Carbon Acquisition in Variable Environments: Aquatic Plants of the River Murray, Australia

Melissa S. Barrett

A dissertation submitted to The University of Adelaide
in partial fulfilment of the requirements for
the degree of Doctor of Philosophy

July 2007

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to my thesis, when deposited in the University Library, being available for loan and photocopying.

________________________________ / /

Melissa S. Barrett

Abstract

This thesis considers the implications of changes in the supply of resources for photosynthesis, with regard for modes of carbon acquisition employed by aquatic plants of the River Murray. Carbon supplies are inherently more variable for aquatic plants than for those in terrestrial environments, and variations are intensified for plants in semi-arid regions, where water may be limiting. In changeable environments the most successful species are likely to be those with flexible carbon-uptake mechanisms, able to accommodate variations in the supply of resources.

Studies were made of plants associated with wetland habitats of the Murray, including *Crassula helmsii*, *Potamogeton tricarinatus*, *P. crispus* and *Vallisneria americana*. The aim was to elucidate the mechanisms of carbon uptake and assimilation employed, and to determine how flexibility in carbon uptake and/or assimilation physiology affect survival and distribution. Stable carbon isotopes were used to explore the dynamics of carbon uptake and assimilation, and fluorescence was used to identify pathways and photosynthetic capacity. The studies suggest that physiological flexibility is adaptive survival in changeable environments, but probably does not enhance the spread or dominance of these species.

V. americana is a known bicarbonate-user, and it is shown here that it uses the Crassulacean Acid Metabolism (CAM) photosynthetic pathway under specific conditions (high light intensity near the leaf tips) concurrently with HCO_3^- uptake, while leaves deeper in the water continue to use the C_3 pathway, with CO_2 as the main carbon source. However, *V. americana* does not use CAM when under stress, such as exposure to high light and temperature. The diversity of carbon uptake and assimilation mechanisms in this species may explain its competitive ability in habitats associated with the Murray. In this way it is able to maximise use of light throughout the water column. In shallow, warm water, where leaves are parallel to the surface, CAM ability is likely to be induced along the length of the leaf, allowing maximal use of carbon and light.

The amphibious *C. helmsii* is shown to use CAM on submergence, even where water levels fluctuate within 24 hours. This allows continued photosynthesis in habitats where level fluctuations prevent access to atmospheric CO_2. It appears that stable conditions are most favourable for growth and dispersal, and that the spread of *C. helmsii* is mainly by the aerial form.

Carbon uptake by *P. tricarinatus* under field conditions is compared with that of *P. crispus* to demonstrate differences in productivity associated with aqueous bicarbonate and atmospheric CO_2 use. *P. tricarinatus* uses HCO_3^- uptake to promote growth toward the surface, so that CO_2 can be accessed by floating leaves. Atmospheric contact provides access to light and removes the limitation of aqueous diffusive resistance to CO_2, thereby increasing photosynthetic capacity above that provided by submerged leaves.

Acknowledgements

There are people I would like to thank for a huge variety of reasons.

Firstly, I would like to thank my supervisors, Assoc Prof Keith Walker, Assoc Prof George Ganf, Dr Jennifer Watling and Prof Howard Griffiths, whose diverse approaches and thoughts have taught me much more than ‘simply’ writing a thesis.

I would like to thank the Cooperative Research Centre for Freshwater Ecology (CRCFE) in Australia and the Australian Postgraduate Award for funding. Special thanks to the Department of Plant Sciences, University of Cambridge and to Jesus College for making me an honorary student and allowing me access to facilities.

I would like to thank all those who helped me with field work including the early mornings, long days and late nights; Neil Cobbett, Amy George, Sue Gehrig, Anne Jensen and my Dad. Thank you to the support staff of the Benham building, Earth and Environmental Sciences at the University of Adelaide for help with all things practical and to all the academic and fellow students in the Benham for your support with what must have seemed like fleeting visits in the lab.

I am grateful for the help provided by the team at the University of Cambridge Botanical Gardens for helping keep my plants alive, moving ponds, and being a source of all manner of information and to the Godwin lab for help with all things ‘mass spectrometer’.

I would especially like to thank the Plant Physiology lab in Cambridge for initially making space for me and then for all the support, friendship and coffees along the way. I am indebted to Barney Davies, Monica Mejia Chang, Nick Betsen, Casandra Reyes Garcia, Wendy Robe, Glyn Jones, Abazar Rajabi, Wanne Kromdijk, Joel Dunn, Gary Lanigan and Andrea Vincent.

Finally, I would like to say a special thanks to Neil Cobbett who supported and helped me throughout the PhD in planning, preparation, field work, lab work and writing. Thank you also to all my family and friends, particularly those in Adelaide and Cambridge, who have put up with me, kept me sane, helped me keep fit, and motivated and supporting me through to the end.

Contents

Abstract V
Acknowledgements VI
Contents VII
Figures X

Chapter 1 INTRODUCTION 1
1.1 Preamble 1
1.2 The Murray-Darling Basin 2
1.2.1 Climate and hydrology 2
1.2.2 Vegetation 4
1.2.3 Photosynthetic diversity 4
1.3 Carbon supply and acquisition 5
1.3.1 Overview 5
1.3.2 Atmospheric CO_2 6
1.3.3 Alternative carbon sources 6
1.3.4 Temperature 8
1.3.5 pH 8
1.3.6 Light 9
1.3.7 Oxygen 10
1.3.8 Salinity 10
1.3.9 Diffusive exchange 10
1.3.10 Heterophylly 11
1.4 Carbon assimilation 12
1.5 Stable carbon isotopes 17
1.5.1 Composition and discrimination 17
1.5.2 Sources of fractionation 17
1.5.3 $\delta^{13}C$ signals in aquatic systems 17
1.6 Fluorescence 19
1.7 Pilot survey of $\delta^{13}C$ in River Murray species 19
1.8 Thesis plan 20

Chapter 2 MATERIALS AND METHODS 22
2.1 Titratable acidities 22
2.2 Chlorophyll 22
2.3 Oxygen evolution 22
2.4 Chlorophyll fluorescence 23
2.4.1 Photosynthetic efficiency 23
2.4.2 Rapid light curves 24
2.4.3 Non-Photochemical Quenching 24
2.4.4 Absorbance 25
2.5 Stable carbon isotope fractionation 27
2.5.1 Plant material 27
2.5.2 Extraction and measurement of carbohydrates 27
2.5.3 $\delta^{13}C$ and Total Inorganic Carbon determination 29
2.6 PEPc assays 30
2.7 Water quality 31
2.7.1 Alkalinity 31
2.8 Microscopy of leaf sections 32
2.9 Statistical analysis 32

Chapter 3 CARBON ACQUISITION IN CRASSULA HELMSII 33
3.1 Introduction 33
3.2 Methods 36
3.2.1 Carbon acquisition in a natural population 36
3.2.2 Impact of water regime on mesocosm plants 37
3.2.3 Statistical analysis 40
3.3 Results 40
3.3.1 Impact of water regime on mesocosm plants 40
3.3.2 Carbon acquisition in a natural population 47
3.4 Discussion 54
3.4.1 Impact of water regime change 54
3.4.2 Carbon acquisition in a natural population 56
3.4.3 Hypotheses Revisited 57

Chapter 4 FIELD STUDIES OF VALLISNERIA AMERICANA 60
4.1 Introduction 60
4.2 Methods 63
4.2.1 Species description 63
4.2.2 Field sites 64
4.2.3 General methods 68
4.2.4 pH drift under field conditions 70
4.2.5 Statistics 70
4.3 Results 71
4.3.1 Characterisation of CAM 71
4.3.2 CAM and bicarbonate use at two sites 74
4.3.3 Bicarbonate use in field incubations 81
4.4 Discussion 83
4.4.1 General Vallisneria system (Brenda Park) 83
4.4.2 CAM and bicarbonate use at Riverglades and Banrock Station 84
4.4.3 Field incubations 86
4.4.4 Hypotheses Revisited 87

Chapter 5 LABORATORY STUDIES OF VALLISNERIA AMERICANA 90
5.1 Introduction 90
5.2 Methods 91
5.2.1 Growth conditions 91
5.2.2 Experimental procedures 91
5.3 Results 94
5.3.1 Characterisation of carbon acquisition 94
5.3.2 Carbon acquisition along the leaf 97
5.3.3 Carbon acquisition at three light levels 99
5.4 Discussion 109
5.4.1 Characterisation of carbon acquisition 109
5.4.2 Carbon acquisition along the leaf 111
5.4.3 Carbon acquisition at three light levels 112
5.4.4 Hypotheses Revisited 117

Chapter 6 PHOTOSYNTHESIS COMPARED IN TWO POTAMOGETON SPECIES 119
6.1 Introduction 119
6.2 Methods 123
6.2.1 Species description 123
6.2.2 Site Description 124
6.2.3 General methods 126
6.2.4 Statistics 127
6.3 Results 128
6.3.1 Morphology of floating and submerged leaves of P. tricarinatus 128
6.3.2 Carbon partitioning between submerged and floating leaves 130
6.3.3 Light responses of floating and submerged leaves 132
6.4 Discussion 135
6.4.1 Morphology of floating versus submerged leaves of P. tricarinatus 136
6.4.2 Light Responses of floating and submerged leaves 136
6.4.3 Carbon partitioning between submerged and floating leaves 139
6.4.4 Hypotheses Revisited 140

Chapter 7 GENERAL DISCUSSION 143
7.1 Study species 143
7.1.1 Crassula helmsii 143
7.1.2 Vallisneria americana 145
7.1.3 Potamogeton tricarinatus 146
7.2 Carbon Dynamics 147
7.2.1 Preamble 147
7.2.2 Carbon acquisition 147
7.2.3 Carbon assimilation 149
7.3 Conclusion 151

Chapter 8 REFERENCES 153

Figures

Figure 1-1..The Murray-Darling Basin, extending over 14% of mainland Australia. 3

Figure 2-1......Calculation of absorbance factor using the leaf-layer technique. (I) is the y-intercept of the fitted least-squares regression, (α) is the slope of the line. ..26

Figure 2-2......Purpose-made CO_2 scrubbing inlet carrier tube attached to IRGA for analysis of CO_2 in headspace from Exetainers. 29

Figure 2-3......... CO_2 purification line, modified with Betson [, 2004 #638], used to purify CO_2 from Exetainers prior to mass spectrometry. 30

Figure 3-1..... Distribution of *C. helmsii* in south-eastern Australia. Red squares are focused on the terminal lakes of the Lower Murray (and SE South Australia); yellow squares are sites in Victoria. Source: New South Wales Flora Online. 33

Figure 3-2... Aerial form of *Crassula helmsii*. Note the roots from nodes dependent on water availability. Source: Center for Aquatic Plants, University of Florida, Gainesville. 34

Figure 3-3..........Five pots of *C. helmsii* per bin were grown and four evenly-grown plants were randomly assigned to three water regimes after two months' growth.39

Figure 3-4........ Water content (a), total length (b) and internodal length (c) after 30 days. Bars are standard errors (pre-regime, n = 16; final, n = 4). a and b signify differences at alpha = 0.05. 41

Figure 3-5. Chlorophyll after 30 days: (a) total chlorophyll, showing chlorophylls a and b and (b) chlorophyll a/b ratio. Pre-regime n = 16, treatments n = 4, where each replicate is the mean of 4 shoots. g and h signify differences at alpha = 0.05. 42

Figure 3-6....Dawn and dusk titratable acidities (a) and overnight accumulated acid (ΔH+) (b) for pre-treatment (day 0) and the control and three treatments (days 1-30). 43

Figure 3-7................Diurnal change in acidity after day 30 for the *control* (■), *fully submerged* (●), *partially submerged* (▲) and *emergent* (◊) treatments (*n* = 4) (a) and titratable acidity in the leaf and stem at dawn on day 30. Bars are standard errors of the mean (n = 4) (b). a and b signify differences at alpha = 0.05............44

Figure 3-8....Dawn and dusk titratable acidities (a) and overnight accumulated acid (ΔH+) (b) pre-treatment (day 0) and for the control and three water regimes (day 1-30). 44

Figure 3-9................$\delta^{13}C$ for pre-treatment and control, fully-submerged, partially-submerged and emergent treatments after day 30. Bars are standard errors (*n* = 4). a and b signify differences at alpha = 0.05. 45

Figure 3-12.... Diurnal optimum quantum efficiency (F_v/F_m) of each treatment after 30 days' treatment and the initial diurnal course pre-initiation of water regime (a) and non-photochemical quenching (NPQ) calculated from the ratio of a change in dark-measured fluorescence (F'_m) to measured fluorescence (F_m) at 9:00 h, 12:00 h and 16:00 h for each treatment (note that NPQ for the control and emergent treatment was zero) (b). Bars are standard errors (*n* = 3). 46

Figure 3-14..........Relative ETR of each treatment after day 30 for four time points.

Control (●), *fully submerged* (▼), *partially submerged* (■) and *emergent* (◆). Bars are standard errors (n = 3)..47

Figure 3-15......Diurnal incident PFD at Riverglades on each sampling occasion (a) and water temperature profiles of micro-sites on 2 January 2004 (b). 48

Figure 3-16...... Analysis of carbon from water samples at Riverglades, midday, pH 10. 48

Figure 3-17......... Titratable acidity in the hour after dusk (PM) and the hour before dawn (AM) of submerged and aerial leaves of different shoots of *C. helmsii* at Riverglades, January 2003. Bars are standard errors (n = 4)................................49

Figure 3-18..δ^{13}C of bulk organic carbon and purified soluble carbohydrates (dawn AM, dusk PM) for submerged and aerial leaves in January 2003 (a). Bars are standard errors (n = 3 for organic samples; no replication for carbohydrates).......50

Figure 3-19..... Diurnal Yield for submerged and aerial tissue (a) effective quantum yield (ΦPSII) and (b) dark-adapted optimum quantum yield (F_v/F_m) (b). Bars are standard errors (n = 10-20). 51

Figure 3-20................Dark-acclimated relative Electron Transport Rate (rETR) for submerged and aerial tissue of *C. helmsii*. Bars are standard errors (n = 4)...........51

Figure 3-21........Diurnal rapid light response curves of *C. helmsii* at three locations over one day. Submerged (●), aerial sheltered site (▼), aerial exposed site (■). Bars are standard errors (n = 4-5). The water level decreased due to wind in the late morning, exposing material that was submerged. One point was removed from the midday reading of aerial exposed site as an outlier. Submerged parts became emergent with decreased water levels late in the day...53

Figure 3-22.. Conceptual model of the stimuli for C_3 and CAM pathways in natural and mesocosm-grown *C. helmsii*...59

Figure 4-2.............Field sites “Brenda Park” near Morgan, “Banrock Station” near Overland Corner and “Riverglades” between Mannum and Wellington. Source: map base from SA Department of Environment & Heritage.................................65

Figure 4-3......... Brenda Park Lagoon, adjacent to the River Murray. Populations of *V. americana* were surveyed along a 100-m stretch of the lagoon. Source: Image ©2005Digitalglobe 66

Figure 4-4. Riverglades wetland, upstream of Murray Bridge. Note that the wetland has only one connection with the River Murray. Source: Image ©2005Digitalglobe 67

Figure 4-5. Banrock Station wetland complex, showing *V. americana* sites. Source: Image ©2005Digitalglobe 68

Figure 4-5...... Water characteristics in *V. americana* populations and in open water and PFD at Brenda Park on 6 December 2003 and 6 January 2004. (a, c) diel temperature, (b, d) PFD at the water surface..72

Figure 4-6..... Titratable acidity at dawn and dusk in *V. americana* leaves at Brenda Park over three periods. Populations (A-D) are shown to indicate the patchiness of sites within sampling periods..72

Figure 4-7...Diel effective quantum yields at Brenda Park on (a) 6 December 2003, (b)18 December 2003 and (c) 6 January 2004. Sites in 6 January 2003 are not the same as those in December. 73

Figure 4-8. Dark-acclimated (20 min) F_v/F_m at 11:00 h for populations of *V. americana*. 74

Figure 4-9....Titratable acidities for Riverglades and Banrock showing (a-c) pooled acidity differences between tip, middle and base of leaves for each site and (d-f) differences in dawn titratable acidity between populations. A-D represent separate populations of *V. americana* within sites..76

Figure 4-10Rapid light response curves at (a) Riverglades at dawn and (b) Banrock at dawn and midday. 77

Figure 4-15..Photochemical efficiency (F_v/F_m) for leaf tips at (a) dawn and midday at Riverglades, (c) at Banrock during January, showing F_v/F_m (black) and Φ PSII (red), and (e) in February, documenting the range within sites at the tip (10 cm depth) of leaves. Non-photochemical quenching (NPQ) at midday (b) at Riverglades and (e) tip and middle of leaves at Banrock in January and (d) variability between sites in February. 79

Figure 4-16.......Dissolved organic carbon in acidified water samples from Banrock Station and Riverglades in 2005. (a) $\delta^{13}C$ of total inorganic carbon (b) TIC and (c) percentage of carbon species calculated using pH, temperature and salinity [Johnston, 2004 #673]..80

Figure 4-17... $\delta^{13}C$ isotope values along the leaf at (a) Riverglades and (b) Banrock Station. 80

Figure 4-18.................Final measurements from the controlled drift experiment on *Vallisneria* at Banrock in February 2005 under natural light or artificial dark, (a) final pH of solutions (b) titratable acidity of leaf tissue at conclusion of experiment, (c) final concentration of inorganic carbon of solution (d) proportions of each carbon species remaining in solution after the incubation and (e) $\delta^{13}C$ of dissolved inorganic carbon in solution. 82

Figure 5-1........... *In situ* rapid light response curves from base, middle and tip (leaf tissue) before dawn (a) and diurnal course of relative ETR (b). Error bars are standard errors...94

Figure 5-2............... Absorbance factor and reflectance calculated from submerged measurements of each leaf section (a), Chlorophyll *a* and *b* content (b) and leaf thickness (c). Bars are standard errors (n = 9). 95

Figure 5-3...........Photosynthesis represented by oxygen evolution at pH 5, 7 and 9. Overlaid is the proportion of each inorganic carbon species [Hutchinson, 1975 #645] over this pH range at 20ºC. Mean±standard error (n = 5). 96

Figure 5-4.*V. americana* with chloroplasts stained in sections from (a) base (b) mid and (c) tip leaf sections at 10X magnification of *V. americana*. 97

Figure 5-5........ Titratable acidity (H^+) for each position on the leaf, measured from samples collected at dawn and dusk. Significant differences are shown by a, b. Bars are standard errors (n = 5). 98

Figure 5-6...pH drift for each position on the leaf in light (○) and dark (●) (b). Bars are standard errors (n = 3)..98

Figure 5-7..............Total DIC (a) and δ13C of incubating medium at conclusion of experiment for leaf sections (b). Bars are standard errors (*n* = 3-5)......................99

Figure 5-8. Titratable acidity representing malate for each of three light regimes for base, middle and tip of leaves for (a) and overnight accumulation (b). Bars are standard errors (*n* = 3)..100

Figure 5-9. pH change over 20 h under three light treatments of 80 (○), 200 (Δ) and 600 (□) μmol m^{-2} s^{-2} for three positions along the length of the leaf (tip, middle and base). From 09:00 until 21:00 h leaves were exposed to light; from 21:00 until 09:00 h leaves were in darkness. Bars are standard errors (*n* = 3). 101

Figure 5-10.....Titratable acidity representing malate for each of three light regimes for base, middle and tip of leaves for (a) and overnight accumulation (b).Bars represent standard errors (n = 3)..102

Figure 5-11................. $F/F_{m'}$ measured before treatment, after 10 h light and after a subsequent 10 h dark period for light levels of 80 μmol $m^{-2}s^{-1}$, 200 μmol $m^{-2}s^{-1}$ and 600 μmol $m^{-2}s^{-1}$. Bars represent standard errors (n=9 for initial measurements, n=3 for treatment measurements). 103

Figure 5-12.........Chlorophyll a and b concentrations for each section of the leaves, treatments were pooled. Bars represent standard errors (n=9). 103

Figure 5-13................. $F/F_{m'}$ measured before treatment, after 10 h light and after a subsequent 10 h dark period for light levels of 80 μmol $m^{-2}s^{-1}$, 200 μmol $m^{-2}s^{-1}$ and 600 μmol $m^{-2}s^{-1}$. Bars represent standard errors (n=9 for initial measurements, n=3 for treatment measurements). 104

Figure 5-14.Calculated NPQ for each position on leaf for the three light treatments. Bars represent standard errors (n = 9)..105

Figure 5-13.... $\delta^{13}C$ of incubating medium at conclusion of experiment (a) and total DIC (b) for each light treatment and collection point. Bars are standard errors (*n* = 3). 106

Figure 5-14................... Inorganic carbon (CO_2, HCO_3^-, $CO_3^=$) as a function of pH, temperature, TIC and conductivity, determined by Johnston's equations (AM Johnston pers. comm.) at the completion of dark or light periods for low (80 μmol m^{-2} s^{-1}), medium (200 μmol m^{-2} s^{-1}) and high (600 μmolm^{-2} s^{-1}) light. Bars are standard errors (*n* = 3-5). 106

Figure 5-15. ...Assimilation calculated from the difference between initial and final DIC concentrations for low, medium and high light incubations, collected after the light period (red) and after the following dark period (green). Bars are standard errors (*n* = 3-5)...107

Figure 5-16.......... $\delta^{13}C$ for carbon species (CO_2, HCO_3^-, $CO_3^{2=}$) for each light level alongside controls. Bars are standard errors (*n* = 3-5)..108

Figure 6-1....*Potamogeton tricarinatus* and *P. crispus*, showing different leaf types 124

Figure 6-2.. Ral Ral Creek with *P. tricarinatus* showing (a) floating leaves in small and large clumps and (b) floating leaves. Turbidity of the water is high and the submerged vegetation cannot be seen in this photograph....................................125

Figure 6-3......Cross-sections of the two leaf types of *Potamogeton tricarinatus*: (a) floating leaf, scale 1 mm and (b) submerged leaf, scale 200 μm. 128

Figure 6-5..................Magnified cross-sections of (a) floating leaved *Potamogeton tricarinatus* and (b) submerged *P. tricarinatus* leaves at 20x magnification. Scale 100 μm. 129

Figure 6-6...Chlorophyll *a* and *b* of floating and submerged leaves of *Potamogeton tricarinatus* (a). Bars are standard errors. 130

Figure 6-7.....Total inorganic carbon (a) and $\delta^{13}C$ of dissolved inorganic carbon (b) from Ral Ral Creek on sampling dates. Bars are standard errors (n = 5). 131

Figure 6-8.. $\delta^{13}C$ of dissolved inorganic carbon and bulk organic leaf material from *P. tricarinatus*; *P. crispus* (Pc) and *P. pectinatus* (Pp) are also included as fully submerged leaves (a), and $\delta^{13}C$ of soluble carbohydrates for *P. tricarinatus* with similar symbols (b). Floating leaves (F), submerged leaves on plants that also had floating leaves (S-f), and submerged leaves on plants that are fully submerged (S-S). S-f leaves are also divided into leaves within 1 m of the surface (<1) and those at greater than 1 m depth (>1). Bars are standard errors (n = 4-5 for organics; n = 2-4 for soluble carbohydrates). 132

Figure 6-9....(a) Optimum (F_v/F_m) and effective (ΦPSII) quantum yield for floating and submerged leaves of *P. tricarinatus* on the 5 January 2005 and (b) F_v/F_m for three forms of *P. tricarinatus* (floating leaves, submerged leaves on stem with floating leaves and submerged leaves on a fully submerged stem) compared with submerged leaves of *P. crispus* measured on the 12 February 2005 measured at PFD of 1093±10.7. Bars are standard errors (n = 5). 134

Figure 6-10...........RLC of *P. tricarinatus* during January 2005; dark-adapted rETR calculated with absorbed light (a) dark-adapted ETR and (b) light-adapted rETR. Dashed lines represent the α for each leaf type. 135

Figure 6-11.......RLC of *P. tricarinatus* and *P. crispus* during February 2005; light-adapted rETR calculated with light absorbed by each leaf type. Bars are standard errors (n = 4). 135