient loadings. Although epiphytic growth was observed to be greatest on *E. canadensis* by the time of harvest, there was no evidence to suggest that the growth of this species was significantly reduced due to the epiphyte presence. Nitrogen and phosphorus concentrations per unit dry weight were, however, found to differ between species and to be greater in *E. canadensis* than in *E. nuttallii*, suggesting that *E. canadensis* may be more successful at removing nutrients from the surrounding water body. However, the slow growth rate of this species may result in only the slow depletion of nutrients from the medium. *E. nuttallii* may compensate for its lower nutrient requirement through a much higher growth rate, and may thus be more successful overall at stripping nutrients. *L. major* had the lowest N and P concentrations. Thus, this species may actually be a potent competitor with indigenous species in upland oligrophic water bodies, as it has also shown considerable tolerance of low temperatures. Its infrequency in these types of water body may simply be a consequence of the geographical isolation of such sites, resulting in its not yet having reached them. This conclusion seems at odds with the subtropical origins of the species, and exemplifies the plasticity of this species in its tolerance of a wide variation in environmental conditions, a factor that may contribute to its success.

Although actually introduced into the UK over 20 years prior to *E. nuttallii*, *L. major* is still far less common than the latter species. In the present study, both species were found able to establish and grow from extremely small shoot fragments, 5 mm in length, if attached to an intact shoot apex. One possible reason for the relatively slow spread of this species may however be the lack of fragmentation in *L. major*. It is a stronger, more robust species and was found to fragment less frequently than *E. nuttallii* in laboratory studies. Thus its potential for spread may be reduced by this characteristic. Furthermore, simply by being a heavier and larger species, its long distances distribution by agents such as birds and animals will be reduced compared to a lighter species. Conversely, the rapid spread of *E. nuttallii* was almost inevitable

in view of the high survival rate of even the smallest shoot lengths and its ready fragmentation and overall smaller, lighter nature.

The initial hypothesis presented in this thesis has similarities with the resource ratio hypothesis put forward by Tilman (1988), in which the differential ability of species to access different limiting resources is the principal driving force determining vegetative community structure. In Tilman's Hypothesis, competition for limiting resources is the most important factor driving species displacements. Evidence from the present study suggests that other factors are contributing to changes in vegetative community structure, such as life history traits and structural characteristics. This is in accordance with the triangular model proposed by Grime

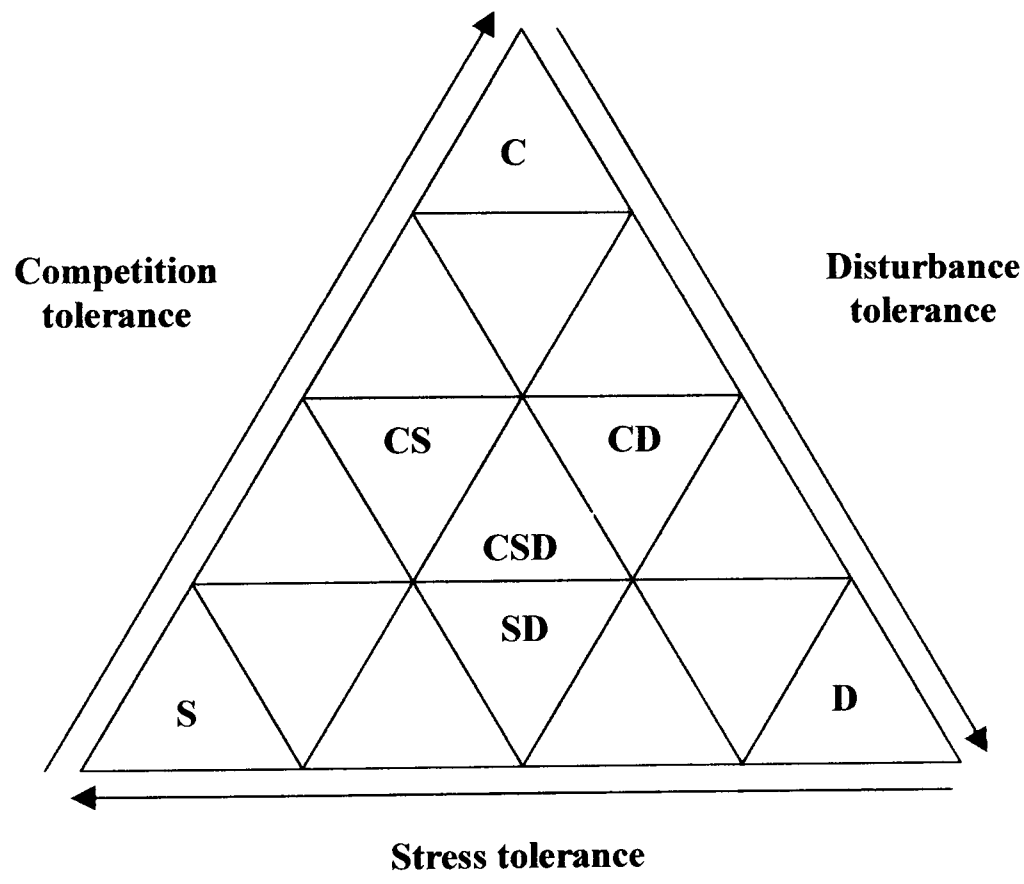


Fig. 9.1 Triangular model of Grime (1979) showing relationship between basic plant traits S (stress tolerance), D (disturbance tolerance) and C (Competition tolerance) and their intermediates.

(1979), in which competition between species is one of three important factors governing community structure, but is only paramount under high nutrient conditions. Grime (1979) defines three principal terrestrial plant types, competition

tolerators, stress tolerators and disturbance tolerators (or ruderals) (Fig. 9.1). These types are characterised by specific traits associated with terrestrial species. In Grime's definition, disturbance tolerance is characterised by specific plant traits that allow the species to survive in habitats that have a high risk of destruction; stress tolerance by traits that allow survival where essential resources may limit production, e.g. low light or low nutrients; and competition tolerance by traits that allow a species to capture resources in a high productivity environment. In view of the different limitations imposed upon species in an aquatic environment, compared to a terrestrial one, plant traits associated with Grime's original groupings are not necessarily those characteristics which may be deemed important in aquatic systems. For aquatic macrophytes, the definitions of these types have been modified by Murphy *et al.* (1990) and Rorslett (1989). This system has been used in grouping *E. canadensis, E. nuttallii* and *L. major* into species types (Table 9.1). *E. nuttallii* is defined principally as a competition tolerator, although it also exhibits many aspects of a disturbance tolerator [CD]. *E. canadensis* has a similar grouping [CD] which is in accordance with the previous classification of this species (Rorslett *et al.*, 1986; Murphy *et al.*, 1990; Abernethy *et al.*, 1996). However, in addition to being a competition tolerator, *L. major* also shows a number of characteristics of a stress tolerator [CDS].

Kautsky (1988) proposes a model similar to that of Grime (1979) but defines two types of stress tolerators in addition to competition and disturbance tolerators. These are species that will survive low disturbance and high stress (biomass storers) and those which occur under both high stress and high disturbance (stunted strategy). The stunted strategy is mainly associated with low growing forms such as rosette-forming species. In general, *L. major* has many attributes considered characteristic of the former plant group (biomass storer), being a clonal macrophyte with extensive lateral spread (in the case of *L. major* at the water surface), robust leaf form, long period of time in establishment, evergreen, a slow growth rate and a slow nutrient uptake and loss rate. At odds with this is collection of attributes is the lower starch concentrations found here for this species compared with the *Elodea* spp.. Nevertheless, this combination of attributes may allow *L. major* to tolerate high stress levels that may ultimately lead to the death of less tolerant species such as *E. canadensis* and *E. nuttallii*.

Results obtained here suggest that it is a combination of plant traits that contribute to the competitive success of a species. Thus it is suggested that, *E. nuttallii* successfully displaces *E. canadensis* as a result of having both:

- a much faster growth rate and hence denser canopy formation and thus the potential ability to shade out *E. canadensis*
- the ability to rapidly adapt to changing CO_2* and bicarbonate availability.

Thus, in the terms of Grime (1979), *E. nuttallii* is a competitive species with a rapid growth rate and can quickly gain an advantage when environmental conditions are conducive to growth. Although *E. canadensis* is similar in some respects, its slower growth rate will result in a slower response to the continuously changing conditions within and around a plant bed.

The success of *L. major* may be explained by this specie's high tolerance to stress. It has many of the traits of stress- and disturbance-tolerant species. While it does not exhibit as rapid a growth rate as *E. nuttallii*, it may nevertheless, through persistence and stress tolerance, ultimately gain an advantage. In addition, lack of significant over-wintering die back gives this species the significant advantage of a large established biomass ready to respond rapidly as temperature increases during spring and may largely contribute to the success of this species.

In defining the three species, *E. canadensis*, *E. nuttallii* and *L. major* in terms their growth strategies it is apparent that it is easy to differentiate between species that exhibit large differences in growth form, i.e. a rosette forming species versus a canopy forming species. However, they are not able to distinguish well between species of similar growth forms, such as the species studied here, as differences between these species are more subtle. It is the relative differences between the species that is actually important in this study. Thus, when one uses the life history characteristics to define the species in terms of their functional groupings, it may be useful to incorporate a simple grading system, as shown in Table 9.1, to express the relative differences between the species within individual characteristics such as the

Table 9.1

Characteristics of the three species in terms of C, S and D strategies modified from Rorslett (1989) and Murphy *et al.* (1990). The species are also defined as to the degree to which they exhibit a particular characteristic, i.e. 3 = greatly, 2 = moderately, 1 = slightly.

*** In overwintering turions**

Strategy	Characteristic	*E. canadensis*	*E. nuttallii*	*L. major*
Competition tolerance (C)	Large peak biomass	2	3	3
	HCO_3- normal C-source for photosynthesis	3	3	3
	CO_2 from air (Floating or emergent foliage)			
	Canopy forming (rapidly elevating monolayer of foliage at or below surface)	2	3	3
	Winter-annual/short lived perennial	3	3	1
	Fast biomass turnover	3	3	
	Low root:shoot ratio	3	3	2
Stress tolerance (S)	CAM-metabolism/CO_2 from water or sediment normal C-source for photosynthesis			
	High root:shoot ratio			
	Perennial or evergreen habit			2
	Starch storage potential	1*		
	Slow biomass turnover			2
	Tolerant of reduced light availability			2
Disturbance tolerators (D)	Vegetative (clonal) reproduction	3	3	3
	Extensive bud or propagule formation	3	3	2
	Fast growth/early reproduction	1	2	2
	Annual, vigorous seed production			

ability to use bicarbonate, or a large peak biomass. This will provide a clearer picture of the relative differences between species with such similar growth forms.

Recommendations for future experimental work

The present study has highlighted the need for further research in some areas. Of benefit would be further studies on plant morphology, with particular emphasis on factors such as light quality and intensity. As morphology, and in particular the ability to form a dense canopy, appears to be important in competition, further competition studies are needed to clarify this. In the current study the containers used may have been too shallow to allow development of a canopy. Future studies using deeper containers to allow species to develop different growth forms.

Another important aspect is the experimental set up. Particular problems are encountered when working with aquatic macrophytes. These mainly involve difficulties in replication under controlled environmental conditions. Due to the necessity of having large volumes of water for each treatment replicate, experiments are often restricted to only a few treatments. Thus, a simple, but interpretable experimental design is paramount if results are to be meaningful. In a future competition design, an expanded version of Competition Experiment 2 design would be recommended with growth of species at least three mixture densities. Spitters (1983) suggests an easily interpretable model for quantification of the effects of intra- and inter- specific competitive effects, and this would be recommend for analysis of the data.

Another feature of the studies, particularly during the comparison of photosynthetic and respiratory rates was the innate physiological variability exhibited by the species. Physiological rates appear dependent upon growth conditions prior to measurements. Thus plant material of different species collected from the field, where a prior growth history is not known, can only very tentatively be compared. It would be recommended that for future studies comparing photosynthetic and respiratory rates of different species, plants are grown under similar controlled environmental conditions for comparative purposes.

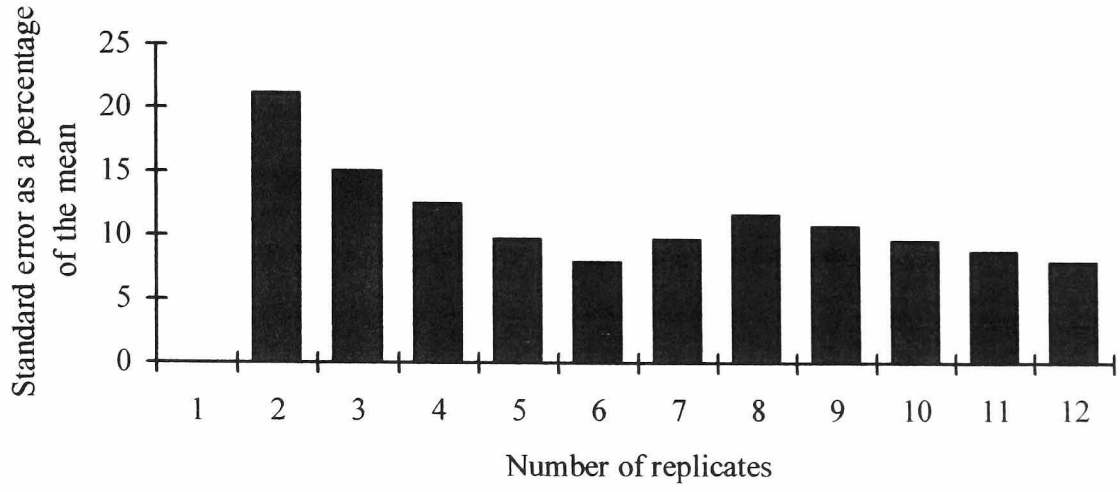
Appendix I

Chemical characteristics of tap water. Values in brackets are 95 % confidence limits.

Conductivity (μS)	210 (n=3)
Alkalinity (meq l^{-1})	0.6 (n=3)
Total CO_2 (g m^{-3})	0.75
CO_2* (g m^{-3})	0.15
HCO_3- (g m^{-3})	0.60
CO_3^2 (g m^{-3})	$2.62 * 10^{-4}$
pH	7.02
Soluble Reactive Phosphorus (PO_4 - P)	558.51 (9.59) μg l^{-1} (n = 5)
Ammonium (NH_4 - N)	18.78 (14.9) μg l^{-1} (n = 5)
Nitrate (NO_3 - N)	1.59 (0.16) mg l^{-1} (n = 3)

Appendix II

(a)



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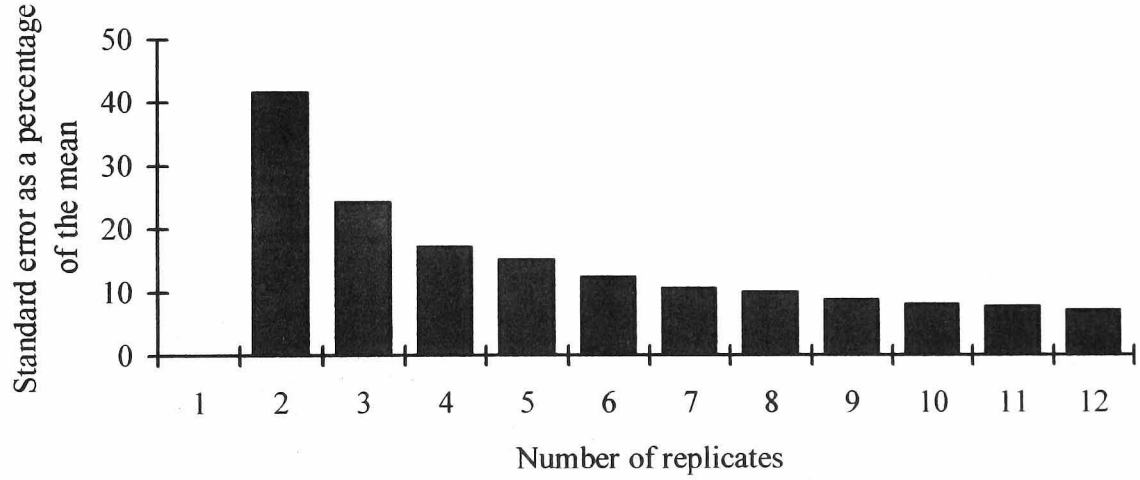

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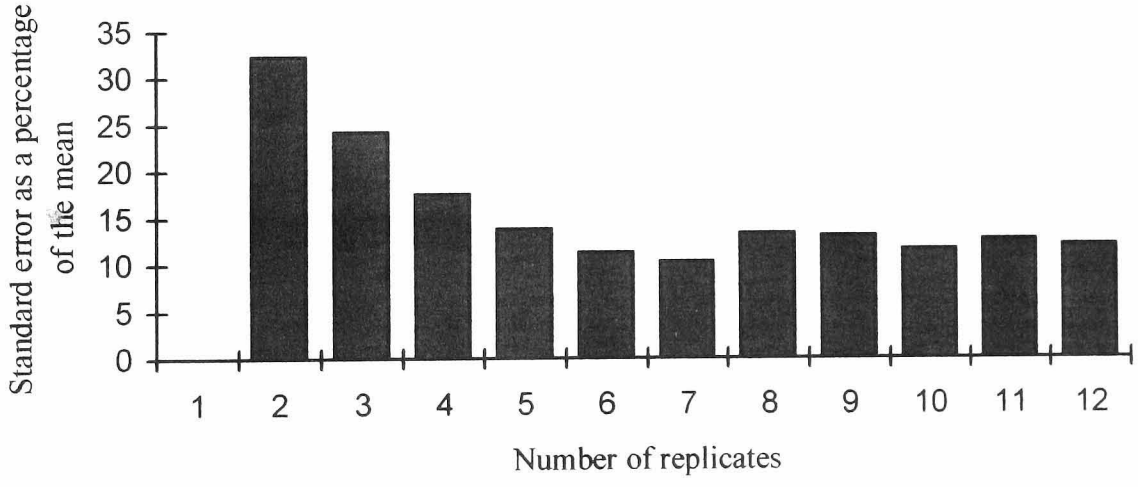


Fig. 1 Change in the standard error of net photosynthesis of a) *E. nuttallii*, b) *E. canadensis*, and c) *L. major*, as a percentage of the mean with increasing replication.

Appendix II

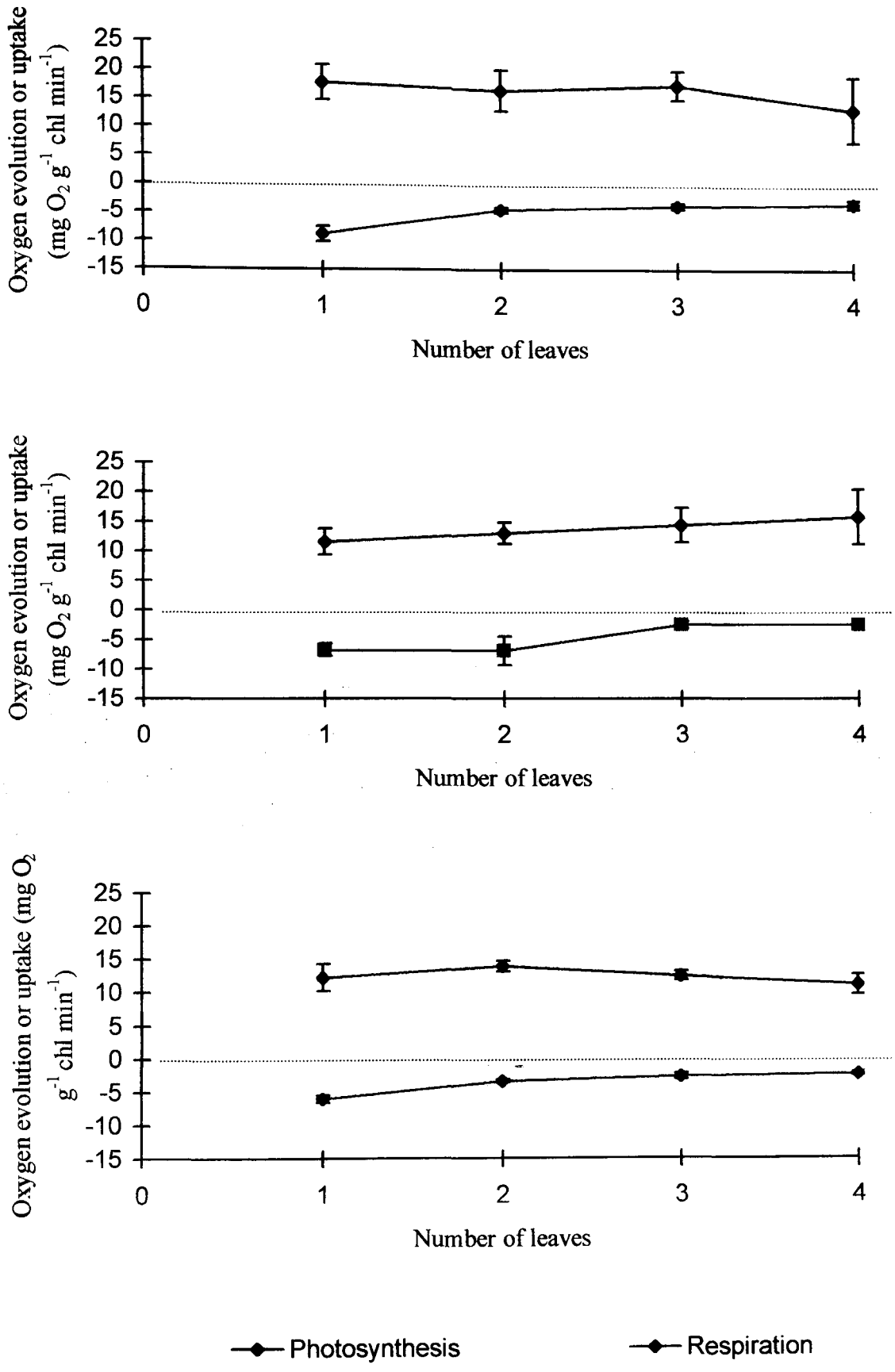


Fig. 2 The effect of leaf number upon net photosynthetic and respiratory rates of a) *E. nuttallii*, b) *E. canadensis*, and c) *L. major*.

Appendix II

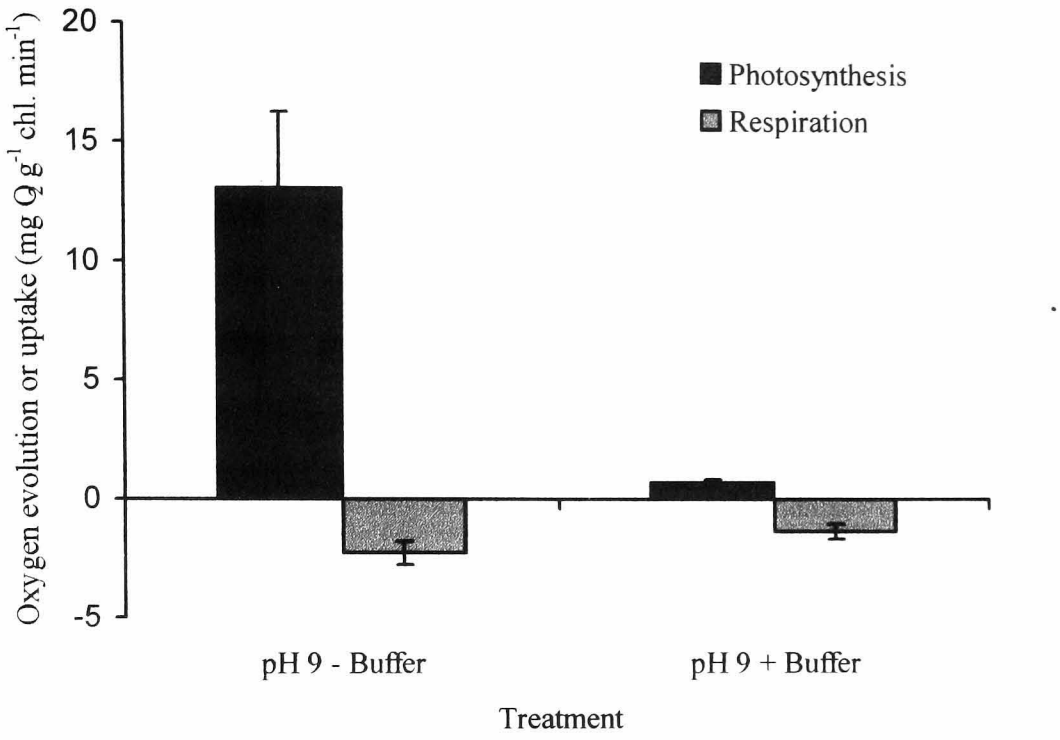


Fig. 3 Effects of buffer on photosynthesis and respiration of *E. canadensis* .Error bars represent 95% confidence limits.

Appendix III

Starch analysis

Introduction

Plant carbohydrates can be divided into two main groups: the carbohydrates whose functions are mainly structural or storage and the simpler sugars generally involved in plant metabolic processes. Many analytical methods are available for the proximate analysis of the carbohydrate fractions. For the analysis of simple sugars a simple cold water extraction can be used as all mono-, di- and tri-saccharid are soluble in water (Allen, 1989). If a hot water extraction is used there may be some hydrolysis of polysaccharides. Allen (1989) states that when extraction takes place at temperatures of 60 ^{o}C and above, a substantial amount of polysaccharides may enter the solution. It may be therefore preferable to use an aqueous ethanol extraction technique as this minimises the possibility of polysaccharide hydrolysis during the extraction.

For the measurement of more complex carbohydrates such as starch the analysis is difficult due to the non-specificity of most available techniques. The anthrone method, commonly used for carbohydrate analysis, is based on a qualitative method first described by Dreywood (1946). Anthrone and carbohydrate react in a strong sulphuric acid solution to produce a green colour that can then be measured spectrophotometrically. In this method polysaccharides present are hydrolysed to monosaccharides during heating. Monosaccharides present from sources other than storage carbohydrate such as metabolic sugars will also be included in the analysis. The anthrone method is however preferable over other methods such as the phenol method of Dubois *et al.* (1956) as little interference is caused by the presence of other substances such as fatty acids and proteins (Gaudy, 1962). However, Koehler (1952) found that different carbohydrates produced different colour intensities. Furthermore, the time needed for maximum colour development differs between carbohydrate fractions. The pentoses and ketohexose type carbohydrates develop maximum colour in a relatively short period of time, 1-2 minutes, while the aldohexose types, particularly glucose, developed maximum colour intensity after 5 to 10 minutes. As the majority of sugars present will be in the form of glucose and glucose derivatives,

most authors suggest an incubation period of between 7.5 and 15 minutes. The temperature at which anthrone and carbohydrate react will also have a significant effect upon final colour. For this study a temperature of 100 ^{o}C was used as the colour development due to glucose is weaker at lower temperatures (Allen, 1989; Koehler, 1952).

Analysis of simple sugars and more complex carbohydrates separately may aid interpretation of results. McCready *et al.* (1950) described a technique for starch analysis in which soluble sugars are first removed with an alcohol before the analysis of the remaining residue. Other simpler procedures involving the heating of samples in water or ethanol may provide a quicker and easier method for the proximate analysis of carbohydrates. The following three comparisons were made for the extraction and measurement of soluble carbohydrates:

(a) Extraction of total soluble carbohydrate using method described by McCready *et al.* (1950).
(b) Extraction of total soluble carbohydrates as by McCready *et al.* (1950), except using ethanol for the extraction of soluble carbohydrates.
(c) Extraction of total soluble carbohydrate using a simple method based on that described by Allen (1989).

Total soluble carbohydrate was extracted using both water and ethanol to assess the possible advantage of ethanol in reducing starch hydrolysis. Following the extraction of soluble carbohydrate, the remaining residue was analysed for starch following the method of McCready *et al.* (1950).

General Methods for the preparation of plant material

For routine analysis, plant material dried at 40 ^{o}C was used. It must be noted however that during the initial stages of drying some enzymatic changes may take place (Allen, 1989). The plant sample was homogenised using a Glen Creston grinder to particle size of less than 1 mm. Unless otherwise stated all parts of the plant except

roots were homogenised together. For preliminary analysis, dried material of *E. nuttallii* was used. A large sample of the plant was mixed and ground together to ensure a homogenous sample for comparison between methods.

Extraction of soluble carbohydrates

In the following method, soluble carbohydrates are extracted using water. As a slight adaptation to this method, ethanol was also used to extract soluble sugars. For further details see McCready *et al.* (1950).

1. 50 mg of dried plant material were measured out
2. 30 ml of water or ethanol were added to the sample and allowed to simmer for 2 hours
3. For measurement of soluble carbohydrate the supernatant was removed and made up to 50 ml in a volumetric flask. This was then analysed following the anthrone method described in Section 1.5.

Starch

Following the extraction of the soluble carbohydrate, the remaining plant residue were solubilised in perchloric acid as described by McCready *et al.* (1950).

1. 5 ml of ice cold water followed by 6.5 ml of 52% perchloric acid were added to the plant residue following the extraction of the soluble sugars. This was stirred rapidly for 5 minutes, and then intermittently for 15 minutes.
2. 20 mls of ice water were then added and the sample centrifuged at 3000 RPM for 5 minutes.
3. The supernatant was removed carefully so as not to disturb the remaining residue and placed in a 100 ml conical flask.
4. The above procedure was then repeated on the remaining residue and place in conical flask containing the first extract.
5. The sample was then made up to 100 ml using distilled water.
6. This sample was then analysed following the anthrone method described below.

The anthrone method for determination of glucose cocentrations

Anthrone reagent: 2 g anthrone / 1 l 70% sulphuric acid (Solution must be replaced every couple of days)

A calibration curve was formed using dilutions of standard glucose solution (1g glucose in 1 litre of either water or ethanol depending upon the method of extraction). For starch analysis, perchloric acid was added to the calibration in quantities equivalent to that in the unknown samples. Following the extraction procedure for either soluble carbohydrate or starch, 2 ml of the unknown sample or calibration standard was pipetted into a boiling tube. 10 ml of anthrone reagent was then carefully added, prepared as described above, to the sample and mixed vigorously in an ice bath. The samples were then heated to 100 ^{o}C in a water bath for 10 minutes. To aid colour development this reaction took place in the dark. Following cooling, the samples were measured spectrophotometrically at 625 nm.

Calculation of soluble carbohydrate:

$$\text{Soluble Carbohydrate}\,(\%) = \frac{\text{C(mg)} * \text{extract volume (ml)}}{10 * \text{aliquot (ml)} * \text{sample weight (g)}}$$

$$\text{Starch}\,(\%) = \frac{\text{C}\,(\bar{\text{m}}\text{g}) * \text{extract volume (ml)}}{10 * \text{aliquote (ml)} * \text{sample weight (g)}}$$

Where C = mg glucose

Table 1.

A comparison of different methods for the extraction of soluble carbohydrates and starch from dried plant material. Error is expressed in brackets as 95% confidence limits.

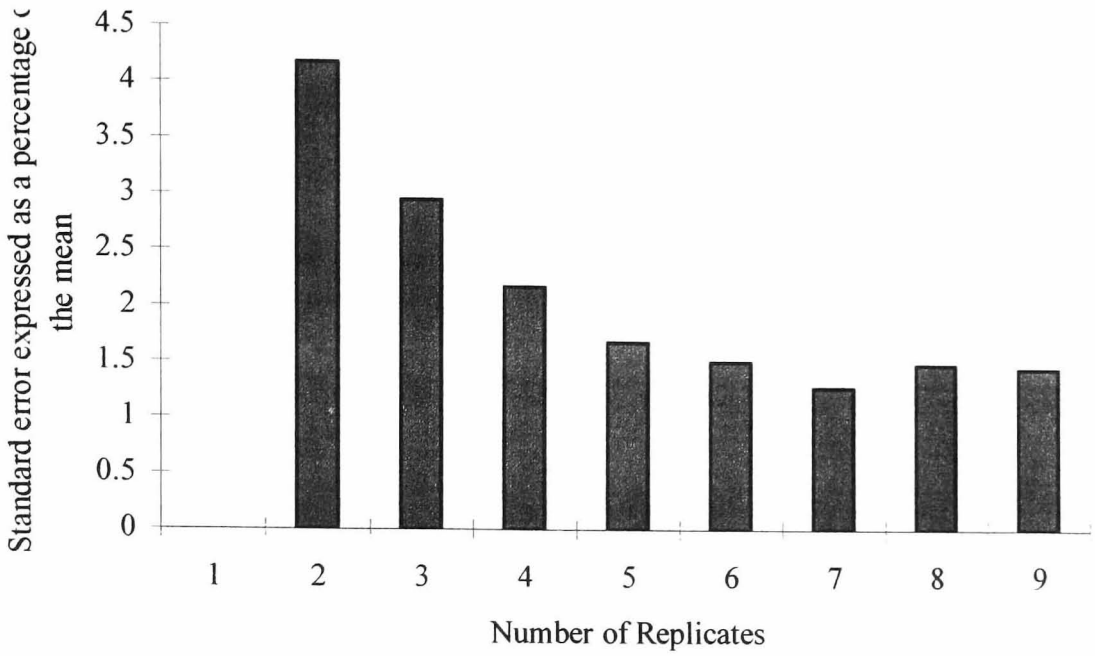
Method of extracting soluble carbohydrates	Amount of dried material used for analysis (g)	Soluble carbohydrate (mg g^{-1} dry weight)	Starch (mg g^{-1} dry weight)	Total carbohydrate (mg g^{-1} dry weight)	Starch as a % of soluble carbohydrate	Reference
Ethanol	0.200	35.24 (1.23)	10.91 (0.80)	46.15 (1.54)	30.99 (2.44)	Mc Cready (1950)
Water	0.05	44.13 (1.85)	7.14 (1.55)	51.27 (2.47)	16.20 (3.51)	Allen (1989)
Ethanol	0.05	27.76 (2.02)	12.01 (2.26)	39.77 (1.71)	40.06 (9.69)	Based on the method of Allen (1989)

Table 2.

Soluble carbohydrate content of dry plant material following use of glucose standards for calibration with three differing reaction times of 2, 10 and 15 minutes.

Time (minutes)	Glucose (mg g^{-1} dry weight)	95 % confidence limits
2	66.24	2.28
10	32.31	0.52
15	31.26	0.49

(a)



(b)

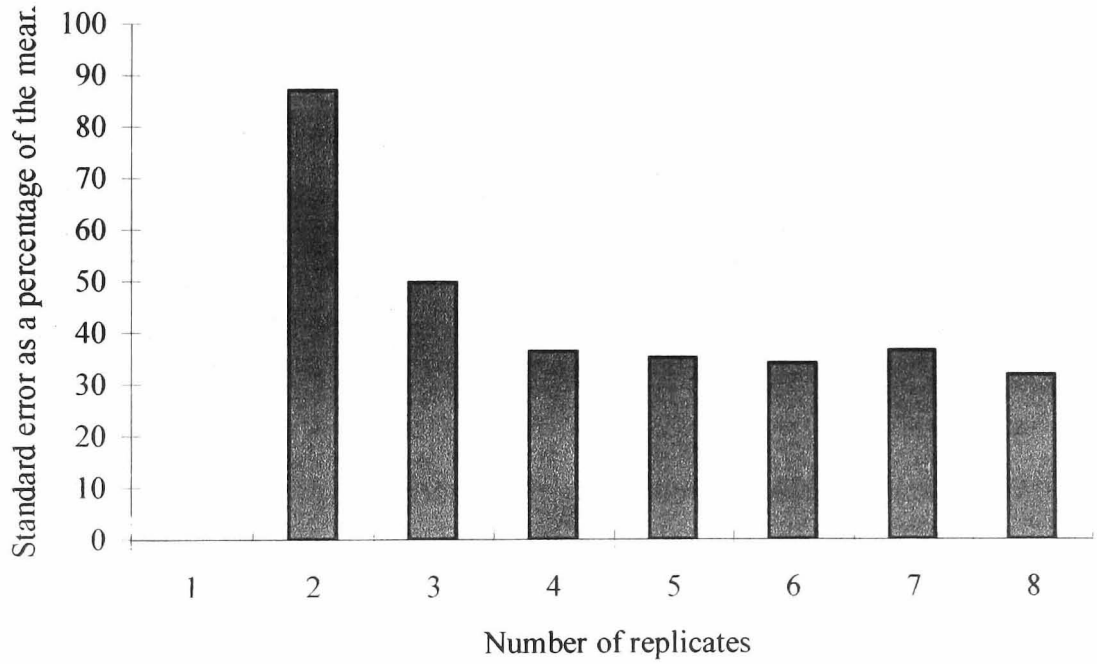


Fig. 1 Standard error expressed as a percentage of the mean for (a) Soluble carbohydrate and (b) starch.

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carbohydrate results was very low. However, results of the starch analysis were extremely variable. It was decided that at least 4 replicates were necessary in the determinations of soluble carbohydrate and starch as this reduces error of starch analysis to below 40 % of the mean.

From the above results a standard procedure for the analysis of soluble carbohydrate and starch was developed as follows:

1. 100 to 200 mg of plant material dried at 40°C was measure out into boiling tubes.
2. 30 ml of 80% ethanol was added and allowed to simmer for two hours at 85°C.
3. The samples were then centrifuge for 5 minutes at 3000 RPM.
4. The supernatant was removed and analysed for soluble carbohydrate if required using the anthrone method (see previous description)
5. To the remaining plant residue, the procedure described in section 1.4 was followed, in which starch was first solubilised in perchloric acid (see previous description) and then reacted with anthrone reagent.

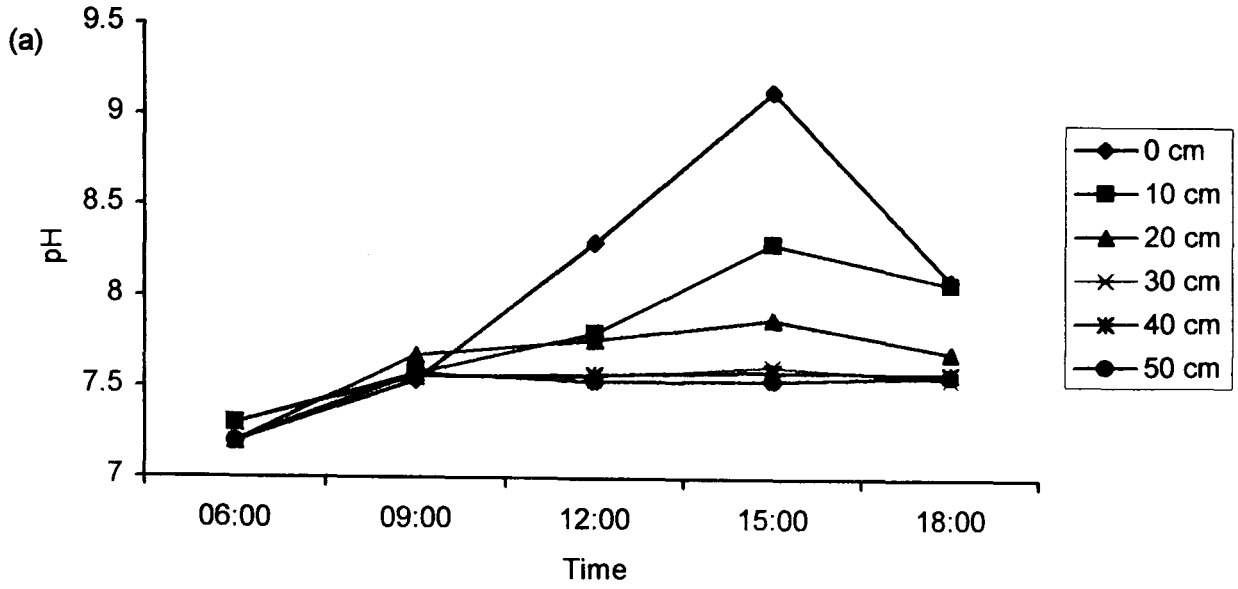
Appendix IV

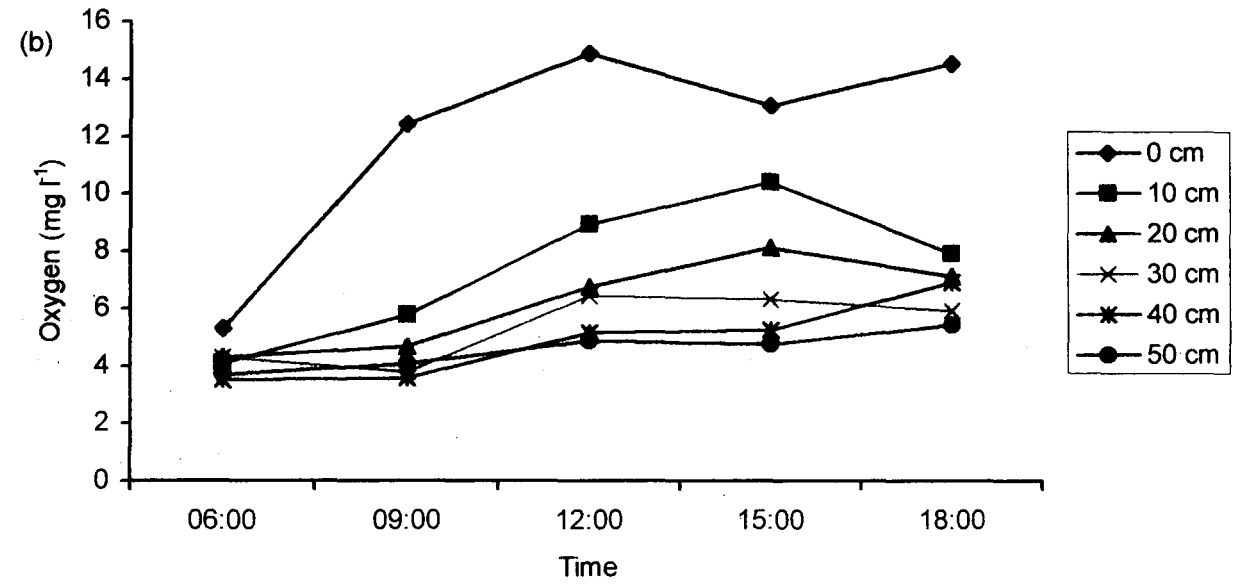
Measurements of vertical profile structure in field populations of *E. nuttallii*

Dense stands of *E. nuttallii* in the Rufford branch of the Leeds and Liverpool canal (SD 460 171) were studied on 18th August 1997, an day with air temperatures in excess of 25 °C and virtually no cloud cover. Vertical profiles of pH (pH Boy-P2, Camlab, Cambridge), O_2 (OXI 126, WTW, Wielheim, Germany) and temperature were made at depths of 0, 10, 20, 30, 40 and 50 cm at 5:00 h, 8:00 h, 11:00 h, 14:00 h and 17:00 h Greenwich Mean Time. In addition a profile was also taken from an adjacent area of open water away from plant stands at 14:30 GMT, a time when it was assumed that any gradients would be close to their day time maximum (Jones, Hardwick and Eaton, 1996). There were no boat movements through this section of the canal during the recordings.

Discussion

The vertical profile was successful in showing the changes that can occur within a dense plant stand, i.e. high pH (and therefore low CO_2* concentrations), temperature and oxygen concentrations. The evidence presented here confirms that *E. nuttallii* stands can result in the development of highly stressful conditions in a similar manner to that reported by Jones *et al.* (1996). These measurements were only made on *E. nuttallii* as dense stands of both *L. major* and *E. canadensis* are relatively rare in canal situations in this country. For comparative measurements to be made between species it would be necessary to have all three species growing in dense clumps at the same field site to avoid confounding the different effects of the species with site differences, a situation which is not known by the present author to occur. As stated during the main thesis. *E. canadensis* occurs rarely now in English canals, and at those sites at which it does occur, it is rarely present in abundance (D. Hatcher, personal communication).





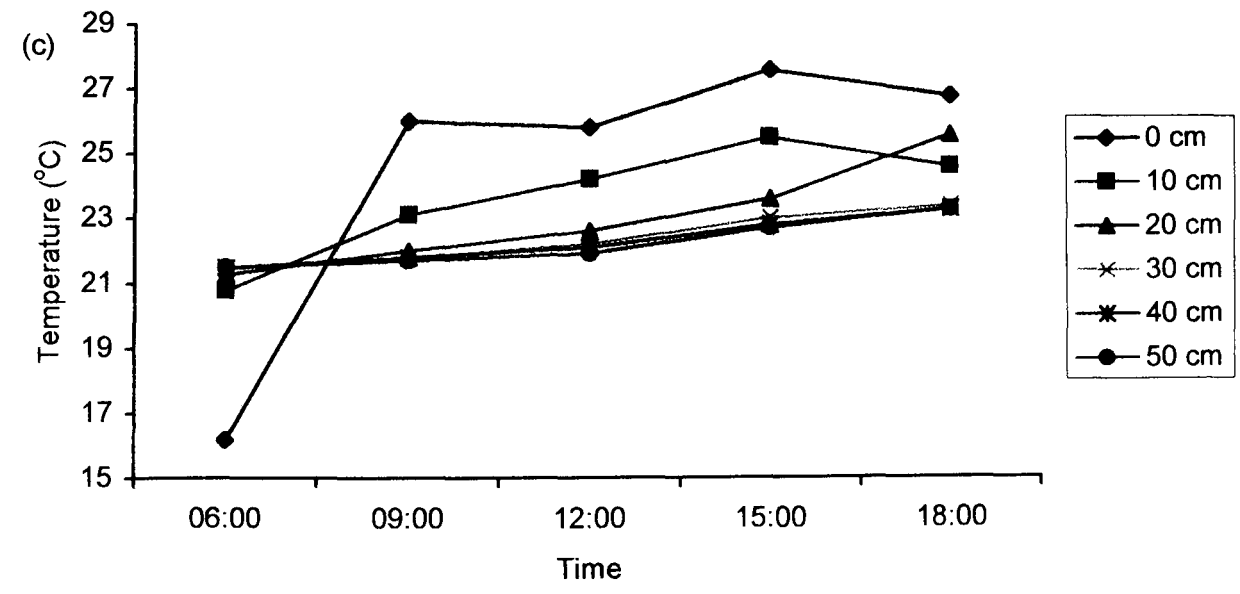
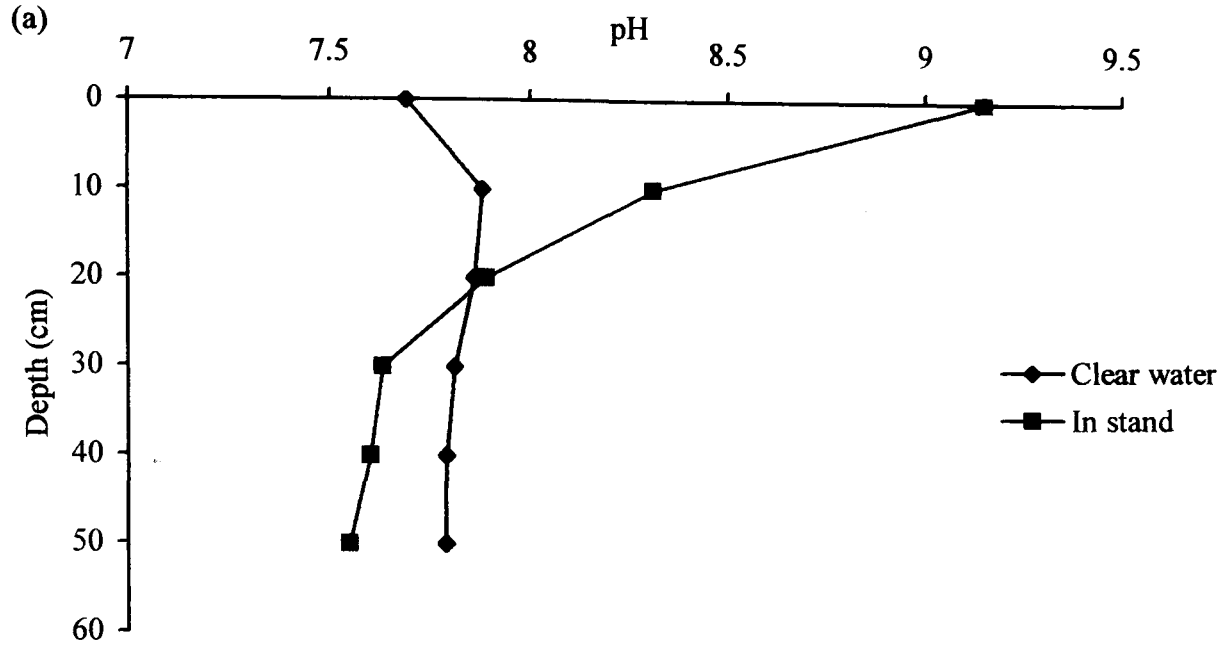


Fig. 1 Vertical profile measurements of water quality (a) pH, (b) oxygen concentration and, (c) temperature, made in a dense weed stand of *E. nuttallii* on the Rufford Branch of the Leeds and Liverpool Canal.



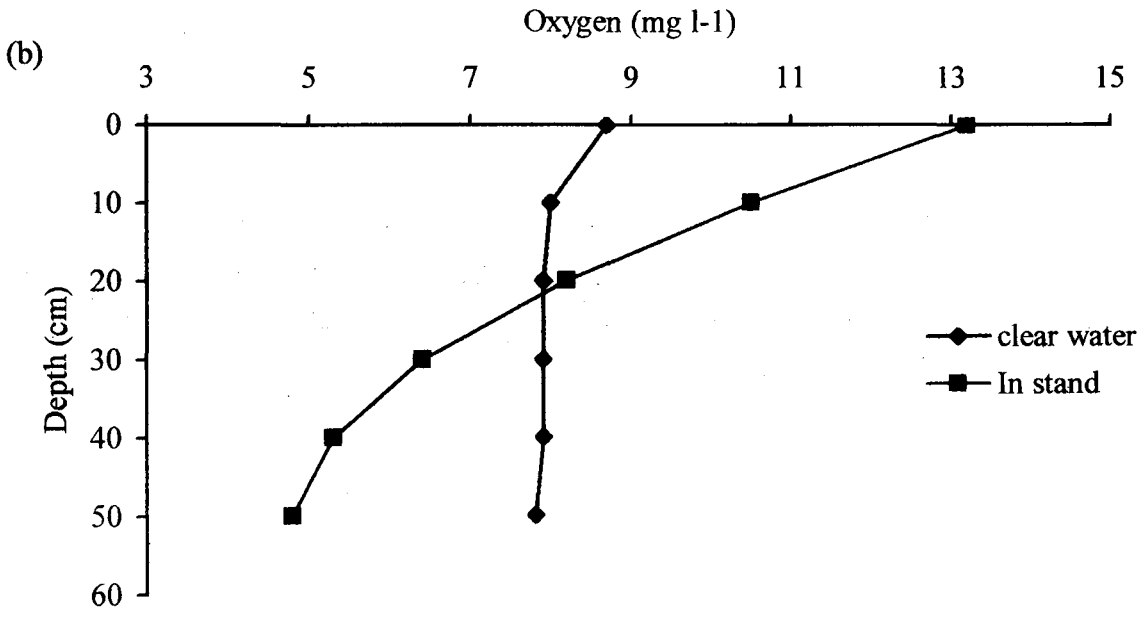


Fig. 2 showing comparison of vertical profile measurements of water quality (a) pH and (b) oxygen concentration a made in a dense stand of *E. nuttallii* and in clear water.

Appendix V

Preliminary measurements of solute leakage

Preliminary measurements of solute leakage into tap water following freezing and chilling temperature treatments are given below. For all treatments with plants, 10 g fresh weight of *E. nuttallii* or *L. major* were placed in 400 ml plastic pots with 300 ml tap water. Control treatments were also run without plants. There were Three replicates of each treatment. Conductivity was measured at the start of each treatment when the tap water medium was at a temperature of 17.7 °C. Plants were then subjected to the stated temperature treatments for 24 hours. Following the cold treatments, the replicates were allowed to warm up to a temperature of 17.5 °C before the conductivity readings were taken again.

Species/ control	Initial cond. (μS)	Temperature treatment (°C)	Final cond. (μS)
Control	213	13.5	232
E. nuttallii	230	13.5	263
L. major	215	13.5	228
Control	217	4.5	157
E. nuttallii	220	4.5	157
L. major	217	4.5	152
Control	227	-20	234
E. nuttallii	230	-20	573
L. major	215	-20	515

Appendix VI

An example is given of continuous monitoring of pH during Competition Experiment 1. pH was monitored using pH electrodes attached to a Data logger (Phillip Harris Scientific, Manchester) and recorded using Data Pro software (Phillip Harris Scientfic, Manchester). The electrode was clamped with the tip 2 cm below the water surface in the maximum biomass cultures (4 plants per container). Measurements were started 4 days after the experiments were set up. *E. nuttallii* and *L. major* recordings were made simultaneously during part 1 of Competition Experiment 1, and *E. canadensis* readings were made during part 2 of Competition Experiment 1.

Appendix VI

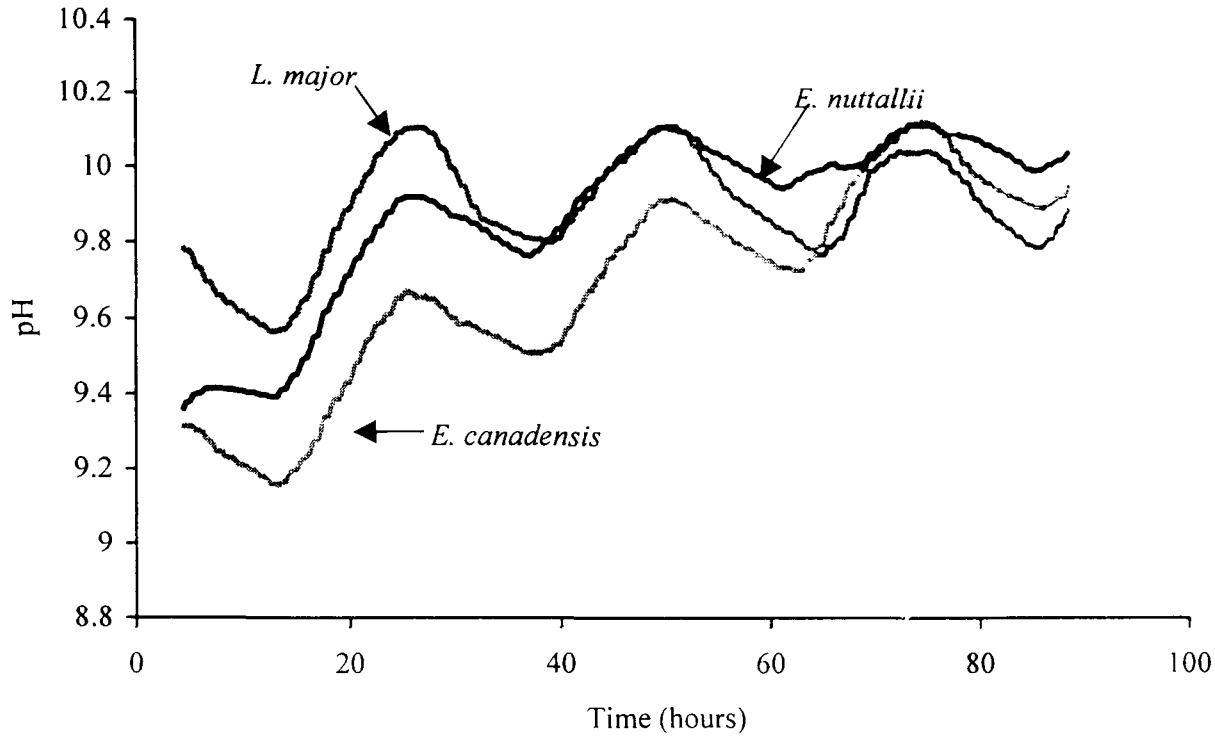


Fig. 1 Graph showing examples of diurnal monitoring of pH shortly after set up of cultures for Competition Experiment 1.

REFERENCES

Abernethy, V. J., M. R. Sabbatini & K. J. Murphy. (1996) Response of *Elodea canadensis* Michx. and *Myriophyllum spicatum* L. to shade, cutting and competition in experimental culture. *Hydrobiologia.* **340;** 219-224.

Adamec, L. (1993). Rapid inhibition of HCO_3^- use by high concentrations of free CO_2 in *Elodea canadensis*. *Aquatic Botany.* **45:** 311-324.

Adams, M. S., P. Guilizzoni & S. Adams. (1978). Relationship of dissolved inorganic carbon to macrophyte photosynthesis in some Italian lakes. *Limnology and Oceanography.* **23 (5):** 912-919.

Agami, M. & K. R. Reddy. (1989). Inter-relationships between *Salvinia rotundifolia* and *Spirodela polyrhiza* at various interaction stages. *Journal of Aquatic Plant Management.* **27:** 97-102.

Allen, E. D. & D. H. N. Spence. (1981). The differential ability of aquatic plants to utilize the inorganic carbon supply in fresh waters. *New Phytologist.* **87:** 269-283.

Allen, S. E. (1989). *Chemical analysis of ecological materials*, 2nd Edition. Blackwell Scientific Publications, Oxford.

Ancibor, E. (1979). Systematic anatomy of vegetative organs of the *Hydrocharitaceae*. *Botanical Journal of the Linnean Society.* **78:** 237-266.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physiologist.* **24:** 1-15.

Ashton, F. M. & S. R. Bissell. (1987). Influence of temperature and light on dwarf spike-rush and slender spike-rush growth. *Journal of Aquatic Plant Management.* **25:** 4-7.

Bain, J. T. & M. C. F. Proctor. (1980). The requirement of aquatic bryophytes for free CO_2 as an inorganic carbon source: some experimental evidence. *New Phytologist.* **86:** 393-400.

Balls, H., B. Moss & K. Irvine. (1989). The loss of submerged plants with eutrophication. I. Experimental design, water chemistry, aquatic plant and phytoplankton biomass in experiments carried out in ponds in the Norfolk Broadlands. *Freshwater Biology.* **22:** 71-87.

Barker, S. B., G. Cumming & K. Horsfield. (1973). Quantitative morphometry of the branching structure of trees. *Journal of Theoretical Biology.* **40:** 33-43.

Barko, J. W., D. Gunnison & S. K. Carpenter. (1991). Sediment interactions with submersed macrophyte growth and community dynamics. *Aquatic Botany.* **41:** 41--65.

Barko, J. W., D. G. Hardin & M. S. Mathews. (1982). Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. *Canadian Journal of Botany.* **60:** 877-887.

Barko, J. W. & R. M. Smart. (1981). Sediment-based nutrition of submersed macrophytes. *Aquatic Botany.* **10:** 339-352.

Barko, J. W. & R. M. Smart. (1982). Comparative influences of light and temperature on the growth and metabolism of selected submersed fresh-water macrophytes. *Ecological Monographs.* **51:** 219-235.

Beer, S. & R. G. Wetzel. (1981). Photosynthetic carbon metabolism in the submerged aquatic angiosperm *Scirpus subterminalis*. *Plant Science Letters.* **21(3):** 199-207.

Belcher, H. & E. Swale. (1990). The South African water plant, *Lagarosiphon major* (Ridley) Moss and its occurrence in Cambridgeshire. *Nature in Cambridgeshire.* **no. 32:** 63-66.

Bell, A. D. (1986). The simulation of branching patterns in modular organisms. *Philisophical Transactions of the Royal Society of London Series B.* **B 313:** 143-159.

Bell, A. D. & A. Bryan. (1991). *Plant Form. An illustrated guide to Flowering Plant Morphology*. Oxford University Press Inc., New York.

Best, E. P. (1977). Seasonal changes in mineral and organic components of *Ceratophyllum demersum* and *Elodea canadensis*. *Aquatic Botany.* **3:** 337-348.

Best, E. P. H. & J. H. A. Dassen. (1987). A seasonal study of the growth characteristics and the levels of charbohydrate and proteins in *Elodea nuttallii*, *Polygonium amphibium* and *Phragmites australis*. *Aquatic Botany.* **28:** 353-372.

Best, E. P. H. & A. K. v. d. Werf. (1986). Respiration in relation to reserve substances in the submerged macrophyte *Ceratophyllum demersum* L. *Aquatic Botany.* **26:** 235-246.

Bhattacharyya, G. K. & R. A. Johnson. (1977). *Statistical concepts and methods*. John Wiley & Sons Ltd, Singapore.

Birch, S. P. (1990). An assessment of the interactions between *Elodea nuttallii* and its epiphyton. Ph.D thesis, University of Liverpool.

Black, M. A., S. C. Maberly & D. H. N. Spence. (1981). Resistances to carbon dioxide in four freshwater macrophytes. *New Phytologist.* **89:** 557-568.

Boston, H. L. & M. S. Adams. (1983). Evidence of crassulacean acid metabolism in two North American isoetids. *Aquatic Botany.* **15:** 381-386.

Bowes, G. (1985). Pathways of CO_2 fixation by aquatic organisms. In *Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms.* (ed. W. J. Lucas and J. A. Berry), American Society of Plant Physiologists, Rockville, MD. pp. 187-210.

Bowes, G. (1987). Aquatic plant photosynthesis: strategies that enhance carbon gain. In *Plant life in aquatic and amphibious habitats* (ed. R. M. M. Crawford), pp. 79-98. Blackwell Scientific Publications, Oxford.

Bowes, G. & M. E. Salvucci. (1989). Plasticity in the photosynthetic carbon metabolism of submersed aquatic macrophytes. *Aquatic Botany.* **34:** 233-266.

Bowmer, K. H., D. S. Mitchell & D. L. Short. (1984). Biology of *Elodea canadensis* Mich. and its management in Australian irrigation systems. *Aquatic Botany.* **18:** 231-238.

Briggs, M. (1977). *Elodea nuttallii. B. S. B. I News.* **15:** 9-10.

Brown, J. M. A., F. I. Dromgoole, M. W. Towsey & J. Browse. (1974). Photosynthesis and respiration in aquatic macrophytes. In *Mechanisms of Regulation of Plant Growth* (ed. R. L. Bieleski, A. R. Ferguson and M. M. Cresswell), pp. 243-249. The Royal Society of New Zealand, Wellington, New Zealand.

Browse, J. A., F. I. Dromgoole & J. M. A. Brown. (1977). Photosynthesis in the aquatic macrophyte, *Egeria densa.* I. $^{14}CO_2$ fixation in natural CO_2 concentrations. *Australian Journal of Plant Physiology.* **4:** 169-176.

Buscemi, P. L. (1958). Littoral oxygen depletion produced by a cover of *Elodea canadensis. Oikos.* **9(11):** 239-245.

Campbell, B. D. & J. P. Grime. (1992). An experimental test of plant strategy theory. *Ecology.* **73(1):** 15-29.

Carignan, R. (1980). Phosphorus sources for aquatic weeds: water or sediment? *Science.* **207:** 987-988.

Carignan, R. (1982). An empirical-model to estimate the relative importance of roots in phosphorus uptake by aquatic macrophytes. *Canadian Journal of Fisheries and Aquatic Sciences.* **39:** 243-247.

Carignan, R. & J. Kalff. (1982). Phosphorus release by submerged macrophytes: significance to epiphyton and phytoplankton. *Limnology and Oceanography.* **27:** 419-427.

Catling, P. M. & W. Wojtas. (1985). The waterweeds (*Elodea* and *Egeria*, Hydrocharitaceae) in Canada. *Canadian Journal of Botany.* **64:** 1525-1541.

Chambers, P. A. (1987). Light and nutrients in the control of aquatic plant community structure II. In situ observations. *Journal of Ecology.* **75:** 621-628.

Chambers, P. A. & J. Kalff. (1987). Light and nutrients in the control of aquatic plant community structure. I. In situ experiments. *Journal of Ecology.* **75:** 611-619.

Chaney, A. L. & E. P. Morbach. (1962). Modified reagents for the determination of urea and ammonia. *Clinical Chemistry.* **8:** 130-132.

Chapman, V. J., J. M. Brown, C. F. Hill & J. L. Carr. (1974). Biology of excessive weed growth in the hydro-electric lakes of the Waikato river, New Zealand. *Hydrobiologia.* **44:** 349-363.

Clayton, J. S. (1982). Effects of fluctuations in water level and growth of *Lagarosiphon major* on the aquatic vascular plants in Lake Rotorua, 1973-80. *New Zealand Journal of Marine and Freshwater Research.* **16:** 89-94.

Coffey, B. T. (1975). Macrophyte distribution in the Waikato lakes. In *New Zealand Lakes* (ed. V. H. Jolly and J. M. A. Brown), pp. 263-270. Auckland/Oxford University Press, Wellington, New Zealand.

Connolly, J. (1986). On difficulties with replacement-series methodology in mixture experiments. *Journal of Applied Ecology.* **23:** 125-137.

Cook, C. D. (1990). *Aquatic Plant Book.* SPB Academic Publishing, The Hague.

Cook, C. D. K. & K. Urmi-Konig. (1985). Revision of the genus *Elodea* (Hydrocharitaceae). *Aquatic Botany.* **21:** 111-156.

Davison, J. R. (1987). Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to changes in growth temperature. *Journal of Phycology.* **23:** 273-283.

de Wit, C. T. (1960). On competition. Institute for Biological and Chemical Research on Field Crops and Herbage, Wageningen.

DeGroote, D. & R. A. Kennedy. (1977). Photosynthesis in *Elodea canadensis* Michx. Four-carbon acid synthesis. *Plant Physiology.* **59:** 1133-1135.

Dendene, M. A., T. Rolland, M. Tremolieres & R. Carbiener. (1993). Effect of ammonium ions on the net photosynthesis of three species of *Elodea*. *Aquatic Botany.* **46:** 301-315.

Denny, P. (1980). Solute movement in submerged angiosperms. *Biological Review.* **55:** 65-92.

Dreywood, R. (1946). Qualitative test for carbohydrate material. *Ind. Endgn. Chem. Analyt.* **18:** 499.

Duarte, C. M. & J. Kalff. (1990). Biomass density and the relationship between submerged macrophyte biomass and plant growth form. *Hydrobiologia.* **196:** 17-23.

Duarte, C. M. & D. A. Roff. (1991). Architectural and life history constraints to submersed macrophyte community structure: a simulation study. *Aquatic Botany.* **42:** 15-29.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers & F. Smith. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry.* **28:** 350-356.

Eaton, J. W. & J. Freeman. (1982). Ten year's experience of weed control in the Leeds and Liverpool Canal. In *European Weed Research Society 6th Symposium on Aquatic Weeds*, pp. 96-104, Novi Sad, Yugoslavia.

Eaton, J. W., K. J. Murphy & T. M. Hyde. (1981). Comparative trials of herbicidal and mechanical control of aquatic weeds in canals. In *Proceedings on Aquatic Weeds and their Control*, pp. 105-116, Oxford, UK.

Elzenga, J. T. M. & H. B. A. Prins. (1987). Light induced polarity of redox reactions in leaves of *Elodea canadensis* Michx.. *Plant Physiologist.* **85:** 239-242.

Engle, S. (1988). The role and interactions of submerged macrophytes in a shallow Wisconsin lake. *Journal of Freshwater Biology.* **4(3):** 329-341.

Eugelink, A. H. (1998). Phosphorus uptake and active growth of *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) St. John. *Water, Science and Technology.* **37(3):** 59-65.

Falk, S., G. Samuelsson & G. Oquist. (1990). Temperature-dependent photoinhibition and recovery of photosynthesis in the green alga *Chlamydomonas reinhardii* acclimated to 12 and 27 °C. *Physiologia Plantarum.* **78(2):** 173-180.

Fitter, A. H. & T. R. Stickland. (1992). Architectural analysis of plant root systems 2. Studies on plants under field conditions. *New Phytologist.* **121:** 243-248.

Fitter, A. H., T. R. Stickland, M. L. Harvey & G. W. Wilson. (1991). Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency. *New Phytologist.* **118:** 375-382.

Forsberg, C. (1965). Nutritional studies of *Chara* in axenic cultures. *Physiologia Plantarum.* **18:** 275-290.

Frodge, J. F., G. L. Thomas & G. B. Pauley. (1990). Effects of canopy formation by floating and submergent macrophytes on the water quality of two shallow pacific northwest lakes. *Aquatic Botany.* **38:** 231-248.

Gaudy, A. F. (1962). Colorimetric determinations of protein and carbohydrate. *Industrial Water Wastes.* **7:** 17-22.

Gerloff, G. C. & P. H. Krombholz. (1966). Tissue analysis as a measure of nutrient availability for the growth of aquatic plants. *Limnology and Oceanography.* **11:** 529-537.

Grace, J. B. (1991). A clarification of the debate between Grime and Tilman. *Functional Ecology.* 583-587.

Grime, J. P. (1979). *Plant Strategies and Vegetative Processes.* Wiley, Chichester.

Gumbricht, T. (1993). Nutrient removal processes in freshwater submersed macrophyte systems. *Ecological Engineering.* **2:** 1-30.

Harper, J. L. (1961). Approaches to the study of plant competition. *In: Mechanisms in Biological Competition.* (F. L. Milthorpe ed.), pp. 1-39. Symposium No. 15, Society for Experimental Biology. Cambridge University Press, Cambridge.

Harper, J. L. (1977). *Population biology of plants.* Academic Press, London, Uk.

Holaday, A. S. & G. Bowes. (1980). C_4 acid metabolism and dark CO_2 fixation in a submersed aquatic macrophyte (*Hydrilla verticillata*). *Plant Physiologist.* **65:** 331-335.

Hough, R. A. (1974). Photosynthesis and productivity in submersed aquatic vascular plants. *Limnology and Oceanography.* **19:** 9912-9927.

Hough, R. A. & R. G. Wetzel. (1975) The release of dissolved organic carbon from submersed aquatic macrophytes: Diel, seasonal and community relationships. Verh

Howard-Williams, C. (1981). Studies on the ability of a *Potamogeton pectinatus* community to remove dissolved nitrogen and phosphorus compounds from lake water. *Journal of Applied Ecology.* **18:** 619-637.

Howard-Williams, C. (1993). Processes of aquatic weed invasions: The New Zealand example. *Journal of Aquatic Plant Management.* **31:** 17-23.

Howard-Williams, C. & B. R. Allanson. (1981). Phosphorus cycling in a dense *Potamogeton pectinatus* L. bed. *Oecologia.* **49:** 56-66.

Howard-Williams, C. & J. Davies. (1988). The invasion of Lake Taupo by the submerged weed *Lagarosiphon major* and its impact on the native flora. *New Zealand Journal of Ecology.* **11:** 13-19.

Hunt, R. (1978). *Plant Growth Analysis*. Edward Arnold, London.

Jacobsen, O. S. (1977). Sorption of phosphate by Danish lake sediments. *Vatten.* **33:** 290-298.

Janauer, G. A. (1981). *Elodea canadensis* and its dormant apices: an investigation of organic and mineral constituents. *Aquatic Botany.* **11:** 231-243.

Johnstone, I. M. (1982). Strategies for the control of macrophytes in hydroelectric impoundments. *Water pollution Management Review 1982*. pp. 65-94.

Jolliffe, P. A., A. N. Minjas & V. C. Runeckles. (1984). A reinterpretation of yield relationships in replacement series experiments. *Journal of Applied Ecology.* **21:** 227-243.

Jones, J. I. (1994). An eco-physiological study of the *Elodea nuttallii*-epiphyton association. Ph.D. thesis, University of Liverpool.

Jones, J. I., J. W. Eaton & K. Hardwick. (1993). Physiological Plasticity in *Elodea nuttallii* (Planch.) St. John. *Journal of Aquatic Plant Management.* **31:** 88-94.

Jones, J. I., K. Hardwick & J. W. Eaton. (1996). Diurnal carbon restrictions on the photosynthesis of dense stands of *Elodea nuttallii* (Planch.) St. John. *Hydrobiologia.* **340:** 11-16.

Kautsky, L. (1988). Life strategies of aquatic soft bottom macrophytes. *Oikos.* **53:** 126-135.

Kautsky, L. (1991). In situ experiments on interrelationships between six brackish macrophyte species. *Aquatic Botany.* **39:** 159-172.

Keeley, J. E. (1990). Photosynthetic pathways in freshwater aquatic plants. *Trends in Evolution and Ecology.* **5:** 330-333.

Keeley, J. E. & G. Bowes. (1982). Gas exchange characteristics of the submerged aquatic crassulacean acid metabolism plant *Isoetes howelli*. *Plant Physiology.* **70:** 1455-1458.

Kirk, J. T. O. (1994). *Light and Photosynthesis in Aquatic Ecosystems*, 2nd Edition. Cambridge University Press, Cambridge, UK.

Koehler, L. H. (1952). Differentiation of carbohydrates by anthrone reaction rate and colour intensity. *Analytical Chemistry.* **24:** 1576-1579.

Kundel, W. (1990). *Elodea nuttallii* (Planchon) St. John in Flussmarschgewassern. *Tuexenia.* **10:** 41-47.

Kunii, H. (1981). Characteristics of the winter growth of detached *Elodea nuttallii* (Planch.) St. John in Japan. *Aquatic Botany.* **11:** 57-66.

Kunii, H. (1982). The critical water temperature for the active growth of *Elodea nuttallii* (Planch.) St. John. *Japanese Journal of Ecology.* **32:** 111-112.

Kunii, H. (1984). Seasonal growth and profile structure development of *Elodea nuttallii* (Planch.) St. John in pond Ojaga-ike, Japan. *Aquatic Botany.* **18:** 239--247.

Kunii, H. & K. Maeda. (1982). Seasonal and long-term changes in surface cover of aquatic plants in a shallow pond, Ojaga-ike, Chiba, Japan. *Hydrobiologia.* **87:** 45-55.

Larcher, W. (1995). Photosynthesis as a tool for indicating temperature stress events. In *Ecophysiology of photosynthesis* (ed. E. D. Schulz and M. M. Cadwell), pp. 261-277. Springer, Berlin Heidelberg, New York.

Li, W. K. W. & I. Morris. (1982). Temperature adaptation in *Phaeodactylum tricornutum* Bohlin - photosynthetic rate compensation and capacity. *Journal of Experimental Marine Biology and Ecology.* **58:** 135-150.

Losee, R. F. & R. G. Wetzel. (1988). Water movement within submersed littoral vegetation. *Verh. Internat. Verein. Limnol.* **23:** 62-66.

Losee, R. F. & R. G. Wetzel. (1993). Littoral flow rates within and around submersed macrophyte communities. *Freshwater Biology.* **29:** 7-17.

Lucas, W. J. (1983). Photosynthetic assimilation of exogenous HCO_3^- by aquatic plants. *Annual Reivew of Plant Physiology.* **34:** 71-104.

Lund, J. W. G. (1979). The mystery of *Elodea* Michx in Great Britain. *Watsonia.* **12:** 338.

Lyons, J. M. (1973). Chilling injury in plants. *Annual Review of Plant Physiology.* **24:** 445-466.

Maberly, S. C. & T. V. Madsen. (1998). Affinity for CO_2 in relation to the ability of freshwater macrophytes to use HCO_3^-. *Functional Ecology.* **12(1):** 99-106.

Maberly, S. C. & D. H. N. Spence. (1983). Photosynthetic inorganic carbon use by freshwater plants. *Journal of Ecology.* **71:** 705-724.

Machena, C., N. Kautsky & G. Lindmark. (1990). Growth and production of *Lagarosiphon ilicifolius* in Lake Kariba - a man-made tropical lake. *Aquatic Botany.* **37:** 1-15.

Mackereth, F. J. H., J. Heron & J. F. Talling. (1989). *Water Analysis: Some methods for limnologists.*, 2nd Edition. Freshwater Biological Association Scientific publication. No. 36.

Madsen, T. V. & H. Brix. (1997). Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. *Oecologia.* **110:** 320-327.

Madsen, T. V., P. Hahn & J. Johansen. (1998). Effects of inorganic carbon supply on the nitrogen requirement of two submerged macrophytes, *Elodea canadensis* and *Callitriche cophocarpa*. *Aquatic Botany.* **62:** 95-106.

Madsen, T. V. & S. C. Maberly. (1991). Diurnal variation in light and carbon limitation of photosynthesis by two species of submerged freshwater macrophyte with a differential ability to use bicarbonate. *Freshwater Biology.* **26:** 175-187.

Madsen, T. V., S. C. Maberly & G. Bowes. (1996). Photosynthetic acclimation of submersed angiosperms to CO_2 and HCO_2^-. *Aquatic Botany.* **53:** 15-30.

Madsen, T. V. & K. Sand-Jensen. (1987). Photosynthetic capacity, bicarbonate affinity and growth of *Elodea canadensis* exposed to different concentrations of inorganic carbon. *Oikos.* **50:** 176-182.

Madsen, T. V. & K. Sand-Jensen. (1991). Photosynthetic carbon assimilation in aquatic macrophytes. *Aquatic Botany.* **41:** 5-40.

Madsen, T. V. & K. Sand-Jensen. (1994). The interactive effects of light and inorganic carbon on aquatic plant growth. *Plant, Cell and Environment.* **17:** 955-962.

Mantai, K. E. & M. E. Newton. (1982). Root growth in *Myriophyllum* - A specific plant response to nutrient availability. *Aquatic Botany.* **13(1):** 45-55.

Marshall, W. (1852). Excessive and noxious increase of *Udora canadensis* (*Anacharis Alsinastrum*). *Physiologist.* **4:** 705-715.

McCready, R. M., J. Guggolz, V. Silviera & H. S. Owens. (1950). Determination of starch and amylose in vegetables. *Analytical Chemistry.* **22(9):** 1156-1158.

McCreary, N. J. & S. R. Carpenter. (1983). Competition among submersed perennial plants: use of reciprocal replacement series in situ. In *Proceedings of the International Syposium on Aquatic Macrophytes*, Njmegen, Holland.

McCreary, N. J., S. R. Carpenter & J. E. Chaney. (1983). Coexistence and interference in two submersed freshwater perennial plants. *Oecologia.* **59:** 393-396.

Milne, L. J. & M. Milne. (1961). *The Balance of Nature*. Macdonald, London.

Moen, R. A. & Y. Cohen. (1989). Growth and competition between *Potamogeton pectinatus* L. and *Myriophyllum exalbescens* Fern. in experimental ecosystems. *Aquatic Botany.* **33:** 257-270.

Moss, B. (1991). The role of nutrients in determining the structure of lake ecosystems and implications for the restoration of submerged plant communities to lakes which have lost them. In *International Conference on N, P and Organic Matter*, pp. 75-85.

Moss, B. (1998). *Ecology of Fresh Waters: Man and Medium, Past to Future*, 3 Edition. Blackwell Science Ltd, Oxford.

Moss, B., H. R. Balls & K. Irvine. (1985). Management of the consequences of eutrophication in lowland lakes in England engineering and biological situations. In *Management Strategies for Phosphorus in the Environment* (ed. J. N. Lester and P. W. W. Kirk), pp. 180-185, London, UK.

Mulligan, H. F. & A. Baranowski. (1969). Growth of phytoplankton and vascular aquatic plants at different nutrient levels. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie.* **17:** 802-810.

Mulligan, H. F., A. Baranowski & R. Johnson. (1976). Nitrogen and phosphorus fertilization of aquatic vascular plants and algae in replicated ponds I. Initial response to fertilization. *Hydrobiologia.* **48(2):** 109-116.

Murphy, K. J. (1980). The ecology and management of aquatic macrophytes in canals. Ph.D. thesis, University of Liverpool.

Murphy, K. J. & J. W. Eaton. (1981). Ecological effects of two herbicides and two mechanical clearance methods used for aquatic weed control in canals. In *Proccedings on Aquatic Weeds and their Control*, pp. 201-217, Oxford, UK.

Murphy, K. J., B. Rorslett & I. Springuel. (1990). Strategy analysis of submerged lake macrophyte communities: an international example. *Aquatic Botany.* **36:** 303-323.

Nichols, S. A. (1991). The interaction between biology and the management of aquatic macrophytes. *Aquatic Botany.* **41:** 225-252.

Nichols, S. A. & B. H. Shaw. (1986). Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. *Hydrobiologia.* **131:** 3-21.

Nielsen, S. L. & K. Sand-Jensen. (1989). Regulation of photosynthetic rates of submerged rooted macrophytes. **81:** 364-368.

Nielsen, S. L. & K. Sand-Jensen. (1991). Variation in growth rates of submerged rooted macrophytes. *Aquatic Botany.* **39:** 109-120.

Ozbay, H. (1998). Interactions between floating and submersed aquatic macrophytes. Ph.D. thesis, University of Liverpool.

Ozimek, T., E. v. Donk & R. D. Gulati. (1993). Growth and nutrient uptake by two species of *Elodea* in experimental conditions and their role in nutrient accumulation in a macrophyte-dominated lake. *Hydrobiologia.* **251:** 13-18.

Phillips, G. L., D. Eminson & B. Moss. (1978). A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. *Aquatic Botany.* **4:** 103-126.

Pokorny, J., J. Kvet, J. P. Ondok, Z. Toul & I. Ostry. (1984). Production-ecological analysis of a plant community dominated by *Elodea canadensis* Michx. *Aquatic Botany.* **19:** 263-292.

Porra, R. J. (1991). Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic and marine organisms, including recalcitrant algae. In *Chlorophylls* (ed. H. Scheer), pp. 31-57. Boca Raton. CRC Press.

Portielje, R. & R. M. M. Roijackers. (1995). Primary succession of aquatic macrophytes in experimental ditches in relation to nutrient input. *Aquatic Botany.* **50:** 127-140.

Preston, C. D. & J. M. Croft. (1997). *Aquatic plants in Britain and Ireland.* Harley Books, Colchester, Essex.

Prins, H. B. A. & R. J. Helder. (1985). HCO_3^- assimilation by *Potamogeton lucens*: polar cation transport and the role of H^+ extrusion. In *Inorganic carbon uptake by photosynthetic organisms.* (ed. W. J. Lucas and J. A. Berry), pp. 271-286. American Society of Plant Physiologists, Rockville, Maryland, USA.

Prins, H. B. A., J. F. H. Snel, P. E. Zanstra & R. J. Helder. (1982). The mechanism of bicarbonate assimilation by polar leaves of *Potamogeton* and *Elodea*. CO_2 concentrations at the leaf surface. *Plant, Cell and Environment.* **5:** 207-214.

Pygott, J. R. (1987). The effects of boat traffic on canal ecosystem. Ph.D. thesis, University of Liverpool.

Rajmanek, M., G. R. Robinson & E. Rejmankova. (1989). Weed-crop competition: experimental designs and models for data analysis. *Weed Science.* **37:** 276-284.

Rattray, M. R., C. Howard-Williams & J. M. A. Brown. (1991a). The photosynthesis and growth responses of two freshwater angiosperms in lakes of different trophic status: responses to light and inorganic carbon. *Freshwater Biology.* **6:** 399-407.

Rattray, M. R., C. Howard-Williams & J. M. A. Brown. (1991b). Sediment and water as sources of nitrogen and phosphorus for submerged rooted aquatic macrophytes. *Aquatic Botany.* **40:** 225-237.

Raven, J. A. (1970). Exogenous inorganic carbon sources in plant photosynthesis. *Biological Reviews.* **45:** 167-221.

Raven, J. A. & W. J. Lucas. (1985). Energy costs of carbon acquisition. In *Inorganic carbon uptake by aquatic photosynthetic organisms.* (ed. W. L. Lucas and J. A. Berry), pp. 305-324. American Society of Plant Physiologists, Rockville, Maryland.

Redfield, A. C. (1963). The influence of organisms on the composition of sea water. In *The Sea* (ed. M. N. Hills), pp. 177-192. John Wiley and Sons, New York, USA.

Rich, T. C. G. & E. R. Woodruff. (1995). Changes in the vascular plant floras of England and Scotland between 1930-1960 and 1987-1988. *Biological Conservation.* **75:** 217-229.

Rolland, T. & M. Tremolieres. (1995). The role of ammonium nitrogen in the distribution of two species of *Elodea*. *Acta Botanica Gallica.* **142:** 733-739.

Rorslett, B. (1989). An integrated approach to hydropower impact assessment. 2. Submerged Macrophytes in some Norwegian Hydroelectric lakes. *Hydrobiologia.* **175:** 65-82.

Rorslett, B., D. Berge & S. W. Johansen. (1986). Lake enrichment by submersed macrophytes: A Norwegian whole-lake experience with *Elodea canadensis*. *Aquatic Botany.* **26:** 325-340.

Roush, M. L., S. R. Radosevich, R. G. Wagner, B. D. Maxwell & T. D. Petersen. (1989). A comparison of methods for measuring effects of density and proportion in plant competition experiments. *Weed Science.* **37:** 268-275.

Sackville-Hamilton, N. R. (1994). Replacement and additive designs for plant competition. *Journal of Applied Ecology.* **31:** 599-603.

St. John, H. S. (1965). Monograph of the Genus *Elodea*: Part 4 and summary. *Rhodora.* **67:** 1-35, 155-181.

Sand-Jensen, K. (1989). Environmental variables and their effect on photosynthesis of aquatic plant communities. *Aquatic Botany.* **34:** 5-25.

Sand-Jensen, K. & D. M. Gordon. (1984). Differential ability of marine and freshwater macrophytes to utilize HCO_3^- and CO_2. *Marine Biology.* **80:** 247-253.

Sand-Jensen, K. & D. M. Gordon. (1986). Variable HCO_3^- affinity of *Elodea canadensis* Michaux in response to different HCO_3^- and CO_2 concentrations during growth. *Oecologia.* **70:** 426-432.

Santamaria, L. & W. v. Vierssen. (1997). Photosynthetic temperature responses of fresh- and brackish-water macrophytes: a review. *Aquatic Botany.* **58:** 135-150.

Schwarz, A. & C. Howard-Williams. (1993). Aquatic weed-bed structure and photosynthesis in two New Zealand lakes. *Aquatic Botany.* **46:** 263-281.

Sculthorpe, C. D. (1985). *The biology of aquatic vascular plants.* Koeltz Scientific Books, Koeltz, Germany.

Silvertown, J. W. & J. L. Doust. (1993). *Introduction to Plant Population Biology*, 3rd Edition. Blackwell Science Ltd, Oxford, UK.

Simpson, D. A. (1984). A short history of the introduction and spread of *Elodea* Michx. in the British Isles. *Watsonia.* **15:** 1-9.

Simpson, D. A. (1986). Taxonomy of *Elodea* Michx in the British Isles. *Watsonia.* **16:** 1-14.

Simpson, D. A. (1988). Phenotypic plasticity of *Elodea nuttallii* (Planch.) H. St John and *Elodea canadensis* Michx. in the British Isles. *Watsonia.* **17:** 121-132.

Simpson, D. A. (1990). Displacement of *Elodea canadensis* Michx by *Elodea nuttallii* (Planch.) H. St John in the British Isles. *Watsonia.* **18:** 173-177.

Simpson, P. S., J. W. Eaton & K. Hardwick. (1980). The influence of environmental factors on apparent photosynthesis and respiration of the submerged macrophyte *Elodea canadensis. Plant, Cell and Environment.* **3:** 415-423.

Simpson, P. S. (1981). Photosynthesis and respiration in *Elodea canadensis* Michx. Ph.D. thesis, University of Liverpool.

Smith, F. A. & N. A. Walker. (1980). Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. *New Phytologist.* **86:** 245-259.

Snaydon, R. W. (1991). Replacement or additive designs for competition studies. *Journal of Applied Ecology.* **28:** 930-946.

Snaydon, R. W. (1994). Replacement and additive designs revisited: comments on the review paper by N. R. Sackville Hamilton. *Journal of Applied Ecology.* **31:** 784-786.

Sondergaard, M. & K. Sand-Jensen. (1979). Carbon uptake by leaves and roots of *Littorella uniflora* (L.) Aschers. *Aquatic Botany.* **6:** 1-12.

Spence, D. H. N. (1964). The macrophytic vegetation of lochs, swamps and associated fens. In *The Vegetation of Scotland* (ed. J. H. Burnett), pp. . Oliver & Boyd, Edinburgh.

Spitters, C. J. T. (1983). An alternative approach to the analysis of mixed cropping experiments. 1. Estimation of competition effects. *Netherlands Journal of Agricultural Science.* **31:** 1-11.

Stace, C. (1991). *New Flora of the British Isles.* Cambridge University Press, Cambridge, UK.

Stumm, W. & J. J. Morgan. (1981). *Aquatic Chemistry an introduction emphasizing chemical equilibria in natural waters.* 2 Edition. John Wiley & Sons, New York.

Thiebaut, G., T. Rolland, F. Robach, M. Tremolieres & S. Muller. (1997). Quelques consequences de l'introduction de deux especes de macrophytes, *Elodea canadensis* Michaux et *Elodea nuttallii* St. John, dans les ecosystemes aquatiques continentaux: exemple de la plaine d'Alsace et des Vosges du nord (nord-est de la France). *Bull. Fr. Peche Piscic.* **344/345:** 441-452.

Tilman, D. (1986). Nitrogen-limited growth in plants from different successional stages. *Ecology.* **67:** 555-563.

Tilman, D. (1987). The importance of the mechanisms of interspecific competition. *American Naturalist.* **129:** 769-774.

Tilman, D. (1988). *Plant strategies and the dynamics of plant communities.* Princeton University Press.

Triest, L. (1982). Patterns of sclerenchyma fibres and air-spaces in the leaves of *Lagarosiphon* (Hydrocharitaceae). In *Studies on Aquatic Vascular Plants* (ed. J. J. Symoens, S. S. Hooper and P. Compere), pp. 70-74. Royal Botanical Society of Belgium, Brussels, Belgium.

Ultsch, G. R. (1973). The effects of water hyacinths (*Eichhornia crassipes*) on the microenvironment of aquatic communities. *Archiv für Hydrobiologie.* **72:** 460-473.

Van, T. K., W. T. Haller & G. Bowers. (1976). Comparison of photosynthetic characteristics of three submersed aquatic plants. *Plant Physiology.* **58:** 761-768.

Van, T. K., W. T. Haller, G. Bowes & L. A. Garrard. (1977). Effects of light quality on growth and chlorophyll composition in *Hydrilla. Journal of Aquatic Plant Management.* **15:** 29-31.

Vollenweider, R. A. & J. J. Kerekes. (1981). Appendix I. Background and summary results of the OECD cooperative programme on eutrophication. In: *The OECD cooperative Programme on Eutrophication: Canadian Contribution.* (comp. Janus, L. L. and R. A. Vollenweider). pp. 251-368. Scientific Series 131, Environment Canada, Ottawa.

Vollenweider, R. A. (1968). Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. OECD, unpublished.

Walker, A. O. (1912). The distribution of *Elodea canadensis* Michaux in the British Isles in 1909. *Proceedings of the Linnean Society.* **124:** 71-77.**Wells, R. D. S., M. D. Welsh, R. P. H. & P. Denny.** (1979). The translocation of ^{32}P in two submerged aquatic angiosperm species. *New Phytologist.* **82:** 645-656.

Wetzel, R. G. (1988). Water as an environment for plant life. In *Vegetation for inland waters* (ed. J. J. Symoens), 1-30. Kluwer Academic Publishers, Dordrecht, Netherlands.

Willby, N. (1994). The management of navigable canals for nature conservation and fisheries. Ph.D. thesis, University of Liverpool.

Zimba, P. V. & M. S. Hopson. (1997). Quantification of epiphyte removal efficiency from submersed aquatic plants. *Aquatic Botany.* **58:** 173-179.

Zar, J. H. (1996). *Biostatistical Analysis.* 3rd ed. Prentice-Hall International Inc..