UNIVERSITY OF BIRMINGHAM

IMPACTS OF ENVIRONMENTAL STRESSORS ON THE RIVER ITCHEN *RANUNCULUS* COMMUNITY

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ABSTRACT

As fundamental components of chalk stream ecosystems, aquatic macrophytes are intrinsically linked to flow regime and physicochemical stability. Assessment of the River Itchen, Hampshire, a classic lowland chalk stream faced with ecosystem degradation, indicates the significance of the discharge regime for controlling both water quality and the spatiotemporal distribution of macrophyte assemblages. Experimental studies using outdoor artificial stream mesocosms signify their effectiveness for macrophyte growth studies and in identifying causality attributed to environmental stressors. In such experiments, the keystone chalk stream macrophyte *Ranunculus pseudofluitans* was identified as having preferences to moderate water velocities, with morphological and physiological trait responses causing distinct morphotypes depending on development in optimal or sub-optimal conditions. Furthermore, when subjected to flow, nutrient and periphytic competitive stressors, main trait responses were categorised as developmental, functional and confounded, respectively, with most traits linked to healthy development associated with flow. In addition, significant filamentous algal growth under low-nutrient conditions, but removal in increased velocities, highlights the importance of flow as a control mechanism. Examination of ontogenetic effects suggest trait variation with age, and overall developmental stage linked to a combination of environmental and plant age effects. This study demonstrates the necessity for good, consistent flow regimes in chalk streams, which enhances macrophyte community diversity, promoting development of keystone taxa, which in turn encourage beneficial heterogeneous flow patterns.

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Plants are so unlike people that it's very difficult for us to appreciate fully their complexity and sophistication.

Michael Pollan
The Botany of Desire

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Chapter I

Submerged aquatic vegetation assemblages in British chalk rivers

1.1. INTRODUCTION

Lowland Cretaceous chalk streams are renowned for having characteristically stable flow conditions, high water quality and high productivity (Heywood & Walling, 2003; Jarvie *et al.*, 2006; Walling *et al.*, 2006), and are intrinsically connected to aquifer-fed discharge regimes that typically account for up to 90% annual river discharge (Berrie, 1992; Mainstone *et al.*, 1999). Consequently, riverine thermal, physical and chemical conditions have greater stability, providing ideal conditions for development of submerged aquatic macrophytes (Berrie, 1992; Sear *et al.*, 1999). A 'macrophyte' is defined as a higher aquatic plant including angiosperms, bryophytes, pteridophytes, and some lichen/charales taxa, but excluding algae (Bornette & Puijalon, 2011). Macrophytes are key components of chalk ecosystems, significantly influencing the physical stream environment and the structure and functioning of stream ecology (Franklin *et al.*, 2008); as a result, they are frequently classified as "biological engineers" (Sand-Jensen, 1998; Cotton *et al.*, 2006; Wharton *et al.*, 2006). However, with macrophytes seen as essential criteria for gaining 'good ecological status' (Bornette & Puijalon, 2011), a more thorough understanding of key controls, stimuli and stressors is long overdue; this is now particularly important, as these habitats have been classified as being 'at greatest risk' from extinction (Ormerod *et al.*, 2010), threatened with community homogenisation (Green, 2005a; Franklin *et al.*, 2008), degradation and loss of macrophytes and supported taxa (Wright *et al.*, 2002, 2003; Green, 2005a).

Chalk streams are highly interrelated with underlying chalk geology, which allows rainwater to rapidly percolate into porous, subterranean, fine-grained limestone aquifers (Berrie, 1992; Haslam, 2006). The slow passage of water through the rock ensures consistent spring-fed discharge regimes, seasonally stable temperatures and neutral-calcareous pH (Berrie, 1992; Bickerton & Petts, 1993; O'Hare *et al.*, 2010). Furthermore, carbon, usually a key limiting factor for plant growth (Haslam, 2006; O'Hare *et al.*, 2010), is in high

concentration in the form of bicarbonate (HCO_3-), with chalk macrophyte taxa able to take advantage of this alternative supply (Newman & Raven, 1999; Lacoul & Freedman, 2006).

As a result of the unique physicochemical conditions in chalk streams, chalk macrophyte communities frequently have correspondingly unique assemblages. Main classifications of chalk communities (Rodwell, 1995; Holmes *et al.*, 1999; Hatton-Ellis & Grieve, 2003), indicate British chalk streams typically contain *Ranunculus penicillatus* ssp. *pseudofluitans* (Syme) S. D. Webster, *Callitriche obtusangula* Le Gall, *Callitriche stagnalis* Scop., *Callitriche platycarpa* Kütz, *Berula erecta* (Huds.) Coville, *Oenanthe fluviatilis* Coleman and, *Rorippa nasturtium-aquaticum* (L.) Hayek, as dominant taxa. General downstream distributions in chalk streams relate to plant dominance associated with flow and channel characteristics (Haslam, 2006), with headwaters characteristically shallower in profile compared to middle or lower river reaches (**Figure 1.1.**). In headwaters, particularly winterbournes (streams that dry irregularly in the summer; Westwood *et al.*, 2006), there are seasonal cycles of plant composition, with generally greater abundance of marginal and emergent taxa, and only

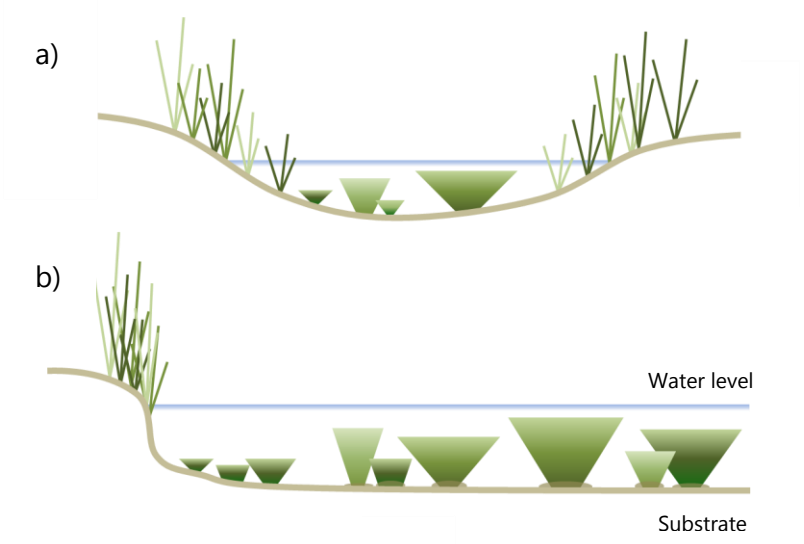

Figure 1.1.

Typical channel cross-sectional profiles of chalk streams. a) represents a shallow profile headwater stream, and b) represents middle/lower reaches, with steeper profiles and less marginal/emergent taxa.

few submerged taxa, occurring in wetted seasons (Haslam, 2006). In contrast, middle and lower reaches are dominated year-round by submerged species, with fewer emergent and marginal species present and commonly only at channel margins (Haslam, 2006).

Submerged assemblages typically form 'patches' or 'stands' of plants, that have little resemblance to terrestrial grass-like vegetation which tends to have a more uniform nature (Green, 2005a; Haslam, 2006). The semi-natural irregularity of the spatial distribution of stands are suggested to have a 'pseudo-braided' effect (Dawson & Robinson, 1984), where flow patterns split around and between stands before rejoining downstream (**Figure 1.2.**).

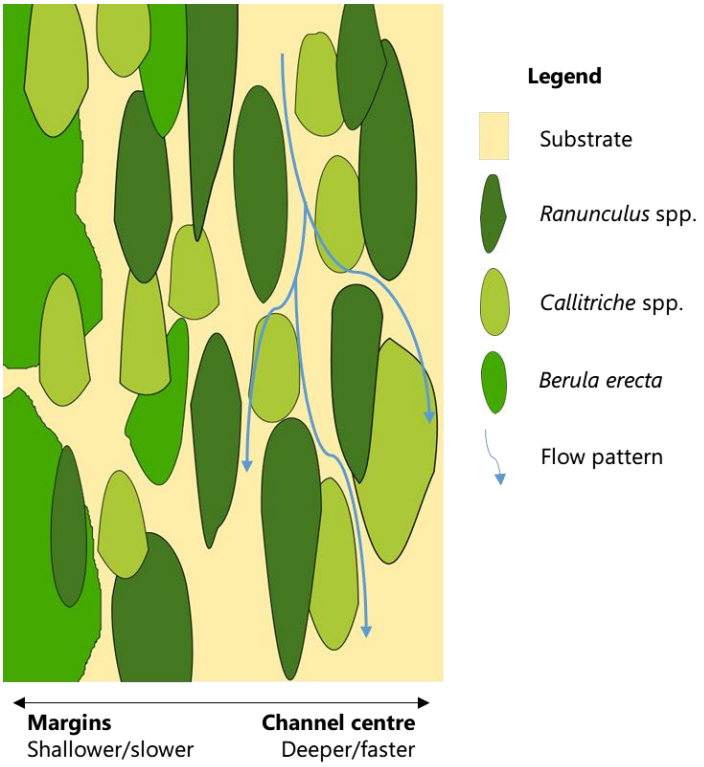

Figure 1.2.

Typical pseudo-braided nature of submerged aquatic macrophytes in chalk streams. Green shapes represent individual macrophyte stands, yellow patches represent river bed substrate, and blue arrows are representative patterns of flow around macrophyte stands.

The typical assemblage presented in **Figure 1.2.** remains relatively consistent along the length of chalk rivers, with largely non-uniform gradients of marginals, emergents and small submergents at channel edges, and larger submerged plants toward the channel centre (Haslam, 2006). The distribution of plants within these assemblages are linked to multiple parameters, including channel cross section, flow direction, riparian shade, substrate types, seasonality, management, and abiotic and biotic interactions (Davis & McDonnell, 1997; Haslam, 2006). In combination, these factors contribute to the biotic and abiotic conditions frequently associated with classic examples of chalk streams (**Figures 1.3. & 1.4.**).

a)

b)

Figure 1.3.

A classic chalk stream - the River Itchen, Hampshire. a) shows a typical open non-headwater reach with limited riparian shading, b) shows typical in-stream submerged macrophyte braided distribution in a non-headwater reach with some riparian shade.

Figure 1.4.

A macrophyte dominated chalk river reach. Limited exposed gravel substrate with abundant macrophyte growth: dark green plants are *Ranunculus* spp., light green plants are *Berula erecta*. Photograph taken through a polarising filter.

Plant life history strategies also contribute to composition of plant assemblages in chalk rivers, particularly as many aquatic macrophytes have evolved life histories in response to flow regimes (Bunn & Arthington, 2002). The frequently dominant *Ranunculus penicillatus* subsp. *pseudofluitans* (**Figure 1.5.**; O'Hare *et al.*, 2010), for example, has a life history that appears well suited to chalk regimes, as a long-lived, perennial, polycarpic species (Rich &

Figure 1.5.

***Ranunculus penicillatus* subsp. *pseudofluitans*.** The macrophyte often found in dominance throughout chalk river reaches.

Jermy, 1998), that has the capacity to reproduce both sexually and asexually, through flowering and vegetative fragmentation respectively. Phenology is most strongly influenced by seasonality, with extension and growth occuring during the spring, maturation in the summer, senescence in the late autumn, and dormancy in the winter (Davis & McDonnell, 1997). This annual growth pattern likely allows *R. pseudofluitans* to adapt to varying conditions by allowing vegetative dispersal, but under normally favourable conditions, maturation and sexual reproduction predominate. Typically, many chalk macrophyte taxa follow similar life history strategies.

However, the semi-natural plant assemblages observed in chalk streams are threatened by extraneous environmental changes (Jarvie *et al.*, 2006), which may result in loss of plant species diversity (Mainstone *et al.*, 1999; Mainstone & Parr, 2002), degradation of abiotic and biotic ecosystem components (Bickerton & Petts, 1993; Baattrup-Pedersen *et al.*, 2006; Cotton *et al.*, 2006; Withers & Jarvie, 2008), encroachment of marginal and emergent

species into channels (Ferreira *et al.*, 2005), alterations to seasonal colonisation patterns (Holmes, 1999) and plant stand and/or assemblage homogenisation (Green, 2005a; Franklin *et al.*, 2008). Threats are principally related to flow regime alteration (Westwood *et al.*, 2006a; Westwood *et al.*, 2006b) and increased pressures on water resources (Ormerod *et al.*, 2010): Generally these include over-abstraction of groundwater (Dunbar *et al.*, 2004; Wheater *et al.*, 2006), greater climate variability and uncertainty (Wheater *et al.*, 2006; Matear *et al.*, 2012), land use change (Pedersen & Friberg, 2009), agricultural intensification (Withers & Jarvie, 2008), and urbanisation (Flynn *et al.*, 2002).

As a result of pressures associated with reducing river flows, chalk rivers have been experiencing water quality deterioration, particularly from enriched nutrients (Heathwaite *et al.*, 1996) and siltation (Heywood & Walling, 2003), algal proliferation (Jarvie *et al.*, 2006), and reductions in macrophyte, macroinvertebrate and salmonid diversity (Acornley & Sear, 1999; Clarke & Wharton, 2001; Wright *et al.*, 2003). In the worst situations, where multiple factors combine, this can cause catastrophic disturbance (Rietkerk *et al.*, 2004; Biggs *et al.*, 2005), with the loss of entire river reach communities (**Figure 1.6.**). However, a significant

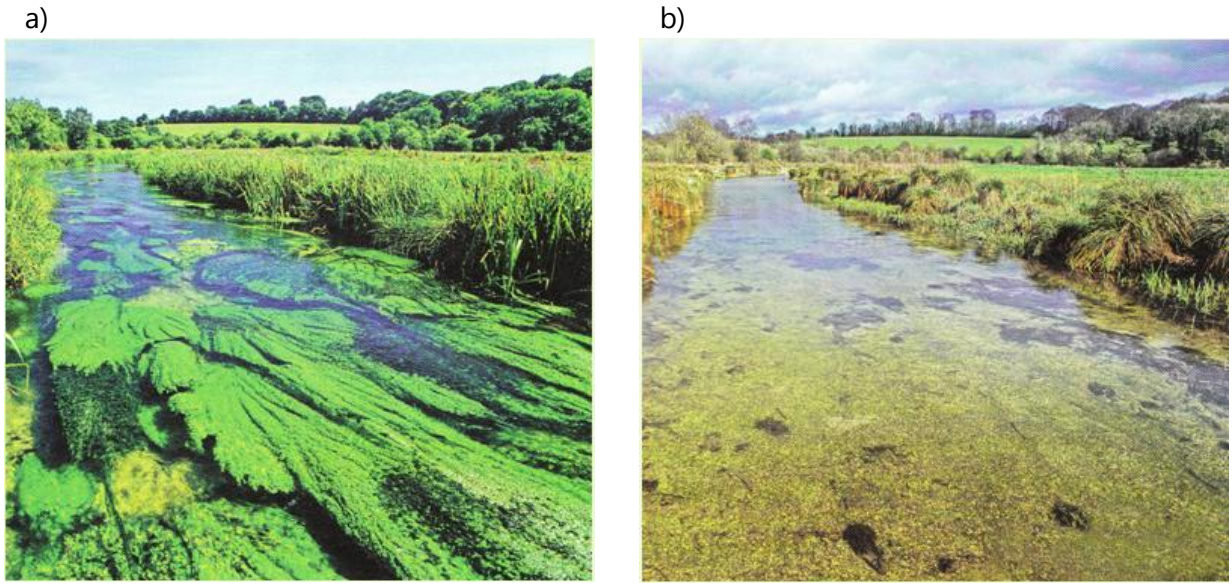

Figure 1.6. **Catastrophic macrophyte loss in the River Itchen.** a) shows a typical lowland reach in July 2003, b) shows the same reach in a highly degraded state in April 2005. Photograph after Glasspool (2007).

research gap exists surrounding the causes of chalk stream degradation (Wright *et al.*, 2002; Jarvie *et al.*, 2006; Stromqvist *et al.*, 2008), and in particular relating to macrophytic responses to environmental stimuli/stressors. Determination of tolerance thresholds of key macrophyte taxa to environmental stressors have also been significantly lacking. With primary and secondary productivity often higher in well managed river reaches (Wright *et al.*, 2003), this information may be highly valuable as guidance for river management and conservation practitioners.

Studies have examined key environmental controls on lowland riverine macrophytes via survey-based work (e.g. Demars & Harper, 1998; Dawson *et al.*, 1999; Jarvie *et al.*, 2002; Onaindia *et al.*, 2005; Raven *et al.*, 2005; Staniszewski *et al.*, 2006), field-based external experimental studies (e.g. Chambers & Kalff, 1987; Carr & Chambers, 1998; Garbey *et al.*, 2006; Puijalon *et al.*, 2008), and laboratory-based experiments (e.g. Westlake, 1967; Sand-Jensen & Madsen, 1991; Gross *et al.*, 2001; Lamberti-Raverot & Puijalon, 2012). Much research focus has been on, but not limited to, the effects of river flow (e.g. Chambers *et al.*, 1991; Champion & Tanner, 2000; Wharton *et al.*, 2006) and velocity (e.g. Sand-Jensen & Pedersen, 1999; Green, 2005b; Albayrak *et al.*, 2012), nutrient impacts (e.g. Carr & Goulder, 1990; Demars & Harper, 1998; Clarke, 2002; Jarvie *et al.*, 2002), competitive interactions (e.g. Barrat-Segretain, 2001; Jones *et al.*, 2002), or combinations of multiple factors (e.g. Puijalon *et al.*, 2007; Heathwaite, 2010; Lamberti-Raverot & Puijalon, 2012), but there is still great need for better understanding of key chalk stream macrophyte responses to environmental factors (in particular flow, nutrient enrichment and algal interactions). Furthermore, impacts of ontogenetic (life-stage) influences on macrophyte success are frequently discussed (e.g. Trémolières, 2004; Mony *et al.*, 2007; Puijalon *et al.*, 2008; Riis *et al.*, 2009), but infrequently assessed, and may represent an important element of habitat development.

The Environment Agency, as a regulatory body, are responsible for enhancing the status of the chalk macrophyte community in the River Itchen, Hampshire (Environment Agency, 2004) as a Site of Special Scientific Interest (SSSI; EEC, 1992) and Special Area of Conservation (SAC; JNCC, 2012). As a result of degrading river conditions and alteration of macrophyte assemblage (Cranston & Darby, 1992, 1995, 1997), the Environment Agency was required to assess the key environmental factors contributing to the decline in the macrophyte community in the River Itchen, and develop an understanding of the driving forces behind key macrophyte stressors in order to promote riverine conditions and enable future resilience for our chalk rivers. This research project represents key research in order to fill current knowledge gaps and fulfil Environment Agency conservation responsibilities.

1.2. STUDY AIMS AND OBJECTIVES

This research project aims to understand the key environmental factors driving variability of chalk river macrophyte assemblages at river catchment, river reach and individual plant scales, thereby examining the impacts of environmental stress at the community level and as direct autecological influences on keystone community taxa.

The study aim is achieved through the assessment of five objectives:

- To determine the environmental controls on the spatiotemporal variability of the chalk stream macrophyte community in the River Itchen, Hampshire.
- To assess the suitability of artificial outdoor experimental stream systems for the examination of keystone macrophyte species.
- To define water velocity tolerance thresholds for the optimum development and growth of the keystone chalk stream macrophyte *Ranunculus pseudofluitans.*

- To understand the effects of the interactive relationship between water velocities, nutrient enrichment and filamentous algae on the development and growth of the keystone chalk stream macrophyte *Ranunculus pseudofluitans.*
- To explain the ontogenetic age-related influences from juvenility into maturity on development of the keystone macrophyte *Ranunculus pseudofluitans* when faced with velocity constraints and naturally variable physicochemistry.

1.3. THESIS OUTLINE

The thesis is comprised of six data chapters, each written in the format of an extended academic journal article, and may therefore feature similar methodologies or repeated elements. Following a brief contextual introduction to the theme of the research, provided by this chapter, the remaining chapters are structured accordingly:

Chapter two examines the spatiotemporal variation of the submerged macrophyte community of a southern English chalk stream (the River Itchen, Hampshire) in relation to changing physicochemical conditions over a six year study period. The river flow regime and water chemistry for this period are characterised, as is the macrophyte community, and the findings of this chapter provide background understanding for the experimental chapters which follow.

Chapters three, four and five present studies that utilise similar experimental setups, and therefore share certain methodological information. The focus of these chapters is on the use of artificial outdoor stream mesocosms. Throughout, the words “mesocosm” and “channel” may be used interchangeably.

Chapter three, the first of the three experimental studies using artificial stream mesocosms, assesses the suitability, realism and replicability of the stream mesocosm

setup for the cultivation and observation of the growth of a keystone submerged chalk stream macrophyte in comparison to a natural stream reach. A large proportion of the fundamental information regarding the experimental design of the mesocosm setup is provided here, with updates only where necessary in the following two chapters.

Chapters four and five comprise the principal experimental studies involving artificial stream mesocosms. **Chapter four** examines the juvenile developmental growth responses of a keystone chalk stream macrophyte under different flow velocities. **Chapter five** investigates juvenile developmental growth responses to a combination of nutrient, flow and unrestricted algal growth treatments. Both chapters examine plant growth by assessing morphological and physiological characteristic traits.

Chapter six explores plant ontogeny over a longer growth period than observed in Chapters three to five. As with preceding chapters, chapter six is also experimental; although, this study involves juvenile specimen cultivation in a natural stream reach originally entirely lacking the target taxon. Planting locations and flow manipulations within the stream reach enable observations of juvenile plants into maturity in conditions previously thought of as 'unfavourable'.

Finally, **chapter seven** provides a synthesis and general discussion of the major preceding manuscripts in this thesis, in an attempt to draw together findings in order to answer the overall aim of this project. Additionally, study limitations and areas of further research are discussed in the context of study findings.

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Chapter II

Flow regime controls on riverine physicochemistry and macrophyte distribution in the River Itchen, Hampshire

2.1. INTRODUCTION

Aquatic macrophytes are fundamental components of many lowland lotic environments, providing key ecosystem functions to abiotic and biotic riverine factors (Franklin *et al.*, 2008; Bornette & Puijalon, 2011). These include providing food (Gross *et al.*, 2001; O'Hare, Stillman, *et al.*, 2007), habitats (Butcher, 1933) and refugia (Westwood *et al.*, 2006a) for riverine fauna, and by influencing biochemical cycles (Carr *et al.*, 1997), hydrological properties (Madsen *et al.*, 2001) and sediment dynamics (Jones *et al.*, 2012). Consequently, macrophytic growth is considered an important control on ecological stability (Franklin *et al.*, 2008), and as sensitive indicators of prevailing environmental conditions (Flynn *et al.*, 2002; Haslam, 2006; Lacoul & Freedman, 2006), the understanding of key factors and processes that influence macrophyte growth and development is vitally important.

Chalk rivers are particularly macrophyte diverse (Butcher, 1933), a feature regarded as a function of their stable, shallow-gradient, low energy hydrological regime, gravel-dominated bed substrate, and nutrient-rich, high quality waters (Berrie, 1992; Harrison, 2000; Heywood & Walling, 2003; Jarvie *et al.*, 2006; Walling *et al.*, 2006). As a consequence, development of rich faunal communities is common, with support provided for abundant macroinvertebrate and fish populations (Sear *et al.*, 1999). However, this natural stability means chalk rivers are also more susceptible to environmental change (Jarvie *et al.*, 2006) due to extraneous influence.

When altered, river conditions can become degraded, showing symptoms that have collectively been termed 'chalk-stream malaise' (Heywood & Walling, 2003; Jarvie *et al.*, 2006; Walling *et al.*, 2006; Gouldson *et al.*, 2008; Stromqvist *et al.*, 2008). Symptoms include deterioration of water quality (Cranston & Darby, 1992), abnormally high nutrient concentrations (Heathwaite *et al.*, 1996), increased suspended sediment loading, siltation

and turbidity (Heywood & Walling, 2003), loss of key macrophyte species (Clarke & Wharton, 2001), decreased macroinvertebrate abundance (Wright *et al.*, 2003), reduction in salmonid reproductive success (Acornley & Sear, 1999), and enhanced growth of filamentous and benthic algae (Cranston & Darby, 1992; Jarvie *et al.*, 2006). Difficulty in identifying causality for many of these symptoms highlights the complexity of these river ecosystems (Carr *et al.*, 1997; Westwood *et al.*, 2006b), along with the need to better understand controlling mechanisms (Franklin *et al.*, 2008) on individual macrophytes, populations and communities.

Major physical and chemical influential factors include flow (as discharge/velocity; Franklin *et al.*, 2008), nutrient availability (Spink *et al.*, 1997), light availability (Sculthorpe, 1967), shading and turbidity (Bornette & Puijalon, 2011), substrate (Barko & Smart, 1986a), and temperature (Barko & Smart, 1986b). In addition the effects of biological interactions, such as competition (Trémolières, 2004), herbivory (Wood *et al.*, 2012) and seasonal management practices (Ham *et al.*, 1982), are also important factors influencing plant distribution. Flow conditions in particular are signified as a key determining factor affecting macrophyte distribution (Haslam, 2006; Franklin *et al.*, 2008), with magnitude, frequency and variability of flows driving disturbance, fragmentation and colonisation dynamics (Riis & Sand-Jensen, 2006; Riis *et al.*, 2008). In chalk streams, distinct spatial differences in flow regime and disturbance patterns occur between middle/lower reaches and headwaters, with the latter often supporting different species compositions (Haslam, 2006; Westwood *et al.*, 2006b) and supporting rarer taxa (Ratcliffe, 1977).

As a consequence of flow controls on macrophytes, main pressures facing chalk streams are those related to flow regime alterations, with droughts and low-flow conditions a prime concern (Westwood *et al.*, 2006a; 2006b). Pressures arise predominantly from land use change (Pedersen & Friberg, 2009), such as agricultural intensification and urbanisation

(Stromqvist *et al.*, 2008; Ormerod *et al.*, 2010) and increased abstraction due to greater water resource demand (Bickerton & Petts, 1993; Dunbar *et al.*, 2004). Furthermore, uncertainty with future climatic change (Wheater *et al.*, 2006) may cause additional pressures on aquatic biodiversity, by intensifying key water cycle processes (Matear *et al.*, 2012) and altering extreme event magnitudes and frequencies. Combined with the effects of increased nutrient and sediment input from agricultural intensification (Withers & Jarvie, 2008), species distribution and composition of aquatic macrophyte communities in chalk rivers could be significantly altered.

During 1989-1992 there was a significant groundwater drought in southern England, related to a shift in winter precipitation patterns (Holmes, 1999; Westwood *et al.*, 2006b), that caused some of the lowest recorded chalk groundwater levels (Price, 1996). Ecological impacts in chalk streams were significant (Giles *et al.*, 1991), and marked differences in plant community recovery were observed to be strongly linked to flow regime recovery (Holmes, 1999), including changes to seasonal colonisation dynamics, community shifts from aquatic to wetland/meadow habitats where flows remained low, and very rapid increases in submerged taxa where flow conditions improved. Typically as flows increase, chalk submerged macrophyte dominance shifts between *Ranunculus* spp., *Berula erecta*, and *Callitriche* spp. depending on flow conditions and other in-stream factors (Wright *et al.*, 2004), but recovery in this manner is not guaranteed after droughts or low-flows.

The importance of longer-term spatiotemporal studies for understanding community responses was an important consideration shortly after the 1989-1992 droughts (Holmes, 1999; Wright & Symes, 1999; Wright *et al.*, 2004). Long-term studies can lead to clearer interpretation of patterns and trends in community responses (Burt *et al.*, 2008), and potentially allow robust inference even when faced with confounding influences (O'Hare, Stillman, *et al.*, 2007). This is particularly important for macrophytic vegetation, where

growth cycles are often seasonally dependent and strongly linked to prevailing conditions over the preceding several months or years (Wilby *et al.*, 1998; Westwood *et al.*, 2006).

The River Itchen in Hampshire, UK, a lowland temperate chalk stream, experienced periods of low flow coinciding with the droughts during the late 1980s and early/mid 1990s (Wilby *et al.*, 1998) and more recently in the early 2000s, which all caused significant deterioration in the macrophyte community. The threat of increased frequency of droughts and hot/dry summers (Marsh & Turton, 1996) due to changing climate, and the associated decline in macrophyte diversity, has prompted the need to determine driving forces behind observed and future potential reductions in macrophyte abundance and richness in the River Itchen. In particular, abundance and distribution of *Ranunculus* spp. (typically *Ranunculus penicillatus* ssp. *pseudofluitans*), a dominant macrophyte in the Itchen and other chalk rivers (Flynn *et al.*, 2002), is of interest due to its important roles in improving flow and habitat heterogeneity (Green, 2005a) and providing refugia and support for macroinvertebrates and other riverine fauna (Flynn *et al.*, 2002).

The Environment Agency performed a series of macrophyte surveys over six years (2004-2009) to monitor the macrophyte assemblage throughout the length of the Itchen, and in conjunction with riverine flow and water quality data, this study aims to determine the spatiotemporal variability of the macrophyte community of the River Itchen in relation to environmental controls during the six year study period. Study hypotheses were: 1) Flow was a key controlling factor for river chemistry and water quality; 2) except for seasonality, river discharge was the predominant controlling factor affecting macrophyte community variability; 3) groundwater reductions increase temporal variability in river physicochemistry, causing shifts in macrophyte assemblage; 4) spatial differences in the macrophyte community are principally dependent on flow regime; 5) abundance of key macrophyte taxa is controlled by competition dynamics and driven mainly by flow regime.

2.2. METHODOLOGY

2.2.1. Study area

The River Itchen, Hampshire is a classic temperate lowland calcareous chalk stream (**Figure 2.1.**). At roughly 28 miles in length and with a catchment area of 470 km² (Halcrow Ltd., 2004), the Itchen is a medium sized chalk stream, rising near the village of New Cheriton (51°2'31"N, 1°9'40"W), travelling past the cities of Winchester (51°3'49"N, 1°18'28"W) and Southampton (50°56'7"N, 1°22'30"W), meeting the sea at Southampton Water (50°53'35"N, 1°23'13"W). The Itchen has two principal tributaries, the River Alre (51°5'17"N, 1°10'59"W) and the Candover Brook (51°5'4"N, 1°11'25"W).

Figure 2.1. **Course map of the River Itchen and the surrounding area** (Poynter, 2011).

The Itchen has undergone hundreds of years of management, creating an artificially complex river with a main channel and a multitude of interconnected carrier channels, canals, water meadows, lakes/ponds, fish farms and watercress beds, keeping it in an unnatural state of succession (Butcher, 1927).

Underlain by approximately 80% chalk geology (Halcrow Ltd., 2004), like many chalk streams the Itchen is groundwater dominated (Berrie, 1992), and yields a stable flow regime, relative high water quality, and high primary and secondary productivity (Harrison, 2000; Heywood & Walling, 2003; Jarvie *et al.*, 2006; Walling *et al.*, 2006).

The Itchen has SAC (Special Area of Conservation) and SSSI (Site of Special Scientific Interest) designation (EEC, 1992; JNCC, 2012), and is an Annex I 'H3260' priority habitat (JNCC, 2010). Six Annex II key species are associated with these habitats, namely *Coenagrion mercurial* (Southern Damselfly), *Cottus gobio* (Bullhead), *Lampetra planeri* (Brook Lamprey), *Salmo salar* (Atlantic Salmon), *Austropotamobius pallipes* (White-clawed Crayfish), and *Lutra lutra* (Otter).

2.2.2. Survey and sampling technique

Study data collection was split into three parts: water quality, flow data, and macrophyte survey data. Water quality parameters were measured monthly by 16 automated water samplers, river discharge was measured daily by 8 automated flow gauging stations, and macrophyte surveys were undertaken at 28 sampling sites, providing coverage of a wide range of sites from river source to mouth (**Figure 2.2.** and **Table A.1.**, Appendix A.). The 28 survey sites were treated as principal sites, and water quality/flow gauging sites approximately correspond to these; in correlative analyses, survey sites were assigned water quality/flow monitoring sites according to proximity and channel associations.

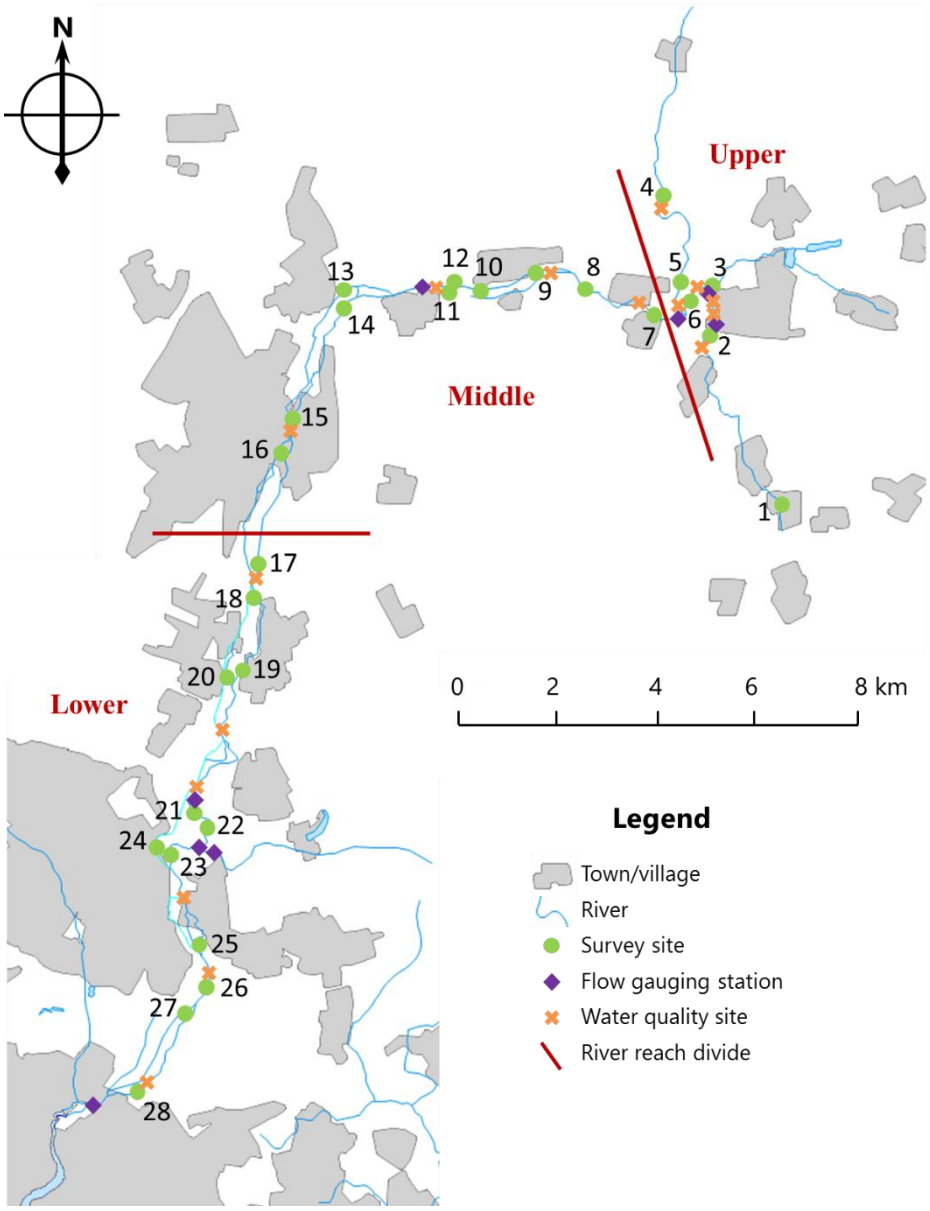

Figure 2.2. **Course map showing the locations of the 28 macrophyte survey sampling sites and the water quality/flow gauging sites along the length of the River Itchen.** Circles are macrophyte survey sites, diamonds are flow gauging stations and crosses are water quality sites. Numbers refer to macrophyte sampling sites. Red lines denote division between sites in upper, middle and lower river reaches.

Spatial variation was assessed by splitting sites into three sections (upper, middle, lower) according to position along the river (**Figure 2.2.**), due to distinct differences between headwaters and lower reaches (Ratcliffe, 1977; Haslam, 2006; Westwood *et al.*, 2006b). Upper reaches were defined as those in first-order headwater streams (with elevation > 40

m above sea level, <7 km downstream of the source), middle reaches were in the second-order main river (*ca.* 25-40 m above sea level, 8-20 km downstream), and lower reaches were downstream sections of the river (*ca.* 0-25 m above sea level, 20-38 km downstream).

2.2.2.1. Water quality and river flow data

Water physicochemistry was measured routinely from September 2004 to July 2009. Physicochemical parameters in this study (**Table 2.1.**) were collected by Environment Agency flow gauging stations and water samplers (section 2.2.2.). Temperature, pH, dissolved oxygen and conductivity were all field-measured using a fully calibrated and maintained multimeter (YSI, Yellow Springs, U.S.A.). Orthophosphate (herein phosphate) was analysed by automated colourimetric analysis and nitrate was analysed using discrete colourimetric analysis in a Konelab Discrete Analyser (ThermoFisher Scientific Inc, Waltham, U.S.A.). Phosphate was determined by reacting samples with ammonium molybdate and antimony (III), reducing with ascorbic acid to form phosphomolybdenum blue, and then analysed colourimetrically (Standing Committee of Analysts, 1981a). To determine nitrate, samples were treated with sulphanilamide and N-1-naphthylene diamine dihydrochloride under acidic conditions, with the resulting pink azo-dye analysed colourimetrically (Standing Committee of Analysts, 1981b).

Physical channel variables were recorded at each macrophyte survey site (see 2.2.2.2.). Mean channel widths and depths were recorded with a tape measure and meter rule respectively. Shade (broken and dense) was recorded as estimated percentage cover for the reach and water clarity as percent cloudiness (0% = clear). Bed substrate and habitat type (pool, run, riffle, slack) were also recorded, but discarded from analysis due to subjectivity and field measurement difficulty due to macrophyte growth in some circumstances.

Daily discharge data were used directly, as current (sample-date) discharge, and to calculate antecedent discharge parameters (**Table 2.1.**) accounting for discharge variability over various plant growth stages. These discharge parameters were calculated over a range of timescales preceding each macrophyte survey date Antecedent time-periods were calculated using each survey date as the starting time-point, and back-calculating the appropriate time-period from that point.

Table 2.1. **Definition of water physicochemistry selected for analysis.** Table split into 'sample date' and 'antecedent' representing parameters corresponding to survey samples and calculated antecedent discharge parameters.

Sample date physicochemistry		**Antecedent discharge**		
Abbreviation	**Physicochemical variable**	**Abbreviation**	**Antecedent discharge parameter***	**Antecedent time-periods**
T	Water temperature (°C)	Q_{mean}	Average discharge	• weekly • 2-weekly • 3-weekly • 4-weekly • 8-weekly • 12-weekly • 16-weekly • 20-weekly • 24-weekly • 32-weekly • yearly • previous summer • previous winter • previous 2-summer's • previous 2-winter's
Q	River discharge (m^3 s^{-1})	Q_{max}	Maximum discharge	
pH	Water pH (pH units)	Q_{min}	Minimum discharge	
N	Nitrate (NO_3-) concentration (mg l^{-1})	Q_{10}	High flows – discharge exceeded only 10% of the time	
P	Orthophosphate (PO_4-) concentration (mg l^{-1})	Q_{25}	Above average flows – discharge exceeded only 25% of the time	
Con	Conductivity (µS cm^{-1})	Q_{50}	Median flows – discharge exceeded 50% of the time	
DO	Dissolved oxygen (% saturated)	Q_{70}	Below average flows – discharge exceeded 70% of the time	
W	River channel width (m)	Q_{90}	Low flows – discharge exceeded 90% of the time	
D	River channel mean depth (m)	Q_{95}	Low flows – discharge exceeded 95% of the time	
ShB	Percentage channel shaded by broken shade (%)	Q_{99}	Very low flows – discharge exceeded 99% of the time	
ShD	Percentage channel shaded by dense shade (%)			
WC	Water clarity (%)			

*Discharge parameters calculated over each antecedent time-period.

In addition to the variables in **Table 2.1.**, channel type (main or carrier), stream order and distance downstream from the source were also investigated. Furthermore, previous

seasonal macrophyte growth (as % cover at the previous season) of the four key taxa (see 2.3.3.) was also used as an explanatory variable.

2.2.2.2. Macrophyte surveys

River macrophyte surveys were performed using techniques in the Mean Trophic Rank (MTR) methodology; for further details, refer to (Holmes *et al.*, 1999). Surveys were conducted three times per year (spring, summer, autumn) from 2005-2008 and in autumn and summer in 2004 and 2009 respectively. All 28 sites were surveyed within three days per season to minimise sampling error, except where weather/access prevented sampling. A 100m river reach at each sample site was surveyed, between two fixed bankside points. Macrophyte taxa (see **Table 2.3.** for most abundant; for full list see **Table A.2.**, Appendix A), including some algae and bryophytes, were recorded as presence/absence and abundance (percentage cover of the stream bed at each survey site). Taxa were identified to species level where possible and difficult to identify taxa in the field were collected and examined in the laboratory. Surveyed aquatic macrophyte taxa, including submergent (plus floating taxa), emergent and marginal species, were recorded in the survey reach within the river channel (including bank area - submerged > 50%, but <85% of the time).

2.2.3. Data analysis

Water chemistry data were presented as collected (monthly) and daily discharge data were averaged over each month to enable comparisons with macrophyte data (monthly).

Before statistical tests were performed, data exploration was undertaken using a range of techniques (Zuur, Ieno, & Elphick, 2009). Boxplots and Cleveland dotplots were used to inspect outliers; pairplots and variance inflation factors (VIFs) to examine collinearity amongst explanatory variables; histograms and QQ-plots tested distributions; and

frequency plots assessed zero inflation. In response to data exploration findings: 1) Major outliers were removed; 2) collinear variables were removed, where VIFs > 3 (Zuur, Ieno, Walker, *et al.*, 2009) – only antecedent discharge parameters were collinear (selection process for discharge parameter inclusion detailed below); 3) appropriate distributions were selected for analyses; 4) zero-inflation was not observed, although data were considerably skewed towards lower values for most macrophyte cover response variables (**Figures A.1.-A.5.**, Appendix A) – this was corrected during model distribution selection.

Temporal variation in physicochemistry during the study period was presented graphically per month. Pearson's Product Moment correlations were used to test discharge influence on river water quality (phosphate, nitrate, conductivity, pH and dissolved oxygen). Data were separated into upper, middle and lower river reaches (as in **Figure 2.2.**) to examine correlations between discharge and chemistry spatially, downstream of the river source. River macronutrients (phosphate and nitrate) were examined using regression analysis and curve estimation to determine best fitting relationships with discharge, using all data.

Community spatial assemblage was assessed by calculating species richness (S_{max}), Shannon-Wiener evenness index (E_H), Berger-Parker dominance index (d) and species turnover (beta diversity) for upper, middle and lower river reaches, as well as for channel type (main/carrier) and stream order. Richness was determined from counts of species present at each sample site and date. For the Shannon-Wiener evenness index, first the Shannon-Wiener diversity index was required:

$$H = \ln N \; - \frac{1}{N} \sum_{i=1}^{\infty} (p_i \; ln \; p_i) n_i$$

where, p_i = proportion of individuals in ith species, n_i = number of species with i individuals. The Shannon-Wiener evenness index was calculated by the following equation:

$$E_H = \frac{H}{\log S}$$

where, H is the Shannon-Wiener diversity index, and S is the total number of species.

The Berger-Parker dominance index was calculated as follows:

$$d = \frac{n_{max}}{n_T}$$

where, n_{max} is the abundance of the dominant species, and n_T, is the proportion of the total number of individuals in the sample.

Classical beta diversity was calculated as:

$$beta = \frac{gamma}{alpha}$$

where beta is species turnover, gamma is total richness, and alpha is per-site richness.

Rank abundance curves were plotted for entire community and submerged taxa, using $\log_{10}$ transformed taxon abundance against $\log_{10}$ transformed ranks ordered in decreasing abundance, to examine diversity between upper, middle and lower river reaches.

A series of unconstrained and constrained ordinations were run to examine general trends in macrophyte assemblage and river physicochemistry. Prior to analysis, data transformations were applied to variables to improve normality. Environmental variables (pH, P, ShB, ShD, Con, Q) were square-root, arcsine square-root or $\log_{10}$ transformed as appropriate, and all species data were square-root transformed. All data were then standardised (0 mean, 1 standard deviation) to correct scale differences. Additionally, macrophyte taxa that were present in < 5% samples were removed from analyses to prevent rare species confounding patterns. For a full list of macrophytes used in

ordinations, refer to **Table A.1.** (Appendix A.) Ordinations were run in the R suite (R Core Team, 2013) using the *vegan* package (Oksanen *et al.*, 2013). Ordinations with explanatory variables underwent variable selection using Monte Carlo permutation tests (1000 unrestricted permutations) to test significance of each component of the model ($p < 0.05$).

Four separate ordinations were performed: unconstrained principal components analysis (PCA) to examine spatial variability of environmental variables, coded as 'species' variables; partial redundancy analysis (pRDA) examining spatial variability of macrophyte taxa constrained to upper, middle and lower river reaches (assigned as dummy variables, 0 or 1); pRDA examining seasonal variability of macrophyte taxa constrained to spring, summer and autumn samples (as dummy variables, 0 or 1); pRDA assessing environmental influence on macrophyte variability. In redundancy analyses, seasonal/environmental, spatial/environmental, and seasonal/spatial influences respectively were partialled out. Redundancy analysis (linear ordination) was used due to short gradient lengths (<3 SD; Lepš & Šmilauer, 2003) during preliminary detrended correspondence analysis (DCA).

To examine associations between physicochemical parameters and key macrophyte taxa (defined in 2.3.3.), binomial generalised additive mixed-effects models (GAMMs), with a logit-link function, were fitted to proportional plant cover data and an Information-Theoretic (IT) multi-model averaging approach was applied (Burnham & Anderson, 2002). GAMMs are a highly useful form of regression for modelling complex non-linear relationships (Austin, 2002), and as residuals showed non-linear patterns during data exploration (Zuur *et al.*, 2007), additive modelling was the preferred choice. Additionally mixed-effects models allow the incorporation of random effects which account for nested data and spatial or temporal correlation and overdispersion (Zuur, Ieno, Walker, *et al.*, 2009). The IT approach allows model parameters to be considered *a priori*, and avoids model selection bias and parameter estimation bias by presenting models from a 'strength

of evidence' perspective (Burnham & Anderson, 2002). GAMMs were fitted in R (R Core Team, 2013) using the packages *mgcv* (Wood, 2004) and *nlme* (Pinheiro *et al.*, 2013), and the IT selection procedure was performed using the package *MuMIn* (Barton, 2013).

Prior to running models, fixed structures, random structures and optimal variance structures (**Table A.7.**, Appendix A) were determined for each taxon, and spatial and temporal auto-correlation was examined. Furthermore, best-fitting antecedent discharge parameters for each taxon were determined using simple binomial generalised linear models and AIC_C (second-order Akaike's Information Criterion) selection in R. Akaike's Information Criterion (Burnham & Anderson, 2002; Zuur, Ieno, Walker, *et al.*, 2009) is a selection criterion used to determine best-fitting models, and second-order AIC (AIC_C), includes corrections for small-sample bias and overdispersion (Burnham & Anderson, 2002). Lowest relative AIC_C score represents the best model. The best-fitting discharge parameter for each taxon was then used as the only discharge parameter in the full models. Data transformations were not performed, as distributions and model structures account for spread of data and heterogeneity (Zuur, Ieno, & Elphick, 2009).

Spatial auto-correlation was assessed using spline correlograms in R (using the package *ncf*; Bjornstad, 2013) by plotting Pearson residuals of linear mixed-effects models (using the package *lme4*; Bates *et al.*, 2013), with all explanatory variables and random effects (sample sites) included to account for correlation explained by environmental and spatial variables. Spatial auto-correlation was not present for any of the key taxa (**Figure A.5.**, Appendix A.) Temporal auto-correlation of data was examined using the auto-correlation function (ACF) visualisation tool in R (Zuur, Ieno, Walker, *et al.*, 2009) by plotting residuals of mixed models and spatial (sampling site) variables. ACFs for all key macrophytes demonstrated temporal auto-correlation, particularly at short time-lags (**Figure A.6.**, Appendix A.). Temporal auto-correlation was resolved by treating sampling dates as evenly spaced time

intervals and adding an auto-regressive correlation structure (AR-1) into models, which resulted in AIC_C improvements for all key macrophytes (*Ranunculus* AIC_C from 1303.57 to 1287.09; *Berula* 1189.88-1188.22; *Callitriche* 1492.74-1458.65; *Cladophora* 1523.71-1383.97). Accounting for temporal autocorrelation also significantly improved residual heterogeneity in model validation.

The IT model selection approach (full models) involves running all possible combinations of explanatory variables (full-suite to individual variables) as a 'candidate set' of possible models and AIC_C was used to select best-fitting models, ranked lowest-highest based on AIC_C score, with lower scores representing better models. Akaike weights (w_i – determined using AIC_C differences, Δ_i) were then used to suggest the likelihood of selection as the best model, with the w_i of a given model (out of 1) is the odds of it being selected as best model. A 95% confidence set was then created and model averaging was applied, in situations where no single model was deemed the best (w_{best} <0.9; Burnham & Anderson, 2002; Johnson & Omland, 2004). The confidence set was created where $\sum w_i \geq 0.95$ of the full set of candidate models, and suggests that there is a significant chance of one of the models in the candidate set being selected (Whittingham *et al.*, 2005). Following this, model averaging is used to indicate the likelihood of selection of single explanatory variables relative to other variables within the candidate set. It is determined by calculating the $\sum w_i$ out of the full set of models containing the given variable, which is classed as the variable 'selection probability' (Burnham & Anderson, 2002). In covariate selection, minimum significance was $p \leq 0.05$ for model parameters, although those close to the significance level should be treated with caution (Zuur, Ieno, Walker, *et al.*, 2009). After best-fit model selection, full model validation was performed to determine if each model still adhered to model assumptions (Zuur, Ieno, Walker, *et al.*, 2009).

2.3. RESULTS

2.3.1. River physicochemistry

Discharge had significant seasonal variation during the study period, with higher flows in winter and spring, and lower flows in summer and autumn (**Figure 2.3.**). There was a marked difference between discharge in 2004-6 (winter mean 2.62 $m^3 s^{-1}$, summer mean 1.79 $m^3 s^{-1}$) and 2007-9 (winter mean 3.69 $m^3 s^{-1}$, summer mean 2.59 $m^3 s^{-1}$) and variability was greater during 2007-9 (range 15.23 $m^3 s^{-1}$) compared to 2004-6 (range 12.96 $m^3 s^{-1}$). Water temperatures were consistent during the study with a distinct seasonal pattern (**Figure 2.3.**). Winter mean temperatures were 7.97 °C and summer means were 14.93 °C, with a summer maximum of 19.51 °C and a winter minimum of 4.6 °C. Variation between sampling sites was minimal during the study period (CV = 5%), supporting the notion of thermal stability. Conductivity was less variable during 2004-6, with a mean of 510 µS cm^{-1} and a range of 71 µS cm^{-1}. 2007-9 shows an increasing trend, with a higher mean of 560 µS cm^{-1} and greater variability with a range of 158 µS cm^{-1}. Water pH was variable but consistently alkaline through the study period, with a winter mean of pH 7.77 and a summer mean of pH 7.98. Phosphate slowly declined throughout the study period (2004-6 mean 0.086 mg l^{-1}; 2007-9 mean 0.056 mg l^{-1}), and had markedly higher variability during 2004-6 (range 0.75 mg l^{-1}) than in 2007-9 (range 0.294 mg l^{-1}). The frequency of high phosphate peaks (> 0.2 mg l^{-1}) is also greater during 2004-6 (n = 33), compared to 2007-9 (n = 4). Nitrate had a fluctuating seasonal pattern and a slight increasing trend from 2004-6 (mean 5.71 mg l^{-1}) to 2007-9 (mean 6.23 mg l^{-1}). Dissolved oxygen exhibited a winter-low, summer-high pattern, although inter-monthly variability was high. There were no overall trends during the study period (mean 99.51%, max 159.9%, min 62.8%).

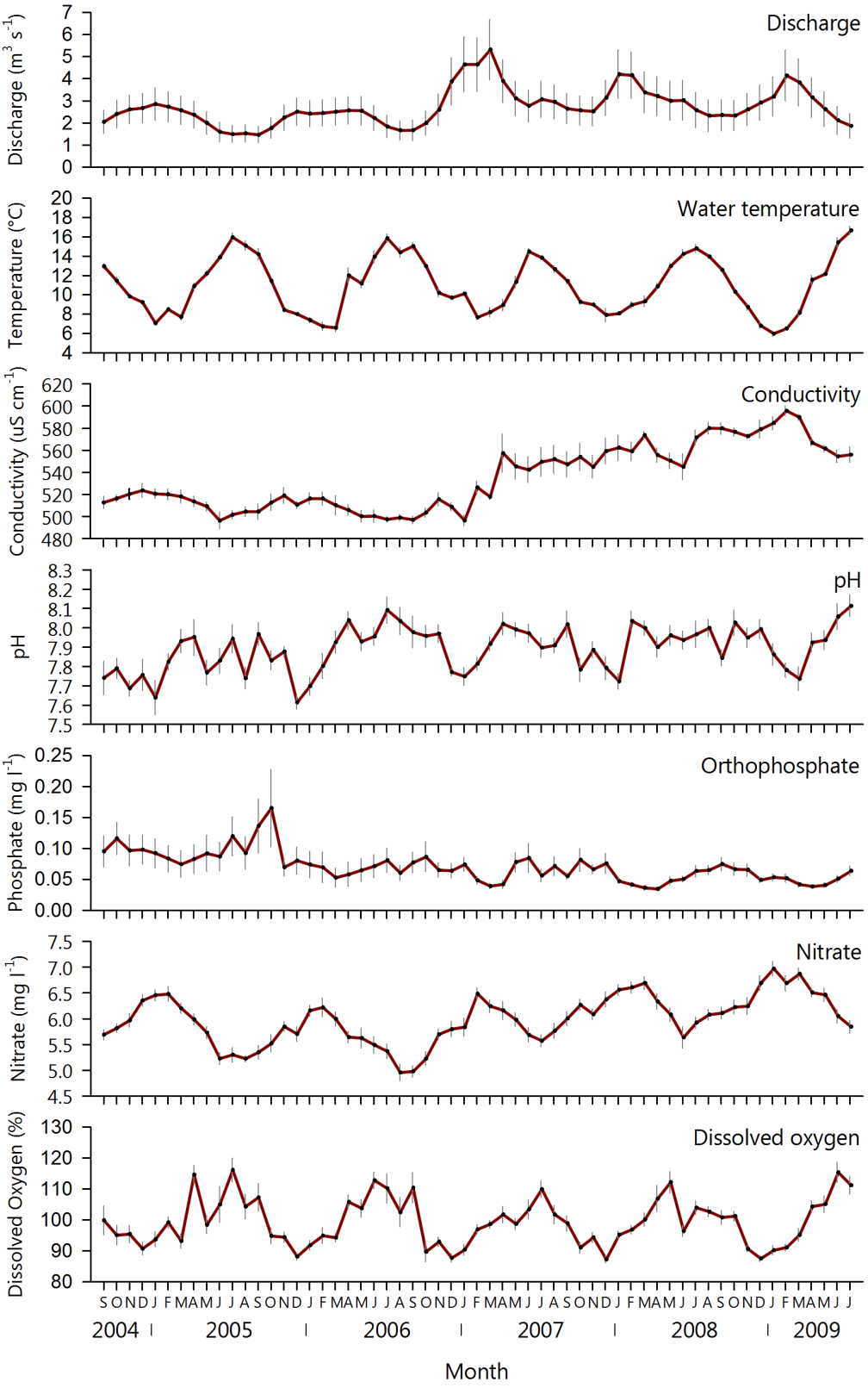

Figure 2.3. **Mean monthly discharge hydrograph, water temperature, conductivity, pH, orthophosphate, nitrate and dissolved oxygen measurements for the River Itchen.** Error bars represent standard error for each month (discharge *n* = 8; water quality *n* = 16).

Spatial differences in discharge between upper, middle and lower river reaches adopt an increasing downstream trend (**Figure 2.4.**). Discharge in upper reaches (mean 0.89 ± 0.04 $m^3 s^{-1}$) was distinctly lower than in the middle (4.05 ± 0.14 $m^3 s^{-1}$) and lower (5.31 ± 0.18 $m^3 s^{-1}$) reaches. Coefficient of variation indicates that discharge in upper reaches is markedly more affected by seasonality (CV = 59%), than middle (26%) and lower (35%) river reaches.

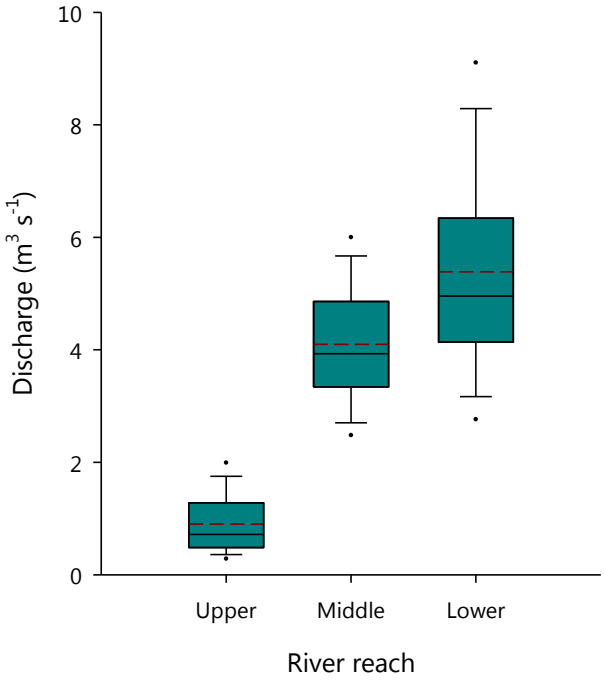

Figure 2.4.

Discharge distribution for the study period in upper, middle and lower river reaches. Boxes represent upper (75%) and lower (25%) quartiles, horizontal solid lines median values and dashed lines mean values. Whiskers represent 95% and 5% percentiles. Dots represent outliers maximum and minimum. $n_{(upper)}$ = 177; $n_{(middle)}$ = 59; $n_{(lower)}$ = 219.

Figure 2.4. indicates flow may have a controlling influence on water chemistry. In particular river macronutrient concentrations are influenced by discharge. Phosphate troughs during high flow periods, and peaks when discharge is lower. This is particularly evident between the 2004-6 and 2007-9 periods, where discharge increases between the two periods, and phosphate declines. Nitrate is also affected by discharge, following a positive correlation, and exhibits greater seasonality in line with increasing discharge.

Pearson's product-moment correlations between discharge and water chemistry (**Table 2.2.**) highlight significant correlations throughout the length of the river. In all river reaches,

phosphate was significantly negatively correlated with discharge, and nitrate and conductivity were significantly positively correlated. pH and dissolved oxygen were not correlated with discharge in upper reaches, but dissolved oxygen was negatively correlated in middle and lower reaches, and pH was negatively correlated in lower river reaches.

Table 2.2. **Pearson's Product Moment Correlations between flow and water quality parameters in upper, middle and lower river reaches.** Significant correlations highlighted in bold. *r* is the correlation coefficient, R^2 is the coefficient of determination. n = 59.

Water quality parameter	Upper		Middle		Lower	
	r	***R²***	***r***	***R²***	***r***	***R²***
P	**-0.324***	0.105	**-0.503****	0.253	**-0.528****	0.279
N	**0.720****	0.518	**0.724****	0.525	**0.490****	0.240
Con	**0.591****	0.349	**0.499****	0.249	**0.324***	0.105
pH	0.054	0.003	-0.075	0.006	**-0.307***	0.079
DO	-0.063	0.004	**-0.356****	0.127	**-0.343****	0.118

** $p < 0.01$; * $p < 0.05$

Phosphate in particular had variable responses to changes in flow that indicate both spatial differences and further support for flow as a controlling mechanism (**Figure 2.5.**). Upper reaches had limited trends with phosphate, although discharge was naturally lower in these reaches. Middle and lower reaches show related trends, with typically higher phosphate concentrations, and phosphate peaks that tend to occur mainly when discharge is low. The highest phosphate peaks (> 0.1 mg PO_4- l^{-1}) also tend to occur mostly in middle and lower reaches.

Spatial variation in water chemistry was further examined using a PCA of monthly water quality data from the study (**Figure 2.6.**). Axes 1 and 2 of the PCA were significant (p <0.05, <1000 permutations) and cumulatively accounted for 42.8% of overall variance. Axis 1 (PC1)

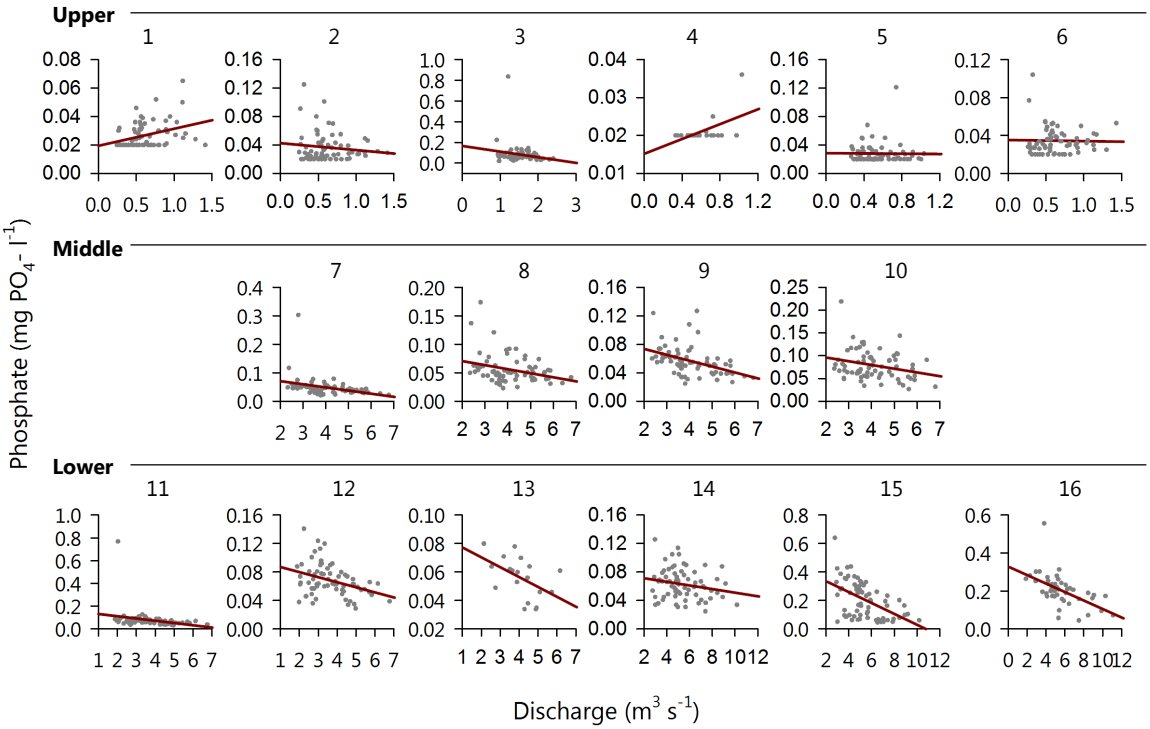

Figure 2.5. **Phosphate-discharge relationships along the length of the river.** Demonstrates general reduction in phosphate under higher flows, particularly in middle-lower reaches. Split into upper, middle and lower reaches. Numbers refer to water quality sampling site – for location information see Table A.1., Appendix A. *n* = 70 (sites 1, 3, 5, 7-12, 14), *n* = 69 (2, 15, 16), *n* = 68 (6), *n* = 20 (4), *n* = 19 (13).

explained 23% variation in water chemistry and indicated a strong gradient with pH, conductivity, discharge and water depth and width positively associated and dense shade negatively associated. Axis 2 (PC2) explained 19.8% variation and highlighted a gradient with nitrate, broken shade and water clarity positively associated and temperature, phosphate and dissolved oxygen negatively associated. This signifies less of a link between river flow and macronutrients, although phosphate and nitrate are at opposing ends of the PC2 gradient. Additionally, sites are positioned in agreement with PC1 and the discharge gradient, with sites in lower reaches generally positively correlated and upper reaches negatively correlated.

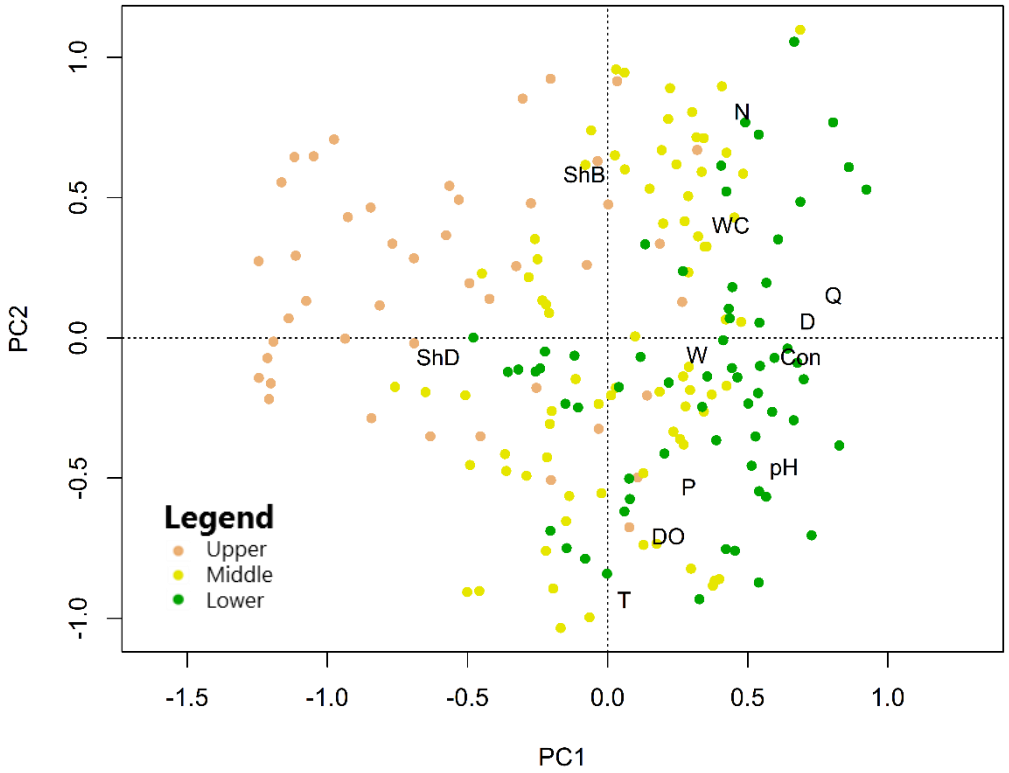

Figure 2.6. **Principal components analysis (PCA) illustrating spatial differences in riverine physicochemistry.** Axis 1 (PC1) explains 23% variance, axis 2 (PC2) explains 19.8% variance. Abbreviated names represent water physicochemical parameters and dots are samples. Con – conductivity, DO – dissolved oxygen, N – nitrate, P – phosphate, pH – water pH, ShB – broken shade, ShD – dense shade, T – temperature, D – channel depth, W – channel width, WC – water clarity, Q – discharge.

A further partial-RDA (**Figure A.7.**, Appendix A) with environmental variables constrained to discharge ($p < 0.001$, $f = 17.049$) suggests that flow regime only explains 8.5% of variation in river physicochemistry, with positive associations to pH, water clarity, conductivity and negative associations with dense shade.

2.3.2. Characterisation of the macrophyte community

A total of 265 submergent, emergent and marginal taxa were recorded along the length of the river (**Table A.2.**, Appendix A). The river was dominated by 15 core taxa (> 1% total abundance; **Table 2.3.**) which collectively accounted for 81% of total macrophyte abundance, with 250 rare taxa (< 1% total abundance) contributing the remaining 19%.

Table 2.3. **Core taxa (> 1% total abundance) recorded during the study.** Ranked according to proportion of total abundance. For full list of taxa recorded see Table A.2., Appendix A.

Taxon name	Proportion of total abundance	Mean cover
Ranunculus penicillatus subsp. *pseudofluitans*	19.9%	16.3 ± 1%
Berula erecta	13%	11.1 ± 0.6%
Callitriche spp.	12.4%	11.6 ± 1%
Cladophora glomerata	12.1%	10.5 ± 1%
Zannichellia palustris	2.9%	9 ± 1.5%
Vaucheria spp.	2.8%	3.8 ± 0.6%
Schoenoplectus lacustris	2.7%	8.1 ± 1.5%
Glyceria maxima	2.6%	5.3 ± 0.9%
Callitriche obtusangula	2.3%	5 ± 1%
Diatom scum	2.2%	32.2 ± 8.1%
Oenanthe fluviatilis	1.9%	5.5 ± 1%
Apium nodiflorum	1.9%	2.6 ± 0.6%
Phalaris arundinacea	1.7%	1.9 ± 0.3%
Hippuris vulgaris	1.5%	5.9 ± 1.1%
Sparganium erectum	1.1%	1.3 ± 0.3%

Seasonality influenced taxon richness and evenness during the study period. Spring generally had fewer recorded taxa (mean richness 25 ± 0.6), compared with summer (30 ± 0.5) and autumn (31 ± 0.6), but had greater evenness as a consequence (mean evenness, spring 0.55 ± 0.01, summer 0.49 ± 0.01, autumn 0.5 ± 0.01) suggesting that progression through the growing season presented conditions for taxa to colonise and grow, but ultimately favoured dominance by a few core taxa. This was reflected by Berger-Parker dominance index scores, showing that summer (mean B-P index 0.51 ± 0.01), and to an extent autumn (0.48 ± 0.01), saw greater contributing abundance of dominant taxa in comparison with spring (0.46 ± 0.02).

Taxon richness also varied with downstream distance (**Figure 2.7.**). In upper reaches, richness increases rapidly with distance from the source. Middle reaches had no discernible pattern (mean richness = 30 ± 0.6), although sites 15 and 16 were anomalous. Sites in lower reaches had similar richness to middle reaches (30 ± 0.5).

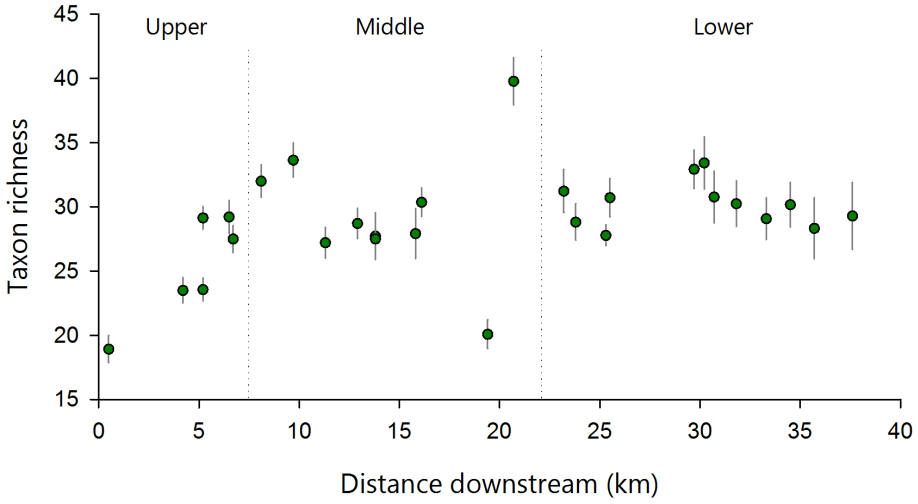

Figure 2.7. **Variation in species richness with distance downstream.** Dotted lines indicate whether sites are in upper, middle or lower river reaches. Error bars represent standard error for each survey site (*n* = 14).

Community structure also varied between upper, middle and lower river reaches (**Figure 2.8.**, **Table 2.4.**). Upper reaches were characterised by communities dominated by 19 core taxa, collectively accounting for 86% of total abundance, with 137 rare taxa contributing the remaining 14%. Middle reaches were dominated by 9 core taxa, accounting for 82% total abundance, and 194 rare taxa contributing 18%. Lower reaches were dominated by 14 core taxa, with 85% of total abundance, and 185 rare taxa making up the remaining 15%. Lower numbers of core taxa in middle reaches are signified by a sharper initial decline in abundance, which would suggest a slightly more disturbed community, dominated by fewer core taxa. 10 out of the 19 core taxa in upper reaches, all 9 core taxa in the middle reaches and 12 out of the 14 core taxa from the lower reaches were core taxa from the full river set (16 originally) showing that, whilst community structure varies spatially, the core

taxa presence remains consistent between river reaches. Major differences include a switch in dominance from *Callitriche* spp. (19.7 ± 3.5%) and *B. erecta* (13.5 ± 1.7%) in the upper reaches, to *R. pseudofluitans* (17.4 ± 1.8%) and *Callitriche* spp. (17.3 ± 1.5%) in the middle reaches, and *R. pseudofluitans* (23 ± 1.7%) and *C. glomerata* (17.4 ± 1.9%) in the lower reaches. Differences in macrophyte assemblage between upper, middle and lower reaches are likely due to plant tolerances to changing river physicochemistry, driven by a downstream increasing gradient of discharge (**Figure 2.4.**).

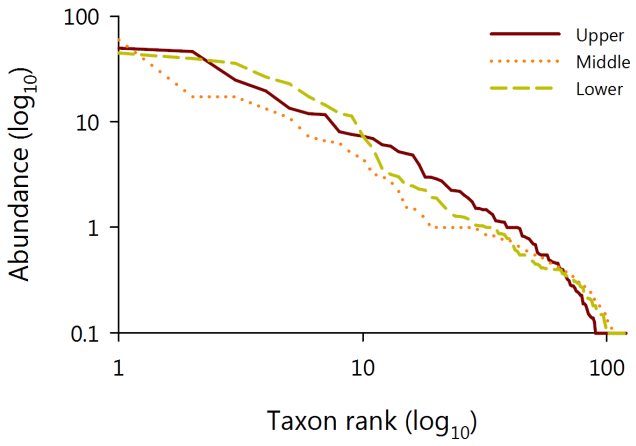

Figure 2.8.

Mean rank abundance (log_{10}) of macrophytes in upper, middle and lower river reaches. Taxa were ranked left to right in order of decreasing abundance.

Beta diversity indicated relatively fast species turnover in all reaches (**Table 2.4.**), with high gamma diversity in upper, middle and lower reaches, yet relatively few species (taxon richness) at individual sites at any one time.

Table 2.4. **Mean site taxon richness, evenness and Berger-Parker dominance index and gamma / beta diversity for upper, middle and lower river reaches** ($n_{(upper)}$ = 84; $n_{(middle)}$ = 137; $n_{(lower)}$ = 149).

	Taxon richness		Taxon evenness		Berger-Parker index		Gamma diversity	Beta diversity	
	Mean ($\bar{x}$)	SE ($\sigma_{\bar{x}}$)	Mean ($\bar{x}$)	SE ($\sigma_{\bar{x}}$)	Mean ($\bar{x}$)	SE ($\sigma_{\bar{x}}$)	Total	Mean ($\bar{x}$)	SE ($\sigma_{\bar{x}}$)
Upper	25	0.58	0.52	0.02	0.50	0.02	156	6.2	1.6
Middle	30	0.60	0.52	0.01	0.45	0.01	203	6.9	1.6
Lower	30	0.51	0.49	0.01	0.51	0.01	199	6.6	0.5

Taxon richness, evenness and B-P dominance index scores did not vary considerably depending on channel type (main channel/carrier channel), or depending on stream order.

Relative contribution of taxon groups (submergent, emergent, marginal) suggested limited spatial variation in taxon richness within the groups (**Figure 2.9.a.**). All reaches had higher counts of marginal taxa (mean richness upper 13, middle 15, lower 15), with emergent taxa accounting for the lowest numbers (mean upper 9, middle 8, lower 10). Submerged taxa had mean richness of 9 in upper reaches, 12 in middle reaches, and 11 in lower reaches. Relative contribution of groups did however highlight differences between upper reaches and middle/lower reaches by abundance (**Figure 2.9.b.**). All reaches were dominated by submergent taxa, but this was less pronounced in upper reaches (42.7% mean relative cover) than in middle (68.3%) and lower (68.7%) reaches. Consequently, emergent and marginal taxa had greater relative abundance in upper reaches (32.7% and 24.6% respectively) in comparison to middle (22.6% and 9.1%) and lower (22.4% and 8.9%) reaches. Variation in submerged taxa temporal presence was also markedly higher in upper reaches (CV = 42%) than emergents (21%) and marginals (26%). In middle and lower reaches, there were no marked differences in variation within taxon groups.

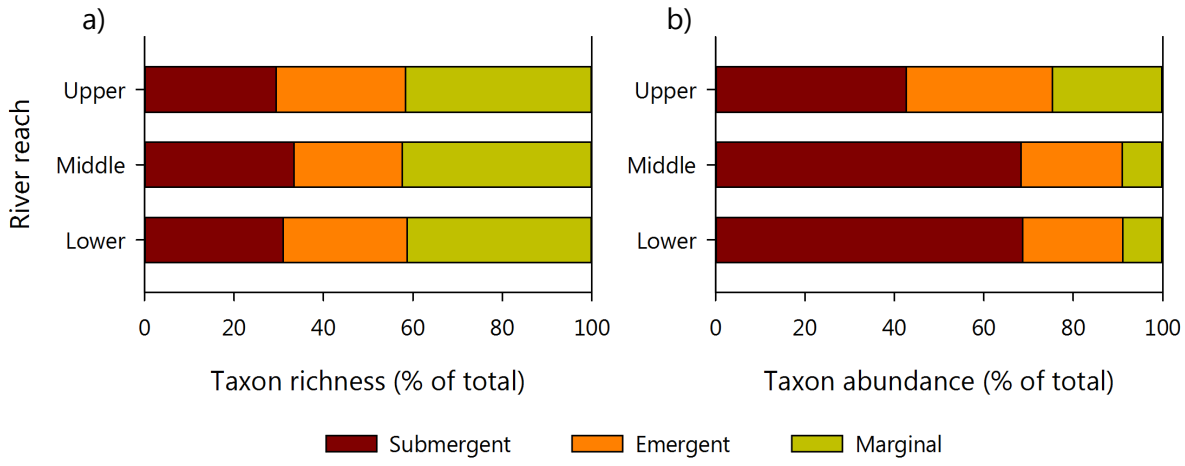

Figure 2.9. **Relative contribution of submergent, emergent and marginal taxa throughout the Itchen.** a) taxon richness and b) taxon abundance, in upper, middle and lower river reaches.

Submerged taxa (total n = 45) were dominant by recorded abundance during the study period, collectively accounting for 77% of total macrophyte richness. 12 of the 16 original core taxa for the overall community were submergents and out of these, 10 collectively accounted for 92% of total submerged macrophyte abundance. Upper reaches were dominated by *Callitriche* spp. and *B. erecta*, which together accounted for 54% of the total macrophyte taxa, reflected by a sharper decline on rank abundance curves (**Figure 2.10.**), as evenness is reduced in headwaters. Middle reaches saw a sharp initial decline in evenness due to dominance by *R. pseudofluitans*, *Callitriche* spp. and *B. erecta* (accounting for 73% abundance). Lower reaches were dominated by *R. pseudofluitans*, *C. glomerata* and *B. erecta*, but community structure was more even than upper and middle reaches. All curves decline rapidly towards the end, suggesting dominance by core taxa throughout.

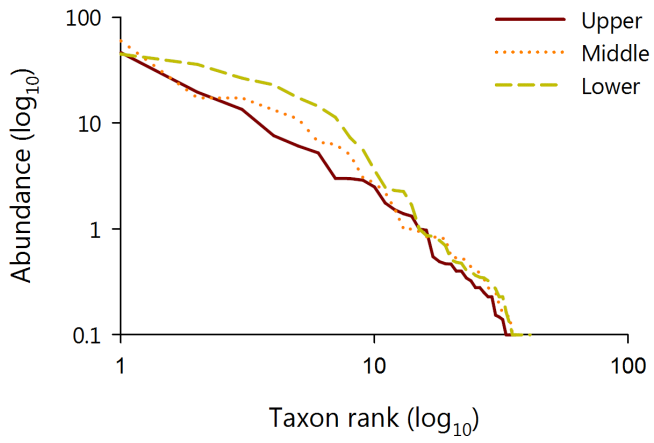

Figure 2.10.

Mean rank abundance (log_{10}) of submergent macrophytes in upper, middle and lower river reaches. Taxa were ranked left to right in order of decreasing abundance.

Spatial variation in the macrophyte community was further assessed with a partial-RDA (**Figure 2.11.**) with species constrained into upper, middle and lower reaches. Axes 1 and 2 were significant (p <0.05, <1000 permutations) and cumulatively accounted for 47.4% of overall variance. Axis 1 (RDA1) explained 27.86% variation in macrophyte coverage and was positively correlated with lower river reaches and negatively correlated with upper and middle reaches. Axis 2 (RDA2) explained 19.54% variation, and was positively correlated

with upper and lower reaches, and negatively correlated with middle reaches. Spatial explanatory variables were significant (upper p <0.05, f = 4.445; middle p <0.05, f = 4.611; lower p < 0.05, f = 5.154). Of the core species, *Callitriche* spp., and *B. erecta* were associated

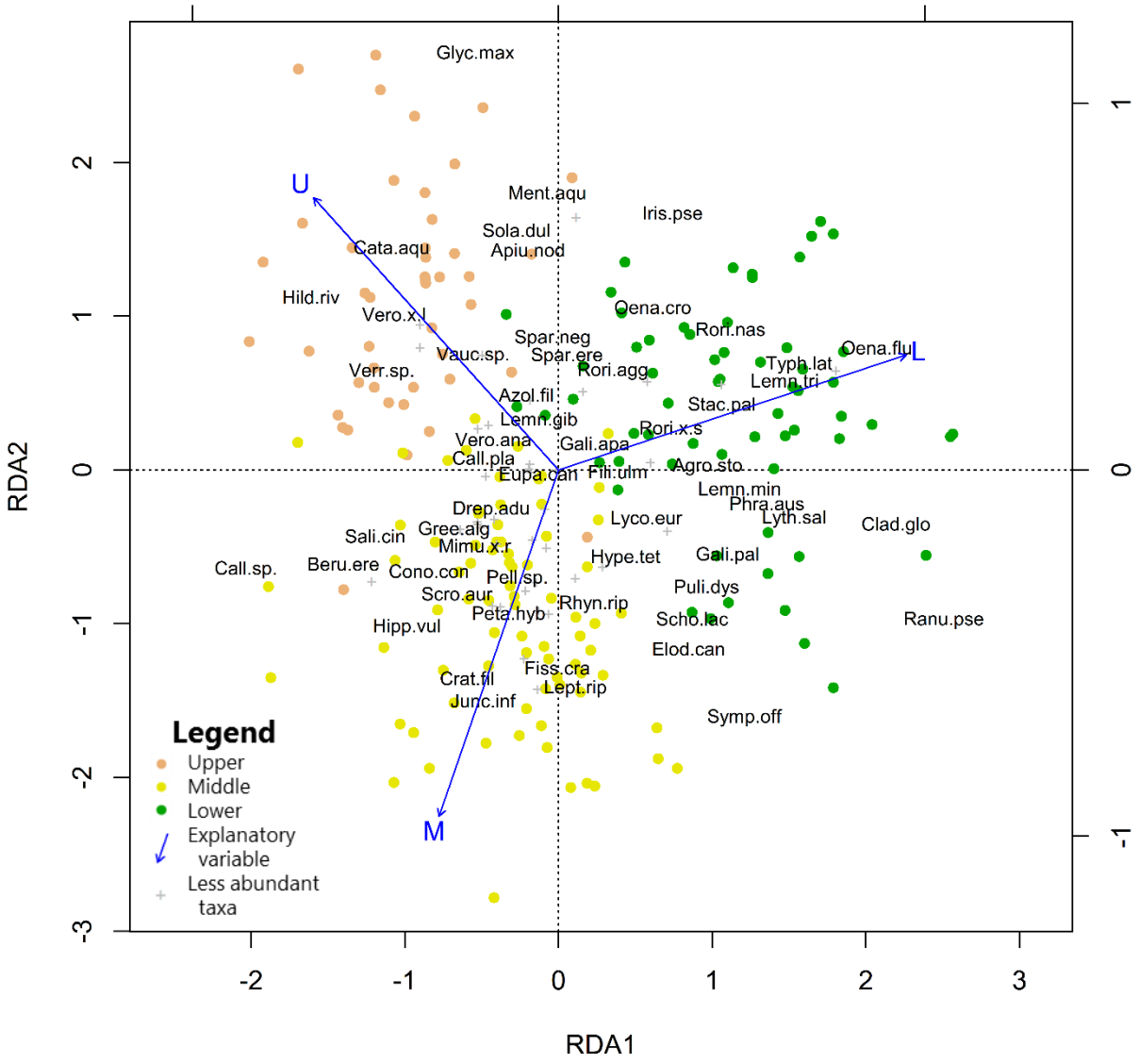

Figure 2.11. **Partial redundancy analysis (pRDA) illustrating spatial differences in macrophyte assemblage downstream of the source, in upper (U), middle (M) and lower (L) river reaches.** Axis 1 (RDA1) explains 27.86% variance, axis 2 (RDA2) explains 19.54% variance. Influence of temporal and environmental variables partialled-out. Arrows represent direction and significance of spatial explanatory variables, abbreviated names are most abundant taxa, crosses represent less abundant taxa and dots are samples. For full taxon names, refer to Table A.1., Appendix A. Where taxon names may overlap, most abundant species are displayed.

with upper and particularly middle river reaches, *R. pseudofluitans* with middle and particularly lower reaches, and *C. glomerata* and *O. fluviatilis* with lower reaches.

Seasonal variation was assessed with a partial-RDA (**Figure 2.12.**) with macrophyte species constrained by spring, summer and autumn seasons. Axes 1 and 2 were significant (p <0.05, <1000 permutations) and cumulatively accounted for 34.24% of overall variance. Axis 1 (RDA1) explained 20.33% variation in macrophyte coverage and was correlated positively with spring and summer, and negatively with autumn. Axis 2 (RDA2) explained 13.91% of variation and correlated positively with spring and negatively with summer and autumn. Seasonal variables were significant (spring p <0.05, f = 2.659; summer p <0.05, f = 3.348; autumn p <0.05, f = 3.103). Of core taxa, *Callitriche* spp. and *R. pseudofluitans* were associated with spring and summer, *B. erecta* most related to spring and autumn, and species synonymous with lower flows and/or enhanced nutrient conditions (*Auduinella* spp., *C. glomerata*, *E. canadesis*, and *Vaucheria* spp.) correlated with summer months.

Variation according to environmental influence was examined with a further partial-RDA (**Figure 2.13.**), with macrophyte species constrained by all environmental variables (excluding antecedent discharge parameters). Axes 1 and 2 were significant (p <0.05, <1000 permutations) and cumulatively accounted for 47.88% of overall variance. Axis 1 (RDA1) explained 29.84% variation in macrophyte coverage and was correlated positively with pH, conductivity, discharge and water depth, and negatively correlated with dense shade. Axis 2 (RDA2) explained 18.04% of variance and was positively correlated with phosphate and negatively with nitrate. Most environmental parameters were significant (pH p <0.05, f = 2.833; temperature p <0.05, f = 3.216; nitrate p <0.05, f = 3.257; phosphate p <0.05 f = 2.674; dense shade p <0.05, f = 2.152; conductivity p <0.05, f = 2.690; discharge p <0.05, f = 2.426; channel-width p <0.05, f = 2.103; water depth p = 0.008, f = 1.528; water clarity = p = 0.013, f = 1.506; dissolved oxygen p = 0.02, f = 1.440) with only broken shade non-

significant (p = 0.815, f = 0.847). Of core taxa, *R. pseudofluitans* was most closely correlated with increasing discharge, *C. glomerata* with increasing phosphate, and *Callitriche* spp. and *B. erecta* negatively with discharge and positively with dense shade and broken shade.

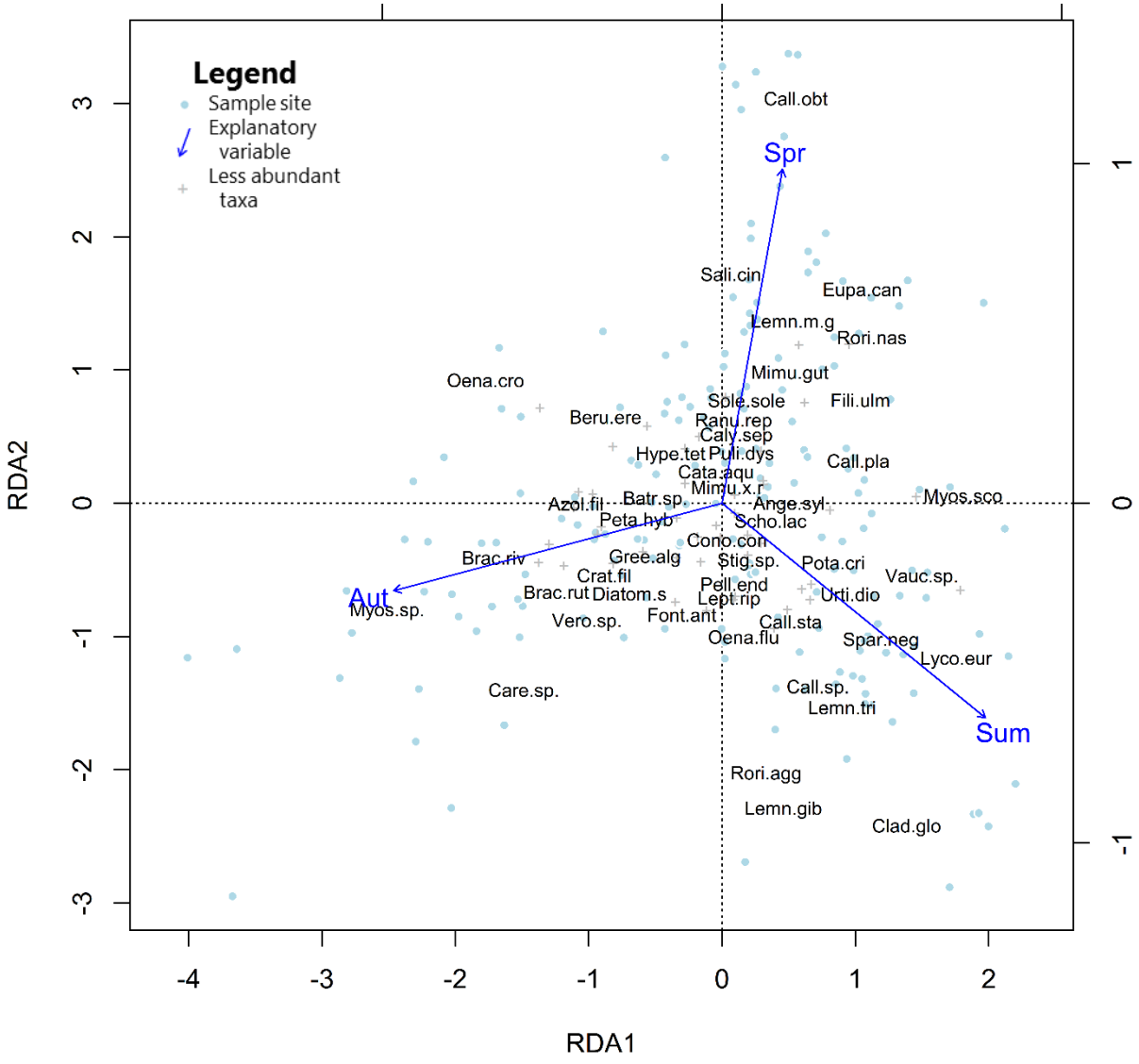

Figure 2.12. **Partial redundancy analysis (pRDA) illustrating seasonal (temporal) differences in macrophyte assemblage between spring (Spr), summer (Sum) and autumn (Aut).** Axis 1 (RDA1) explains 20.33% variance, axis 2 (RDA2) explains 13.91% variance. Influence of spatial and environmental variables partialled-out. Arrows represent direction and significance of seasonal explanatory variables, abbreviated names are most abundant taxa, crosses represent less abundant taxa and dots are samples. For full taxon names, refer to Table A.1., Appendix A. Where taxon names may overlap, most abundant species are displayed.

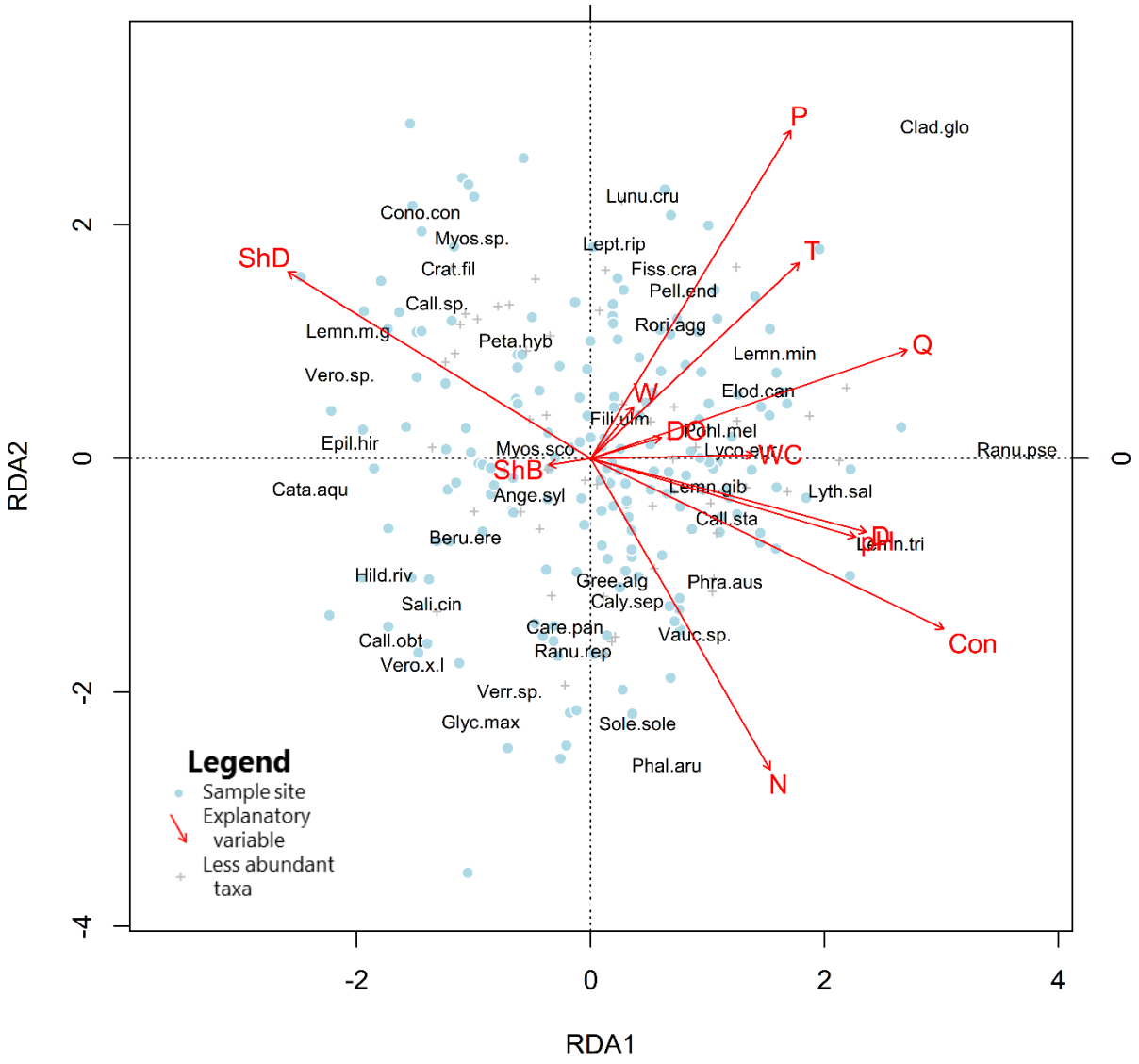

Figure 2.13. **Partial redundancy analysis (pRDA) illustrating differences in macrophyte assemblage according to environmental variables.** Axis 1 (RDA1) explains 29.84% variance, axis 2 (RDA2) explains 18.04% variance. Influence of spatial and temporal variables partialled-out. Arrows represent direction and significance of environmental explanatory variables, abbreviated names are most abundant taxa, crosses represent less abundant taxa and dots are samples. Con – conductivity, DO – dissolved oxygen, N – nitrate, P – phosphate, pH – water pH, ShB – broken shade, ShD – dense shade, T – temperature, D – channel depth, W – channel width, WC – water clarity, Q – discharge. For full taxon names, refer to Table A.1., Appendix A. Where taxon names may overlap, most abundant species are displayed.

2.3.3. Relationships between key taxa and environmental parameters

Out of the core taxa for the river, *R. pseudofluitans*, *B. erecta*, *Callitriche* spp., and *C. glomerata*, all submerged taxa, collectively accounted for 57% of total macrophyte abundance during the study period, and all four contribute to core taxa in upper, middle and lower river reaches. The dominant taxon in all river reaches was one of these four taxa. Due to their dominance, they can be classified as key species within the plant community.

The discharge hydrograph and environmental variable profiles (**Figure 2.3.**) reflect some of the general trends seen in mean abundance of these key taxa (**Figure 2.14.**). Discharge was lower and phosphate higher in 2004-6, and converse during 2007-9. *R. pseudofluitans* was variable throughout the study period, but typically saw lower abundance in 2004-6 (mean 12 ± 1.2% cover) compared to 2007-9 (18 ± 1.5%). Coverage is also seasonally dependent in 2007-9, with spring lows (12 ± 2.3%), summer highs (22 ± 2.5%) and autumn tail-off periods (18 ± 2.9%). *Callitriche* spp. varied minimally between 2004-6 (9 ± 1.2%) and 2007-9 (10 ± 1.2%), however seasonal variability was greater in the 2004-6 period. Spring was characterised by low abundance (7 ± 1%), with summer crashes of *Callitriche* spp. (0.4 ± 0.1%) and considerable regrowth by the autumn (16% ± 2.4%). In contrast 2007-9 saw an increasing trend from spring to autumn (spring 7 ± 1.9%, summer 9 ± 1.5%, autumn 14 ± 2.8%). *B. erecta* varied between 2004-6 (8 ± 0.7%) and 2007-9 (11 ± 1%), although range was greater in 2004-6 (min-max 8.4) compared to 2007-9 (3.7 - excluding spring), suggesting increased seasonal stability in plant abundance in the latter half of the study period. *C. glomerata* had the most marked difference between the two time periods. 2004-6 had a mean of 14 ± 1.5% cover and 2007-9 had a mean of 4% ± 0.6%. Substantially higher abundance during the first half of the study period is due to excessive peaks of *C. glomerata* cover predominantly during the autumn (mean 23 ± 2.8%, max 90%).

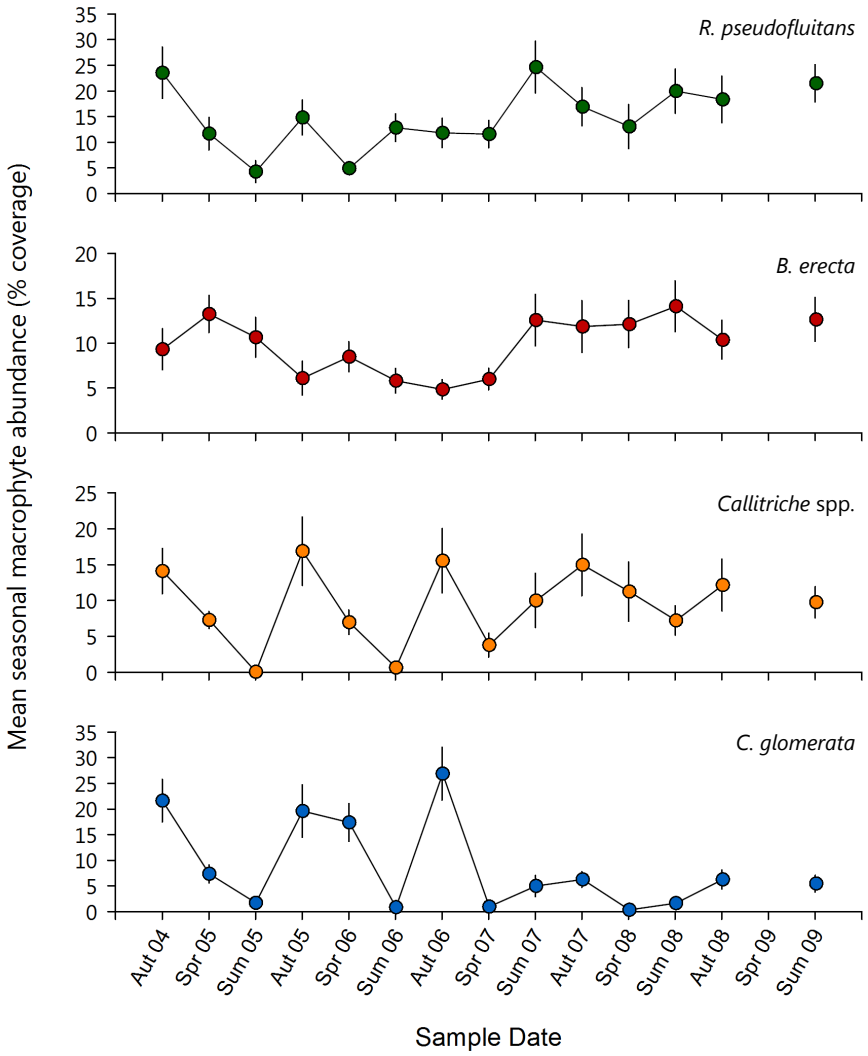

Figure 2.14. **Key macrophyte mean seasonal abundance during the study period.** Averaged across all sample sites. Macrophyte taxa include *Ranunculus pseudofluitans*, *Berula erecta*, *Callitriche* spp. and *Cladophora glomerata*. Error bars represent standard error (n = 28).

Results from mixed-modelling support the correlative trends between macrophyte abundance and environmental parameters. Firstly, best-fitting discharge parameters were calculated for key taxa (**Table 2.5.** & **Table A.3. - A.6.**, Appendix A.): *R. pseudofluitans, Callitriche* spp. and *C. glomerata* were all best represented by antecedent parameters and *B. erecta* was best represented by current study date discharge.

Table 2.5. **Best-fitting discharge parameters for the four key macrophyte taxa.**

	Macrophyte taxa			
	R. pseudofluitans	*B. erecta*	*Callitriche* spp.	*C. glomerata*
Selected best-fit discharge parameter	Q_{25} since the previous winter	Q	Q_{max} over the preceding 12 weeks	Q_{max} since the previous 2 summers
Abbreviation	pwq25	Q	w12max	p2smax

Following discharge parameter selection, the IT model selection approach was taken with all possible environmental explanatory variables included. The following additive mixed model was fitted for each taxon and applied using the IT approach:

$$M_{is} = \alpha + f(E_{is}) + a_i + \varepsilon_{is} \qquad 2.1.$$

$$\varepsilon_{is} \sim B(\pi, N) \qquad 2.1.a.$$

$$\varepsilon_{is} = \rho\varepsilon_{i,s-1} + \eta_{is} \qquad 2.1.b.$$

$$\mathrm{cor}(\varepsilon_{is}, \varepsilon_{it}) = \begin{cases} 1 & \text{if } s = t \\ \rho^{|t-s|} & \text{else} \end{cases} \qquad 2.1.c.$$

where 2.1. is the main model: M_{is} is the key macrophyte taxon for observation i in sample site s, E_{is} is the corresponding explanatory term, $f(\ )$ is the smoother, α is the intercept, α_i is the normally distributed random component, and ε_{is} represents residuals (2.1.a.), binomially distributed with a variance defined by the associated structure in **Table A.7.** (Appendix A), with an auto-regressive correlation structure of order 1 (2.1.b.) with ρ being unknown and requiring estimation from the data and ε_{s-1} residuals at time i,s as a function of residuals at time $i,s-1$ (2.1.c.). Any additional covariates, as smothers and/or parametric terms, were added into Eq. 2.1. after the first smoothing term in the form ...+ $f(E_{is})$... or ...+ E_{is}... respectively (see **Table 2.6.** for additional model components).

All four key macrophyte species were strongly associated with 2 or more environmental variables that approximate correlative trends and patterns in multivariate tests. In all cases, one of these variables was a discharge parameter fitted as a key smoothing term (**Table 2.6.** & **Figures 2.15. – 2.18.**). Best-fit models, whilst none individually had significant chances of selection (**Table 2.6.**; all w_i <0.95), best-fit model parameters for each macrophyte taxon also exactly matched high (> 0.6) explanatory variable selection probabilities (**Table 2.7.**) from the 95% candidate set during model averaging. Models for each taxon support the hypothesis that river discharge plays an important role in controlling abundance, but obvious differences between controlling relationships (**Figures 2.15. – 2.18.**) highlight possible important differences in plant adaptive and evolutionary life-history characteristic traits.

Table 2.6. **Key macrophyte taxa vs. environmental variable best-fit model summaries.** Using binomial generalised additive mixed models. Model statistics: $-\Delta_i$ is the AIC_C difference between the best-fit model and the next best (not shown), w_i is the Akaike weight, $R^2_{(adj.)}$ shows the adjusted R^2 value, *F* is the *F*-statistic, *edf* is the estimated degrees of freedom, and *p* is the *p*-value of the smoothing terms. In best-fit model components, *ƒ*( ) represents smoothing terms. For full model statistics, see Appendix A. Where w_i > 0.9, model is significantly likely to be chosen (highlighted in bold). Where w_i < 0.9, the best fit model does not have a significant chance of selection – in this case see Table 2.7. for model averaged individual explanatory variable selection probabilities.

Macrophyte taxa (genus)	Total number of models run	Best-fit model components	Model statistics					
			$-\Delta_i$	w_i	$R^2_{(adj.)}$	*F*	*edf*	*p*
R. pseudofluitans	1527	ƒ(pwq25) + Con + D	0.35	0.13	0.264	23.81	2.766	<0.001*** + <0.001*** + <0.05*
B. erecta	1908	ƒ(Q) + ƒ (Con) + ƒ(ShD)	0.84	0.087	-	3.486 1.669 4.817	1.896 1.801 1.709	0.035* + 0.188 + 0.023*
Callitriche spp.	1873	ƒ(w12max) + ƒ(ShD) + Con + T + W + N	0.69	0.288	-	8.843 3.463	1 1.846	0.003** + 0.037* + 0.178 + 0.002** + 0.008** + 0.002**
C. glomerata	1890	ƒ(p2smax) + ƒ(ShB) + ƒ(P) + Con + DO	2.03	0.248	-	2.096 0.748 6.050	1.764 1.620 1	0.129 + 0.433 + 0.01** + 0.459 + 0.014*

*** p <0.001; ** p <0.01; * p <0.05

Table 2.7. **Model selection probabilities (SPs) of explanatory variables.** For macrophyte taxa where no single best model was determined (w_{best} < 0.9). SPs calculated as the $\sum w_i$ of models from the set in which the explanatory variable occurs. High SPs (> 0.6) are highlighted in bold. For high SPs, (+) signifies a positive linear relationship, (-) a negative linear relationship and (±) a quadratic relationship.

Explanatory variable	Macrophyte taxa			
	R. pseudofluitans	***B. erecta***	***Callitriche* spp.**	***C. glomerata***
Conductivity	**0.999 (+)**	**1 (±)**	**1 (+)**	**1 (+)**
Temperature	0.235	0.182	**1 (+)**	0.208
Dissolved oxygen	0.162	0.245	0.163	**0.776 (-)**
Nitrate	0.175	0.301	**1 (-)**	0.019
Orthophosphate	0.425	0.106	0.04	**0.654 (+)**
Broken shade	0.027	0.241	0.424	**0.845 (±)**
Dense shade	0.047	**0.745 (±)**	**1 (±)**	0.267
Water depth	**0.845 (+)**	0.349	0.308	0.242
River channel width	0.259	0.173	**0.982 (-)**	0.057
Water clarity	0.178	0.213	0.082	0.133
Discharge (Q)	-	**1 (±)**	-	-
Discharge (psq25)	**0.937 (±)**	-	-	-
Discharge (w12qmax)	-	-	**1 (-)**	-
Discharge (p2sqmax)	-	-	-	**1 (±)**

R. pseudofluitans was fitted with a quadratic relationship with previous-summers Q_{25} flows (**Figure 2.15.**), with abundance increasing rapidly in low-moderate flows (0 - 4 m^3 s^{-1}) before plateauing at higher flows (4+ m^3 s^{-1}). There is no indication that abundance decreases as flows become very high (6+ m^3 s^{-1}), but model uncertainty increases here due to fewer

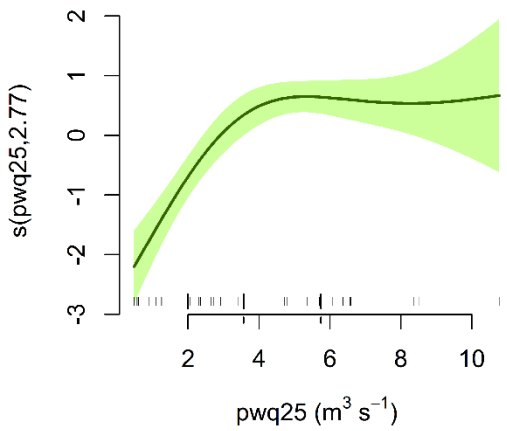

Figure 2.15.

Estimated smoother for best-fit generalised additive mixed model of *R. pseudofluitans*.

Smoother shown is for Q_{25} since the previous winter. Solid line is estimated smoother and shaded area is 95% point-wise confidence bands. *x* axis shows Q_{25} in m^3 s^{-1} and *y* axis is smoother contribution to fitted values.

residuals. Additional positive relationships with conductivity and water depth improve the fit of this model.

Model parameters for *B. erecta* indicate abundance is best explained by three smoothing terms: all quadratic with current discharge, conductivity and dense-shade (**Figure 2.16.**).

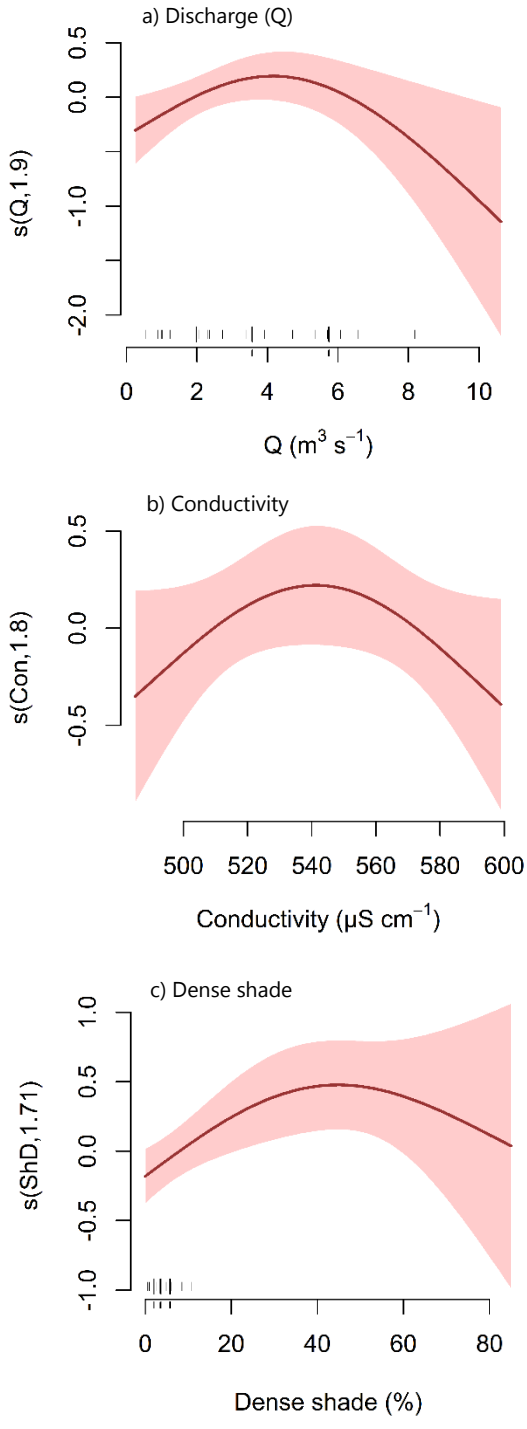

Figure 2.16.

Estimated smoothers for best-fit generalised additive mixed model of *B. erecta*. Smoothers shown are a) Q, b) conductivity, and c) dense shade. Solid lines are estimated smoothers and shaded areas are 95% point-wise confidence bands. *x* axes show a) Q in $m^3 s^{-1}$, b) conductivity in µS cm^{-1}, c) dense shade (%), and *y* axes are smoother contributions to fitted values.

Current discharge acts to restrict growth at higher velocities (4+ m^3 s^{-1}) while promoting development at low-moderate velocities (0 – 4 m^3 s^{-1}). In addition, conductivity suggests limits to development when dropping lower than, or rising higher than, ~ 540 µS cm^{-1}. Furthermore, *B. erecta* has tolerance to increasing levels of dense-shade, up to ~ 50% shaded conditions, where abundance is then limited as shade increases.

Callitriche spp. was best explained by two smoothing terms: 12-weekly Q_{max} and dense-shade (**Figure 2.17.**). Although the smoothing term was significant, the relationship with 12-weekly Q_{max} suggests a negative linear pattern, with a distinct controlling mechanism whereby development is inhibited by spatey flows, and particularly where flows are very high. Dense-shade suggests an unusual situation where growth is minimal in open conditions but increases rapidly in more shaded (> 40%) conditions. In addition, model fit

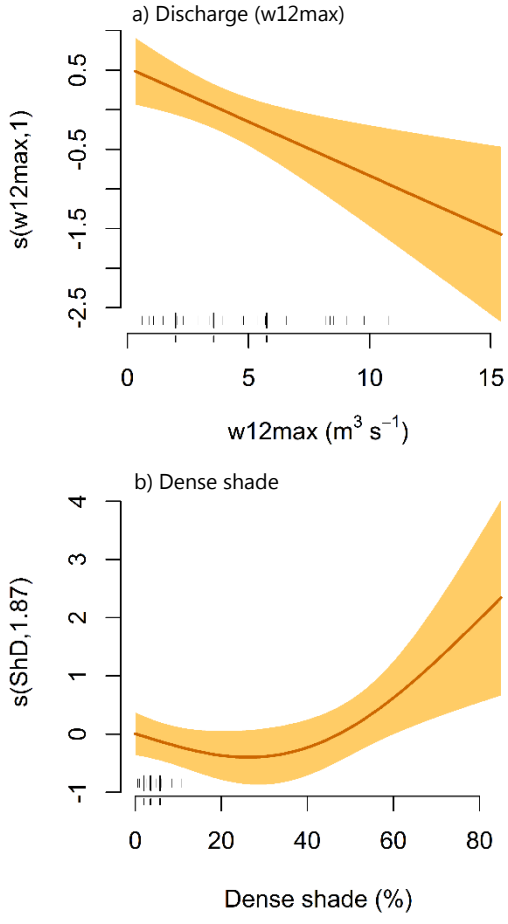

Figure 2.17.

Estimated smoothers for best-fit generalised additive mixed model of *Callitriche* spp. Smoothers shown are a) Q_{max} over the preceding 12 weeks, and b) dense shade. Solid lines are estimated smoothers and shaded areas are 95% point-wise confidence bands. *x* axes show a) Q_{max} in m^3 s^{-1}, b) dense shade (%), and *y* axes are smoother contributions to fitted values.

is enhanced by the influence of positive associations with conductivity and temperature, and negative with nitrate and increasing channel width.

Abundance of *C. glomerata* was best explained with three smoothing terms (**Figure 2.18.**): previous 2-summers Q_{max}, broken-shade and phosphate concentrations. Abundance increases rapidly in low-moderate flows, but declines sharply at higher flows (> 4 m^3 s^{-1}). In particular, the association with prev. 2-summers Q_{max} suggests a controlling mechanism

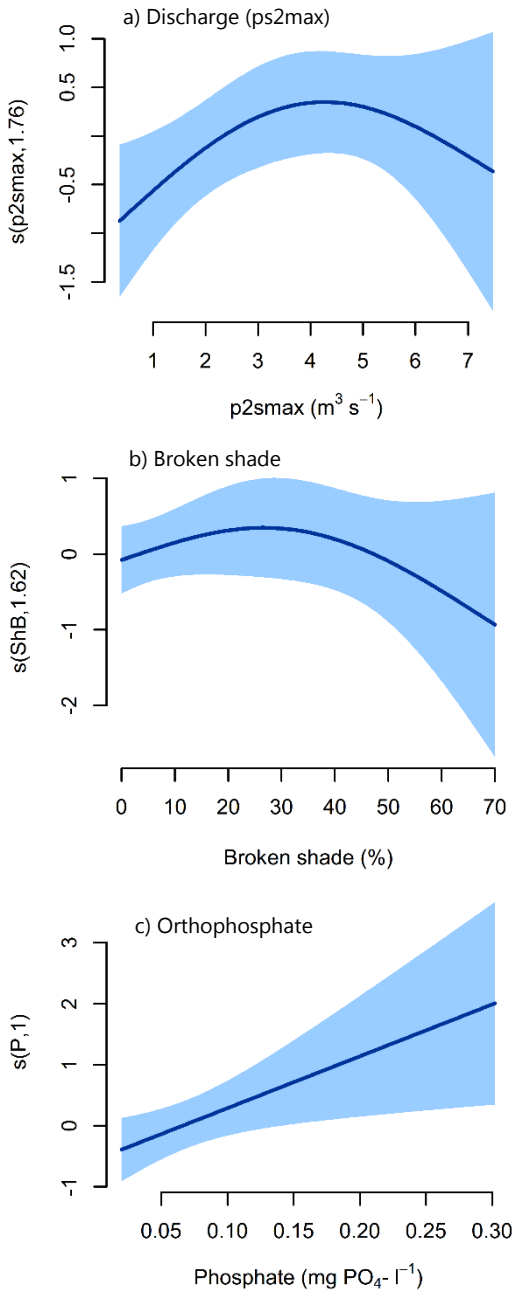

Figure 2.18.

Estimated smoothers for best-fit generalised additive mixed model of *C. glomerata*. Smoothers shown are a) Q_{max} over preceding 2 summers, b) broken shade, and c) orthophosphate. Solid lines are estimated smoothers and shaded areas are 95% point-wise confidence bands. *x* axes show a) Q_{max} in m^3 s^{-1}, b) broken shade (%), c) orthophosphate in mg PO_4- l^{-1}, and *y* axes are smoother contributions to fitted values.

with very high, spatey flows flushing algae out, particularly in summer/post-summer flows when algal abundance is at its highest. Abundance is generally higher in less shaded conditions (< 30%) and phosphate, whilst a significant smoothing term, has linear control on *C. glomerata* abundance, with increasing concentrations enhancing growth.

2.4. DISCUSSION

Throughout the study period, discharge was highlighted as a principal controlling factor on river chemistry and also for directly and indirectly affecting macrophytic assemblage. Water chemistry was significantly influenced by river discharge, varying consistently both spatially and temporally. In particular, phosphates were diluted, and nitrates concentrated, under increasing river flow. Similarly, macrophyte assemblage varied spatially and temporally according to river discharge. However, whilst key macrophyte species were strongly related to river flow, reach assemblage composition was often confounded by the influence of additional environmental variables.

As a consequence of the main findings, discussion of results has been split into three topics: discharge as a controlling mechanism on river chemistry; spatiotemporal distribution and variability of the plant community; and, physicochemical influences on key macrophytes.

2.4.1. Flow controls on river chemistry

River physicochemistry differed markedly during the study period, with distinct differences between the first three years (2004 to 2006) and the latter three years (2007 to 2009) that were particularly pronounced for river discharge, conductivity and phosphate.

Physical parameters - Annual peak discharge periods occurred between January and April with lowest periods between July and October, which is comparable to patterns observed

in the Itchen between 1974-97 (Phillips *et al.*, 2003), although mean discharge in 2004-6 was considerably lower than in 2007-9 or the mean for 1974-97. Water temperature ranges were typically low variation, similar to other chalk streams (Ham *et al.*, 1981; Wright & Symes, 1999; Allen *et al.*, 2010; Howden *et al.*, 2010), with winter lows averaging ~8°C and summer highs averaging ~15°C. Temperatures were well within known tolerance ranges of several macrophytes (Cook, 1969; Carr *et al.*, 1997), indicating thermal-tolerance was unlikely a growth limiting factor during the study.

Chemical parameters - Conductivity was also similar to other chalk streams (Allen *et al.*, 2010), except during 2004-6 where values were much lower due to reduced discharge. Mean pH approximated observations on the Itchen by Butcher (1927) and those seen in other chalk streams (Allen *et al.*, 2010; Howden *et al.*, 2010). Mean phosphate concentrations throughout the river were comparable to sites exposed to diffuse nutrient input on other chalk streams (Jarvie, Neal, *et al.*, 2002; Howden *et al.*, 2010), although some high peaks, particularly abundant in 2004-6, were indicative of nutrient enrichment. Nitrate concentrations were considerably higher than observed on the Itchen by Butcher (1927), although this increase may be attributed to increased agricultural sources (Howden *et al.*, 2010) from more intensive forms of farming than seen in 1925. Dissolved oxygen concentrations were frequently supersaturated due to high primary productivity and comparable to other chalk streams (Allen *et al.*, 2010; Howden *et al.*, 2010).

Although seasonal variability was distinct for some environmental parameters (e.g. discharge, relating to aquifer recharge; Grapes *et al.*, 2005) and was the primary source of variation in others (e.g. water temperature, related to air temperature variability and natural temperature stability of chalk aquifers; Mackey & Berrie, 1991), some differences in water chemistry were more pronounced between the two time periods. Discharge,

controlled by winter aquifer recharge rainfall (Phillips *et al.*, 2003) was the main reason for differences in water chemistry. In particular, phosphate and nitrate were significantly affected by alterations in river discharge, with dilution of phosphates and elevation of nitrates under higher flows. This control mechanism has been discussed in previous studies (Flynn *et al.*, 2002; Hanrahan *et al.*, 2003; e.g. Ballantine *et al.*, 2008; Bowes *et al.*, 2009; Howden *et al.*, 2010), although concentrations vary depending on contributions of point and diffuse sources. Spatial similarity in correlations between discharge and phosphate, nitrate and conductivity further support this concept: Upper river reaches had considerably lower average year-round discharge and higher variability than middle and lower reaches, as expected in spring-fed winterbourne reaches that are more susceptible to groundwater level changes (Westwood *et al.*, 2006b). Nitrate and conductivity was more highly correlated with this headwater flow variability, which may also have been due to greater diffuse agricultural and groundwater influences (Mainstone & Parr, 2002; Howden *et al.*, 2010), with phosphate being more highly correlated in lower river reaches, where greater sources of phosphate exist. In particular, most high phosphate peaks occurred in lower river reaches, close to major point-source inputs. However, throughout the river phosphate was rarely measured in concentrations lower than 0.02 mg PO_4- l^{-1}, with most reaches frequently above the 0.06 mg l^{-1} target (well within the range where changes in ecology is expected; Environment Agency, 2000; UKTAG, 2008), highlighting significant elevation above 'natural' levels in chalk rivers (Mainstone & Parr, 2002).

2.4.2. Temporal and spatial variability of the macrophyte community

The macrophyte community of the River Itchen was highly spatially and temporally variable during the study period: Temporal variation was principally attributed to combinations of natural seasonal fluctuations and environmental driven changes, with

spatial variation connected to differences in environmental conditions rather than physical characteristics at site level. Submerged taxa were the most abundant macrophytes recorded on the River Itchen, accounting for a majority of core taxa for the entire plant community.

Recorded gamma diversity was lower during springtime because of early growth season (Flynn *et al.*, 2002) controls (e.g. temperature, Davis & McDonnell, 1997) meaning fewer arriving colonists and lesser post-winter dormancy regrowth. Greater spring evenness and lower dominance by key species also suggests that typically dominant taxa are only present in low abundance during the spring (Flynn *et al.*, 2002). Gamma diversity increased during summer and autumn surveys. This seasonal pattern was expected, however, inter-annual variability was the main influence on macrophyte abundance. This was clearest between 2004-6 and 2007-9, coinciding with distinct differences in river discharge. Submerged taxa found in greater abundance in 2004-6 were those related to lower river discharge and poorer water quality (e.g. *C. glomerata*, *E. canadensis*), and those in greater abundance in 2007-9 were taxa that preferred improved flow (e.g. *B. erecta*, *O. fluviatilis*, *R. pseudofluitans*). This conforms with Wilby *et al.* (1998) and Flynn *et al.* (2002), who observed high discharge and low phosphate conditions correlated with increased *Ranunculus* spp. growth.

Submerged taxon variability was higher in upper river reaches compared to middle and lower reaches, corresponding with equally variable discharge. Taxon evenness and dominance remained spatially similar throughout, although individual site richness, gamma and beta diversity was reduced in upper reaches. High beta diversity throughout suggests high rates of species turnover at all sites. Lower relative abundance of submergent taxa, greater proportions of marginal/emergent taxa, and limitations to specific taxa in upper reaches, including switches in dominance to *Callitriche* spp. and *B. erecta* from *R.*

pseudofluitans in middle/lower reaches, may explain reductions in taxon richness. These taxon assemblage differences highlight cross-sectional profile differences, with narrower channels and gently sloping marginal gradients, associated with headwater streams (Haslam, 2006). Headwater streams are typically associated with taxa more tolerant of drought and low-flows (Westwood *et al.*, 2006a; 2006b), with taxa preferring higher flows generally less abundant, as observed in this study.

Downstream spatial changes in macrophyte assemblage were considerably different to those observed by (Butcher, 1927), where upper reaches were dominated by *R. penicillatus* (likely *R. pseudofluitans*), *A. nodiflorum* and *B. erecta*. In this study, dominant upper-reach species were *Callitriche* spp., *B. erecta*, *G. maxima* and *A. nodiflorum*. Whilst there are some similarities, *R. pseudofluitans* was in far lower abundance during 2004-9, and *Callitriche* spp. was dominant in its place. As *Callitriche* spp. is thought to be more silt tolerant (Butcher, 1927; Haury & Aidara, 1999), this could indicate more silted conditions in upper reaches during this study, which may be explained by lower discharges affecting water velocity and therefore allowing sediment deposition encroachment into the river channel (Heppell *et al.*, 2009; Stubbington *et al.*, 2009). In middle reaches, Butcher (1927) observed dominance by *H. vulgaris*, *Sparganium simplex*, *E. canadensis* and *C. stagnalis*, with *R. pseudofluitans* and *B. erecta* occurring in swifter reaches. During 2004-9, *R. pseudofluitans* is dominant, with lower presence of *Callitriche* spp., *B. erecta, C. glomerata*, and *H. vulgaris*. In lower reaches, *E. canadensis* and *C. stagnalis* were dominant in Butcher's study, but again *R. pseudofluitans* was dominant in this study, with *C. glomerata* and *B. erecta* also in high abundance. Butcher's brief description of flow indicates that flow conditions were improved during 2004-9 for middle and lower river reaches, with dominance by taxa preferring swifter flows (e.g. *R. pseudofluitans*), rather than silted conditions. However, lack of *C. glomerata* during Butcher's study suggests conditions with

much higher water quality and less nutrient inputs than can be seen today on the river; this is supported by markedly lower nitrate concentrations (phosphate was not discussed). Furthermore, observations infer that macrophyte assemblages on the Itchen in 1925 were principally affected by river flow conditions.

In contrast to the findings by Butcher (1927), macrophyte assemblage was similar to this study in surveys performed by Cranston & Darby (1992, 1995, 1997) between 1991-6, although fewer sites were assessed on the Itchen. In particular, emphasis was placed on growth and recession of filamentous algae, which in 1991 and 1992 were high in abundance due to lack of higher discharges acting as a removal mechanism, similar to this study (Cranston & Darby, 1992). By 1994 considerable rainfall had increased river flows, reducing algal growth and allowing higher macrophytes to grow more successfully (Cranston & Darby, 1995). This trend reversed again by 1996, with enhanced algal growth again correlating with reduced river flows (Cranston & Darby, 1997). In all studies, indications of declines in *R. pseudofluitans* and replacement by *B. erecta* and *Callitriche* spp. are given; this is only observed in upper river reaches in this study, with little evidence to support this trend since the 1990s.

In this study, anomalies between two adjacent river study sites (**Figure 2.7.**) close to Winchester (sites 15 and 16) meant these sites had unusually low and high alpha diversities respectively. Site 15 is a canalised section of river in Winchester city centre, so reductions in taxon richness are expected. Site 16 has unusually high taxon richness, due to a large number of rare taxa (114) accounting for only 20% of macrophyte abundance throughout the study. Reasons for high numbers of low abundance taxa are unknown, but site 16 runs through secluded, private grounds, with a rich history of water meadow use, which may account for the increased richness.

2.4.3. Abundance of key macrophyte taxa

Four submerged taxa (*R. pseudofluitans*, *B. erecta*, *Callitriche* spp., *C. glomerata*) accounted for a majority of total macrophyte abundance during the study, and were therefore key community species. *R. pseudofluitans* is particularly well known as a keystone chalk stream species (Flynn *et al.*, 2002; Green, 2005a; O'Hare, Stillman, *et al.*, 2007). Generally *B. erecta* and *Callitriche* spp. are readily abundant in chalk streams (Wilby *et al.*, 1998; Wright & Symes, 1999), and *C. glomerata* is not usually found in abundance in unimpacted chalk streams (Wilby *et al.*, 1998) and is indicative of degraded or eutrophic ecosystems (Demars & Harper, 1998).

R. pseudofluitans was most dominant, and at its maximum occupied 95% of river reaches it was recorded in; this dominance is common (Barko *et al.*, 1986; Wright *et al.*, 2003), with *Ranunculus* spp. proliferating from bank to bank under optimum conditions. Similar maxima were seen for *Callitriche* spp. and *C. glomerata* (both 90%), with *C. glomerata* related to episodes of eutrophication (Carr & Goulder, 1990; Hilton & Irons, 1998; Mainstone & Parr, 2002). Consequently, as during 2004-9, *C. glomerata* is highly variable during the study period; a phenomenon also noted by Wilby *et al.* (1998) on the same river. *B. erecta* only had a maximum of 60% during the study period. Generally restricted to shallower waters, being stoloniferous and unable to develop a large suspended canopy (Barrat-Segretain, 2001), it can be easily light-restricted by canopies of larger submerged macrophytes, which likely accounts for lower observed maximum than in other key taxa.

In models explaining key macrophyte abundance, relationships with environmental factors suggest different controlling mechanisms: *R. pseudofluitans* was correlated with increasing river discharge, *B. erecta* with moderate discharge, conductivity and dense shade, *Callitriche* spp. with decreasing discharge and increasing dense shade, and *C.*

glomerata with moderate discharge, broken shade and increasing phosphate concentrations. However, two influential variables had common effects on all key macrophytes: Conductivity was positively influential in all models, with all species typically related to increasing conductivity – this may be because conductivity is considered a surrogate for availability of important plant cations (Demars & Edwards, 2009); no correlations were found between previous seasonal macrophyte cover – this is quite unusual, as Wilby *et al.* (1998) indicate this is one of the most significant factors affecting macrophyte distribution, as growth and residual plant parts throughout seasons are important for determining plant success (Cranston & Darby, 1992). It is unknown why no correlations were found with previous seasonal cover here, but it may be related to high beta diversity at each site suggesting high rates of species turnover.

R. pseudofluitans - The best-fit relationship with Q_{25} discharge since the previous winter indicates preference for higher flows throughout each annual growth cycle, and lack of reductions at very high discharge (although greater model uncertainty) does not suggest an upper flow threshold. Wilby *et al.* (1998) note that a large coverage of residual plant parts remain overwinter, potentially explaining how discharge over preceding annual growth periods can positively influence abundance and regrowth. High discharge was the reason for *Ranunculus* spp. regrowth in observations by Wright *et al.* (2002), and other studies also note relationships between the distribution and growth of *Ranunculus* spp. and increasing discharge (Ham *et al.*, 1981, 1982; Giles *et al.*, 1991; Wilby *et al.*, 1998), although limited evidence of *Ranunculus* spp. flow preferences are given other than positive relationships with 'high flow'. It is possible discharge is also indirectly influencing *R. pseudofluitans* distribution through nutrient dilution and sediment dynamics (Jarvie, Lycett, *et al.*, 2002; Cotton *et al.*, 2006; Ballantine *et al.*, 2008), via algal growth (Wade *et al.*, 2002), washouts and deposition (Bornette & Puijalon, 2011), although this was not

observed in this study. Competitive interaction between filamentous algae such as *C. glomerata* have been suggested as being highly important for the successful growth of *Ranunculus* spp. (Franklin *et al.*, 2008).

B. erecta – Best explained by a model including three smoothers (current Q, conductivity and dense shade) appeared restricted to particular ranges. Direct responses to current discharge conditions reflects lower tolerances of *B. erecta* to increasing flows, as plants rapidly reduce overall size when flow increases as an avoiding strategy to drag force stress (Puijalon *et al.*, 2005). Under increasing flow, plants are likely susceptible to shading by taxa with larger canopies, which may explain its preference to moderate (~ 2-5 m^3 s^{-1}) flow conditions. *B. erecta* is often correlated in a lag-response with increases in *R. pseudofluitans* cover and river flow (Flynn *et al.*, 2002), perhaps due to slowing of velocities in localised patches, as *R. pseudofluitans* can affect flow conveyance and promote heterogeneous flow conditions (Green, 2005a), although these patterns were not observed here. Relationships with conductivity also indicate an optimal growth range between ~530-550 µS cm^{-1}, which may be the range at which there are enough plant-available cations for successful growth (Demars & Edwards, 2009), and above which may indicate nutrient enrichment and/or reduced water quality. The relationship with dense shade again suggests an optimal range for growth between ~20-60% shaded conditions. Typically, as *Ranunculus* spp. is less shade tolerant, *B. erecta* is able to grow successfully in reaches otherwise dominated by *R. pseudofluitans* (Wright *et al.*, 1982).

Callitriche **spp.** – Models suggest *Callitriche* spp. reduces in abundance with increasing discharge, particularly Q_{max} over the preceding 12 weeks, suggesting seasonal responses to highest flows. A preference for lower flows has been determined in previous research (Wright *et al.*, 2002, 2003), however studies investigating drag forces acting on macrophyte stands suggest *C. stagnalis* has comparable resistance to *R. pseudofluitans* (O'Hare,

Hutchinson, *et al.*, 2007). It is possible there may therefore be a form of competitive interaction between *R. pseudofluitans* and *Callitriche* spp. that may explain this relationship, particularly as *Callitriche* spp. is thought to be more silt tolerant (Butcher, 1927; Wright *et al.*, 2003). Similar to *B. erecta*, *Callitriche* spp. is often outcompeted by *Ranunculus* spp. in unshaded conditions (Flynn *et al.*, 2002), and likely has competitive advantages in shaded reaches. A preference for shaded sites was also observed by Wilby *et al.* (1998).

C. glomerata – Of the three principal explanatory variables determined for *C. glomerata* in this study, the relationship with discharge is unusual, as a relatively linear decrease with increasing flows was expected. Instead, abundance appears to be limited at lower Q_{max} over the previous 2 summers, peaking at ~3-4 $m^3 s^{-1}$ and thereafter reduces rapidly. The rapid reductions, particularly related to maximum discharge during summer months, is indicative of flushing events removing much algal growth. This was also noticed by Wilby *et al.* (1998), with summer storms acting as the primary cause of flushing events, although they note the role of winter flows preventing overwintering of algae is also important. As complex filamentous algae, *C. glomerata* lack sufficient anchoring capability, and high discharge flushing events have been noted as a key controlling mechanism for algal growth (Wright *et al.*, 1982; Cranston & Darby, 1995). Additionally, dense shade was determined as an additional controlling factor, with reductions in algal growth as shade increases significantly. As a photosynthetic periphytic algae, *C. glomerata* requires unshaded sunlight to proliferate (Comte *et al.*, 2005). A particularly significant association with *C. glomerata* was the positive increase with phosphate concentrations. Branching filamentous algae, such as *C. glomerata* are limited to utilising only water column nutrients (Hilton *et al.*, 2006), and as phosphate is typically in lower concentrations in chalk rivers (Mainstone & Parr, 2002), tends to be the primary limiting factor to periphytic algae.

Furthermore, filamentous algae have been well documented being synonymous with eutrophic conditions (Demars & Harper, 1998; Wilby *et al.*, 1998), but as there is an interconnected relationship with discharge, particularly relating to nutrient dilution (Ballantine *et al.*, 2008) and flushing-out of algae (Hilton *et al.*, 2006; Franklin *et al.*, 2008), filamentous algal dynamics are more complex than associations with single parameters.

Principal associations between river discharge, phosphate concentrations and potential competition relating to riparian shading suggest that these are key driving physicochemical variables affecting abundance of the key macrophyte taxa in the River Itchen. The discharge influence on water quality is considerable, so either directly or indirectly, river flow is signified as the dominant environmental factor determining aquatic macrophyte assemblages throughout the river. Spatial patterns can to an extent be explained by physical conditions downstream of the source, but river discharge is better for explaining macrophyte distribution between sites.

However, it should be noted that there is a distinct lack of studies on key taxa to suggest direct responses to environmental pressures. For example, as a keystone species, *R. pseudofluitans* should be a conservation and management priority, yet little is known about impacts on plant form and function. In-stream and experimental studies have investigated certain features of *R. pseudofluitans* in relation to environmental variables, for example eutrophication (O'Hare *et al.*, 2010), low-velocities (Westlake, 1967), sediment nutrients (Clarke & Wharton, 2001) and flow resistance (Green, 2005b; O'Hare, Hutchinson, *et al.*, 2007), but studies have not attempted to examine tolerance and threshold ranges to particular environmental conditions as an aid for management and conservation. As many variables directly affect plants at a local stand/individual plant scales (e.g. water flow; Franklin *et al.*, 2008) further work is required to establish guidelines for macrophyte community management and conservation.

The basis of the findings in this study do enable some practical advice however. As a management tool, the importance of maintaining good river flow must be highlighted, not only as a control for poor water quality, algal growth and unfavourable river conditions, but also to promote the healthy growth of key macrophyte species, particularly *Ranunculus* spp., which are well known to encourage heterogeneous flow conditions and affect bed substrate and plant community heterogeneity, benefitting macroinvertebrate and associated faunal communities. However, it is also clear from past observations on the River Itchen (Butcher, 1927), where water quality was significantly higher, algal growth (e.g. *C. glomerata*), was not present on the river, so the requirement for more stringent controls on water quality standards may be required regardless of flow management strategies. For flow management, the use of localised manipulations/modifications of river channels through river engineering may help improve river hydraulics by altering channel gradient and channel width/depth ratios. A combination of these local management techniques and catchment-wide management plans aimed at river water conservation (e.g. assessments of water use, abstraction, future climate change) will help combat the issue of low flows in the River Itchen and provide future resilience for this classic chalk river.

2.4.4. Study constraints

Main study limitations were related to variables that affect macrophyte growth that could not be accounted for. Channel gradients, important for influencing macrophyte assemblage (Westwood *et al.*, 2006a), were not recorded. Furthermore, while rudimentary assessments of substrate were carried out (and discarded from analysis due to poor quality/subjectivity), substrate composition is an important consideration for macrophyte success (Franklin *et al.*, 2008). Light availability is another variable known to play a key role in controlling macrophyte abundance (Franklin *et al.*, 2008), but again there were no

recordings of light availability (other than shading). Velocity, whilst linked to river discharge, has multiple local micro-scale effects on plants (Franklin *et al.*, 2008), but the nature of the sampling technique meant assessments of local stand velocities would not be possible. Additionally suspended sediment and siltation dynamics are a potentially key factor affecting macrophyte success (Heywood & Walling, 2003; Jones *et al.*, 2012), but were not recorded for assessment in this study. Grazing/herbivory is another important variable that can have a significant effect on plant growth and biomass (O'Hare, Stillman, *et al.*, 2007), and similarly the effects of management, such as weed cutting (Ham *et al.*, 1982; Baattrup-Pedersen & Riis, 2004), were not available for assessment, but likely have significant influence on macrophyte assemblages.

Additionally, if surveys were performed more frequently throughout the study, the significance of plant-environment relationships would be improved and several relationships may have been improved due to increased resolution of samples.

Finally, although the uncertainty of changing climate have been briefly mentioned (Wheater *et al.*, 2006), the impacts of future climate change were not considered in this study. This is an important consideration, as climate change threatens to change variability, frequency and magnitude of weather systems (Matear *et al.*, 2012) and therefore river flow variability (Biggs *et al.*, 2005), and rising air temperatures could have distinct implications for macrophyte communities in chalk streams (Barko & Smart, 1981). A majority of key taxa within the chalk stream environment have little supporting evidence for the effects of temperature on plant growth, although there are suggestions that high temperatures may impair plant physiology (Westlake, 1969).

2.5. CONCLUSION

Aquatic macrophytes form a fundamental part of the chalk stream environment, responsible for driving primary productivity, influencing key riverine processes and providing refugia for faunal components of the ecosystem. However, changes in key riverine physicochemical parameters causes significant alterations to macrophyte community assemblage that may exacerbate degraded river conditions. In this study, a valuable six year dataset for the River Itchen, Hampshire, covering observed periods of low flow and average flow, has been assessed to determine key macrophyte-environment interactions. Whilst a lack of congruence between macrophyte abundance and some water quality parameters was observed, the study has signified the overarching importance of river flow as a control mechanism for determining water chemistry and macrophyte distribution and assemblage. River physicochemistry displayed marked differences between the low-discharge period of 2004-6 and the moderate-discharge period of 2007-9. Differences in discharge significantly influenced water chemistry. In particular, phosphate decreased and nitrate increased with increasing discharge in 2007-9. Seasonality had a significant control on macrophyte community abundance, but general spatial and temporal trends in community assemblage were driven by variations in river discharge. Moreover, spatial variation was related to distribution of groundwater and discharge, with upper reaches characterised by low-flow preferring, semi-aquatic and emergent/marginal taxa, and middle/lower reaches principally by submergent taxa tolerant of swifter flows. In 2004-6, discharge was lower and phosphate concentrations were higher, with taxa preferring enriched nutrient conditions (e.g. *C. glomerata*) in greater abundance. In 2007-9, phosphates were reduced and taxa that preferred higher flows were in dominance (e.g. *R. pseudofluitans*). Four key taxa were dominant during the study period: *R. pseudofluitans*, *Callitriche* spp., *B. erecta* and *C. glomerata*. Relationships with key environmental variables

indicate subtle niche partitioning in response to competition and tolerance to specific environmental tolerances. *R. pseudofluitans* was significantly positively related to antecedent discharge, preferring improved flows, and was the overall dominant taxon. *B. erecta* was preferred lower discharges and areas with increasing shade, and was therefore likely confined to channel margins. *Callitriche* spp. was also limited by increasing magnitude discharges and tolerated heavy shade, but negative relationships with river width suggests preference for headwater reaches. *C. glomerata* was related to maximum discharges over multiple preceding seasons, suggesting control by flushing mechanisms with high flows, particularly important after summer seasons and overwintering. Additionally, *C. glomerata* was positively related to increasing phosphate and negatively associated with shaded conditions, highlighting the importance of nutrient enrichment and sunlight. The study was limited by missing some important influential factors, but signifies the importance of flow management and nutrient regulation for improving macrophytic growth. In addition, understanding of keystone species tolerances and thresholds to key environmental parameters requires further investigation.

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Chapter III

Artificial stream mesocosms as realistic and replicable tools for submerged aquatic macrophyte growth experiments

3.1. INTRODUCTION

The use of artificial stream mesocosms (other synonyms include experimental channels or artificial streams) has become increasingly important for freshwater ecological experiments (Odum, 1984; Lamberti & Steinman, 1993; Ives *et al.*, 1996; Ledger *et al.*, 2006, 2008; Harris *et al.*, 2007; Mohr *et al.*, 2007), particularly due to the levels of control and flexibility they offer over natural whole-ecosystem manipulations (Petersen *et al.*, 1999; Petersen & Englund, 2005; Ledger *et al.*, 2009). Mesocosms have been used widely as research tools to assess disturbance (Steinman, 1992; Cardinale & Palmer, 2002; Ledger *et al.*, 2006, 2008), drought impacts (Ledger *et al.*, 2011, 2012), biofilm ecology (Battin *et al.*, 2003; Trudeau & Rasmussen, 2003), macroinvertebrate growth, survivorship and colonisation (Hauer, 1993; Ledger *et al.*, 2006), food web complexity (Brown *et al.*, 2011) and aquatic macrophyte responses to changing environmental conditions (e.g. Short *et al.*, 1995; Taylor *et al.*, 1995; Kercher & Zedler, 2004; Goulet *et al.*, 2005; Chase & Knight, 2006; Coors *et al.*, 2006; Mohr *et al.*, 2007). Studies on macrophytes in artificial lotic systems are lacking, however, and seldom attempt to determine the suitability of stream mesocosms for growing plants in comparison to their natural habitat (Beklioglu & Moss, 1996; Carr & Chambers, 1998; Lindig-Cisneros & Zedler, 2002; Roussel *et al.*, 2007; Knauer *et al.*, 2008).

Ledger *et al.* (2009), states that mesocosm effectiveness requires both realism (i.e. similarity to natural systems) and replicability (i.e. the extent of replicate mesocosm physicochemical and biological inter-variability). However, replicability and realism are elements of mesocosm design which are rarely reported, with many studies seemingly assuming that such experiments are both realistic and replicable, and therefore reflective of natural systems (Petersen & Hastings, 2001). This concern has been, to some extent, addressed in studies of calcareous macroinvertebrate communities (Harris *et al.*, 2007; Ledger *et al.*, 2009), where model systems were paralleled amongst replicates and realistic analogues of

natural systems, but similar studies involving aquatic macrophytes are particularly lacking. Moreover, a majority of macrophyte oriented mesocosm studies are either indoor laboratory-based (Sand-Jensen & Madsen, 1991; Mohr *et al.*, 2007), use recirculating channels (Matthews *et al.*, 1990; Craig, 1993) or focus on lentic systems (Mckee *et al.*, 2002; Morris *et al.*, 2003; Wolfer *et al.*, 2006; Feuchtmayr *et al.*, 2009), with few looking at lotic systems (Mohr *et al.*, 2007) and even fewer sourcing water directly from a local natural stream or aquifer (Garbey *et al.*, 2006; Mony *et al.*, 2007; Puijalon *et al.*, 2007). Furthermore, some indoor studies have time constraints due to potential fluctuation of water physicochemistry (Mony *et al.*, 2007), and introduction of unexpected artefacts, even when water has been obtained from the natural river system; this is a particular constraint of closed, recirculating systems. This raises additional concerns regarding the realism of such studies, as physicochemistry may vary from the optimum growing conditions of the relevant macrophyte (Carr *et al.*, 1997; Mony *et al.*, 2007), and without assessment against a natural river system, it would be difficult to take this into account.

In many cases realism and replicability testing should be required for the taxa and river type in question, as different biota likely show variations in response that could otherwise not be accounted for. All lotic mesocosm studies should, therefore, be examined for ecological realism and replicability to ensure experimental robustness. For example, a recent study on macroinvertebrate community realism in stream mesocosms was performed by Ledger *et al.* (2009), who assessed taxonomic composition in relation to a parent stream, and determined that the mesocosms were a good representation of the source stream for studied physicochemical conditions and fauna. Whilst this outcome indicates the usefulness of mesocosms for the taxa studied, it may be less applicable to other taxa in the same river habitat (e.g. floral), or for studies of riverine systems other than lowland chalk streams. Similarly, Harris *et al.* (2007), examined replicability of a

comparable invertebrate community, and found that channel water chemistry and biota were highly replicable; again, this may not be an appropriate assumption to apply for other, untested biota. Furthermore, experimental designs by Ledger *et al.* (2009) and Harris *et al.* (2007) were fed from naturally fluctuating river-sourced water; water source may therefore play an important role in mesocosm robustness. Research objectives must also be considered; for example, it is possible that stream mesocosm designs are better suited to particular biota (e.g. floral or faunal) due to physical constraints (channel widths/depths), or studies of single or multiple species (e.g. individual macrophytes or macroinvertebrate communities).

In this chapter, an array of outdoor once-through mesocosms were used to test the suitability of artificial streams for growing the keystone macrophyte *Ranunculus penicillatus* subsp. *pseudofluitans* in comparison to growth in an adjacent naturalised chalk stream. Physicochemistry and macrophyte growth were examined both between mesocosm channels to determine synchronicity (thus testing replicability) and compared to the stream reach to assess congruence with mesocosm plant growth (thus testing realism). The aim of this chapter, therefore, is to determine the suitability of stream mesocosms for growing juvenile specimens of the aquatic macrophyte *Ranunculus penicillatus* subsp. *pseudofluitans*. This will be achieved by testing the following hypotheses: 1) water physicochemical conditions and macrophyte specimen growth in artificial stream mesocosms is comparable to conditions and growth in a naturalised stream; 2) once-through artificial stream mesocosms are highly replicable systems for water physicochemistry and testing submerged macrophyte growth; and, 3) water depth does not affect macrophyte development.

3.2. METHODOLOGY

3.2.1. Study area

The study was performed over a 28-day period in April and May 2011 using a series of outdoor artificial stream mesocosms at Fobdown Watercress Farm, Vitacress Ltd, near New Alresford, Hampshire, U.K. (51°06′08.57″N, 1°11′06.33″W), and a comparison 'natural stream site' on the Candover Brook, a small chalk stream, adjacent to the mesocosm plot (**Figure 3.1.**). The Candover Brook is a tributary of the River Itchen, a Site of Special Scientific Interest and Special Area of Conservation (Natural England, 2013; JNCC, 2013).

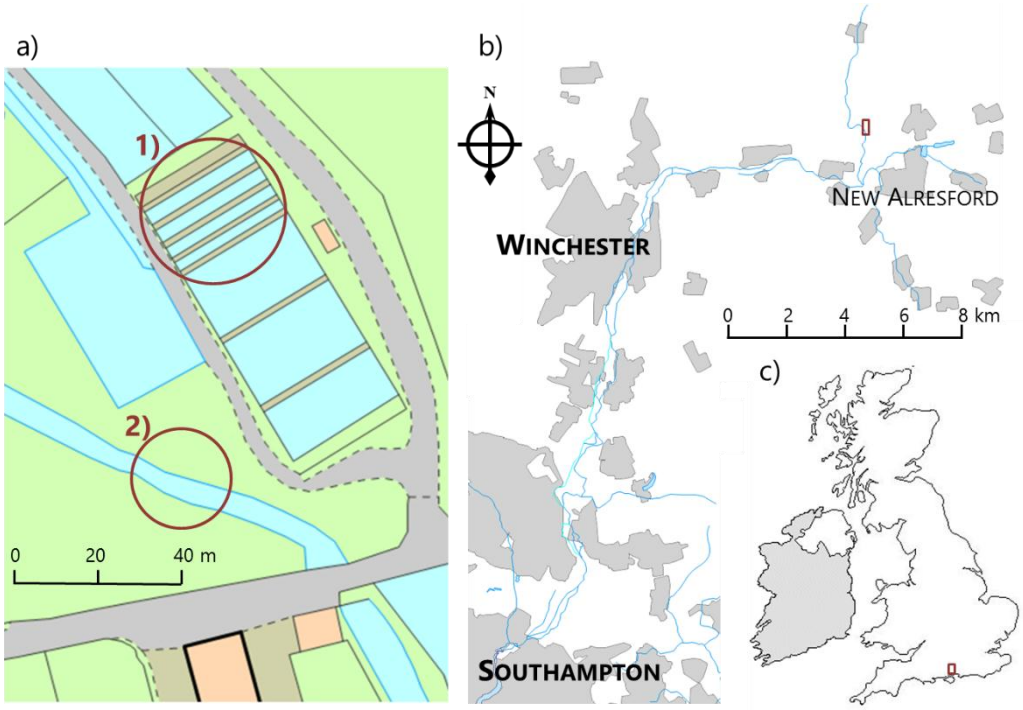

Figure 3.1. **Location of the study site at Fobdown Farm, Hampshire.** Showing a) the location on the watercress farm, with 1) signifying mesocosm locations and 2) denoting the Candover brook sample site, b) the location compared to the course of the River Itchen, and c) the location within the U.K. (Poynter, 2012).

3.2.2. Study taxon

Ranunculus penicillatus subsp. *pseudofluitans* (Syme) S.D. Webster (herein *R. pseudofluitans*), a divergent, fine-leaved, submerged aquatic macrophyte, was chosen due

to its keystone role in the chalk stream ecosystem (O'Hare *et al.*, 2010), by providing refugia for riverine fauna and as a biological engineer of river flow, and for its potential ability to show rapid plastic responses over a short time period (Garbey *et al.*, 2004). Close relatives can double initial biomass within one month (Sand-Jensen & Madsen, 1991; Madsen & Brix, 1997), supporting the decision to conduct this study over a 28-day period.

3.2.3. Stream mesocosms

Mesocosms were arranged in two blocks into pre-existing experimental beds, with four mesocosms per block (**Figure 3.2.a.**, **Figure 3.3.**). Each mesocosm (**Figure 3.2.b.**) was constructed from a half-pipe twin-wall sewage pipe (width 0.3 m x depth 0.15 m x length

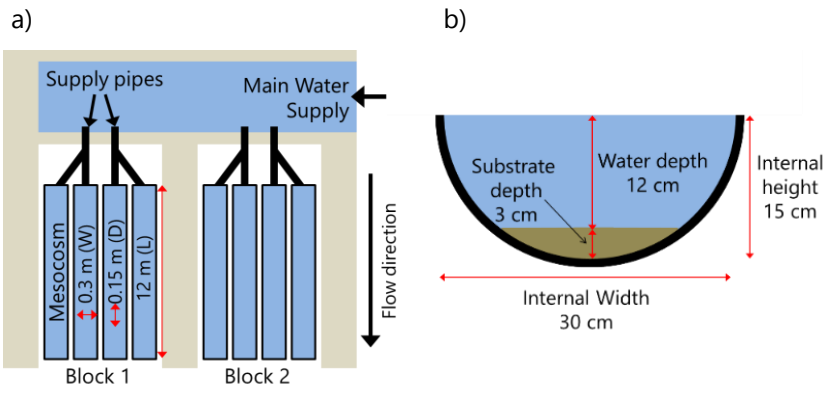

Figure 3.2.

Schematic diagram of the stream mesocosm setup. Showing a) blocks, and b) individual mesocosm cross sectional dimensions.

Figure 3.3.

Photograph of an example 'block' of four mesocosms. Photograph taken shortly after initial construction.

12 m) fed by branched 110 mm plastic waste-pipes. Each 110 mm waste-pipe was fitted with a butterfly valve to control water supply. Unfiltered water, originating from an

adjacent aquifer-fed artesian borehole, was directed to the mesocosms via a main supply channel. Upper ends of the mesocosm channels were sealed, and lower ends were open and drained into a "waste" channel, which was located approximately 8-10 cm below each channel outlet to prevent cross contamination. Channel gradient was low, with water free-draining under gravity. Flow velocities were low-moderate (**Table 3.2.**, **Figure 3.5.**) Mesocosms were filled with a layer of washed substrate (particle sizes: 80% 11-22 mm; 12% 2-11 mm; 6% 0.35-2 mm; 2% <0.35 mm) to a depth of 3 cm to allow rooting medium and was comparable with substrate found in the Candover Brook (see 3.2.4.). Water depth was 118-120 mm in each channel, which was assumed a suitable growing depth for juvenile *R. pseudofluitans* specimens and water physicochemistry was also consistent throughout the study and congruent between mesocosms (see 3.3.1.).

3.2.4. Natural stream site: The Candover Brook

The stream site in the adjacent Candover Brook was located slightly downstream of the mesocosm plot, in a 30 m stretch of the stream (**Figure 3.4.**). This site was chosen due to its

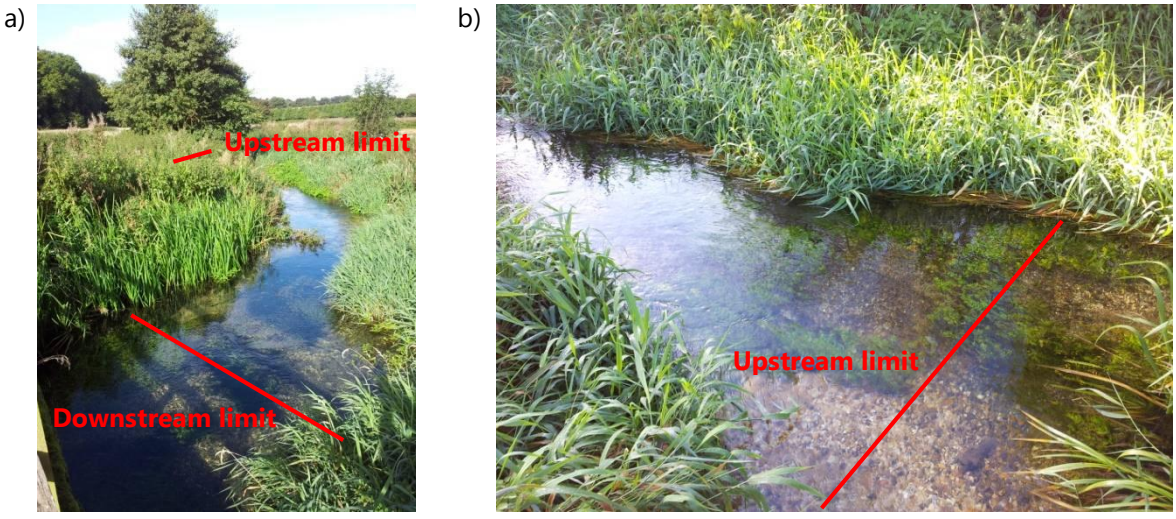

Figure 3.4. **Photographs of the location of the 30 m in-stream planting reach on the Candover Brook.** Showing a) the downstream limit of the 30 m reach with the rough location of the upstream limit, and b) a close up of the upstream limit.

open shallow, yet varied, profile (depth range 15-49 cm), predominantly gravel substrate (83% 11-22 mm; 7% 2-11 mm; 7% 0.35-2 mm; 3% <0.35 mm), a varied velocity profile and limited natural submergent macrophyte growth during the study period. Variation in water depth enables additional opportunities to test water depth-effects on macrophyte development. Four different water depth categories (i) 11-20 cm; ii) 21-30 cm; iii) 31-40 cm; and, iv) 41-50 cm) were therefore selected to determine influence of water depth on juvenile plant development. All plants in the first depth category were planted at ~12 cm depth, to correspond with those grown in the mesocosms. Planting sites were chosen by surveying the 30 m reach of the stream and randomly assigning plants to one of the four identified areas of stream bed present at each type of depth category. Stream velocity was measured at these planting sites to confirm that they were within velocity thresholds chosen for the 8 mesocosms (0.25-0.3 m s^{-1}) for consistency.

3.2.5. Sampling process

To test variation in plant responses between mesocosm and natural stream conditions, specimens of the submerged aquatic macrophyte *R. pseudofluitans* were grown in comparative conditions at each location (except for water-depth tests, see 3.2.4.).

3.2.5.1. Initial specimen harvesting and planting

Fragments of *R. pseudofluitans* were grown in each mesocosm and in the Candover Brook to simulate the important juvenile establishment and development phase after vegetative propagation of this species by allofragmentation (Riis *et al.*, 2009). Allofragments occur due to stem breakage from disturbance and can be naturally or anthropogenically caused (Riis *et al.*, 2009). Fragments were harvested from a local mature parent plant stand from the Candover Brook by lightly pulling on the branches to mimic physical-resistance stress

breakage. On the first experimental day, 80 genetically similar clonal specimens were harvested at random from the parent plant using the above method at 4 internodes length from the apex, with adventitious roots still present at the internode closest to the break. Specimen apices needed to be healthy, otherwise they were discarded, and another selected. Fragment lengths were congruent (176 ± 2 mm), as were initial biomass readings (4.77 ± 0.13 g f_w).

Fragments were assigned planting positions at random within mesocosms and the stream planting site. Plants were spaced at 2 m intervals within the channels, and were planted in a close line (with approx. 10 cm between each plant), perpendicular to the direction of flow in the stream. Fragments were then planted by carefully burying the lower 50 mm of the specimen (so at least the lowest adventitious roots were also buried) into mesocosm and stream substrate.

3.2.5.2. Water physicochemistry

Mesocosm and stream water physicochemistry measurements were recorded at both sites at the start of the study period, and were sampled weekly until the end of the study. Water temperature (°C), pH, dissolved oxygen (%) and conductivity (µS cm^{-1}) were measured in the field using a calibrated multimeter (YSI Pro Plus, Yellow Springs, U.S.A.) on each sample date. Water samples were taken in each mesocosm and at each stream planting site using 500 ml plastic bottles on each sample date. Water samples were refrigerated at 6°C for no more than 2 days, and were subsequently analysed for soluble reactive phosphorus (mg PO_4- l^{-1}), nitrate (mg NO_3- l^{-1}) and suspended sediments (mg l^{-1}). Water samples for SRP and nitrate were analysed using ion chromatography (Dionex ICS2000 with AS40 Autosampler, Thermo Fisher Scientific, Leeds, U.K.). Suspended sediment concentrations were analysed by filtration. Velocity readings (m s^{-1}) were taken in the field using a Sensor-RC2 water

velocity meter (Aqua Data Services Ltd, Lyneham, U.K.) for each mesocosm and at each stream planting site at 60% of water depth from the surface. Discharge was calculated by using velocity readings and mesocosm/stream channel parameters using the following equation:

$$Q = a \times u \qquad 3.1.$$

where Q = discharge ($m^3\ s^{-1}$), a = cross-sectional area (width x depth), u = velocity ($m\ s^{-1}$).

Reynolds numbers, to determine flow characteristics (turbulent/transitional/laminar), were calculated for each mesocosm and stream planting site using the equation:

$$Re = \frac{u\ x\ r}{\mu} \qquad 3.2.$$

where Re = Reynolds number, u = velocity ($m\ s^{-1}$), r = hydraulic radius (see below), μ = kinematic viscosity. μ is temperature dependent and was scaled to water temperature measurements (e.g. at 10.5 °C, μ = 1.2498 x 10^{-6} kg $m.s^{-1}$). Re <500 = laminar flow, 500-2000 = transitional flow, >2000 = turbulent flow. Hydraulic radius was calculated using the following equation:

$$r = \frac{a}{P_w} \qquad 3.3.$$

where r = hydraulic radius, a = cross-sectional area (width x depth), P_w = wetted perimeter (width + (2 x depth)).

3.2.5.3. Plant characteristic traits

Macrophyte traits (**Table 3.1.**), including morphological traits and biomass (initial fresh weight, f_w) were recorded for each specimen at the start of the study, and morphology was then taken weekly. In addition, the final sample involved uprooting plant specimens to

record end f_w, which was performed immediately after removal from the water (post dab-drying for 30 seconds per specimen with a paper towel). All uprooted specimens were then oven dried at 60°C for at least 8 hours on the same day they were removed from the planting sites. Total d_w and d_w of roots and shoots were also recorded for root:shoot (r:s) allometric coefficients. Morphological 'length' traits were measured using a vernier measuring caliper, 'angles' measured with a plastic semi-circular protractor, and remaining traits were simply counted. Biomass measurements were performed using a portable electronic precision balance (Fisher Scientific SG-202, Loughborough, U.K.).

Morphological traits (**Table 3.1.**; **Figure 3.5.**) were selected to characterise vegetative growth and fragmentation (vegetative reproduction), relevant to *R. pseudofluitans* and the study's experimental timescale. No sexual reproductive traits were measured (study time was assumed too short for development). Traits were similar to those investigated by Garbey *et al.* (2004, 2006) in studies on a close relative (*Ranunculus peltatus*).

Table 3.1. **List of characteristic traits selected for sampling.**

Identifier	Abbreviation	Plant characteristic trait	Measure of
i	L	Length of main stem	Speed of elongation and overall development.
ii	PI	Position of longest internode (on main stem)	Plant stress – longest internode thought to remain constant during growth. Changes may indicate stress.
iii	LI	Length of longest internode (on main stem)	Internode development – length indirectly reflects number of new internodes.
iv	LL	Length of longest leaf (on main stem)	Leaf development on main stem – length proportional to internode length for plant variety. Deviance from this may indicate unfavourable conditions.
v	SB	Length of longest secondary branch	Lateral development – short secondary branches or slow growth may indicate unfavourable conditions.
vi	AB	Angle of branches	Lateral development – a reflection of plant form. Manipulated principally by flow conditions.
vii	PR	Position of first adventitious roots	Vegetative reproductive ability – healthy plants less likely to fragment, adventitious roots further from apex.
I	B	Biomass (f_w & d_w)	Overall development and productivity.
II	R:S	Root:shoot allometric coefficient	Root development proportional to above-ground biomass

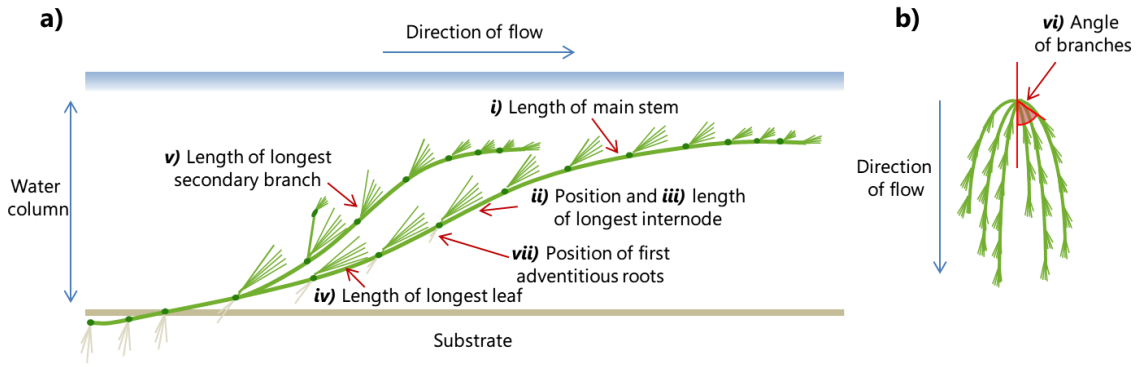

Figure 3.5. **Diagrammatic representation of *R. pseudofluitans* showing measured morphological traits.** a) cross section of plant length, b) aerial view of plant growth.

3.2.6. Data analysis

To test realism and replicability of mesocosm physicochemistry and plant growth, correlative analyses (Spearman's Rank Correlation (Sp)/Pearson Product Moment (Pe) Coefficients) were performed between mesocosm and stream data. Initial data exploration assessed normality and homogeneity of variance assumptions. Significant correlations were identified where $p < 0.05$ and $r > 0.35$. Regression analysis was performed to examine relationships in plant traits, with significance at the $p < 0.05$ level.

Additionally, a series of partial ordinations were conducted using CANOCO 4.56 (ter Braak & Šmilauer, 2002), to compare mesocosm variation attributed to spatial (mesocosm) and temporal (study period) effects on physicochemistry and plant growth. Short axes gradient lengths (<2 SD, Lepš & Šmilauer, 2003), using exploratory detrended correspondence analysis (DCA), suggest the need to use redundancy analysis (RDA). Physicochemical variables and morphological traits were treated as 'species' data, and were centred and standardised (ter Braak & Šmilauer, 2002), and mesocosms (1-8) and sampling days (5 days) were treated as dummy environmental variables (0 or 1). A total of four ordinations were run for both physicochemistry (7 variables, $n = 7$) and morphological traits (7 variables, $n = 7$) in order to determine the percentage variation explained by: i) mesocosms and time

(both as explanatory variables); ii) mesocosms only (with time as covariables); iii) time only (with mesocosms as covariables); and, iv) mesocosm blocks (1 or 2, coded as dummy variables) and positions within blocks (left, centre-left, centre-right, right, coded as dummy variables). In all models, Monte Carlo permutation tests (with 1000 permutations), were used to test statistical significance.

3.3. RESULTS

3.3.1. Water physicochemistry

3.3.1.1. Realism - Stream site and mesocosm inter-variability

The Candover Brook was characterised by alkaline waters (pH range 7.34-7.89) and relatively ion and nutrient rich conditions (range 521.2-542.3 µS cm^{-1}; mean 0.02 mg PO_4- l^{-1}; mean 7.63 mg NO_3- l^{-1}; **Table 3.2.** & **Figure 3.6.**).

Table 3.2. **Mean, minimum and maximum physicochemistry of the stream site and mesocosms during the experimental period.**

	Candover Brook			**Mesocosms**		
	Mean	Min	Max	Mean	Min	Max
pH	7.6	7.34	7.89	7.57	7.31	7.92
Dissolved Oxygen (DO)	106.26	98.7	112.8	107.88	101.1	113.4
Temperature (°C)	11.6	10.3	14.2	10.4	10.3	10.4
Conductivity (µS cm^{-1})	533.3	521.2	542.3	386.1	385.1	386.9
Velocity (m s^{-1})	0.255	0.24	0.281	0.256	0.243	0.273
Discharge (m^3 s^{-1})	0.259	0.146	0.327	0.011	0.011	0.012
SRP (mg l^{-1})	0.02	0.02	0.02	0.02	0.02	0.02
Nitrate (mg l^{-1})	7.63	7.45	7.95	7.00	6.98	7.02

Water temperatures were relatively warm and consistent (range 10.3-14.2 °C) and highly oxygenated throughout (mean 106.26%). Water velocities were swift and consistent

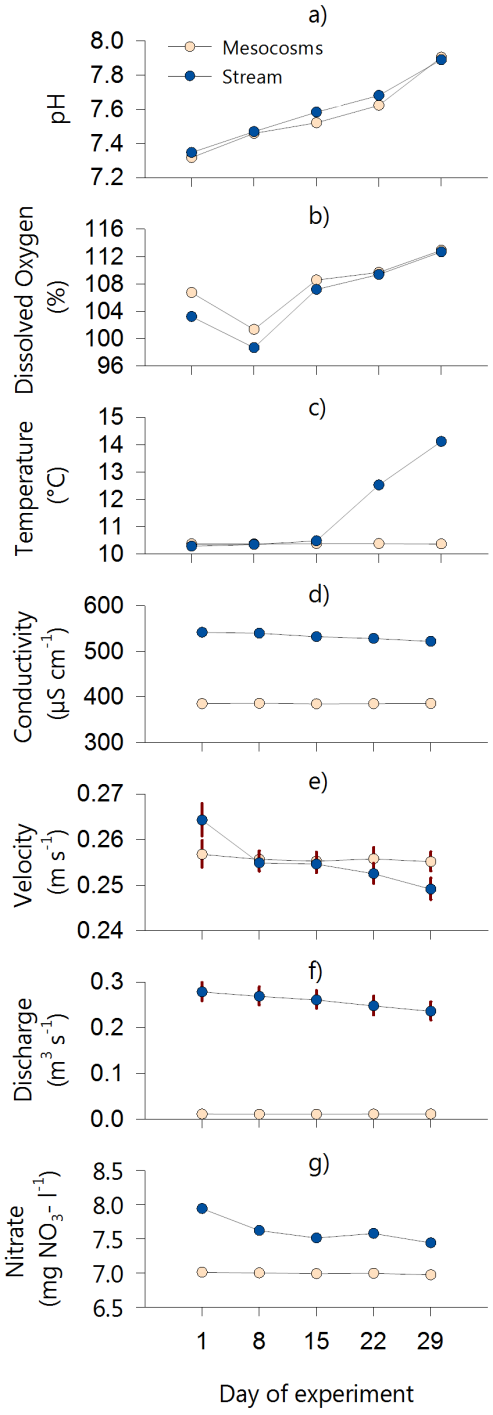

Figure 3.6. **Mesocosm and stream physicochemistry for the study period.** Parameters shown include a) pH, b) dissolved oxygen, c) temperature, d) conductivity, e) water velocity, and f) nitrate. Error bars represent standard error (n = 8). Phosphate not displayed (see Table 3.2.).

throughout (range 0.24-0.281 m s^{-1}), and discharge was low (mean 0.259 m^3 s^{-1}). Suspended sediment concentrations in all samples were non-detectable (n.d.).

In comparison, mesocosm conditions were generally similar to those seen in the stream reach (**Table 3.2**. & **Figure 3.6.**). They were also characterised by alkaline conditions (pH range 7.31-7.92) but had lower conductivity (range 385.1-386.9 µS cm^{-1}). Waters were also similarly nutrient rich (mean 0.02 mg PO_4- l^{-1}; mean 7 mg NO_3- l^{-1}). Temperatures were consistent (range 10.3-10.4 °C) and waters were well oxygenated (mean 107.88%) throughout. Flow velocities were also continuously moderate (0.243-0.273 m s^{-1}), and discharge low (mean 0.011 m^3 s^{-1}). As with stream samples, suspended sediment concentrations were n.d.

Spearman's rank correlation coefficient was used to compare temporal variation between the Candover Brook and stream mesocosms. Statistically significant correlations for all mesocosm units exposed contemporaneous changes in pH (r = 0.984-1, $P < 0.01$), DO (r = 0.981-1, $P < 0.01$), SRP (r = 1, $P < 0.001$) and

nitrate (r = 0.82-1, P < 0.05). Temperature, conductivity, velocity and discharge showed no statistically significant correlation, likely due to differences in water sources, residency and physical channel parameters between stream and mesocosm (**Figure 3.6.**). Temperature remained consistent throughout within mesocosms, but shows an increasing trend towards the end of the study in the stream reach. Conductivity also remains stable in mesocosms, and shows a slow decreasing trend throughout the study period in the stream reach. Velocity appears steady in mesocosms, but shows a decreasing trend during the study period in the stream reach. Furthermore, velocity appears more variable throughout in comparison to other physicochemical variables (**Figure 3.6.**), but small y axis scale indicates limited relative fluctuation.

Flow characteristics between mesocosms and the Candover Brook site also showed no significant correlations. Reynolds numbers (Re) were high in mesocosms (15,292 ± 76) and in stream sites (52,828 ± 2,402) signifying highly turbulent water flow, but higher numbers in the stream account for the lack of congruence. Enhanced variability in Re in stream sites is due to increased hydraulic radius from greater water depth.

3.3.1.2. Replicability - Inter-mesocosm variability

Physicochemistry was statistically highly synchronous between mesocosms during the study period (**Figure 3.7.**) for pH (Pe, r = 0.996-1, p < 0.001), DO (Pe, r = 0.997-1, p < 0.001), water temperature (Sp, r = 1, p < 0.01), conductivity (Pe, r = 0.806-0.995, p < 0.05), SRP (Sp r = 1, p < 0.01) and nitrate (Pe r = 0.821-1, p < 0.05). Velocity, discharge and Re had no statistically significant inter-mesocosm correlations.

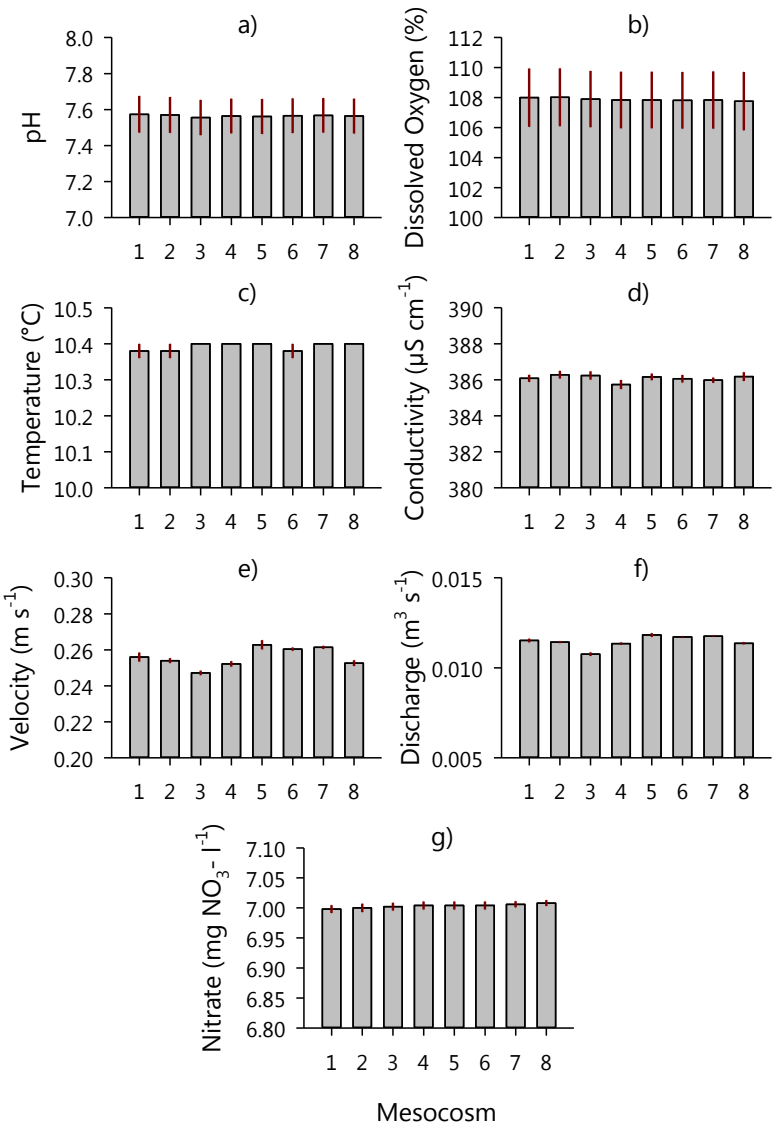

Figure 3.7. **Mesocosm physicochemistry compared between the 8 studied stream mesocosms.** Parameters shown include a) pH, b) dissolved oxygen, c) temperature, d) conductivity, e) water velocity, f) discharge, and g) nitrate. Error bars represent standard error (*n* = 5). Phosphate excluded.

A partial-RDA of mesocosm physicochemistry supports this replicability, with limited spatial variation accounting for only 0.2% of overall variation (**Table 3.3.**, analyses 1, 3). Furthermore, there was no significant effect of mesocosm positioning (**Table 3.3.**, analysis 4) observed on physicochemistry. Consequently, considerable variation was attributed to temporal variability, with 88.5% of the variation in the model connected to study period time (**Table 3.3.**, analyses 1, 2).

Table 3.3. **Partial redundancy analysis (RDA) results for spatial and temporal variation in physicochemistry in mesocosms.** Individual model significance tested with Monte Carlo permutation tests (1000 permutations).

Analysis	Explanatory variables (covariables)	*F*-ratio	*P*-value	Percent variation explained
1. Physicochemistry	Time, mesocosms	227.84	0.001	88.7
2. ...	Time (mesocosms)	312.62	0.001	88.5
3. ...	Mesocosms (time)	36.5	0.001	0.2
4. ...	Mesocosm block and position	0.1	0.752	-

3.3.2. Macrophyte morphometry

3.3.2.1. Realism - Stream site and mesocosm inter-variability

Macrophyte specimens growing in the Candover Brook elongated steadily throughout the study period (**Figure 3.8.**), but experienced rapid growth during the last week (mean end L 282 ± 2 mm). Leaves also grew progressively, but did not extend as rapidly in-line with length towards the end (mean LL start 68 ± 2, end 80 ± 2). Longest internodal position (PI) had a narrow range throughout, but remained close to the rooting zone as the plant elongated (range start 3-4, end 4-6), and the length of longest internode (LI) grew steadily in line with elongation (mean length start 67 ± 1, end 82 ± 1), and in particular leaf length. Secondary branch lengths (SB), and consequently branching angle (AB) only began developing at the start of the third week, and showed limited variation for the remainder of the study (SB range 13-40, AB mean 12 ± 0°). Position of first adventitious roots (PR) was close to the apex after the first week (mean 1 ± 0), but was not in this position by the study end (mean 3 ± 0), remaining further from the apex in line with stem elongation.

Specimens growing in mesocosms closely correspond to those in stream sites. Elongation was in line with plants in the stream; however, growth was much slower in the last week by comparison (mean final L 218 ± 2 mm). Leaf length was similar (mean start LL 60 ± 2,

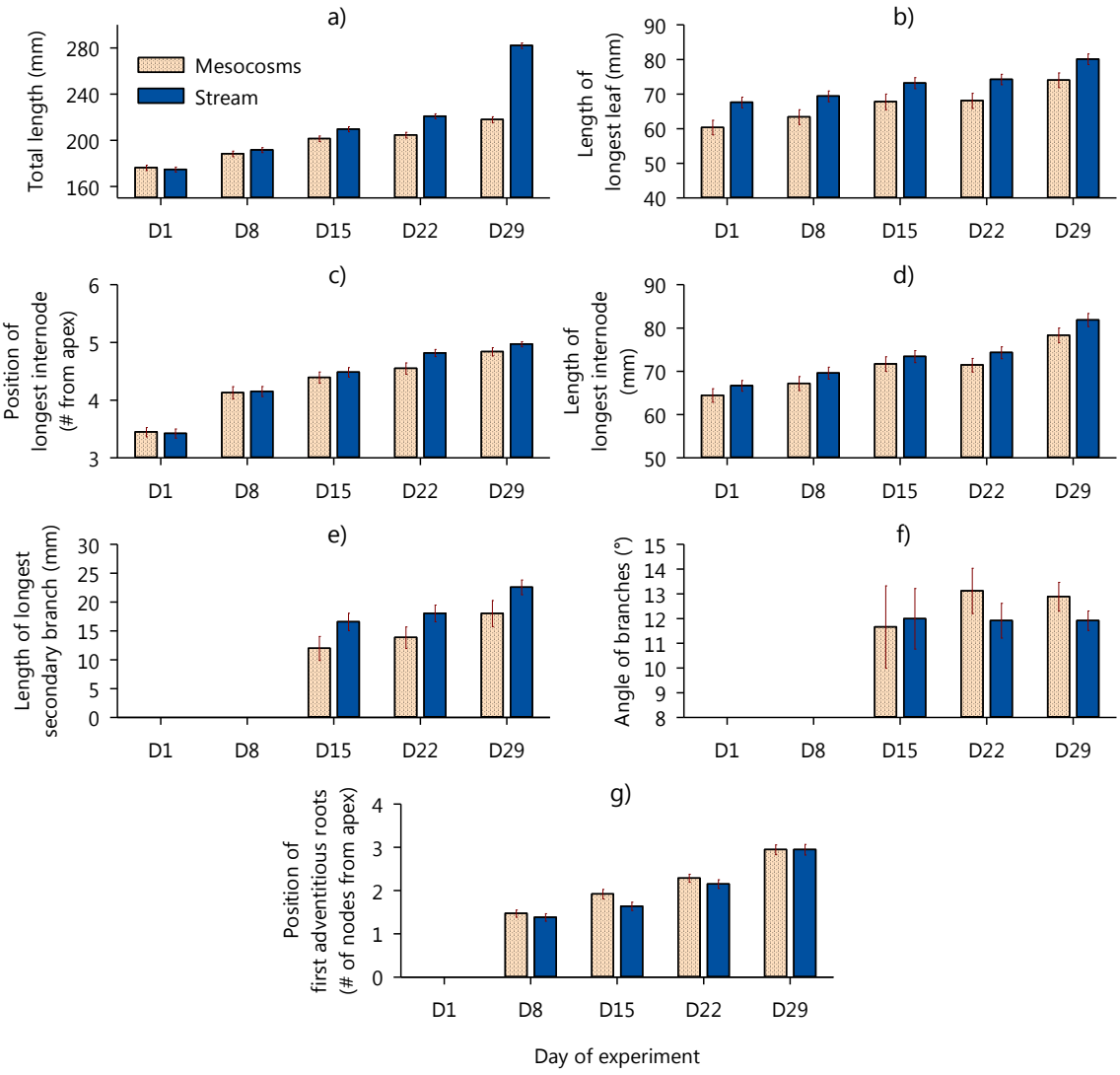

Figure 3.8. **Morphological traits of *R. pseudofluitans* in mesocosms (dark bars) and stream sites (light bars).** Traits displayed include a) total length, b) length of longest leaf, c) position of longest internode, d) length of longest internode, e) length of longest secondary branch, f) angle of branches, and g) position of first adventitious roots. Error bars represent standard error. For a), b), c), d) and g), D1 *n* = 40 (Mesocosm), *n* = 40 (Candover); D8, 15, 22, 29 *n* = 38 (M), *n* = 39 (CB). For e) and f), D15 *n* = 3 (M), *n* = 5 (CB); D22 *n* = 8 (M), *n* = 13 (CB); D29 *n* = 19 (M), *n* = 39 (CB).

end 74 ± 2), and although initially shorter on average, grew more quickly than in the stream by the end. PI and LI were comparable to the growth seen in the stream (PI range start 3-4, end 4-6; LI mean length start 64 ± 2, end 78 ± 2), with LI again consistent with LL. SB and AB first developed during the third week and were similar to stream plants (SB

range 9-36, AB mean 13 ± 0°). PR did not differ from the stream sites either (mean position start 1 ± 0, end 3 ± 0).

Comparisons between mesocosms and stream sites were supported by significant statistical correlations using Sp for L (r = 0.44-0.88, $p < 0.05$), LL (3 mesocosms only r = 0.43-0.83, $p < 0.05$), PI (r = 0.39-0.8, $p < 0.05$), LI (4 only r = 0.45-0.73, $p< 0.05$), and AR (7 only r = 0.52-0.78, $p < 0.05$). SB and AB, whilst showing similar ranges and means between mesocosms and stream sites did not show any significant correlation, likely attributed to the limited number of observations, with traits only observed after week three without patterns.

There were also no statistically significant correlations for total end dry weights or total end fresh weights due to final enhanced extension in stream plants (end mean weights: fresh mesocosm 9.61 ± 0.16 g, stream 12.7 ± 0.08; dry mesocosm 0.57 ± 0.01, stream 0.76 ± 0.01). Root:shoot (r:s) allometric coefficients were low (mean mesocosm 0.11 ± 0.01, stream 0.12 ± 0.01), but were highly statistically significantly similar between the stream site and mesocosms (Pearson's r = 0.918-1, $P < 0.01$).

3.3.2.2. Replicability - Inter-mesocosm variability

Morphological traits were highly synchronous throughout the study (**Figure 3.9.**), with statistically significant correlations for L (Pe r = 0.413-0.948, $p < 0.05$), LL (Pe r = 0.465-0.869, $p < 0.05$), PI (Sp r = 0.453-0.724, $p < 0.05$), LI (Pe r = 0.431-0.815, $p < 0.05$), and PR (Sp r = 0.530-0.902, $p < 0.01$). Again, SB and AB were not significantly related.

Plant d_w & f_w were not significantly correlated between channels. This is likely due to only a loose connection between overall length and plant weight. Linear regression analysis also indicates that end weight is closely related to specimen weight at the start of the study

period (R^2 = 0.479, p < 0.001), which explains the lack of spatial correlation, as specimens were assigned randomly at the start.

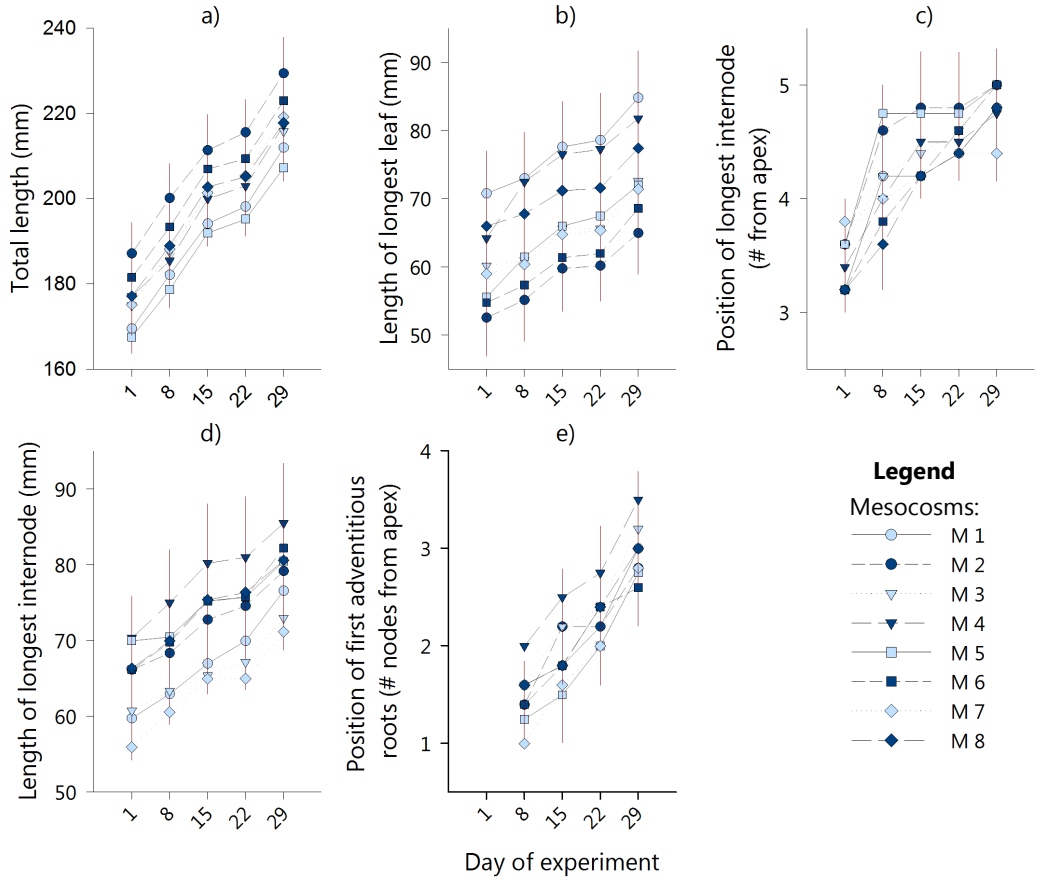

Figure 3.9. **Morphological traits of *R. pseudofluitans* compared between the 8 studied stream mesocosms.** Morphological traits: a) total length, b) length of longest leaf, c) position of longest internode, d) length of longest internode, and e) position of first adventitious roots. Error bars represent standard error. Plants per mesocosm: D1 n = 5; D8, 15, 22, 29 n = 5 (Mesocosm 1, 2, 3, 6, 7, 8), n = 4 (M 4, 5).

Additionally, a partial RDA of morphological traits also indicates high replicability, with a majority of variation (82.2%) explained by temporal influence (**Table 3.4.**, analyses 1, 2). Only limited variation (0.4%) was attributed to spatial variability (**Table 3.4.**, analyses 1, 3), and there was no statistically significant influence of mesocosm positioning on morphological traits (**Table 3.4.**, analysis 4).

Table 3.4. **Partial redundancy analysis (RDA) results for spatial and temporal variation in morphological traits in mesocosms.** Individual model significance tested with Monte Carlo permutation tests (1000 permutations).

Analysis	Explanatory variables (covariables)	*F*-ratio	*P*-value	Percent variation explained
1. Plant traits	Time, mesocosms	85.25	0.001	82.6
2. ...	Time (mesocosms)	88.02	0.001	82.2
3. ...	Mesocosms (time)	6.65	0.003	0.4
4. ...	Mesocosm block and position	1.49	0.226	-

3.3.2.3. Depth effect on traits

Stream specimens were planted at four different water depths, and congruence in correlative analysis of plant traits (**Figure 3.10.**) suggests that depth does not have an effect on macrophyte morphological traits. Significant correlations between macrophyte traits were seen for L (Pe $r = 0.94\text{-}0.97$, $p < 0.001$), LL (Sp, 2 sites $r = 0.41$, $p < 0.05$), PI (Pe $r = 0.69$-

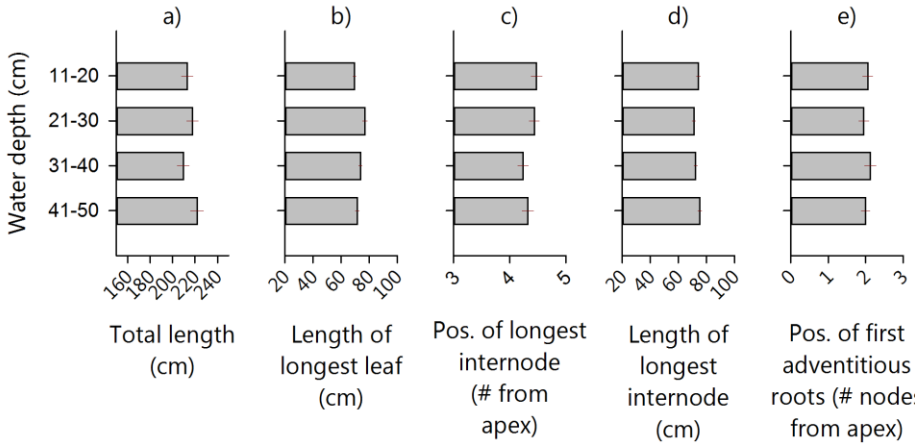

Figure 3.10. **Mean plant morphological traits at different water depth categories in the Candover Brook stream site.** Morphological traits: a) total length, b) length of longest leaf, c) position of longest internode, d) length of longest internode, and e) position of first adventitious roots. Error bars represent standard error. 11-20, *n* = 46 (e) = 36); 21-30, 31-40, 41-50, *n* = 50 (e) = 40).

0.81, $p < 0.001$), LI (Sp 3 sites $r = 0.71$-0.87, $p < 0.001$), and PR (Sp $r = 0.46$-0.86, $p < 0.05$). SB and AB did not show significant correlations, again due to limited observations (see 3.3.2.1.).

End d_w and f_w did not show any significant correlations with depth, but like mesocosm specimens, significant relationships between end and start f_w (linear regression $R^2 = 0.394$, $p < 0.001$) suggests initial biomass is a more important determining factor for end biomass, mainly where limited environmental variation exists.

Coinciding with changing water depth, discharge tends to be higher and Re was considerably higher with increasing water depth, although increasing water volume and turbulence had a limited effect on plant growth.

3.4. DISCUSSION

During the study, it was evident that both mesocosm physicochemistry and plant characteristic traits were closely paralleled to measurements taken in the adjacent stream reach, indicating that the artificial channels used were realistic analogues of natural chalk systems. Furthermore, physicochemistry and plant traits were highly congruent amongst mesocosm replicates, suggesting highly replicable conditions.

Mesocosms are useful tools for bridging the gap between highly experimental systems, which enable causality at the expense of realism, and in-situ field studies, which provide full biological complexity, but typically only allow correlative inference (Stewart *et al.*, 2013). Stream mesocosms, similar to those used in this study, are frequently utilised in aquatic research (e.g. Short, 1987; Short *et al.*, 1995; Taylor *et al.*, 1995; Beklioglu & Moss, 1996; Kercher & Zedler, 2004; Goulet *et al.*, 2005; Ledger *et al.*, 2006, 2008, 2009, 2011, 2012; Mohr *et al.*, 2007; Harris *et al.*, 2007; Brown *et al.*, 2011), but with studies commonly failing to address their suitability or realism (i.e. how well they mimic natural river

conditions), concerns should be raised until appropriate evaluations of ecological and physicochemical realism have been conducted. Likewise, whilst replicates are used regularly, analysis of replicability is rarely performed.

In this study, juvenile specimens of the aquatic macrophyte *Ranunculus pseudofluitans* were artificially planted in stream mesocosms (resembling a gravel-dominated substrate reach, with moderate flow) and an adjacent stream to simulate vegetative dispersal, allofragmentation and colonisation (Riis *et al.*, 2009), and trait development and biomass were assessed to provide an indication of plant health. Water physicochemistry was also analysed, and data were used to determine realism and replicability, and therefore suitability of artificial channels for macrophyte growth experiments.

Realism between model and natural systems is a vital component for consideration in the design of stream mesocosms (Cooper & Barmuta, 1993; Englund & Cooper, 2003; Ledger *et al.*, 2009). Basic physicochemical conditions observed in the nearby Candover Brook were comparable to those seen in the studied mesocosms. Water pH, SRP, dissolved oxygen and velocity all closely corresponded to natural stream conditions, and whilst differences were detected for conductivity, nitrate and temperature, relative differences were minimal and unlikely to influence plant growth. Greater temporal consistency in mesocosms for conductivity and nitrate, compared to the stream reach, are likely due to greater stability of the mesocosm source water (Berrie, 1992), and variation in temperature during the last two weeks of the study in the stream can be attributed to rising air temperatures (Mackey & Berrie, 1991) and increased water residency times within the stream channel. Reynolds numbers were high in mesocosms and the stream, both considerably above the 'turbulent' threshold, indicating that plants may have been subjected to significant battering and tangling (Haslam, 2006), although observational evidence would suggest otherwise. Reynolds numbers were somewhat higher in the stream reach, suggesting greater

turbulence, and whilst consideration should be given to this difference (Craig, 1993), it is unlikely this influenced plant growth.

Ledger *et al.* (2009) suggest that closely paralleled conditions are likely attributed to mesocosm design, with open-ended channels allowing through-flow of water, limiting water residency times and providing comparable exposure to light and air temperature experienced by the natural stream. The use of once-through channels may be a key element of the mesocosm design, as other experimental systems have shown a tendency for physicochemistry to diverge from natural conditions (Schindler, 1998). This is particularly the case for lentic mesocosm systems (i.e. lake/pond mesocosms) and "closed" circulating stream setups, which tend to introduce artefacts from enclosure (Petersen & Englund, 2005; Harris *et al.*, 2007), imposing limitations on hydrodynamics, air-water gas exchange, catchment-derived nutrient sources and periphyton accumulation on mesocosm walls (Schindler, 1998).

An additional benefit of the design of this study in comparison to Ledger *et al.* (2009), was the use of borehole water direct from the aquifer, which allowed greater control and less variability in water physicochemistry, whilst retaining chemical characteristics similar to the Candover Brook. However, limitations of using borehole water rather than direct from parent source meant that water temperatures, conductivity and nitrate remained constant in mesocosms despite variation within the stream site. Whilst this causes minor problems for comparative purposes, as in this study, the physicochemical consistency imposed by an aquifer/groundwater-fed water source may be considerably better for controlled manipulative outdoor experimentation, and for statistical power in determining treatment-effect causality (Cooper & Barmuta, 1993; Kennedy *et al.*, 1999), more akin to levels of indoor experimental control (Brooks *et al.*, 1996). However, it should be noted that, for macroinvertebrate drift and colonisation, as in Ledger *et al.*, (2009), borehole

sourced water would severely impair taxon migration, and would therefore be unsuitable unless overcome by using an alternative method of 'seeding'; in this study, this experimental consideration was not a concern.

Assessment of ecological realism in this study focused on morphological variation and changes in biomass of the keystone macrophyte *R. pseudofluitans*. In fact 'realism' was foremost a test of suitability for growing submerged macrophytes in artificial streams, and consequently realism was not tested at any other level of organisation (e.g. community assemblage as in Ledger *et al.*, 2009).

Plant morphological traits on specimens growing in the Candover Brook were comparable to those in specimens within stream mesocosms. Despite these strong similarities, small differences were detected in some traits; overall plant elongation, leaf length and secondary branch lengths were all marginally larger in stream specimens throughout the study, and total length also exhibited greater elongation at the last sample date. Shorter total length at the last sample date may signify a form of temperature constraint on plant elongation within mesocosms, when compared with stream specimens under increasing temperature (Carr *et al.*, 1997). However, similarities in other morphological traits may suggest that plants in mesocosms were not unduly stressed by lower temperatures, which may only account for inhibition of elongation by reducing rates of chemical reactions (Carr *et al.*, 1997).

Position and length of longest internode remained consistent between stream sites and mesocosms, with both traits increasing steadily throughout the study period. The increase in these traits was expected, and agrees with Garbey *et al.*, (2004), who suggest that position and lengths of the longest internodes reflect development of the length of the main stem. Lengths of secondary branches and branching angles were also comparable between the

stream and mesocosms, suggesting equivalent plant lateral development (Garbey *et al.*, 2004). Branching angles on all specimens saw low divergence from the main stem under moderate flow velocities, as might be expected with increasing drag forces (Sand-Jensen, 2008), although this may require testing under a variety of velocities to determine the usefulness of measuring this trait. Position of first adventitious roots was highly congruent, with moderate position movement away from the apical tip showing a change in potential vegetative dispersal ability (Garbey *et al.*, 2004). It is possible that when plant adventitious root position remains further from the apex as the plant develops, there is a lesser requirement for vegetative dispersal, and hence indicates good growing conditions for the plant. It must be noted that this may not remain true once the plant reaches maturity; as this study only examined juvenile development, there was limited ability to test this.

Final fresh and dry weights saw limited correspondence between stream sites and mesocosms, probably due to differences in total final length; assessments of the relative growth rate (RGR) would account for this difference and allow better assessment of causality in treatment based studies. The closeness of the root:shoot ratio, however, may represent similarity of observed flow conditions, and the impact this corresponding mechanical resistance has on plant resource allocation (Madsen, 1991; Puijalon *et al.*, 2008) for the purpose of anchoring.

Water depth had limited effect on plant growth, even with the impact of increasing turbulence. This may be due to the limited developmental time (28 days), and may also only be applicable to juvenile growth; mature specimens (or larger, older juveniles) may find the limited depths used in the mesocosms detrimental to growth, as indicated by Garbey *et al.*, (2006), who noticed that shallower depths (17 cm) induce below-optimum growth in a related species (*R. peltatus*). Water depth may influence flowering (Garbey *et al.*, 2006), suggesting a change in resource allocation that could affect growth.

If extraneous variation is kept to a minimum, a series of well-designed mesocosms is capable of allowing inference of causality between treatment applications and biotic responses (Cooper & Barmuta, 1993), particularly with the use of appropriate statistical tests (Kennedy *et al.*, 1999; Harris *et al.*, 2007). In this study, both physicochemical conditions and plant growth characteristics were comparable between stream mesocosms, with limited spatial difference (between channel position or block) and strong temporal variation among mesocosms. Parallel physicochemistry was representative of good mesocosm design, location (Harris *et al.*, 2007), and to some extent, the water source. Water velocity was not fully congruent between mesocosms, however; this was thought to be due to, a) difficulty establishing corresponding velocities at the beginning of the experiment, and b) minimal variation of temporal within-channel velocities during the study. This is an aspect of mesocosm design which could be improved. However, the between-mesocosm range was relatively small, so the impacts on plant growth were likely negligible. Furthermore, water residency times were minimal in all channels owing to the once-through mesocosm design, restricting deviation in water temperature and water chemistry.

All juvenile plant traits were found to be highly synchronous between mesocosms. This is with the exception of length of longest secondary branch and angle of branches; both these traits were observed first during the middle sample day, suggesting that a longer period of observation may be useful to ascertain whether these traits were significantly similar between mesocosms. Final fresh and dry weights were also not significantly correlated, although this is due to the dominance of temporal effects and the correspondence of individual plant weight increases to initial start weights, rather than because of differences in positioning within and between mesocosms. Assessments of relative rates of growth may therefore be more appropriate.

3.5. CONCLUSION

Artificial stream mesocosms have long been useful tools for ecological experimentation, and once-through mesocosms, supplied from a stable water source, appear particularly well suited to lotic macrophyte growth studies due to the high levels of tractability and manipulation that they offer. Physicochemical conditions and plant morphology closely parallel those in natural stream reaches, owing to the once-through design, meaning mesocosms are less likely to suffer from 'closed' system limitations. In particular, a stable aquifer-fed water source is beneficial for experimentation with macrophytes, as less temporal variation is experienced, and therefore levels of control are raised nearer to indoor experimental setups. The statement by Harris *et al.* (2007), that stream mesocosms are more representative of small scales in natural river reaches (e.g. habitat patches), is supported by the findings of this study. This may be even more appropriate for studies involving submerged aquatic macrophytes due to difficulties in the capacity to increase complexity; for example, the mesocosms in this study had a relatively uniform gravel bed, fairly homogeneous flow conditions, limited ability for erosional and depositional processes to occur, and a lack of herbivory and competitive interaction. However, whilst some of the above issues may be easier to rectify than others, the overall simplicity of the mesocosm design does lend itself well to directly examining the impact of changing environmental conditions on the plastic responses of individual macrophytes. This in turn may allow greater understanding of why plant community changes occur under increasingly pressured river ecosystems.

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CHAPTER IV

Optimum growth at moderate flows: Velocity tolerance of the aquatic macrophyte *Ranunculus pseudofluitans*

4.1. INTRODUCTION

The distribution of submerged aquatic macrophytes is particularly associated with riverine flow regimes and localised flow patterns in river channels (Chambers *et al.*, 1991; Riis & Biggs, 2003; Franklin *et al.*, 2008), with presence of plant species being strongly linked to flow tolerance at specific river reaches (Haslam, 2006). However, facing increasing periods of drought, low flow, and greater flow regime variability (Holmes, 1999), the impact changing flows may have on the distribution of macrophyte communities in lowland chalk streams is little understood. Furthermore, with climate change and over-abstraction likely to exacerbate low-flows (Holmes, 1999; Wright *et al.*, 2002; Hatton-Ellis & Grieve, 2003; Clayton *et al.*, 2008), maintaining ecologically healthy flows may be of even greater concern in the future management and conservation of chalk stream ecosystems.

Flow regime is considered the main driver of many in-stream processes (Wood & Armitage, 1999; Withers & Jarvie, 2008). Aquatic macrophytes are often constrained by the environmental heterogeneity created by river flow, and often occur in transitional communities due to regular disturbance (Hatton-Ellis & Grieve, 2003). Chalk stream macrophyte communities, by contrast, are often present in a state of patchy equilibrium, resulting in greater community stability and higher species diversity (Berrie, 1992). Ultimately this is attributed to the continuity of the chalk aquifer-fed flow regime (Berrie, 1992; Mainstone *et al.*, 1999). This stability also makes chalk communities particularly susceptible to extraneous environmental influences (Jarvie *et al.*, 2006).

Water movement is a mechanical stimulus for aquatic plants (Puijalon *et al.*, 2005). Flow (particularly velocity) impacts on macrophytes can be either direct (e.g. via physical/mechanical damage) or indirect (e.g. via rates of mass transfer; Haslam, 2006; Franklin *et al.*, 2008), and are roughly categorised into biomass gain or loss processes (Riis

et al., 2008). Biomass gain is linked to reaches of limited disturbance in low and median flow conditions, whereas loss can be attributed to stress caused by higher, more variable flows (Riis *et al.*, 2008). However, local velocities at the plant-stand scale are thought to be one of the most significant forms of environmental stimuli for plant growth (Puijalon & Bornette, 2004; Franklin *et al.*, 2008), with growth usually increasing in low to medium velocities, and becoming limited when velocities are higher (Chambers *et al.*, 1991; Madsen *et al.*, 2001). Consequently, the consistency of discharge regimes in chalk streams, usually conducive of high biomass gain, if altered may result in biomass loss processes (Franklin *et al.*, 2008; Riis *et al.*, 2008), changing community assemblage and dynamics.

Riverine macrophytes are highly phenotypically plastic, and many possess specialist morphological adaptations suited to particular flow conditions (Miler *et al.*, 2012). Adaptations often correspond with mechanical forces induced by water velocity, such as parallel/lateral drag and lift (Koehl, 1982a), with responses typically involving reductions in surface area and streamlining of form to minimise physical resistance (Albayrak *et al.*, 2012). Consequently, prior research focus has been on biomechanical reconfiguration of leaf and stem form (e.g. Sand-Jensen, 2003; O'Hare *et al.*, 2007a; Albayrak *et al.*, 2012). Reconfiguration acts to limit chances of damage or uprooting (Koehl, 1982b). although exact responses vary between taxa (Puijalon & Bornette, 2004). Puijalon *et al.* (2005) recognise that morphological variability drives reconfiguration, therefore minimising hydrodynamic resistance, by creating sturdier/more flexible forms or avoiding stress by reducing size. Furthermore, Puijalon *et al.* (2008) suggest links between morphological variability and plant performance (in terms of fitness, survival and reproductive capability) and indicate these are commonly induced by environmental stimuli.

Studies have examined variations in plant biomass due to different water velocities (e.g. Chambers *et al.*, 1991; Schutten & Davy, 2000; Sand-Jensen, 2008), and investigated

feedback mechanisms of changing plant biomass and plant form on water conveyance and turbulence (Dodds & Biggs, 2002; Stephan & Gutknecht, 2002; Green, 2005, 2006). However, few have investigated the phenotypic plasticity of multiple morphological plant traits (e.g. leaf form, branching structure, root development) in changing velocities (e.g. Puijalon & Bornette, 2004; Puijalon *et al.*, 2005). Furthermore, very few studies have attempted to determine whether aquatic plants have environmental stress tolerance thresholds to water velocities and drought conditions by examining morphological traits and using experimental gradients (Puijalon *et al.*, 2005), and in particular by incorporating physiological assessments using keystone taxa. This latter point is particularly important for riverine conservation and management strategies, which often rely on research data that may be inadequate to make appropriate decisions; as Franklin *et al.* (2008) highlight, there is still a significant need to better understand the impacts of flow on key components of macrophyte communities in order to aid river management.

The submerged perennial chalk stream macrophyte *Ranunculus penicillatus* ssp. *pseudofluitans* var. *pseudofluitans* (herein *R. pseudofluitans*) is a dominant keystone species (O'Hare *et al.*, 2007b; O'Hare *et al.*, 2010; Wood *et al.*, 2012) due to its fundamental roles in influencing sediment dynamics (Gurnell *et al.*, 2006), creating flow and habitat heterogeneity (Green, 2005), driving ecosystem productivity by supporting large numbers of macroinvertebrates (Wright *et al.*, 2002) and raising river depths during low-flows in summer (Hearne & Armitage, 1993). However, whilst studies (e.g. Garbey *et al.*, 2004; Mony *et al.*, 2007) have used related taxa to examine morphological and physiological plastic trait responses to changing environmental conditions, the potential benefits for the use of *R. pseudofluitans* as an indicator of environmental change, have not yet been fully realised.

The aim of this chapter is to determine whether growth of the keystone chalk stream macrophyte *R. pseudofluitans* is constrained by mechanical stress from a velocity (flow)

gradient and reduction in water levels. The following hypotheses were tested: 1) optimal development of plant traits are constrained to moderate water velocities, 2) distinct differences in macrophyte form occur when growing in optimal or sub-optimal conditions, 3) drought and reduced flow conditions are significantly detrimental to macrophyte development. The testing of these hypotheses were achieved by using an expanded version of the experimental mesocosm setup (Chapter 3) and by incorporating a mixed modelling approach to examine trends in plant growth relative to changing water flow.

4.2. METHODOLOGY

4.2.1. Study site and stream mesocosms

The experiment was undertaken over a 28-day period in August and September 2012 using a series of outdoor artificial stream mesocosms, as described in Chapter 3.

Mesocosms were the same once-through design as in Chapter 3, and were arranged in three blocks, with four mesocosms per block (12 mesocosms total; **Figure 4.1.**). Mesocosm realism and replicability was expected to be high, and block and channel position had limited effect on plant growth (see Chapter 3). Inflowing water physicochemistry was consistent throughout the study period and similar among the mesocosms (see 4.3.1.).

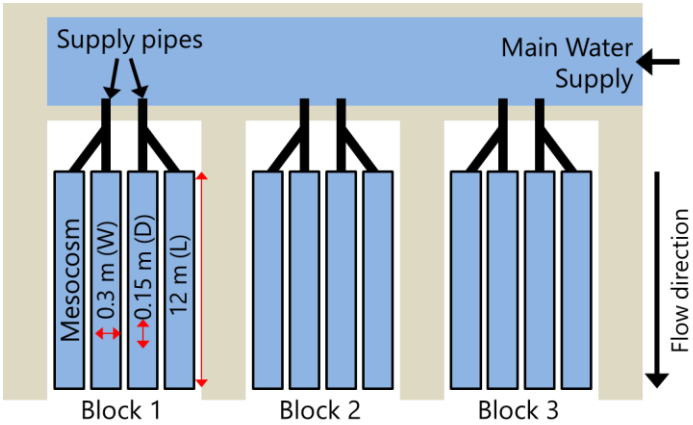

Figure 4.1.

Schematic diagram of the stream mesocosm blocks.

4.2.2. Experimental design

R. pseudofluitans was chosen as a model taxon due to its role as a keystone chalk stream species (O'Hare *et al.*, 2010), for its potential plasticity in trait responses to changing conditions (Garbey *et al.*, 2004) and for its ease of cultivation and sampling (Chapter 3).

4.2.2.1. Experimental treatments

On day 1, immediately after planting, specimens were subjected to a gradient of varying velocities and states of dewatering. Twelve treatments were selected (3 dewatering states, 9 velocity treatments; **Table 4.1.**), and randomly assigned to the 12 mesocosms. Flow treatments were achieved by altering the volume of water entering each channel via supply-pipe valves (**Figure 4.1.**). Water depths were maintained at full channel height (12 cm) for all treatments except reduced water treatments (**Table 4.1.**). Water velocities within each channel remained consistent during the experimental period (**Figure 4.3.** & **Table 4.4.**).

Table 4.1. **Flow gradient treatment velocities.** For recorded treatment velocities, see Figure 4.3.

Treatment label	Treatment velocity	Notes
a)	0 m s^{-1}	No Water, substrate wet.
b)	0 m s^{-1}	Dewatering. Limited water depth (~5-6 cm), limited flow.
c)	0 m s^{-1}	Standing water. Limited flow.
d)	0.025 m s^{-1}	Full channel water depth (~12 cm).
e)	0.075 m s^{-1}	
f)	0.1 m s^{-1}	
g)	0.15 m s^{-1}	
h)	0.25 m s^{-1}	
i)	0.35 m s^{-1}	
j)	0.45 m s^{-1}	
k)	0.55 m s^{-1}	
l)	0.6 m s^{-1}	

4.2.3. Sampling process

4.2.3.1. Initial specimen harvesting and planting

As in Chapter 3, plant specimens were grown from fragments to simulate the important juvenile establishment and development phase after vegetative propagation of this species by allofragmentation (Riis *et al.*, 2009). Five fragments of *R. pseudofluitans* were grown in each mesocosm. Due to mesocosm space constraints, measurements from each of the five specimens per channel were treated as replicates, with statistical corrections accounting for potential nested replication issues (see 4.2.4.).

Fragments were harvested using the procedure in Chapter 3 (3.2.5.1). On day 1 of the experiment, 60 genetically similar clonal specimens were harvested from a local stream and planted 5 per mesocosm. Initial fragment lengths were similar (315 ± 4 mm), as was total biomass (10.63 ± 0.27 g f_w).

4.2.3.2. Water physicochemistry

Mesocosm water physicochemistry was recorded and analysed weekly in each mesocosm from day 1 of the experiment (see Chapter 3 for method details). Physicochemical variables include: Velocity (m s^{-1}), water temperature (°C), pH, dissolved oxygen (%), conductivity (µS cm^{-1}), nitrate (mg NO_3- l^{-1}), soluble reactive phosphorus (mg PO_4- l^{-1}).

In addition, discharge, turbulence (Reynolds numbers) and water residency times were measured and included in initial exploratory analyses (see 4.2.4.), but subsequently excluded due to a) strong collinearity with velocity, and b) weaker relationships with response variables. In addition, suspended sediment was recorded, but excluded due to non-detectable concentrations throughout (see Chapter 3).

4.2.3.3. Plant characteristic traits

Morphometric traits (Table 4.2., Figure 4.2.) - Macrophyte trait measurements were recorded weekly, as per the method in Chapter 3 (3.2.5.3.). In addition to those in Chapter 3, the following morphological traits were also included in this study: Number of leaves, number of branches, and length of longest tertiary branch. Numbers of damaged plants, and washouts were also considered, but not measured during the experiment.

Table 4.2. **List of characteristic traits selected for sampling.** Updated from Chapter 3 – additions are highlighted in bold.

Identifier	Abbreviation	Plant characteristic trait	Measure of
i	L	Length of main stem	See 3.2.5.4. in Chapter 3.
ii	LL	Length of longest leaf (on main stem)	
iii	**NL**	**Number of leaves**	Reflects productivity and photosynthetic capability.
iv	PI	Position of longest internode (on main stem)	See 3.2.5.4. in Chapter 3.
v	LI	Length of longest internode (on main stem)	
vi	SB	Length of longest secondary branch	
vii	**TB**	**Length of longest tertiary branch**	Lateral development – short secondary branches or slow growth may indicate unfavourable conditions.
viii	**NB**	**Number of branches**	Lateral development and overall plant form. Also reflects biomass gain.
ix	AB	Angle of branches	See 3.2.5.4. in Chapter 3.
x	PR	Position of first adventitious roots	
I	**RGR**	**Relative growth rate**	Indication of relative accumulation of biomass to start mass.
II	R:S	Root:shoot allometric coefficient	See 3.2.5.4. in Chapter 3.
III	**SD**	**Stem densities**	Indication of stem tissue density.
IV	**LD**	**Leaf densities**	Indication of leaf tissue density.
V	**Ca**	**Chlorophyll a**	Chlorophylls reflect photosynthetic capacity of plant tissues. Carotenoids are protective plant pigments that may indicate senescence in high concentrations.
VI	**Cb**	**Chlorophyll b**	
VII	**CAR**	**Carotenoids**	

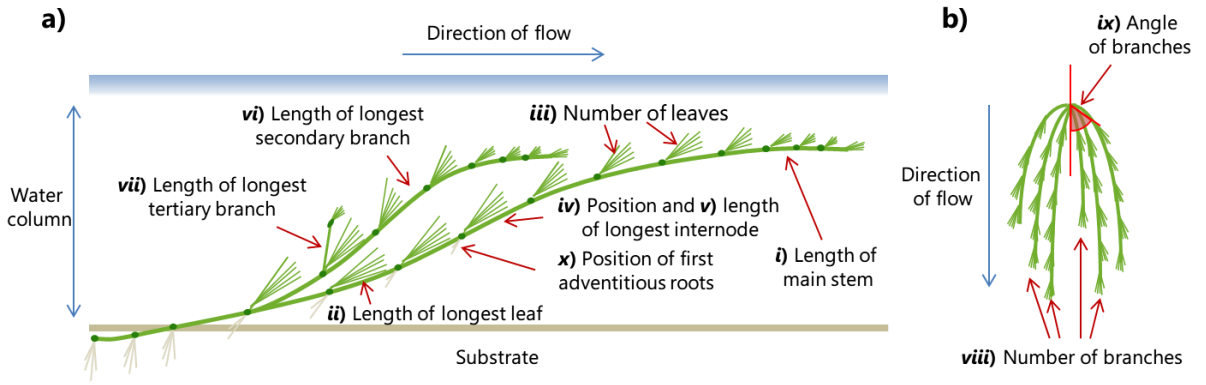

Figure 4.2. **Diagrammatic representation of *R. pseudofluitans* showing measured morphological traits.** a) cross section of plant length, b) aerial view of plant growth. Updated from Figure 3.2.

Trait measurements were then used to calculate weekly rates of change/growth, using a modified version of the equation stated by Hunt (1978) for biomass growth, to determine the relative rate of extension (*RRE*) per week for each trait:

$$RRE\ (\hat{r}_2) = \frac{(M_2) - (M_1)}{t_2 - t_1} \qquad 4.1.$$

where $RRE\ (\hat{r}_2)$ is the relative rate of extension (in mm mm^{-1} week^{-1} (continuous variables) or increase(+)/decrease(-) week^{-1} (count variables)), (M_1) and (M_2) are the morphological trait measurements at times t_1 and t_2. Unlike the original equation (Eq. 4.2.), data for morphological traits were better represented without a $\log_e$ transformation (Eq. 4.1.).

Biomass (Table 4.2.) **–** As in Chapter 3, final fresh and dry weights were recorded for plant components (leaves, stems, roots) and total weights. End root:shoot allometric coefficients were also calculated to determine plant resource allocation to above and below ground biomass. Weekly relative growth rates (*RGR*) for the study period were then calculated for total fresh weights using the classical growth equation (Eq. 4.2.) in Hunt (1978):

$$RGR\ (\hat{r}_2) = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \qquad 4.2.$$

where $RGR\ (\hat{r}_2)$ is the relative growth rate (in g f_W g^{-1} day^{-1}), ln(W_1) and ln(W_2) are the $\log_e$ transformed fresh weights (f_W) at times t_1 and t_2.

In RGR calculations, Hoffmann & Poorter (2002) suggest use of an estimator involving sample means of $\log_e$ transformed data. However, this was not possible due to experimental constraints meaning measurements were only available from individuals (rather than samples from a population), so the equation by Hunt (1978) was used, with $\log_e$ transformations to minimise bias as much as possible.

Biomechanical tissue properties (Table 4.2.) - Densities of stem and leaf tissues were recorded as content of dry matter per fresh matter (d_W/f_W) for each organ respectively (Puijalon *et al.*, 2008). More dense tissues are thought to contain higher proportions of material in cell walls (Garnier & Laurent, 1994), indicating increased strength and resilience to mechanical damage, but lower flexibility (Puijalon *et al.*, 2008).

Determination of leaf pigment concentrations (Table 4.2.) – To assess photosynthetic capacity, leaf pigment extraction was conducted using the method by Lichtenthaler & Buschmann (2005a) on fresh leaf samples in 100% acetone. Additional samples were collected and dried to determine dry weight. Spectral readings were taken immediately after extraction at 662 nm (Chlorophyll *a*), 645 nm (Chlorophyll *b*), 470 nm (Carotenoids) and at 750 nm and 520 nm (for turbidity) using a Jenway 6305 UV-VIS spectrophotometer (Bibby Scientific, Stone, U.K.). All absorbance readings were between the specified 0.3 – 0.85 range, and measurements at 750 nm were negligible and at 520 nm were all <10% of the readings at 662 nm (Lichtenthaler & Buschmann, 2005b). Pigment concentrations were then calculated according to Lichtenthaler (1987), using the following equations:

$$Chl\ a\ (\mu g\ mL^{-1}) = 11.24\ x\ A_{662} - 2.04\ x\ A_{645} \qquad 4.3.$$

$$Chl\ b\ (\mu g\ mL^{-1}) = 20.13\ x\ A_{645} - 4.19\ x\ A_{662} \qquad 4.4.$$

$$Carotenoids\ (\mu g\ mL^{-1}) = \frac{1000\ x\ A_{470} - 1.9\ x\ A_{662} - 63.14\ x\ A_{645}}{214} \qquad 4.5.$$

where A_{662}, A_{645} and A_{470} are sample absorbance at 662, 645 and 470 nm respectively. Final concentrations were then converted and expressed as mg g^{-1} d_W of Chlorophyll a, Chlorophyll b and Carotenoids.

4.2.4. Data analysis

Preceding analysis, a range of data exploration techniques were employed (Zuur *et al.,* 2009a) to determine distribution of the data variables. Outliers were examined using boxplots and Cleveland dotplots; explanatory variable collinearity was assessed using pairplots and variance inflation factors (VIFs); response variable distributions were inspected with histograms and QQ-plots; and zero inflation was examined with frequency plots. The following actions were taken in response to data exploration: 1) Major outliers removed; 2) collinear variables removed (VIFs > 3 (Zuur *et al.*, 2009b) - dissolved oxygen, discharge, turbulence, water-residency); 3) appropriate distributions were selected; 4) zero-inflation was not observed. Explanatory variables included in models were therefore: velocity, temperature, pH, dissolved oxygen, conductivity, nitrate and phosphate.

Between-mesocosm water chemistry was analysed using repeated measures analysis of variance (RM ANOVA) in order to assess variation and therefore levels of environmental control among the stream mesocosms. Parameters which did not differ significantly ($p > 0.05$) between mesocosms were assumed to have limited variation during the study period and were therefore unlikely to affect treatment influence on plant trait responses.

Associations between plant growth characteristics and environmental variables were modelled using generalised additive mixed models (GAMMs) in R (R Core Team, 2013). GAMMs were run with the package *mgcv* (Wood, 2004). Additive modelling was used because non-linear patterns in residuals were detected during data exploration (Zuur *et al.*,

2007) for all growth characteristics. GAMMs are particularly useful for modelling non-linear relationships (Austin, 2002), and allow the incorporation of variance structures and mixed-effects to account for nested experiments (Zuur *et al.*, 2009b), which traditional statistical methods (e.g. ANOVA, basic regression) fail to account for.

All plant morphological and physiological traits were modelled individually, as traits were thought to exhibit different responses to environmental stimuli due to variations in form and function. Only final sample morphological data were used, to correspond with physiology data and prevent the need for temporal autocorrelation structures in models. Each model underwent a protocol to determine fixed structures, random structures, and optimal variance structures (**Table B.1.**, Appendix B.; after checking heteroscedasticity by plotting standardised residuals against fitted values). Data transformations were avoided in order to maintain as much original information as possible (Zuur *et al.*, 2009a).

In all models, "channel" was defined as a random effect to account for within-channel repeat (nested) measurements. Interactions between covariates were unlikely, as indicated by multipanel scatterplots, and therefore not included. Best-fit models were selected with Akaike's Information Criterion *second-order* (AIC_C) using a top down stepwise selection approach, where model parameters were dropped depending on significance, and AIC_C was assessed until the best (lowest AIC_C) model with all significant parameters was determined (Burnham & Anderson, 2002; Zuur *et al.*, 2009b). AIC_C was used rather than AIC as it includes a small-sample bias correction (Burnham & Anderson, 2002). In model parameter selection, minimum significance $p \leq 0.05$, although *p*-values close to the 95% significance level should be treated with caution (Zuur *et al.*, 2009b). After selecting the best-fit model for each trait, model validation (plotting QQ-plots, histograms, fitted vs. residuals, fitted vs. response) was performed to determine if each model still adhered to model assumptions (Zuur *et al.*, 2009b).

4.3. RESULTS

4.3.1. Water physicochemistry

Velocity treatments were significantly different between mesocosms (**Table 4.3.**, **Figure 4.3.**), but did not affect physicochemical conditions, which were temporally similar and comparable between-mesocosms throughout the study period, with the exception of outliers skewing results for temperature and conductivity.

Table 4.3. **Mean, minimum and maximum physicochemistry and results of Repeated Measures Analysis of Variance tests.** Performed between all stream mesocosms, unless stated.

				RM ANOVA Test Results		
	Mean	Min	Max	F	d.f.	*p*
Velocity (m s^{-1})	0.218	0	0.63	55.000†	11	<0.001**
Temperature (°C)	10.7	10.4	12.2	2.759	11	0.008*
pH	7.83	7.68	8.03	1.161	11	0.341
Dissolved oxygen (%)	99.32	96	103.8	1.845	11	0.075
$Conductivity (µS cm^{-1})	875.1	571	932	10.056†	11	0.436
Nitrate (mg l^{-1})	7.00	6.94	7.04	1.394	11	0.210
SRP (mg l^{-1})	0.02	0.02	0.02	-	-	-

** p <0.001; * p <0.01

† RM ANOVA on Ranks performed. In this case, F value represents Chi-square value (H). $ ANOVA result excludes 'No water' mesocosm – samples in this treatment were significantly different due to difficulty in measuring conductivity. See also Figure 4.4.

Each treatment category maintained velocities close to the treatment mean throughout the study (**Figure 4.3.a.**), and provided a range of flow conditions from stagnant waters to swift, flushing flows. Water temperatures were low but consistent across all mesocosms (mean 10.7 ± 0.06 °C), although slightly elevated temperatures in some channels, with higher residency times toward the study end, caused significant differences between mesocosms in the ANOVA (**Table 4.3.**). Whilst this also caused minor collinearity with velocity categories, it was below the VIF threshold for models (≤ 3), and was therefore still included in the models.

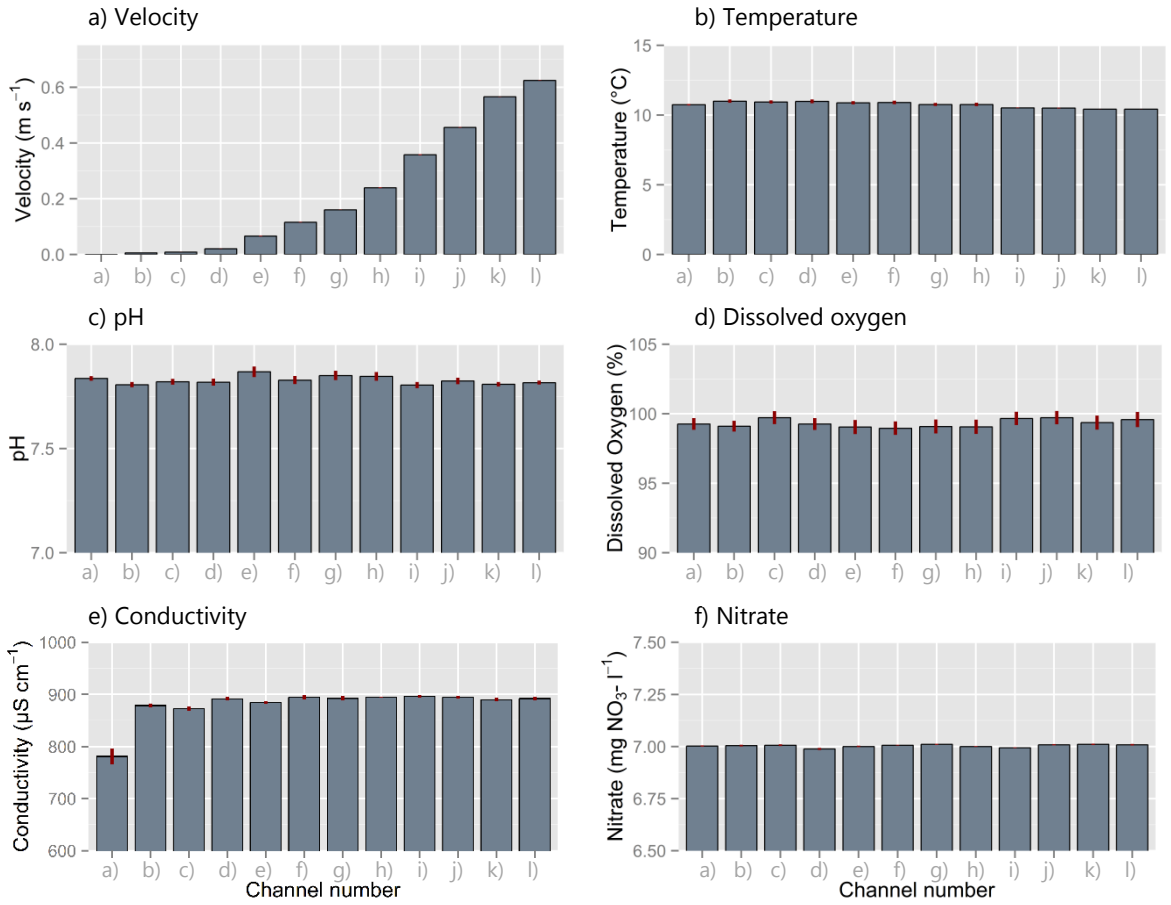

Figure 4.3. **Mesocosm physicochemistry compared between the 12 stream mesocosms.**

Parameters shown include a) Velocity, b) temperature, c) pH, d) dissolved oxygen, e) conductivity, and f) nitrate. In all charts, error bars represent standard error (n = 25) – non-visible error bars signify limited temporal variation. Phosphate not displayed (see Table 4.3.). Channel numbers represent treatment types: a) no water, b) dewatering, c) standing water, d) 0.25 m s^{-1}, e) 0.75 m s^{-1}, f) 0.1 m s^{-1}, g) 0.15 m s^{-1}, h) 0.25 m s^{-1}, i) 0.35 m s^{-1}, j) 0.45 m s^{-1}, k) 0.55 m s^{-1}, and l) 0.6 m s^{-1}.

Conductivity was consistently high (mean 875 ± 7 µS cm^{-1}) apart from a single low erroneous measurement in mesocosm e); upon removal of the outlier, conditions parallel the other mesocosms. Mesocosm a) had considerably lower conductivity readings (mean 781 ± 34) as the probe could not be fully submersed in water, and were excluded from the ANOVA on this basis. Inflowing water was continually alkaline (pH range 7.68 – 8.03), highly oxygenated (DO range 96 – 103.8%) and relatively nutrient rich (nitrate range 6.94 – 7.04 mg NO_3- l^{-1}; SRP 0.02 mg PO_4- l^{-1} in all channels).

4.3.2. Plant morphometry and physiology

Plant morphological and physiological characteristics were significantly influenced by flow velocities, and overall plant form was markedly altered as a consequence (**Figures 4.4., 4.5. & 4.6.** and **Tables 4.4. & 4.5.**).

Morphometric trait relative rates of extension - Many morphological trait RREs exhibited similar temporal trends between all channels, although PI and AB did not show any appreciable trends (**Figure 4.4.**). For all traits, specimens in the 'no water' channel showed limited, if any, extension over the study period; observable decreases in extension in some traits (e.g. leaf/branch lengths) over time suggest plant senescence.

Plant length in all channels increased steadily each week (L 53 ± 3 mm mm^{-1} $week^{-1}$), although rates in slower flowing channels (b – e) in the last week were considerably higher (184 ± 6 mm mm^{-1} $week^{-1}$). Plants growing in slower flows were markedly longer than those in faster velocities by the end of the experiment.

Leaf and internode lengths were concurrent throughout (LL 10 ± 1 mm mm^{-1} $week^{-1}$; LI 12 ± 1 mm mm^{-1} $week^{-1}$), with rates in medium/fast flowing channels (h – l) declining from week 2 to 3 and levelling-off thereafter; slow flowing channels (b – e) had rapid initial increases, followed by fluctuating, but higher rates. Plants in slower flows therefore had longer leaves and internodes compared to plants in faster velocities.

Leaf and branch numbers also demonstrated corresponding steady increasing temporal trends (NL +2 $week^{-1}$; NB +1 $week^{-1}$), with plants in moderate flowing channels (i & j) having the most rapid leaf/branch production, followed by faster flowing channels, and slow flow channels exhibiting the slowest rates. Fastest development in moderate flows meant that these specimens were more multi-branched and leaved than specimens in high or low flows.

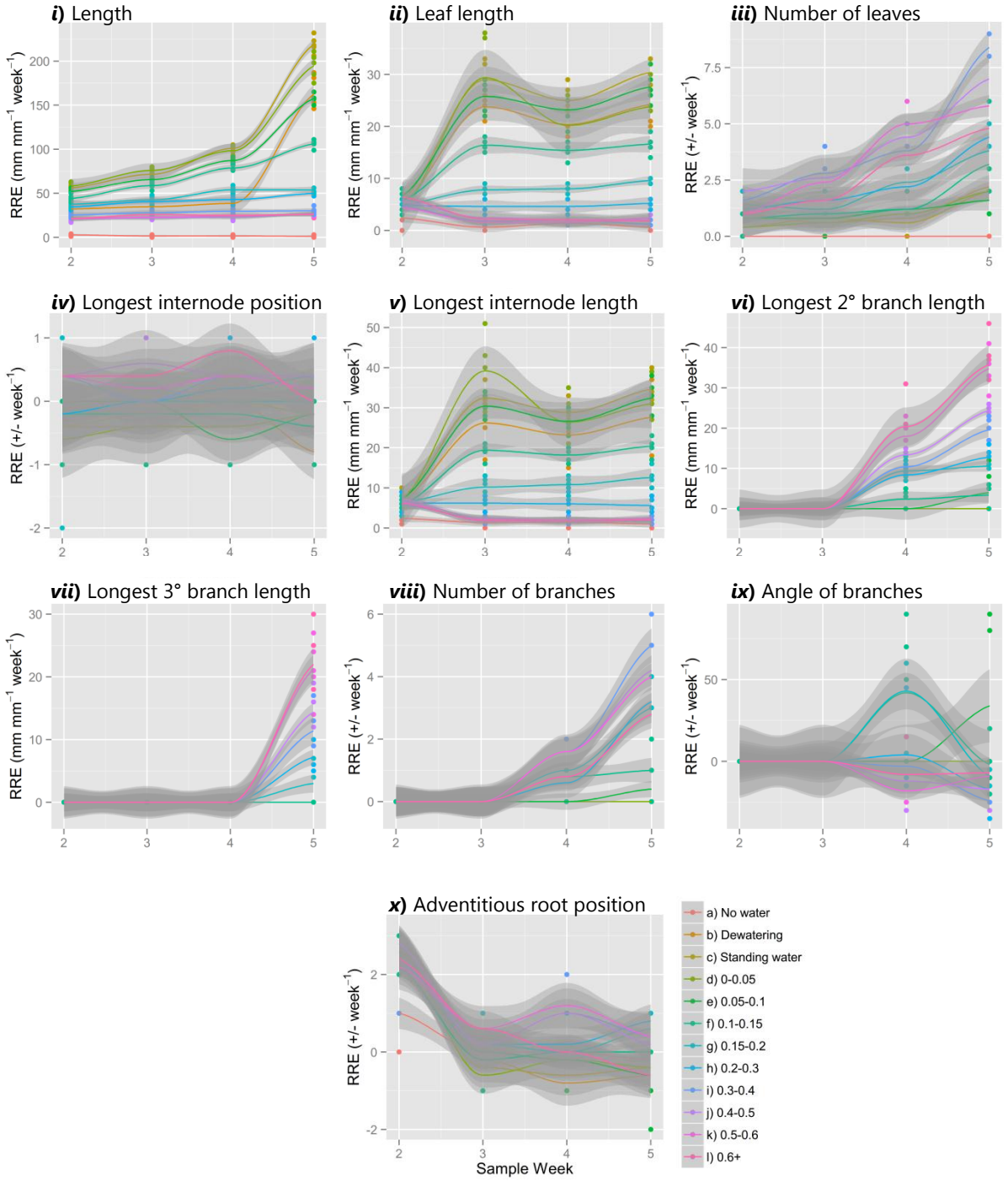

Figure 4.4. **Temporal changes in relative rates of extension (RRE) of morphological traits.** Solid lines are estimated loess smoothers and shaded areas are 95% point-wise confidence bands. *x* axis shows the sample week and *y* axis is the RRE of the following morphological traits: *i*) length, *ii*) leaf length, *iii*) number of leaves, *iv*) position of longest internode, *v*) length of longest internode, *vi*) length of longest secondary branch, *vii*) length of longest tertiary branch, *viii*) number of branches, *ix*) branching angle, and *x*) position of first adventitious roots.

No particular trends related to flow were identified for the position of longest internodes, as these appeared to fluctuate throughout, although moderate flow channels did exhibit a slight increasing trend for internodal positions away from plant apices.

Lengths of longest secondary/tertiary branches also displayed matching trends, though tertiary branches were typically 1 week behind in showing responses. Clear patterns of increasing growth rates alongside increasing flows are observable (secondary, fast (l) 28 ± 3 mm mm^{-1} $week^{-1}$; moderate (i) 15 ± 2 mm mm^{-1} $week^{-1}$; slow (e) 2 ± 1 mm mm^{-1} $week^{-1}$). Typically, plants in faster flows had longer lateral branches.

Branching angles and positions of first adventitious roots show little in the way of temporal trends, although PR do decrease slightly in positioning throughout the study.

Best-fit model selection - In all models, velocity was the only covariate observed to have a significant smoothing parameter. Therefore, the following additive mixed model was fitted to data for each morphological trait:

$$T_{is} = \alpha + f(Velocity_{is}) + a_i + \varepsilon_{is} \qquad 4.6.$$

$$\varepsilon_{is} \sim N(0, \sigma_i^2) \qquad 4.6.a.$$

where T_{is} is the morphological/physiological trait for observation i in mesocosm s, $Velocity_{is}$ is the corresponding mesocosm velocity, $f(\ )$ is the smoother, α is the intercept, α_i is the random component, normally distributed with expectation 0 and variance σ_a^2, and ε_{is} represents residuals (4.6.a.), normally distributed with mean 0 and a variance defined by the associated structure in **Table B.1.** (Appendix B.). Some models had additional explanatory covariates (E) incorporated as parametric terms, which were added into Eq. 4.6. after the smoothing term in the form ...+ E_{is}... (see **Table 4.5.** for additional model components). These additional parametric terms influenced trait responses, but smoothing terms were most important.

Morphometric trait model outcomes **-** Best-fit model predictions for individual morphological traits loosely agreed with the trends of week-by-week RRE observations (**Table 4.4.**; **Figure 4.5.**). Smoothing terms for all models were highly non-linear, with estimated degrees of freedom (*edf*) well above1 (where 1 represents a linear smoother; Zuur *et al.*, 2009b). In all models (except AB and PR), velocity was a highly significant smoothing variable, considerably dictating morphological trait growth responses. Additionally, LL and LI were somewhat influenced by conductivity, and NL by pH, although this influence is only a minor modification to the smoothing function, rather than any significant effect.

Table 4.4. **Morphological vs. environmental variable trait best-fit model summaries.** Using generalised additive mixed models. Model statistics: $R^2_{(adj.)}$ shows the adjusted R^2 value, *F* is the *F*-statistic, *edf* is the estimated degrees of freedom of the smoothing terms, and *p* is the *p*-value of the smoothing terms. In best-fit model components, *ƒ*( ) represents smoothing terms. "-" indicates no significant parameters were present in any model. Additional explanatory terms included in best-fit model components are significant ($p < 0.05$).

Response variable (morphological trait)	Best-fit model components	Model statistics			
		$R^2_{(adj.)}$	*F*	*edf*	*p*
L	*ƒ*(Vel)	0.948	65.35	3.50	<0.001
LL	*ƒ*(Vel) + Cond	0.958	198.5	5.25	<0.001
NL	*ƒ*(Vel) + pH	0.858	67.56	4.00	<0.001
PI	*ƒ*(Vel)	0.321	9.90	2.21	<0.001
LI	*ƒ*(Vel) + Cond	0.951	195.2	5.25	<0.001
SB	*ƒ*(Vel)	0.843	122.6	2.86	<0.001
TB	*ƒ*(Vel)	0.881	135.6	3.99	<0.001
NB	*ƒ*(Vel)	0.845	72.42	4.19	<0.001
AB	*none*	-	-	-	-
PR	*none*	-	-	-	-

The smoothing curves of fitted values (**Figure 4.5.**) produced for L, LL and LI indicate that plants in lower velocities have more rapid extension of these traits, which quickly reduces for plants at velocities of ~ 0.3+ m s^{-1}. This agrees with separation of trends examined week-by-week (**Figure 4.4.**), and supports the notion that plants growing in slow flows had considerably elongated features compared with those in moderate-fast flows.

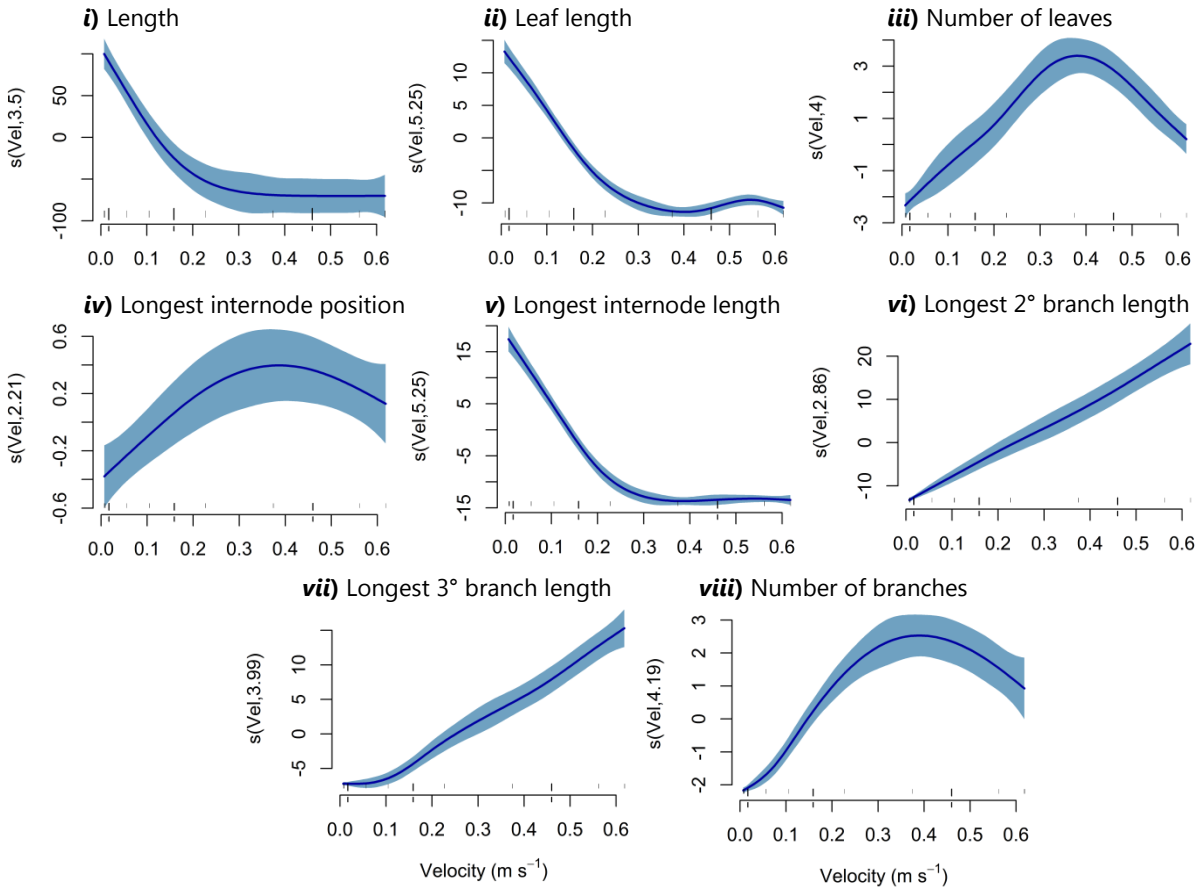

Figure 4.5. **Estimated velocity smoothers for best-fit generalised additive mixed models of morphological traits.** Solid lines are estimated smoothers and shaded areas are 95% point-wise confidence bands. *x* axis shows water velocity in m s^{-1} and *y* axis is smoother contribution to fitted values. Each smoother represents a separate model for each morphological trait: *i*) length, *ii*) leaf length, *iii*) number of leaves, *iv*) position of longest internode, *v*) length of longest internode, *vi*) length of longest secondary branch, *vii*) length of longest tertiary branch, *viii*) number of branches, and *ix*) branching angle.

NL, NB, and to some extent PI, had comparable relationships with velocity. Smoothers signify a sharp rise in leaf and branch production as velocity increases, peaking at around 0.4 m s^{-1}, and a subsequent decline as velocities increase higher. At a similar peak, PI positions are further from the apex than in lower or higher velocities, though larger confidence bands suggest greater model uncertainty. As a result, specimens growing between 0.3 – 0.5 m s^{-1} had numerous leaves and branches compared to specimens in slower or faster velocities.

Lengths of SB and TB had different relationships with velocity to previously mentioned traits, with an almost linear smoother in appearance (although *edf* suggest highly non-linear smoothers; **Table 4.5.**). However, there is a subtle difference in the smoothing curves at very low velocities 0 – 0.1 m s^{-1}; tertiary branches appear to have a flat area where there is little change in rates of extension, although this difference is caused by lack of growth during the study period by plants in lower flowing channels. The results show that as flow increases, more resources are allocated to the production of lateral branches.

AB and PR did not have any models with significant explanatory parameters, agreeing with the lack of patterns seen in **Figure 4.4.**

Physiologic trait model outcomes - The best-fit model outcomes for biomass, biomechanical tissue properties and leaf chlorophyll are displayed in **Table 4.5.** and **Figure 4.6.** All smoothing terms were again non-linear, and all models were fitted only with velocity as a highly significant smoothing variable, which had a significant control on plant functioning. However, most models were also partially influenced by water temperature, except R:S and SD which were influenced by nitrate and conductivity/pH respectively.

Table 4.5. **Physiological trait vs. environmental variable best-fit model summaries.** Using generalised additive mixed models. Model statistics: $R^2_{(adj.)}$ shows the adjusted R^2 value, *F* is the *F*-statistic, *edf* is the estimated degrees of freedom of the smoothing terms, and *p* is the *p*-value of the smoothing terms. In best-fit model components, *ƒ*( ) represents smoothing terms. Additional explanatory terms included in best-fit model components are significant ($p < 0.05$).

Response variable (physiological trait)	Best-fit model components	Model statistics			
		R^2 (adj.)	*F*	*edf*	*p*
RGR	*ƒ* (Vel) + Nit	0.762	21.72	2.81	<0.001
R:S	*ƒ*(Vel) + Temp	0.953	110.3	4.61	<0.001
SD	*ƒ*(Vel) + Cond + pH	0.394	7.05	1.14	0.008
LD	*ƒ*(Vel) + Temp	0.715	3.73	1.23	0.030
Ca	*ƒ*(Vel) + Temp	0.839	63.11	3.75	<0.001
Cb	*ƒ*(Vel) + Temp	0.9	51.92	5.08	<0.001
CAR	*ƒ*(Vel) + Temp	0.544	3.88	2.08	0.025

RGR closely corresponds with relationships seen between velocity and the production of leaves and branches. Rates of biomass gain peak at ~ 0.4 m s^{-1} and reduce either as velocities reduce or increase. Plants in moderate velocities were therefore typically larger, bushier specimens, albeit shorter.

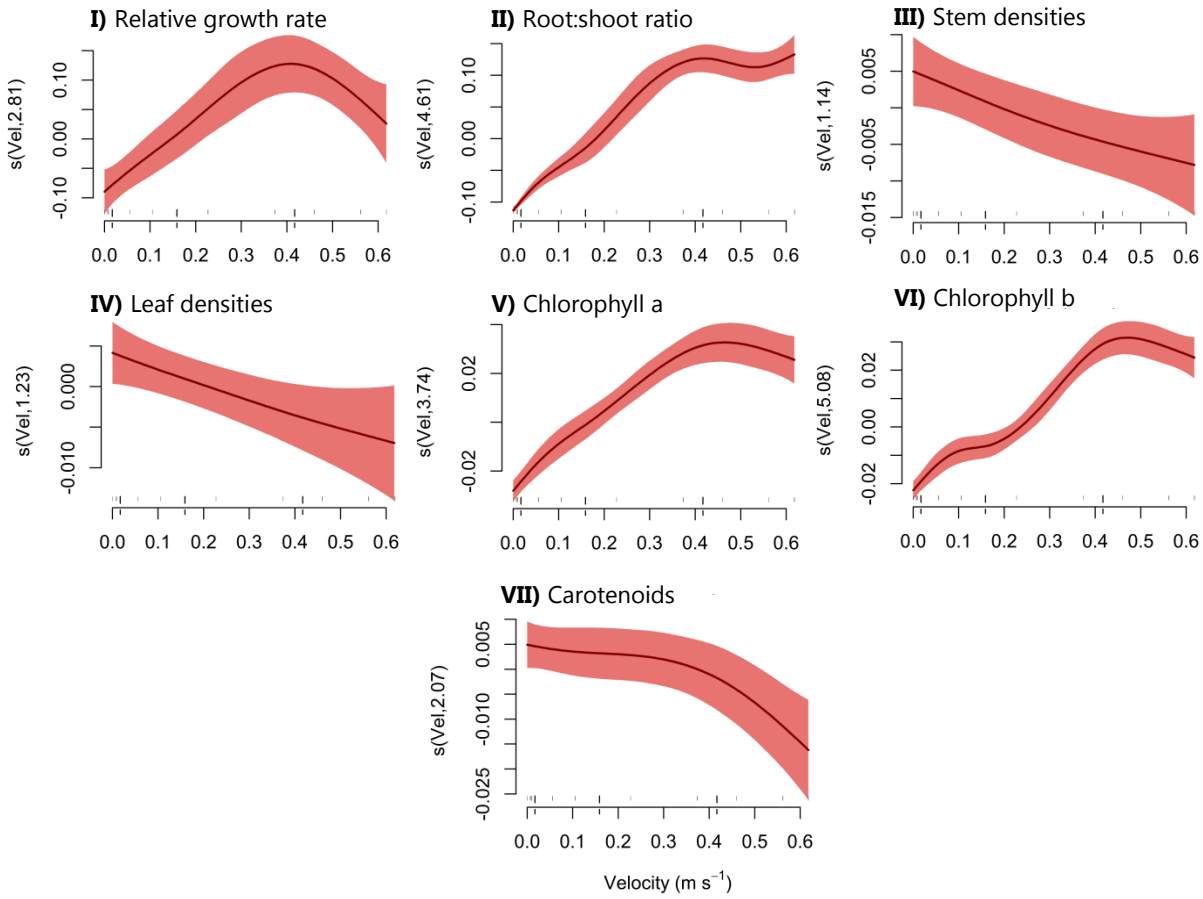

Figure 4.6. **Estimated velocity smoothers for best-fit generalised additive mixed models of physiological traits.** Solid lines are estimated smoothers and shaded areas are 95% point-wise confidence bands. *x* axis shows water velocity in m s^{-1} and *y* axis is smoother contribution to fitted values. Each smoother represents a separate model for each physiological trait: I) root:shoot ratio, II) relative growth rate (RGR), III) stem densities, IV) leaf densities, V) chlorophyll a, VI) chlorophyll b, and VII) carotenoids.

R:S ratios highlight increasing root production and therefore resource partitioning to root biomass as velocities increase. The ratio has a minor peak at ~ 0.4 m s^{-1} and slight reduction until ~ 0.5 m s^{-1} which may suggest some form of trade-off threshold, preventing additional

resources being allocated to roots. However, other measured traits do not indicate what is causing this, and thus it may be related to unmeasured physiological functioning.

SD and LD have almost linear negative relationships with velocity, with decreasing tissue densities occurring as flow velocities increase, although confidence intervals suggest some uncertainty with these models.

Concentrations of Ca and Cb follow a similar increasing pattern with increasing velocities to R:S, but chlorophylls peak at ~ 0.45 m s^{-1} before slowly reducing at very high velocities, and in the case of Cb, a minor peak occurs at ~0.08 m s^{-1}, and follows a sharper incline thereafter. This indicates greater photosynthetic potential at 0.4 – 0.5 m s^{-1}, and reduced productivity in lower or higher velocities. Furthermore, CAR differs from both Ca and Cb by being present in higher concentrations at low velocities and declining gradually until ~ 4 m s^{-1} where concentrations decline even more rapidly.

4.4. DISCUSSION

Mesocosm units in this study were found to be highly replicable under experimental treatments, and were therefore ideal for testing environmentally induced responses in the aquatic macrophyte *Ranunculus pseudofluitans*. Significant differences were observed in plant trait responses according to the velocity treatments specimens were subjected to. A majority of traits were observed at their optimum at moderate flow velocities, which was reflected by substantial differences in overall plant form, compared to specimens growing in slower or faster flow velocities.

Submerged riverine macrophytes are well documented as having high phenotypic plasticity (Garbey *et al.*, 2004, 2006; Onaindia *et al.*, 2005; Puijalon *et al.*, 2005, 2008; Puijalon & Bornette, 2006; Mony *et al.*, 2007; Kohler *et al.*, 2010) and are readily able to

adapt morphological and physiological characteristics in response to environmental stimuli. Whilst this plasticity is well studied, there is need for improved understanding of changes to form and function of macrophyte taxa under a range of conditions, particularly when the studied taxon is a keystone in that community. This is especially important in the face of increasing pressures to river ecosystems, for example from climate change (Ormerod *et al.*, 2010) and over-abstraction of groundwater (Wilby *et al.*, 1998; Westwood *et al.*, 2006), and to aid management decisions, where healthy development of keystone species is typically extremely beneficial (Franklin *et al.*, 2008).

In this study, juvenile specimens of the aquatic macrophyte *Ranunculus penicillatus* subsp. *pseudofluitans* var. *pseudofluitans* were artificially planted in stream mesocosms and subjected to a range of flow constrained treatments that represented a gradient of different water velocity and drought conditions. Morphological and physiological growth characteristic traits were then modelled against a range of physicochemical parameters in an attempt to examine the development of the juvenile specimens and determine if water velocities are a significant driving factor for individual macrophyte success.

The physicochemical conditions in the extended artificial mesocosm setup were similarly consistent, as observed in the suitability study (Chapter 3). Temperature, pH, dissolved oxygen, nitrate and soluble-reactive phosphorus were all roughly comparable in conditions to the study in Chapter 3, but temperatures were elevated towards the end of this study and conductivity was unexpectedly higher in this study. The elevated temperatures were likely related to water residency times in the slower flowing channels allowing higher air temperatures to have greater influence in those channels (Armengol *et al.*, 1999). Higher conductivity readings are more difficult to interpret, but are likely related to greater influence of the chalk aquifer signature, with increased concentration of groundwater constituents such as calcium and bicarbonate, due to lower summer baseflows (Griffiths *et*

al., 2007; Howden *et al.*, 2010). Nevertheless, between-mesocosm water physicochemistry was considerably more comparable than between-mesocosm velocity treatments. Whilst treatments were significantly different from each other, the within-mesocosm variability of treatments was low.

All plant characteristics exhibited significantly different responses along the velocity gradient, with the exception of two morphological traits (branching angle and position of first adventitious roots), and in all models velocity was defined as key covariate and smoothing term, and therefore the main influential environmental factor.

R. pseudofluitans experienced slower overall length, leaf length and internode length elongation when subjected to increasing velocities. This observation corresponds with the concept that mechanical stimulation by increasing water flow rates triggers a reduction in plant size and form in order to become more streamlined, reduce contact area and reduce drag forces (Koehl, 1982b; Albayrak *et al.*, 2012). This concept is consistent with other macrophyte species that are often found occurring alongside *R. pseudofluitans*, such as *Berula erecta* (Puijalon & Bornette, 2004; Puijalon *et al.*, 2005), although unusually no variation in length according to different velocities was observed by Garbey *et al.* (2006) on a close relative (*Ranunculus peltatus*). These changes in form highlight evolved polymorphism in addition to phenotypic plasticity (Puijalon & Bornette, 2004), with the potential for many morphologically distinct specimens to be present within small reaches.

The dewatered channel experienced very different results overall. Plants had very limited growth, with most traits not developing at all, or increasing at very minimal rates. Similar reduced size features are a notable occurrence on an amphibious relative (*R. trichophyllus*; (Germ & Gaberscik, 2003). However, importantly, plants did not senesce during the study, adopting a markedly different semi-amphibious form, that was stumpy with short, stunted,

untidy leaves. This highlights a potential drought coping mechanism that, at least in the short term, may prove a useful strategy in dealing with low flow situations.

In fully submersed specimens, converse to decreases in main length-measurement traits correlating with increasing velocities, longest secondary and tertiary branches had an almost linear increase, with no sign of a plateau or tailing off. These traits are a reflection of plant lateral development (Garbey *et al.*, 2004) and such development can be explained in this study by two possible factors: 1) the specimens were only observed during early juvenile growth, meaning this relationship may change when plants reach maturity; and/or 2) the increased lengths of lateral branches represent a greater capability for plant resource uptake and allocation, and therefore better overall plant growth rates with increasing velocities.

Also in contrast to length-measurement traits were the number of leaves and branches, and position of the longest internode. These traits all saw steady positive increases in line with velocity up to a peak of around 0.3-0.4 m s^{-1}, and thereafter a steady decrease in line with further increasing velocities. This suggests *R. pseudofluitans* has an optimum for the production of new leaves and branches, and a preservation of longest internodal position at velocities of between 0.3 and 0.4 m s^{-1}. Typically, aquatic plants are thought to reduce leaf and lateral biomass allocation when faced with mechanical stress (Puijalon *et al.*, 2007), in order to streamline and minimise physical resistance, yet *R. pseudofluitans* opposes this concept in this study, when faced with moderate velocities. Tolerance to higher drag forces, induced by increasing plant surface area (Sand-Jensen, 2008), give *Ranunculus* spp. a competitive advantage under higher flows, but weaker/reduced growth in slow flows, as observed in this study, may indicate why *R. pseudofluitans* typically occurs in lower abundance when flows are low; in these situations less flow tolerant species may gain competitive advantage.

Root:shoot ratios, RGR and chlorophyll content displayed somewhat comparable responses to the number of leaves/branches and position of internodes, except that observed peaks were at ~0.4 m s^{-1} for root:shoot ratio and RGR, and ~0.4-0.5 m s^{-1} for chlorophyll content. Root:shoot ratio, however, differs slightly in that it continues to see a positive relationship with velocity after this peak, although at a slower pace.

An increase in root allocation indicates partitioning of resources to enhance root biomass for anchorage and in order to resist uprooting in higher flows, as seen in other aquatic macrophyte species (Idestam-Almquist & Kautsky, 1995; Puijalon *et al.*, 2005). As *R. pseudofluitans* is adapted to cope with higher flows, this trait is consistent with other observed form changes due to mechanical stimuli, and supports potential for evolved polymorphism in this species. However, there are multiple studies that have suggested overall macrophyte root biomass decreases in increasing flows, in line with overall form reduction (Chambers *et al.*, 1991; Madsen *et al.*, 2001), although this mainly relates to taxa that have lower tolerance for fast flows, and may indicate a need for improved dispersal for these taxa under such conditions (Puijalon *et al.*, 2005). This study shows that the reverse is true for *R. pseudofluitans*, which thrives in moderate flows, yet shows signs of improving dispersal ability in low flows by reducing root biomass.

Increases in biomass represent the most obvious sign of healthy plant development, and can be an indicative surrogate of individual traits. Relative growth rates in this study peaked, suggesting optimum biomass accumulation, at velocities of around 0.4 m s^{-1}. Whilst there is little in the literature to suggest optimum biomass development, Halcrow Ltd. (2004) discuss results from experiments that indicate optimal growth of *Ranunculus* spp. is between 0.2-0.35 m s^{-1}. This is lower than the findings presented here, although it should be noted that the experimental conditions presented by Halcrow Ltd. are unknown, therefore growth may be affected by confounding environmental or biological factors.

Stem and leaf tissue densities showed less convincing responses to the flow velocity gradient, although both were negatively correlated with increasing velocities. A reduction in stem and leaf densities is associated with enhanced tissue flexibility (Puijalon *et al.*, 2008) meaning greater streamlining can occur, reducing drag forces in higher flowing waters, although at the expense of less mechanical damage resistance (Sand-Jensen, 2003). This is a likely adaptive response for *R. pseudofluitans*, as a streamlined flow tolerant macrophyte, as breakage resistance may be a worthwhile trade-off for enhanced flexibility, until very high flows, where fragmentation and dispersal may be favoured. However, the reconfiguration experienced by a combination of flexibility (from reduced stem/leaf densities) and increased surface area (from increased leaf and branch development) under higher flow velocities (Sand-Jensen, 2003, 2008) means stands of *R. pseudofluitans* will have reduced frontal areas exposed to approaching flow and greater numbers of shoots and leaves in a shielded-submerged canopy (Sand-Jensen, 2003), thus greatly reducing overall drag-forces. This may also explain why leaf and branch production of specimens in this study is considerably higher under faster water velocities, and may indicate an adaptive response of *R. pseudofluitans* to higher flows.

Of the major plant pigments, chlorophylls are responsible for light absorption for photosynthesis, and carotenoids function as both an energy transfer mechanism and to minimise photo-oxidative damage from excess incident light (Demmig-Adams & Adams, 1996). In this study, chlorophyll a and b production was stimulated by increasing water velocity steadily up to peaks at ~0.4-0.5 m s^{-1}, indicating enhanced photosynthetic ability of leaf tissues around such velocity levels. As plants approaching flowering phase are believed to have the greatest photosynthetic capacity (Simova-Stoilova *et al.*, 2001), plants in moderate velocities may therefore be growing under optimal conditions for faster development into maturity. An alternative explanation is that under higher velocities,

plant leaves 'stack up' on top of each other causing self-shading (Puijalon *et al.*, 2005), which may stimulate chlorophyll pigment production. Carotenoid response was different, however, decreasing slowly until ~0.4 m s^{-1}, where they began to decrease more rapidly. The relationship between plant chlorophylls and carotenoids can provide additional information regarding the health of plants. Lower chlorophyll content and higher carotenoid content can be indicative of plant senescence, as carotenoids tend to persist in plant cells for longer, with chlorophylls breaking down more rapidly (Sims & Gamon, 2002). Specimens of *R. pseudofluitans* in this study showed that at lower velocities, this was occurring, suggesting plant poor growth and even senescence in slow flowing, or still waters.

There were no apparent relationships between the position of the first adventitious roots and flow velocity which strongly agrees with the findings of Garbey *et al.* (2004) for *R. peltatus*. However, whilst Garbey *et al.* suggest the likely correlative link between increasing flows and increasing root biomass and adventitious root production for anchoring purposes, the findings of this study suggest that in reality there may be no link between the two different forms of root production. Increases in root allocation, as indicated by significant changes to the root:shoot ratio, do occur under increasing flow velocities; however, this is likely unrelated to adventitious root positioning, as *R. pseudofluitans* may only need to make use of them for additional anchorage during rapid elongation. Moreover, it is possible that the main role of adventitious roots is the anchoring of new fragments when undergoing vegetative reproduction.

In addition to the influence of velocity, it was observed that temperature fluctuations had minor influence on responses of plant functioning. Water temperatures are known to act as a control for chemical reaction and respiratory rates in aquatic plants (Barko & Smart, 1986; Kirk, 1994; Carr *et al.*, 1997), so this influence may explain a small portion of the

variation for plant physiological responses, although velocity was still the overwhelming driver.

The variety of different responses observed in this study by characteristic traits of *R. pseudofluitans* enables postulation about overall plant form and function in different velocities. According to Sultan (1987), flow stress should induce morphological trait responses that enhance plant functioning. Puijalon & Bornette (2004) add that principal trait changes under increasing velocities should include increases in rooting ability (for better anchorage) and profile changes in order to reduce drag. These suggestions well-represent the changes observed by *R. pseudofluitans* in this study: both plant functioning and form change significantly depending on velocity.

On the basis of these findings, it can be proposed that, for *R. pseudofluitans,* optimal juvenile development occurs at between 0.3-0.5 m s^{-1} (**Figure 4.7.**). Below and above this, form and/or function appear to change such that growth appears sub-standard. This optimum range is also suggested by Riis & Biggs (2003) for other macrophyte taxa, who indicate this is due to balance of the conflicts between mass transfer and drag forces.

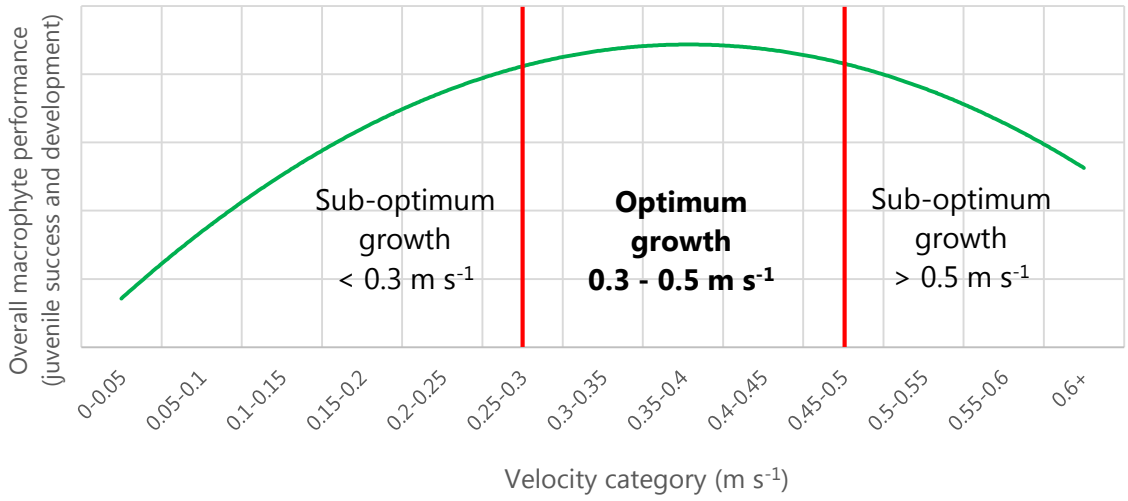

Figure 4.7. **Conceptual optimum growth curve for R. pseudofluitans in chalk rivers.**

In the case of *R. pseudofluitans*, the following changes to form and function are most apparent as velocities become closer to the 'optimum':

- Overall size reduces – plant length, leaf length and internode lengths elongate less.
- Biomass is allocated to leaves and branches – to compensate for reduced length, enhancements occur to lateral development and photosynthetic potential.
- Biomass is allocated to roots – as a requirement for improved anchoring ability.
- Tissues become more flexible – to improve streamlining in faster flowing water.
- Leaf chlorophyll content rises – providing further photosynthetic ability.

This materialises as visibly distinct differences in growth form that can be broadly categorised as 'elongated' in slower flows, or 'compact' in faster flows (**Figure 4.8.**). When

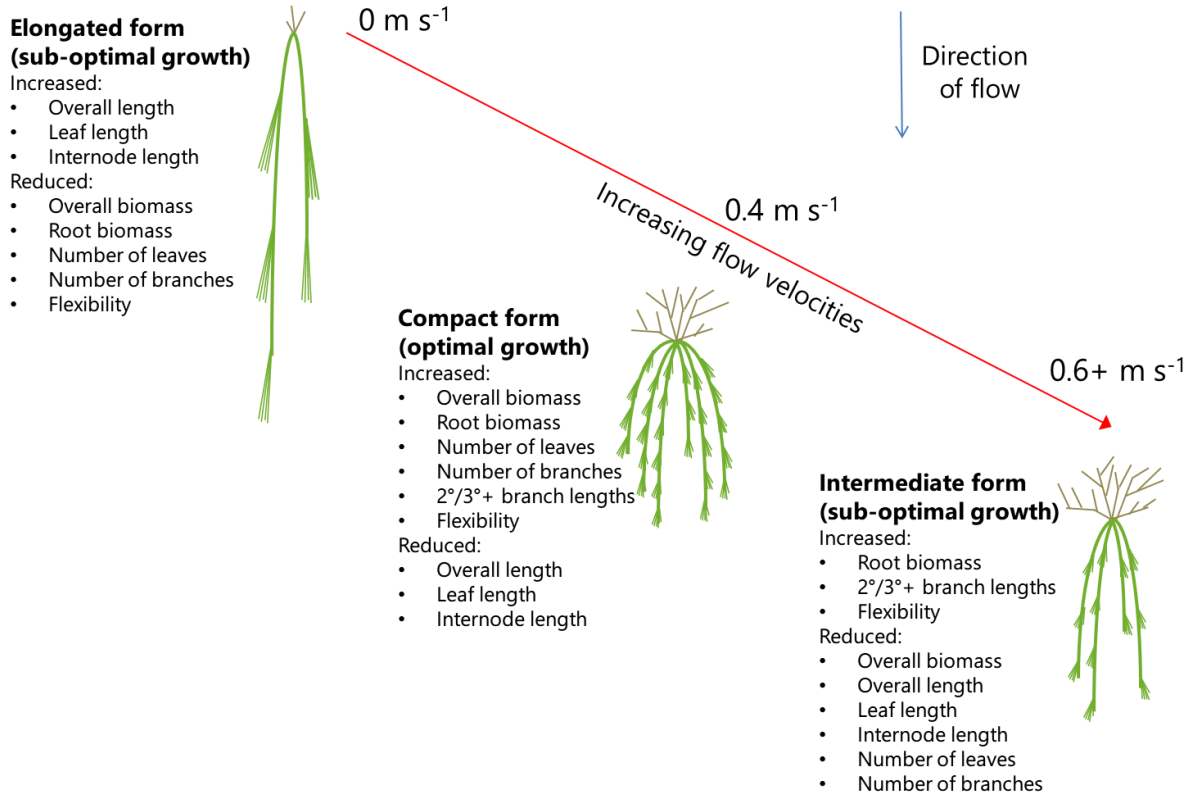

Figure 4.8. **Hypothesised changes to the growth form of juvenile *R. pseudofluitans* depending on a velocity gradient.**

flow velocities accelerate past the optimum, *R. pseudofluitans* then appears to take on an 'intermediate form', with characteristics of both 'elongated' and 'compact' specimens.

These findings may provide complications for identification, where it can often be difficult to discriminate between the two varieties of *R. pseudofluitans,* var. *pseudofluitans* and var. *vertumnus* (Rich & Jermy, 1998), as juvenile *R. pseudofluitans* in intermediate flowing channels very closely resembled the appearance of var. *vertumnus*. However, var. *vertumnus* is said to have compact, globose leaves, whereas specimens in this study had relatively untidy leaves, a feature known of var. *pseudofluitans* (Rich & Jermy, 1998).

The impact of these findings on practical management and conservation are also worth consideration. Macrophyte monitoring may benefit from the recording and assessment of plant form; as this study indicates, wide variations in form can occur depending on velocity gradients, so assessing form may act as a rapid indicator of riverine conditions. Additionally, these findings may benefit members of regulatory bodies or private river keepers, for example, who are often required to assess, monitor and modify features of chalk rivers for the healthy maintenance of riverine conditions for ecological and/or commercial purposes. It is therefore useful to have benchmarks for components of river ecosystems that are often fundamental for keeping river reaches in healthy states. *R. pseudofluitans*, as a keystone species, potential indicator species, and known biological engineer (Franklin *et al.*, 2008), is one such component that is severely lacking in targets. For river management, it is recommended that flows are maintained such that velocities are kept in the 0.3-0.5 m s^{-1} range (**Figure 4.7.**). This range should promote growth of R. pseudofluitans, which will hopefully in turn improve heterogeneous flow conditions and allow additional macrophyte species to colonise and grow healthily (Green, 2005). However, consideration should always be given to the wider community in specific river reaches, so this range may not be applicable in all situations.

A final comment must be given regarding the unaccounted for influence of ontogeny (plant life stage) and additional environmental variables. This study focused on growth of juvenile specimens of *R. pseudofluitans* from day 1 of their (artificial) allofragment colonisation up to 28 days. Whilst observed responses were noteworthy, findings and suggestions may relate only to plants in this stage of development. Further work will be required to determine if similar responses are seen in mature plants. Furthermore, riverine conditions are unique in space and time, so these responses may be considerably different where plants are impacted upon by variations in other environmental variables (e.g. changes in nutrient status, algal competition). Further work is therefore also necessary to quantify the impacts of multiple environmental stimuli on the form and functioning of the keystone macrophyte *R. pseudofluitans*.

4.5. CONCLUSION

River flow is an important controlling factor for macrophytic growth, but survey based studies are often inadequate for determining causality with changing flows, and many autecological and experimental studies fail to determine direct responses to the range of conditions macrophytes may be exposed to. Artificial stream mesoscosms are a reliable way of assessing changes in form and function in keystone macrophytes, and when arrayed as a gradient of treatments, allow for the assessment of optimal and sub-optimal growth according to water velocity preferences. In this study, velocity – as a mechanical stimulus – was found to significantly influence the early juvenile developmental growth of a keystone chalk stream macrophyte (*Ranunculus pseudofluitans*). Through highly plastic physiological and morphological traits, which involve combinations of streamlining and size reductions to enable reconfiguration in response to drag forces, and alterations in resource allocation, macrophytes experienced optimal growth under moderate flow

velocities, with lower flows and higher flows producing sub-optimal growth. However, the study also highlights that growth of *R. pseudofluitans* was not observed to reach upper or lower threshold points where plants were unable to maintain original biomass and senesce, even where drought conditions occurred and a semi-terrestrial form was adopted. This indicates that *R. pseudofluitans* is a highly adaptable macrophyte capable of persisting in sub-optimal conditions; nevertheless, additional work is required to determine whether macrophytes can continue to exist in these conditions throughout ontogeny, or when environmental conditions vary, both in frequency and magnitude.

4.6. REFERENCES

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CHAPTER V

Flow, nutrient and periphytic algae individually influence form and function of the aquatic macrophyte *Ranunculus pseudofluitans*

5.1. INTRODUCTION

In riverine ecosystems, submerged biota are exposed to the influence of multiple environmental variables (Ormerod *et al.*, 2010), with community structure and function defined by tolerance to particular combinations of variables. The growth and development of riverine macrophytes is particularly influenced by mechanical stimuli (e.g. water flow; Bornette & Puijalon, 2011), resource stimuli (e.g. nutrient limitation; Carr *et al.*, 1997) and competitive interactions (e.g. algal smothering; Wade *et al.*, 2002), which can act independently or in synergism (Franklin *et al.*, 2008; Bornette & Puijalon, 2011). Chalk river macrophytes grow in atypical conditions as they are rarely mechanically confined, due to the overall stability of the chalk flow regime (Walling *et al.*, 2006), or nutrient resource-limited, due to a large supply of carbon in the form of bicarbonate (HCO_3-) from the chalk aquifer (Spence & Maberley, 1985; Neal, 2001) and nitrogen from prevailing land use (Neal *et al.*, 2008). Phosphorus is typically present in low concentrations in chalk rivers (Mainstone *et al.*, 1999), preventing algal proliferation, but rarely limits higher plant growth (Westlake, 1981). Common growth patterns in chalk streams are therefore mainly constrained by seasonal variability involving factors such as temperature, light availability and herbivore densities (Wood *et al.*, 2012).

Chalk stream flow regimes and water quality are likely to be affected by changing climate (Milly *et al.*, 2005), increased water abstraction (Heathwaite, 2010), and greater inputs of nutrients and sediments due to anthropogenic activities (Franklin *et al.*, 2008). As a consequence, macrophyte community assemblages may be altered (Westwood *et al.*, 2006), particularly in response to lower flow velocities, therefore reduced hydrodynamic forces (O'Hare *et al.*, 2007) as 'mechanical stimuli' (Puijalon & Bornette, 2004), and higher nutrient concentrations, therefore reduced likelihood of nutrient resource-limitations (Madsen & Cedergreen, 2002) as a 'resource stimuli' (Puijalon *et al.*, 2007). However, the added impact

of enhanced competition from filamentous and benthic algae under enriched nutrient conditions (Carr & Goulder, 1990), which can be termed 'competition stimuli/stress'. Shifts in assemblages may include reductions in species diversity, as increases in nutrient levels and reductions in flow are said to favour highly competitive species (Bornette & Puijalon, 2011), predominantly those species that are epiphytic and therefore rely on water column conditions for success (Biggs & Close, 1989).

In natural riverine conditions, plants are often subjected to the combined effects of multiple environmental stimuli or stressors (Ormerod *et al.*, 2010). Rarely, however, do plants respond predictably according to the cumulative effect of each independent stimuli (Puijalon *et al.*, 2007), with more complex interactions occurring than often anticipated. This is likely because changes in particular stimuli can have both direct effects on plant characteristics, and indirect effects that may cause variation in the influence of another stimuli. Flow is considered the principal factor in determining macrophyte distribution (Haslam, 2006; Franklin *et al.*, 2008) and directly influences plant growth through hydrodynamic drag forces induced by changing water velocities (Chapter 4; Schutten & Davy, 2000; Puijalon & Bornette, 2004; O'Hare *et al.*, 2007; Sand-Jensen, 2008), but the movement of water can also affect chemical flow across plant boundary layers (Crossley *et al.*, 2002; Mommer & Visser, 2005), typically with increases favouring mass transfer. This passage of chemicals across boundary layers is also dependent on the concentration of the chemical in question (for example key macronutrients), which may in itself be controlled by flow dilution (Withers & Jarvie, 2008). Concentrations of these key nutrients (e.g. phosphorus, nitrogen) within the water column are a direct limiting factor to plant growth (Mainstone & Parr, 2002), particularly if sediment nutrients are poor and macrophytes must satisfy nutrient uptake via leaf and stem tissues (Madsen & Cedergreen, 2002). Furthermore, algal growth is recognised as an inhibitor to plant development, smothering

leaves, causing shading and reducing mass transfer uptake (Franklin *et al.*, 2008), is directly dependent on nutrient concentrations, yet constrained by river flow (Hilton *et al.*, 2006). Consequently, varied synergism between multiple influential variables can cause considerable differences in plant trait responses (such as reductions in morphological traits and restrictions to plant functioning; Puijalon *et al.*, 2007), which may in turn exhibit marked differences in community assemblage. This combined impact can often signify environmental degradation relating to climate change and/or enhanced anthropogenic influence (Ledger *et al.*, 2012).

Previous research examining multiple stressors has focused on terrestrial plant responses, principally on multiple resource limiting stimuli (e.g. Urbas & Zobel, 2000; Sack, 2004), but few aquatic studies exist, particularly examining combined resource and mechanical stimuli (although see Puijalon *et al.*, 2007; Lamberti-Raverot & Puijalon, 2012). Moreover, studies investigating combinations of resource, mechanical and competition stressors are lacking, particularly when examining morphological and physiological plant responses. The importance of the interactive effects of water flow, nutrients and algal growth are one such dynamic which is frequently studied and discussed, but rarely generates convincing evidence, despite the conception of numerous theories and hypotheses (see Hilton *et al.*, 2006). In many river systems, this relationship is commonly debated by practitioners and landowners involved with conservation and management, but there is little scientific evidence available to guide decisions on whether to focus on flow or nutrient management.

The aim of this chapter is to examine the combined effects of flow (mechanical stimuli), nutrient concentration (resource stimuli) and algal growth (competition stimuli) on the growth characteristics of juvenile specimens of the keystone chalk stream macrophyte *Ranunculus penicillatus* ssp. *pseudofluitans* var. *pseudofluitans* (herein *R. pseudofluitans*). The following hypotheses were tested to satisfy this aim: 1) the interaction between water

flow, nutrient concentrations and algal growth is more important to plant trait characteristics (morphological and physiological) than the impacts of individual factors; 2) plant growth is optimal at moderate flows, with low nutrients and limited algal growth; 3) the growth of algae is determined by the combined effect of flow and nutrient concentrations; 4) the removal of algal growth has a significant positive impact on the growth of *R. pseudofluitans*. These hypotheses will be tested using a further expanded version of the experimental mesocosm setup as used in Chapter 4, and will again employ the use of mixed modelling to examine plant growth patterns in relation to controlled treatments of water flow, nutrients and algal growth.

5.2. METHOD

5.2.1. Study site and stream mesocosms

Research was conducted in a series of outdoor artificial stream mesocosms over a 28-day period in July and August 2011.

Mesocosms were the same once through design as in Chapters 3 and 4, and were arranged in four blocks, with four mesocosms per block (16 mesocosms total; **Figure 5.1.**). Positions of blocks and channels had limited effect on plant growth (see Chapter 3). Inflowing water physicochemistry was temporally consistent and replicable among mesocosms (see 5.3.1.).

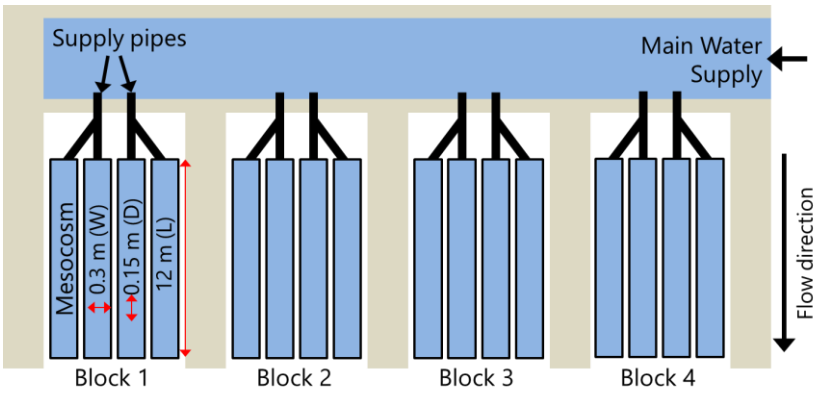


Figure 5.1.

Schematic diagram of the stream mesocosm blocks.

5.2.2. Experimental design

The chalk stream macrophyte *R. pseudofluitans* was used because of its importance as keystone species (O'Hare *et al.*, 2010), and for highly plastic trait responses and ease in cultivation (see Chapters 3 &4).

5.2.2.1. Experimental treatments

Treatments were arranged in a 2x2 factorial design with the inclusion of an additional uncontrolled effect (**Table 5.1.**). Key factorial treatments were velocity ('high' ~0.35 m s^{-1} and 'low' ~0.1 m s^{-1}) and phosphate ('high' ~0.2 mg PO_4- l^{-1} and 'low' ~0.02 mg PO_4- l^{-1}). The uncontrolled effect was algal influence, which was allowed to establish naturally in each channel. Each factorial treatment had 4 replicate mesocosms, out of which 3 were assessed in conjunction with the uncontrolled algal effect, and 1 was a comparison 'control' channel, where algal growth was removed as much as possible. Velocity treatments represent flows conducive of good (high) and sub-optimal (low) plant growth (Chapter 4) and phosphate treatments represent unimpacted 'baseline' levels (low) and impacted 'threshold' levels (high) where changes in ecology might be expected (Environment Agency, 2000; UKTAG, 2008). Algal growth, a product of the interaction between flow and enriched nutrients (Hilton *et al.*, 2006), is a characteristic sign of degraded water quality.

Treatments were assigned randomly to mesocosms, and were set up to begin on day 1, coinciding with planting and maintained throughout the study. Flow treatments were controlled by altering the volume of water entering channels with butterfly valves. In dosed nutrient treatments, phosphates (added as soluble H_3PO_4) were fed to each mesocosm water inlet supply pipe via capillary tubing attached to a peristaltic dosing pump (201-Aquadoser-SC-050, Williamson Pumps, Poynings, U.K.), pumping from a well-mixed 600L tank. Slow-flowing mesocosms were supplied at a rate of ~ 40 ml min^{-1}, and fast mesocosms

at ~ 70 ml min^{-1} to achieve the desired in-channel concentrations. A turbulent mixing zone at the top 0.5m of each channel ensured nutrient concentrations were well mixed.

Table 5.1. **Flow, nutrient and algal treatment categories.** Treatment acronyms: HN = high nutrient; LN = low nutrient; HF = high flow; LF = low flow; c = control (no algae). Shaded cells highlight where a particular treatment was applied. Numbers represent the number of replicate mesocosms per treatment category.

Treatment label	Treatment category	Nutrient treatment		Flow treatment		Algal growth	Number of replicate channels
		High (~0.2 mg l^{-1})	Low (~0.02 mg l^{-1})	High (~0.35 m s^{-1})	Low (~0.1 m s^{-1})		
a)	HN:LFc					*Control*	1
b)	HN:HFc					*Control*	1
c)	LN:LFc					*Control*	1
d)	LN:HFc					*Control*	1
e)	HN:LF						3
f)	HN:HF						3
g)	LN:LF						3
h)	LN:HF						3

Water velocities and phosphate concentrations within each channel remained consistent during the experimental period (**Figure 5.3.** & **Table 5.4.**).

5.2.3. Sampling process

5.2.3.1. Initial specimen harvesting and planting

As per Chapters 3 & 4, small plant fragments were used to simulate juvenile establishment after allofragmentation (Riis *et al.*, 2009). Five fragments of *R. pseudofluitans* were grown in each mesocosm. Measurements from each of the five specimens per channel were treated as replicate response variables, with statistical corrections accounting for potential nested replication issues (see 5.2.4.).

At the start of the experiment, 80 genetically similar clonal specimen fragments were harvested from a local stream and planted 5 per mesocosm using the method reported in Chapter 3 (3.2.5.1.). Initial fragment lengths were congruent (172 ± 3 mm), as were biomass readings (3.59 ± 0.53 g f_w).

5.2.3.2. Water physicochemistry

Mesocosm water physicochemistry was recorded and analysed weekly in each mesocosm from day 1 of the experiment as per the method in Chapter 3, and the same suite of parameters were measured: Velocity (m s^{-1}), water temperature (°C), pH, dissolved oxygen (%), conductivity (µS cm^{-1}), nitrate (mg NO_3- l^{-1}), soluble reactive phosphorus (mg PO_4- l^{-1}).

Due to findings in Chapters 3 and 4, discharge, turbulence (Reynolds numbers), water residency and suspended sediments were not measured.

5.2.3.3. Plant morphometry and physiology

Morphometric traits (Table 5.2.) - Macrophyte trait measurements were recorded weekly as per the method in Chapter 3 (3.2.5.3.). In addition to those in Chapter 4, the following morphological traits were also included in this study: the number of damaged plants, and number of washouts.

Measurements were then used to calculate the weekly relative rates of extension, using the equation given in Chapter 4 (Eq. 4.1.).

Biomass (Table 5.2.) - Fresh and dry weights were also recorded for plant components (leaves, stems, roots) as in Chapter 4, and used to calculate the root:shoot allometric coefficients and relative growth rates (*RGR* – Chapter 4, Eq. 4.2.).

Table 5.2. **List of characteristic traits selected for sampling.** Updated from Chapter 4 – additions are highlighted in bold.

Identifier	Abbreviation	Plant characteristic trait	Measure of
i	L	Length of main stem	See 3.2.5.4. in Chapter 3.
ii	LL	Length of longest leaf (on main stem)	
iii	NL	Number of leaves	See 4.2.3.3. in Chapter 4.
iv	PI	Position of longest internode (on main stem)	See 3.2.5.4. in Chapter 3.
v	LI	Length of longest internode (on main stem)	
vi	SB	Length of longest secondary branch	
vii	TB	Length of longest tertiary branch	
viii	NB	Number of branches	See 4.2.3.3. in Chapter 4.
ix	AB	Angle of branches	See 3.2.5.4. in Chapter 3.
x	PR	Position of first adventitious roots	
xi	**D**	**Number of damaged plants**	Indication of stem breakage from physical and/or stressful environmental conditions.
xii	**W**	**Number of washouts**	Poor root anchorage due to physical and or/stressful environmental conditions.
I	RGR	Relative growth rate	See 4.2.3.3. in Chapter 4.
II	R:S	Root:shoot allometric coefficient	See 3.2.5.4. in Chapter 3.
III	SD	Stem densities	See 4.2.3.3. in Chapter 4.
IV	LD	Leaf densities	
V	Ca	Chlorophyll a	
VI	Cb	Chlorophyll b	
VII	CAR	Carotenoids	
VIII	**TP**	**Tissue phosphorus**	Phosphorus storage capability. Correlation with environmental phosphorus may signify nutrient limitations.

Biomechanical tissue properties (Table 5.2.) - Stem and leaf tissue densities were also calculated, as in Chapter 4, to assess tissue strength and flexibility.

Chlorophyll pigment concentrations (Table 5.2.) - Leaf chlorophyll concentrations were determined as per the method in Chapter 4 (Eq's. 4.3., 4.4., 4.5.), in order to provide information on Chlorophyll a, Chlorophyll b and Carotenoid concentrations in leaf tissues.

Tissue nutrient concentrations (Table 5.2.) - Total organic phosphate in plant tissues were extracted using the wet oxidation method detailed by Parkinson & Allen (1975), and were determined spectrophotometrically and expressed as SRP g^{-1} d_W.

5.2.3.4. Algal biomass

Biomass of periphytic algae present within each mesocosm was determined using a simple dry weight sampling regime. To avoid disturbance to plant specimens, three between-plant spaces within each mesocosm were selected at random, and algal samples were collected at the end of the study by inserting a 0.1 m^2 open-ended tube vertically into the mesocosm to 'trap' a sample area of algae. Samples were then removed from the mesocosms and bagged. In the laboratory, samples were washed to remove any sediments or litter, then oven dried at 65°C for 24 hours and weighed to determine dry weight. Algal biomass was expressed as g d_W m^{-2}. Algal taxonomy was not investigated in the laboratory, but field observations indicate it predominantly comprised *Cladophora* spp. (likely *C. glomerata*).

5.2.4. Data analysis

Data exploration was conducted as in Chapter 4 (Zuur, Ieno, & Elphick, 2009), including: outlier examination; collinearity amongst explanatory variables; distribution; and zero inflation. Following this, outliers and collinear variables were removed (VIFs > 3 (Zuur, Ieno, Walker, *et al.*, 2009) - dissolved oxygen, discharge, turbulence, water-residency, nitrate, pH and conductivity); appropriate distributions were selected for analyses; and zero-inflation was not observed and needed no action. Phosphate was collinear with nitrate, therefore nitrate was removed to avoid confounding influence on phosphate treatments. Conductivity was collinear with phosphate and nitrate, being clearly influenced by them (**Figure 5.3.**), and was therefore not modelled. pH was also removed for

showing minor collinearity with multiple variables. Consequently, final explanatory variables for assessment in models were: velocity, dissolved oxygen, phosphate, algal biomass and their interactions (see below).

Between-mesocosm water chemistry was analysed using repeated measures analysis of variance (RM ANOVA) to assess variation among stream mesocosms.

Associations between plant growth characteristics and environmental variables were modelled using linear mixed-effects models with gaussian distributions (LMMs) in R (R Core Team, 2013). LMMs were run with the package *nlme* (Pinheiro *et al.*, 2013). Linear models were used because residuals showed linear patterns during data exploration (Zuur *et al.*, 2007) for all growth characteristics. Mixed-effects models were used as they allow the incorporation of variance structures and mixed-effects to account for nested experiments (Zuur *et al.*, 2009b), which traditional statistical methods (e.g. ANOVA, basic regression) fail to account for. In addition, a mixed-model approach is a more robust method of analysing experiments such as this, as treatment level effects and additional influences (algal) can be easily separated, allowing better inference of environmental influence. As in Chapter 4, models only included final sample morphological data, avoiding incorporation of temporal autocorrelation structures into models.

All plant morphological and physiological measurements were modelled separately and for each model, fixed structures, random structures, and optimal variance structures (**Table C.1.**, Appendix C) were determined. Data transformations were avoided where possible (Zuur, Ieno, & Elphick, 2009). Damaged plants (*xi*) and washouts (*xii*) were not modelled due to the limited number of response variables for these traits, and are only presented graphically (**Figure 5.4.**) and as basic in text descriptive statistics.

In all models, "channel" was defined as a random effect to account for within-channel repeat (nested) measurements. Two-way interactions were included for velocity:algae and phosphate:algae, as these variables were likely to exhibit interactive effects (**Figure 5.2.**). A three-way interaction including velocity:phosphate was excluded, as algal growth was collinear with the interaction term and therefore used as an interactive product of the linkage between velocity and phosphate.

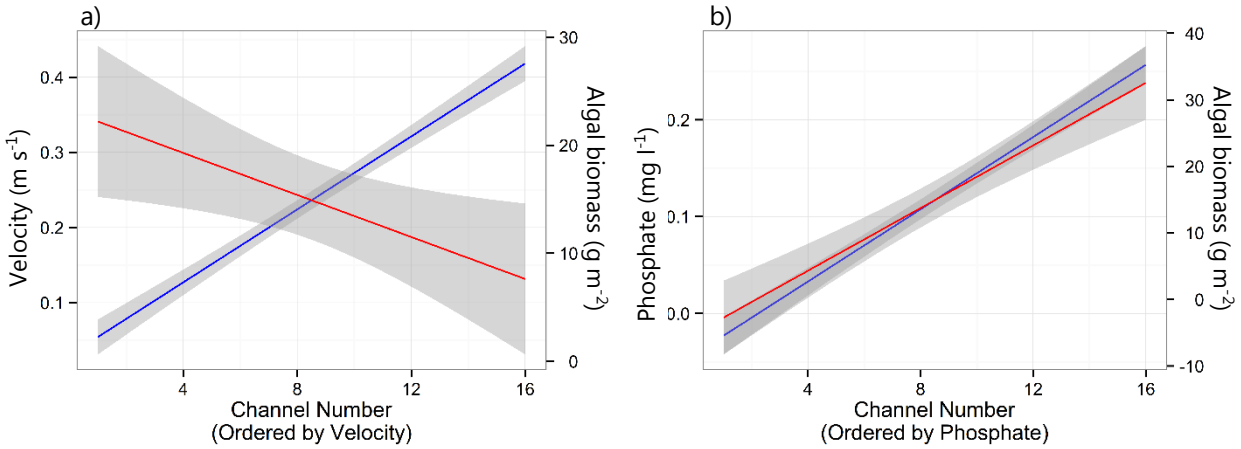


Figure 5.2. **Interactions between velocity/algae and phosphate/algae.** Plots show linear regression trendlines (solid lines) and standard error (shaded areas) for a) water velocity (blue line) and algal biomass (red line), and b) phosphate concentration (blue line) and algal biomass (red line). In both plots, *x* axis represents the channel number (1-16) ordered by a) velocity (lowest-highest), and b) phosphate (lowest-highest).

Best-fit models were determined using Akaike's Information Criterion *second-order* (AIC_C) using a top down stepwise selection approach, where model parameters were dropped depending on significance, and AIC_C was assessed until the best (lowest AIC_C) model with all significant parameters was determined (Burnham & Anderson, 2002; Zuur *et al.*, 2009b). AIC_C was used rather than AIC as it includes a small-sample bias correction (Burnham & Anderson, 2002). Coefficients of variation (R^2) were calculated using the mixed-effects models method by Nakagawa & Schielzeth (2013). This involves calculating two R^2 values: a marginal R^2 (R^2_{mar}) which shows variation explained by the fixed effects, and a conditional R^2 (R^2_{con}) which shows variation explained by fixed and random effects. In covariate

selection, minimum significance was chosen to be $p \leq 0.05$ for model parameters, although those close to the significance level should be treated with caution (Zuur, Ieno, Walker, *et al.*, 2009). After best-fit model selection, model validation was performed to determine if each model still adhered to model assumptions (Zuur, Ieno, Walker, *et al.*, 2009).

5.3. RESULTS

5.3.1. Water physicochemistry and algal biomass

Both velocity and phosphate treatments were significantly different between treatment mesocosms, but post-hoc Tukey tests revealed that replicate treatment mesocosm were synchronous (**Table 5.3.**, **Figure 5.3.**). Non-treatment physicochemical conditions were highly comparable, temporally and between-mesocosms. Waters inflowing to mesocosms were alkaline (mean pH 7.5 ± 0.03), nutrient rich (nitrate 7.08 ± 0.004 mg NO_3- l^{-1}, SRP 0.02 ± 0 mg PO_4- l^{-1}, conductivity 390 ± 5 S cm^{-1}) and well oxygenated (DO 79 ± 4%) throughout the study.

Table 5.3. **Mean, minimum and maximum physicochemistry and results of Repeated Measures Analysis of Variance on Ranks tests.** Performed between all stream mesocosms.

				RM ANOVA on Ranks Test Results		
	Mean	Min	Max	H	d.f.	*p*
Velocity (m s^{-1})	0.233	0.071	0.392	68.268	15	<0.001***
Between 'Low' channels	0.113	0.071	0.173	13.182	7	0.068
Between 'High' channels	0.354	0.319	0.392	2.011†	7	0.084
SRP (mg l^{-1})	0.12	0.02	0.24	61.162	15	<0.001***
Between 'Low' channels	0.02	0.02	0.02	0	7	1.000
Between 'High' channels	0.21	0.24	0.19	1.687	7	0.975
Nitrate (mg l^{-1})	7.08	7.02	7.15	69.909	15	<0.001***
pH	7.5	6.98	7.7	0.958	15	1.000
Dissolved oxygen (%)	79.66	65.6	118.4	2.141	15	1.000
Conductivity (µS cm^{-1})	389.76	202.8	579	24.286	15	0.060
Algal biomass (g m^{-2})	14.91	0	56.3	75.786	15	<0.001***

*** p <0.001; ** p <0.01; * p <0.05

† Normality assumption passed and standard ANOVA run instead; 'H' represents F value in this case.

Algal biomass was significantly different both between- and within-treatment categories, suggesting variation according to differences between treatments. Biomass was highest in HN:LF channels (43.9 ± 1.2 g m^{-2}), relatively high in HN:HF (24.2 ± 0.6 g m^{-2}), relatively lower in LN:LF (10.8 ± 0.3 g m^{-2}), and in LN:HF treatments, growth was negligible (0.15 ± 0 g m^{-2}).

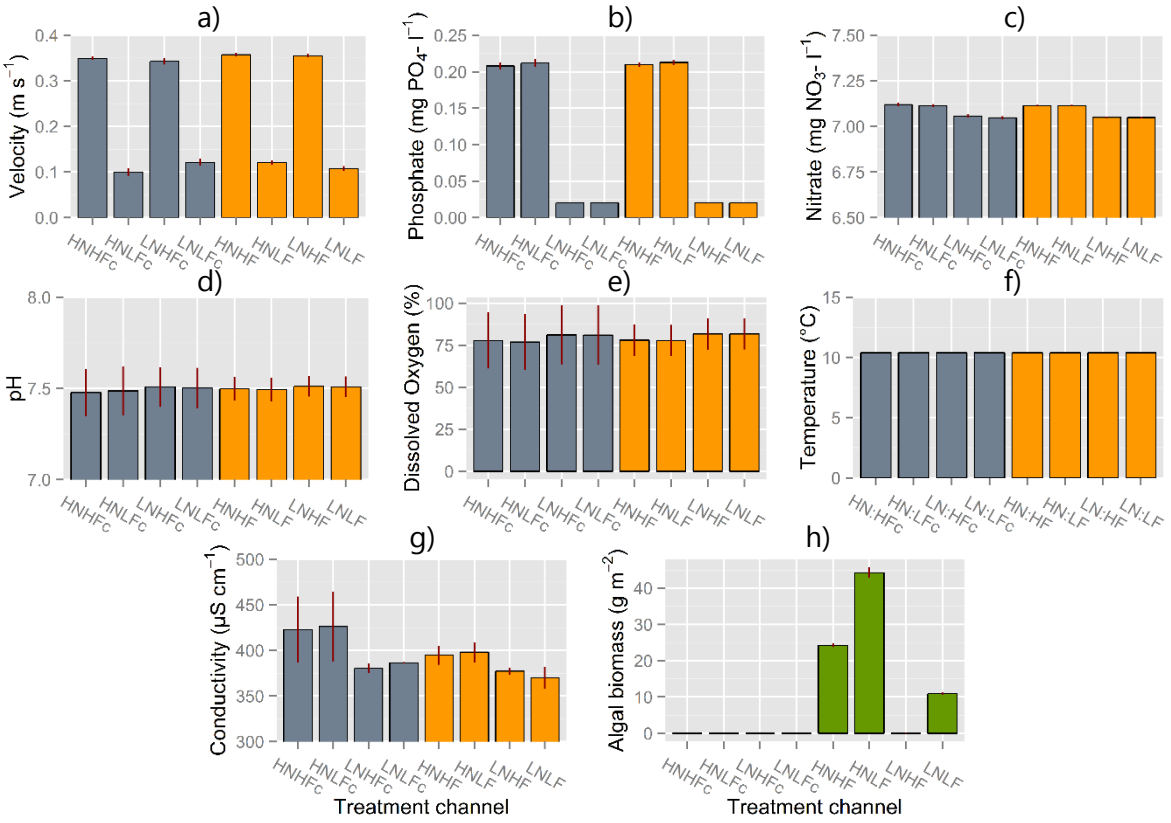


Figure 5.3. **Mesocosm physicochemistry and algal biomass compared between the 8 treatment categories.** Dark (left four) bars represent control treatments, light (right four) bars represent full treatments. Parameters shown include a) Velocity, b) phosphate, c) nitrate, d) pH, e) dissolved oxygen, f) temperature, g) conductivity, and h) algal biomass. Error bars represent standard error (treatment *n* = 75, control *n* = 25).

5.3.2. Plant morphometry and physiology

Plant trait characteristics varied markedly (**Figures 5.4., 5.5. & 5.6.**), and were significantly influenced by treatment effects (**Tables 5.4. & 5.5.**), although plant form predominantly

responded to hydraulic forces from velocity, with most functional traits responding to combinations of phosphate and/or algal influence.

Morphometric trait relative rates of extension - Few traits exhibited similar temporal trends in morphological trait RREs among replicate treatment channels, although numerous displayed different patterns between treatments (**Figure 5.4.**).

All channels, regardless of treatment saw steady increases in RRE each week for overall plant lengths, particularly LN treatments (mean L – LN 64 ± 10 mm mm^{-1} $week^{-1}$, HN – 45 ± 9), with fastest rates occurring at the study end. Typically, plants in LN treatments were longer than those in HN, with control channels having marginally longer specimens than algal influenced specimens.

Numbers of leaves and branches had comparable treatment influences, although temporal trends differed. HF treatment specimens had markedly higher rates of development (mean – NL 3 ± 0.4 $week^{-1}$, NB 0.4 ± 0.1) than in LF treatments (NL 1.7 ± 0.3, NB 0.2 ± 0.1), irrespective of nutrient treatment or algal presence. This development meant plants in HF channels had more leaves and were developing laterally faster than low flow plants. Similar treatment effects can be seen for PI (HF 0.7 ± 0.2 $week^{-1}$, LF 0.3 ± 0.2), TB (HF 7.5 ± 2.5 mm mm^{-1} $week^{-1}$, LF 2.3 ± 1.8), and to a lesser extent PR (HF 0.8 ± 0.3 $week^{-1}$, LF 0.6 ± 0.2), with all three traits showing particular differences between HF and LF in non-control channels. Plants in HF treatments therefore were also producing new nodes faster, growing new branches more rapidly and producing less adventitious roots than counterparts in LF.

Unexpectedly, SB did not mirror the treatment responses of TB, and whilst showing a similar increasing overall temporal trend, instead had greatest extension in LN treatments (mean – LN 41 ± 8 mm mm^{-1} $week^{-1}$, HN 30 ± 6). Consequently, by the study end, plants in LN treatments had marginally longer secondary branches than HN treatment specimens.

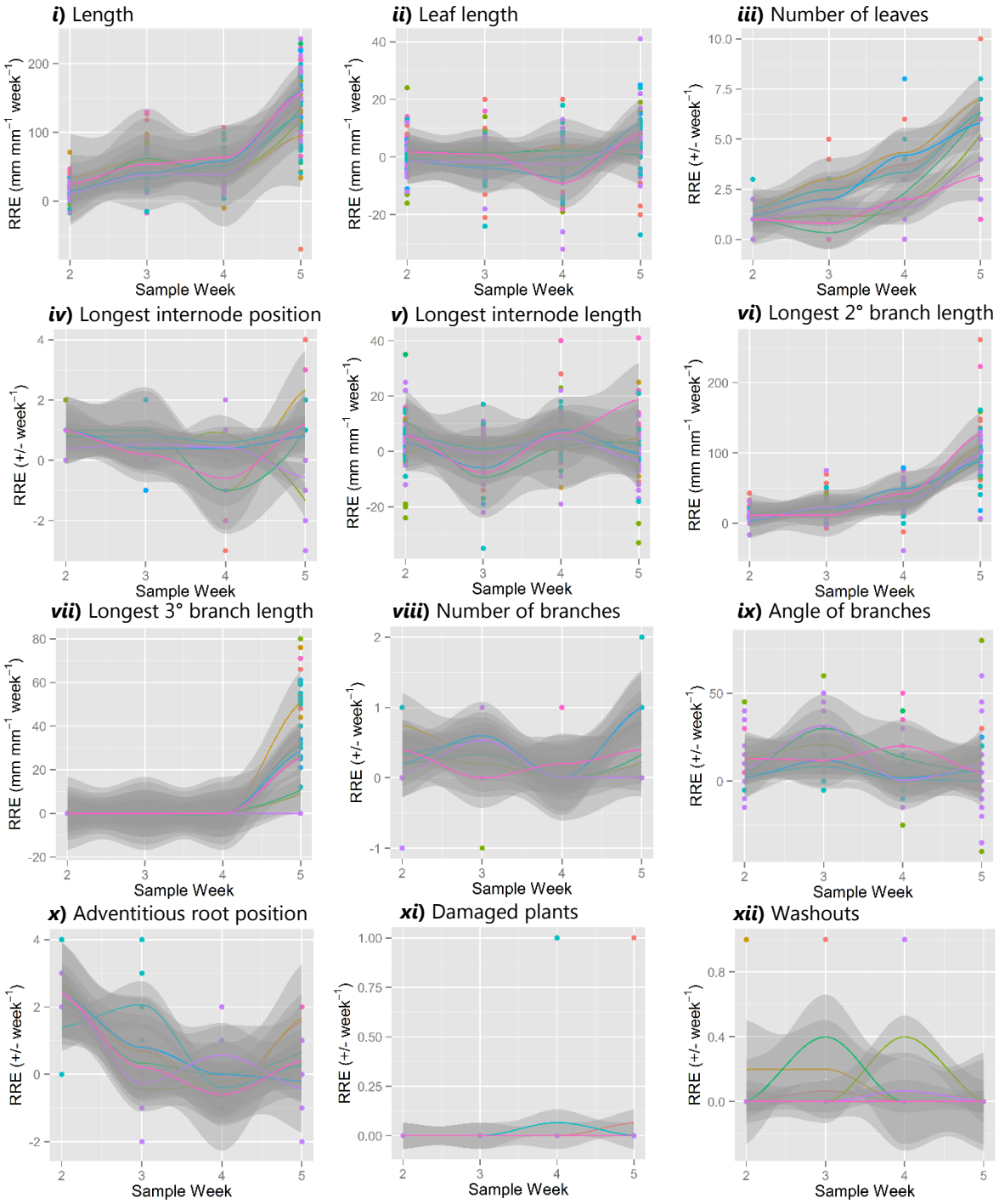


Figure 5.4. **Changes in relative rates of extension (RRE) of morphological traits.**

Solid lines are estimated loess smoothers and shaded areas are 95% point-wise confidence bands. Horizontal axis shows the sample week and vertical axis is the relative rate of extension (RRE) of the following morphological traits: *i*) length, *ii*) leaf length, *iii*) number of leaves, *iv*) position of longest internode, *v*) length of longest internode, *vi*) length of longest secondary branch, *vii*) length of longest tertiary branch, *viii*) number of branches, *ix*) branching angle, *x*) position of first adventitious roots, xi) number of damaged plants, and xii) number of washed away plants.

Leaf lengths (LL) were difficult to interpret due to significant variability, yet many specimens had reducing leaf RREs each week, for example specimens in LN treatments (LL mean -0.1 ± 2.3 $week^{-1}$). Additionally, LI was similarly variable, although this trait saw differences possibly attributable to algal development (control 2.8 ± 2.4 $mm\ mm^{-1}\ week^{-1}$, non-control 2.1 ± 1.7). Furthermore, branching angles were also difficult to determine due to variability, but specimens in LF treatments tended to have branches with greater angles (FF 4.5 ± 2 $° week^{-1}$, LF 11 ± 3).

Damaged and washed out plants had few observations (D – 2 visibly damaged; W – 12 washouts), therefore no major temporal trends. However, damaged plants occurred in HF treatments, and a majority of washouts were linked with HN treatments (11) with considerable overlap with LF treatments (9).

Best-fit model selection **-** The following linear mixed model was fitted to data for each characteristic trait:

$$T_{is} = \alpha + \beta_1 \times X_{1is} + a_i + \varepsilon_{is} \qquad 5.1.$$

$$\varepsilon_{is} \sim N(0, \sigma_i^2) \qquad 5.1.a.$$

where T_{is} is the morphological/physiological trait for observation i in mesocosm s, X_{1is} is the associated explanatory variable, α is the intercept, β is the slope, a_i is the random component, normally distributed with expectation 0 and variance σ_a^2, and ε_{is} represents residuals (**Figure 5.1.a**), normally distributed with mean 0 and a variance defined by the associated structure in **Table C.1.** (Appendix C.). Models with multiple explanatory variables were incorporated by adding additional covariate elements as required after the first explanatory term in the form ...+ $\beta_2 \times X_{2is}$ + ... (see **Table 5.5. & 5.6.** for included explanatory variables).

Morphometric trait model outcomes **-** Overall, the morphological trait best-fit models roughly correlate with RRE observations (**Figure 5.4**) and match closely the visual changes to end-study morphology in relation to treatment effects (**Figure 5.3. & 5.5.**). Generally, most trait responses were driven by the main treatment effects (velocity and phosphate), but several were influenced by algal growth, or the interaction with main treatments. In addition, much of the variance explained for each model can be attributed to treatments (R^2_{mar}; fixed effects), with limited variation explained by nested data (R^2_{con}; random effects).

NL, TB and NB were positively related to velocity, with specimens in higher flowing treatments having more leaf and lateral development and longer tertiary branch lengths. The model for NL also includes the velocity:algae interaction, phosphate and algae as significant terms, and with a high coefficient of variation (R^2_{mar} = 0.549), velocity, supported by these variables, explains much of the variation in this leaf production.

Table 5.4. **Morphological trait vs. environmental variable best-fit model summaries.** Using linear mixed models. Model statistics: R^2_{mar} is the marginal R^2, R^2_{con} is the conditional R^2, and *p* signifies the *p*-values of the model components. "-" indicates no significant parameters were present in any model.

Response variable (morphological trait)	Best-fit model components	Model statistics		
		R^2_{mar}	R^2_{con}	*p*
L	-	0.029	0.037	-
LL	Phos	0.314	0.382	0.039*
NL	Vel + Vel:Alg + Phos + Alg	0.549	0.549	<0.001*** + 0.004*** + 0.016* + 0.042*
PI	Alg + Phos + Vel	-0.386	-0.423	0.002** + 0.038* + 0.05*
LI	Alg + Phos:Alg + Vel + Vel:Alg	-0.184	-0.184	<0.001*** + 0.003** + 0.007** + 0.015*
SB	-	-	-	-
TB	Vel	0.235	0.235	<0.001***
NB	Vel	0.334	0.334	<0.001***
AB	Vel:Alg + Alg	-0.966	-0.966	<0.001*** + 0.034*
PR	Vel:Alg + Alg + Phos	-0.385	-0.468	<0.001*** + 0.001** + 0.015*

*** p <0.001; ** p <0.01; * p <0.05

LL, as observed in RRE temporal plots, is significantly positively influenced by higher phosphate concentrations suggesting enhanced leaf elongation in higher nutrient conditions, though due to large variability in lengths, explained variance by this model is low (R^2_{mar} = 0.314).

PI best-fit model negatively relates internodal lengths to algae but positively to phosphate and velocity, meaning that nodal production occurs faster for plants in high flows under higher nutrients, and without the influence of algae.

LI is also mainly negatively related to increasing algae, although limited variance explained (R^2_{mar} = -0.184) highlights the uncertainty connected with this model, as supported by visual observations (**Figure 5.5.**). Typically, however, plants had slightly longer internodes where algae was not present.

Both AB and PR were negatively influenced by the velocity:algae interaction and the presence of algae, and in the case of PR, positively related to increasing phosphate. For branching angle, which had significant amounts of variation explained by this model (R^2_{mar} = -0.966), angles were significantly reduced, with branches more parallel to the main stem, when algal biomass was increased and under high flow situations. Relationship with PR may indicate a reduction of resource allocation to producing adventitious roots in competitive conditions, and an increase in nutrient rich situations.

Overall length and secondary branch lengths did not have any significant explanatory variables, which agrees with study RRE observations (**Figures 5.4 & 5.5.**) and end lengths.

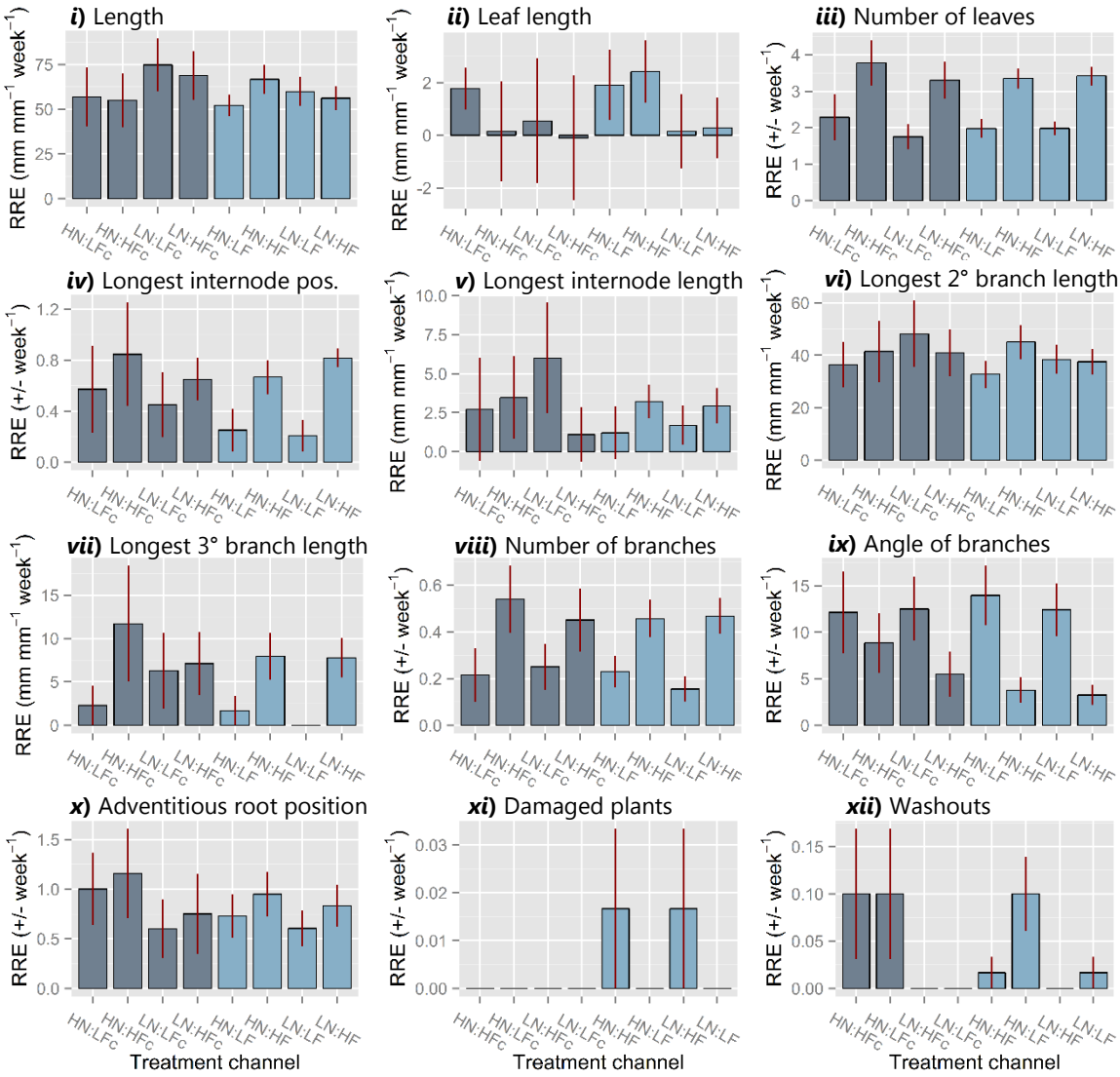


Figure 5.5. **Plant morphological traits compared between the 8 treatment categories.** Dark (left four) bars represent control treatments, light (right four) bars represent full treatments. Morphological traits include *i*) length, *ii*) leaf length, *iii*) number of leaves, *iv*) position of longest internode, *v*) length of longest internode, *vi*) length of longest secondary branch, *vii*) length of longest tertiary branch, *viii*) number of branches, *ix*) branching angle, *x*) position of first adventitious roots, *xi*) number of damaged plants, and *xii*) number of washouts. Error bars represent standard error (treatment n = 15, control n = 5).

Physiologic trait model outcomes **-** Best-fit model outcomes for biomass, biomechanical tissue properties, leaf chlorophyll and tissue phosphorus concentrations are displayed in **Table 5.5.** The models vary in their ability to explain variance, but are generally well associated to observed trait responses (**Figure 5.6.**). As with morphology, models were

significantly influenced by main treatment effects, although several models included algal and interaction terms.

R:S ratios, SD and LD were all significantly affected by velocity treatment effects. In particular, stem tissue densities were considerably (R^2_{mar} = -0.911) driven by velocity, with plants in high flows having more flexible stems as a result. R:S ratios, whilst significantly influenced, were less well explained by velocity (R^2_{mar} = 0.308), suggesting unaccounted for influences may be reducing root allocation in certain situations.

Table 5.5. **Physiological trait vs. environmental variable best-fit model summaries.** Using linear mixed models. Model statistics: R^2_{mar} is the marginal R^2, R^2_{con} is the conditional R^2, and *p* signifies the *p*-values of the model components. "-" indicates no significant parameters were present in any model.

Response variable (morphological trait)	Best-fit model components	Model statistics		
		R^2_{mar}	R^2_{con}	*p*
RGR	-	-	-	-
R:S	Vel	0.308	0.316	0.019*
SD	Vel	-0.911	-0.911	<0.001***
LD	Vel	0.248	0.248	<0.001***
Ca	Phos + Vel:Alg + Alg + Phos:Alg	-0.999	-0.999	<0.001*** + <0.001*** +<0.001*** +<0.001***
Cb	Vel:Alg + Alg + Phos:Alg + Phos	-0.492	-0.494	<0.001*** + <0.001*** +<0.001*** +<0.003**
CAR	Phos:Alg + Alg + Phos + Vel:Alg	0.431	0.543	<0.001*** + <0.001*** + 0.002** + <0.01**
TP	Phos + Vel + Alg	0.961	0.965	<0.001*** + <0.001*** + <0.001***

*** *p* <0.001; ** *p* <0.01; * *p* <0.05

Leaf chlorophyll (Ca, Cb) and carotenoids (CAR) were all explained by models that include combinations of phosphate, algae, velocity:algae and phosphate:algae. In the case of Ca explained variance is significant (R^2_{mar} = -0.999), while Cb and CAR are explained less than half, although still a large portion of variation in these traits. Both Ca and Cb were influenced by the presence of algae, as increasing algae resulted in reductions in concentrations of leaf chlorophylls; in these situations presence of phosphates increased

Ca and Cb concentrations, suggesting a complex competitive interaction, as denoted by the inclusion of the interaction terms. Conversely, CAR increased with algal biomass and enhanced nutrients.

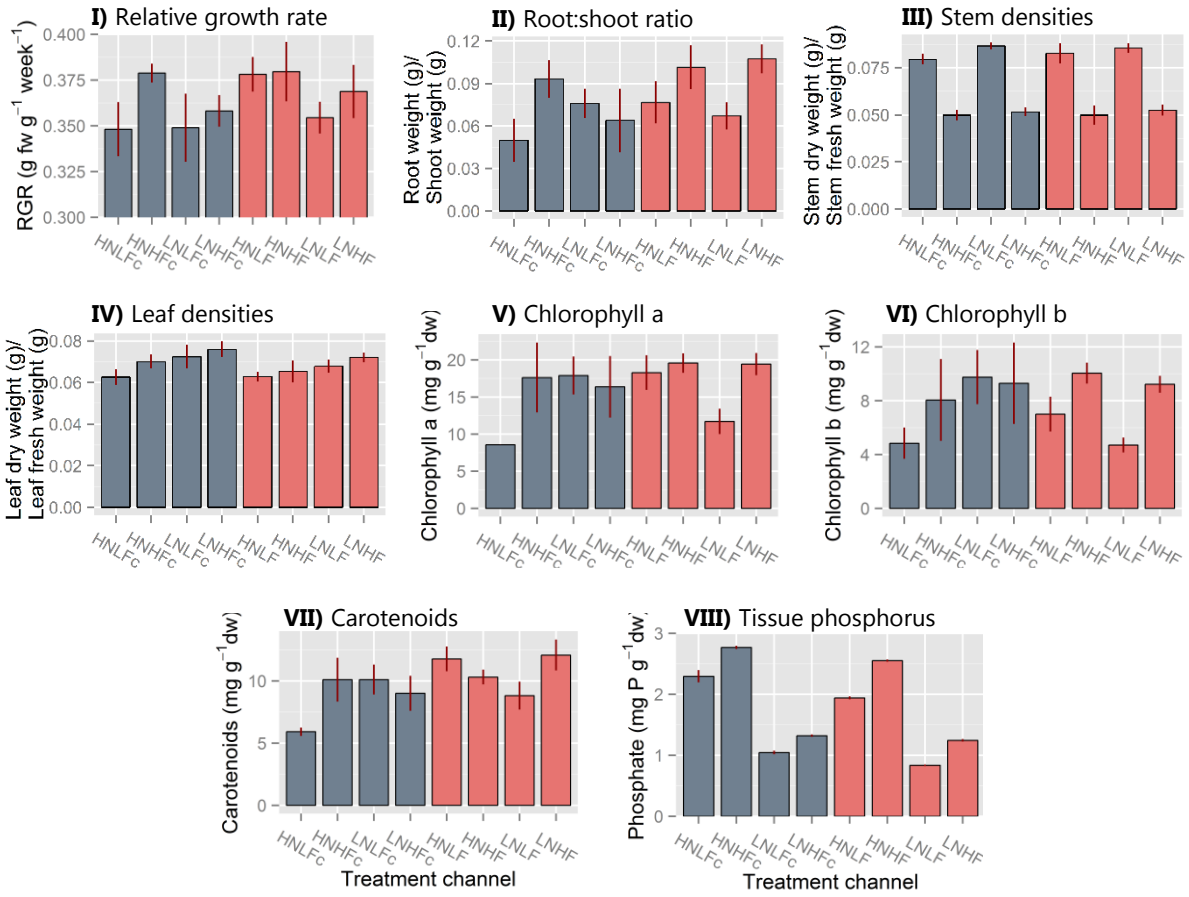


Figure 5.6. **Plant physiological traits compared between the 8 treatment categories.** Dark (left four) bars represent control treatments, light (right four) bars represent full treatments. Physiological traits include I) Root:shoot allometric coefficient, II) relative growth rate, III) stem densities, IV) leaf densities, V) chlorophyll a concentrations, VI) chlorophyll b concentrations, VII) carotenoid concentrations, and VIII) tissue phosphorus concentrations. Error bars represent standard error (treatment n = 15, control n = 5).

TP concentrations were highly significantly explained (R^2_{mar} = 0.961) by a positive relationship with increasing phosphate concentrations and higher flows, and negatively with increasing algae. Plant tissues are therefore uptaking greater quantities of soluble

phosphorus from the water column where phosphates are in greater abundance, flows are higher and algal growth is reduced.

5.4. DISCUSSION

Multiple environmental stimuli have the potential to exert interactive effects on macrophyte taxa, however, specimen responses throughout the study were mainly related to the influence of individual treatment variables. Velocity was found to affect traits that were related to the development of form (e.g. lateral development), phosphates were seen to affect functional attributes (e.g. plant tissue storage), and algal growth provided a confounding influence on some results, and altered photosynthetic and competitive traits.

Riverine macrophyte growth responses to environmental stimuli are well documented (e.g. Demars & Harper, 1998; Riis & Biggs, 2003; Puijalon *et al.*, 2008; O'Hare *et al.*, 2010), but often focus on the effects of single environmental parameters. Where studies examine combinations of environmental stimuli on plant growth (Idestam-Almquist & Kautsky, 1995; Crossley *et al.*, 2002; Baldy *et al.*, 2007), typically growth responses are reported as measures of biomass, size or abundance, with morphological trait plasticity either overlooked or presented with less convincing responses (although see for example Chapter 4, Garbey *et al.*, 2006; Puijalon *et al.*, 2007).

Some lotic macrophytes, such as *Ranunculus peltatus,* as in Garbey *et al.* (2004, 2006), and *Ranunculus pseudofluitans*, as in this study, show such rapid plastic morphologic responses they can be considered ideal for testing the impacts of multiple environmental stimuli and act as indicators of environmental change (Onaindia *et al.*, 2005; Lacoul & Freedman, 2006; Sondergaard *et al.*, 2010). However, such responses are often confounded by lack of control on environmental factors, with robust experimental designs required to

minimise the impacts of varying abiotic measurements (Puijalon *et al.*, 2007). Consequently, establishing initial cause and effect for key macrophyte trait responses in-situ at river study sites may prove difficult, as environmental factors may vary widely in space and time. Furthermore, plants are rarely solely exposed to one stimuli, with great importance placed on the interactive effects of multiple environmental factors (Ormerod *et al.*, 2010). In particular, understanding multi-factorial systems is essential for conservation and management decisions.

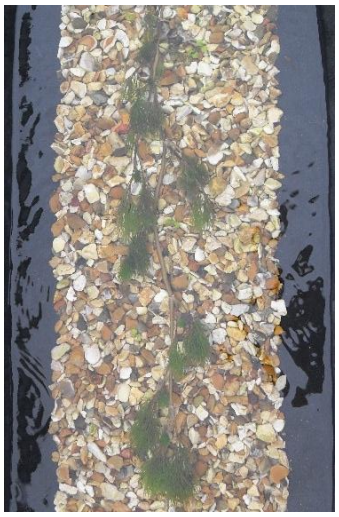
In this study, allofragments of the aquatic macrophyte *Ranunculus penicillatus* subsp. *pseudofluitans* var. *pseudofluitans* were artificially planted in stream mesocosms and subjected to a series of combination treatments of velocity and phosphate, with the influence of natural algal growth. Characteristic morphological and physiological growth traits were examined against key abiotic (physicochemistry) and biotic (algal growth) parameters to determine the main driving factors for healthy macrophyte development particularly when faced with stressful conditions.

Water physicochemistry was highly consistent throughout the study period, and replicable amongst mesocosms, with the exception of the treatments, which were congruent within-treatment replicates, but significantly different from each other. Temperatures were low due to the presence of the aquifer fed water, pH remained continuously slightly alkaline, and nitrate was consistently between the 'very low' and 'low' grades of the Environment Agency General Quality Assessment (Environment Agency, 2013). Dissolved oxygen varied over the study period, but remained consistent between channels. DO was 'high' towards the start of the study, but slowly reduced below 'good' levels toward the end in some channels (UKTAG, 2008). It is unknown why this occurred, but could be related to a build-up of decaying organic matter from senescing algae/diatoms. Conductivity was generally lower than expected for chalk waters (Allen *et al.*, 2010), although higher concentrations

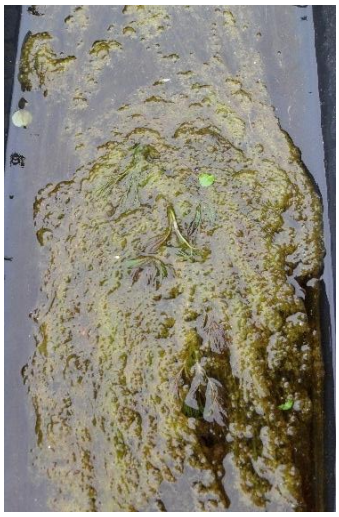
in the nutrient dosed channels, and collinearity with phosphates and nitrates, indicate correlations between these. Of the phosphate treatments, the low treatments remained well below the revised 'high quality' Water Framework Directive standards (UKTAG, 2013), and high treatments were significantly above the revised 'moderate quality' standards, and within the 'threshold' levels (where changes in ecology are expected) for chalk rivers set by UKTAG (2008). Water flows were maintained at velocities roughly correlating with optimal plant growth in the 'high' flow treatments (Chapter 4; Halcrow Group Ltd., 2004), and sub-optimal in the 'low' flow treatments.

In the algal treatment channels, the growth of filamentous algae differed considerably. There was a stark difference between low and high nutrient channels, with substantially more algal and diatom growth where nutrient levels were elevated. In addition to this, within nutrient treatments, there was a large difference between low and high flowing channels, with greater growth under lower flows. Visually, there was a noticeable difference between these channels (**Figure 5.7.**). Elevated phosphorus is known to cause

a) High flow, low nutrient



b) Low flow, high nutrient



c) Low flow, low nutrient

Figure 5.7. **Algal development at the end of the study period.** Pictures shown include a) an example of limited algal development around a specimen growing in a 'high flow' channel in 'low nutrient' conditions, b) an example of extensive algal development around a specimen growing in a 'low flow' channel in 'high nutrient' conditions, and c) an example of algal development in a 'low flow' channel in 'low nutrient' conditions.

enhanced growth of algae (Mainstone & Parr, 2002), and higher velocities are recognised as a removal mechanism of epiphytic algae (Wright *et al.*, 1982; Bergey *et al.*, 1995; Wilby *et al.*, 1998). However, substantial growth of epiphytic algae is less-well documented when nutrient levels are low, even under low-flow conditions. The development observed in the low-nutrient, low-flow channels in this study presents an unusual situation that may be explained by the homogeneity of flow conditions within each mesocosm (lack of riffles, pools etc). Whilst this will need further investigation, it should certainly raise concern in rivers with reducing flows, even in those free from the impact of nutrient pollution.

In general, plant characteristic trait responses observed in this study can be categorised depending on three main relationships: 1) velocity driven traits, 2) phosphorus driven traits, 3) algal interactive effects (which tend to include velocity or phosphate influences). Several traits were principally influenced by either velocity or phosphate as a single treatment stimulus (leaf length, longest tertiary branch, number of branches, root:shoot ratio, stem/leaf densities), with most being determined by more complex interactions that involve algae (number of leaves, longest internode length/position, branching angle, positions of adventitious roots, chlorophyll a/b, carotenoids, tissue phosphorus), and a few were unexplainable (length, longest secondary branch, relative growth rate), but likely related to furthermore complex interactions.

1) Velocity driven traits – Those traits that were driven primarily by velocity involved structural components that, a) signified greater lateral development (branch production; Garbey *et al.*, 2004a) and higher productivity (leaf production; Bloom *et al.*, 1985), and b) indicated enhanced plant survivability by improving anchorage (increasing resource allocation to roots; Puijalon *et al.*, 2005) and causing reconfiguration (reduction of stem and leaf densities; Sand-Jensen, 2008) under increasing drag forces.

Increases in root/stem allocation and reduction in leaf production is suggested under increasing nutrient concentrations for some macrophyte taxa in lower flows (Gedroc *et al.*, 1996; Madsen & Cedergreen, 2002; Puijalon *et al.*, 2007), and for other taxa, nutrient stresses can significantly reduce root production (e.g. *Berula erecta*; Puijalon *et al.*, 2007). However, this was not experienced for *R. pseudofluitans*, as all these traits increased with higher velocities, regardless of nutrient concentration, suggesting velocity effects overpowered any nutrient influence. The principal role of increasing root biomass is anchorage under increasing flows (Chapter 4; Idestam-Almquist & Kautsky, 1995; Puijalon *et al.*, 2005), and for a highly streamlined flow-tolerant macrophyte such as *R. pseudofluitans*, this may therefore be a resource allocation priority.

Such a strong positive relationship between flow and rates of leaf growth opposes the concept of increased mechanical stimuli reducing leaf production (Puijalon & Bornette, 2004; Puijalon *et al.*, 2007). In addition, increases in leaf number, and elongation of tertiary branches and number of branches, both reflections of lateral development (Garbey *et al.*, 2004a), meant plants in this study presented greater surface areas to oncoming flow, having to resist higher drag forces (Sand-Jensen, 2003). However, as observed in Chapter 4, this rapid production of leaves and lateral branches coinciding with greater tissue flexibility may be an adaptive response to maximise productivity (Sand-Jensen, 2003, 2008).

Tissue densities, and in particular stem densities, decreased under higher flow conditions, enabling greater flexibility in higher flows (Puijalon *et al.*, 2008). There were no significant increases in density related to low-nutrient conditions, as discussed by Puijalon *et al.* (2007) and Lamberti-Raverot & Puijalon (2012). This is unusual, as denser tissues are purported to improve nutrient conservation (Ryser, 1996; Lamberti-Raverot & Puijalon, 2012), which is less necessary in high-nutrient conditions. As no changes in density were experienced between nutrient levels, this signifies that *R. pseudofluitans* may already meet its

phosphorus requirements under 'low' phosphorus conditions, or it may simply represent primary adaptive responses to enhance reconfiguration under higher flows (Sand-Jensen, 2003; O'Hare *et al.*, 2007)

2) Phosphate driven traits – Unexpectedly, only two traits were principally driven by phosphate concentrations: rates of leaf length elongation and tissue phosphorus concentrations. While the latter may be an obvious link, the model for tissue phosphorus also suggests influence from water velocities and algal growth, whereas leaf lengths were solely influenced by phosphate.

Tissue phosphorus correlation with velocity may be explained by the influence of diffusion boundary layers, which are less restricting in intermediate flows (Crossley *et al.*, 2002; Mommer & Visser, 2005), however, correlation between riverine SRP concentrations and tissue phosphorus is suggested to indicate P-limiting conditions (Spink *et al.*, 1997), which is unusual as chalk water adapted macrophytes are not typically phosphate limited (Westlake, 1981). However, whilst this correlation exists, it is unlikely phosphorus was limiting to plants, as all tissue phosphorus concentrations were well above the critical minimum threshold for maximum yield of 1.3 mg P g^{-1} (Gerloff & Westlake, 1982). Differences in concentrations may therefore represent variations in storage patterns within plant tissues (Thiébaut, 2005), and increased tissue phosphorus concentrations may not necessarily relate to enhanced growth.

Leaf length associations with phosphate are more difficult to interpret; whilst the model only suggests phosphate explains a small amount of variation in leaf lengths, it is still a significant driver. In other aquatic macrophytes it has been shown that leaf areas and lengths increase in response to increasing nutrient levels (Crossley *et al.*, 2002). It is possible this occurs to aid nutrient uptake via leaves in nutrient poor waters (Madsen & Cedergreen,

2002), but this is unlikely in this experiment, and as suggested by observed increased tissue phosphorus concentration, may just signify correlation between increasing tissue mass and phosphorous storage (Thiébaut, 2005; Thiébaut & Muller, 2009)

3) Algal interactive influenced traits – Where algal development was present in models, there were significant links between treatment effects (both phosphate and velocity), algal biomass and plant responses. Plant traits that were affected by these interactions included positions and lengths of internodes, angles of lateral branches, and positions of adventitious roots, as morphological responses, and tissue pigments (chlorophylls a and b, carotenoids), as physiological changes.

Internodal lengths and positions were influenced by more complex relationships with phosphate, algae and their interaction, although graphical outputs suggested some form of velocity control also. Elongation was faster and internode position was retained further from the apex in specimens grown without the impacts of algae. The longest internode and its position are thought to represent new internode development (Garbey *et al.*, 2004a), and situations where longest internodes are present close to specimen apices may indicate unfavourable conditions. The findings in this study highlight limitations to overall development and elongation when in competition with algae, although overall length was unaffected by algal presence.

Lateral branching angles were considerably modified by the presence of algae and interactions with velocity. Where algae was present in large quantities, angles were close to being parallel with the main stem, suggesting periphytic algae attached to plants are creating additional drag forces and resistance with added mass. However, periphytic algae attached to macrophytes have the potential to reduce drag forces by smoothing surfaces and reducing micro-roughness (Green, 2005), although this theory is yet to be tested.

Alternatively, if periphytic algae increases resistance, it is possible a combination of greater uprooting/breakage likelihood, alongside the weakening effects of algal smothering (Wade *et al.*, 2002), may reduce macrophyte success in certain situations.

First adventitious root positions were determined by a complex relationship between phosphate, algae and the interaction between velocity and algae. Generally, in the absence of algae, and also where velocities were higher, adventitious roots developed further from the plant apex. The change in adventitious roots, when growing in conditions with increased algal growth, may indicate a response to gain competitive advantage by increasing nutrient uptake (Garbey *et al.*, 2004b), either from sediments or the water column. Phosphate concentrations of plant tissues may provide limited support for this notion, as algal influence was also deemed important for this response. Mony *et al.* (2007) hypothesise that the creation of adventitious roots should be enhanced in low nutrient situations, perhaps in order to increase sediment rooting potential for additional resource acquisition. The results in this study agree with this hypothesis, as adventitious roots were present close to plant apices under low nutrient treatments.

Plant leaf chlorophyll and carotenoid concentrations were all linked to the phosphate/algae interaction. This general connection between pigment concentration, phosphates and algae may indicate potential competition. The presence of algae corresponds with reductions in chlorophyll, although this response is unusual, as typically freshwater plants increase levels of chlorophyll when presented with decreased irradiance (Barko & Filbin, 1983). However, algal presence corresponds with higher carotenoid concentrations, which can be indicative of senescence (Sims & Gamon, 2002), possibly suggesting algal smothering and competition for nutrients, if not light.

The main aspects of plant plasticity discussed by Mony *et al.* (2007) for nutrient availability, and the findings of Puijalon *et al.* (2007) and Lamberti-Raverot & Puijalon (2012) relating to combinations of flow and nutrients, present interesting paradigms relating to plant development, and in particular, costs and benefits of different plant characteristics under varying conditions. In the case of *R. pseudofluitans* in this study, there is some overlap in agreement with previous studies, but specimens also show some contrasting responses, which are likely related to differences in taxon phenotype. As observed here, *R. pseudofluitans* is capable of rapid growth over a short period of time, and appears to allow for a greater plastic capacity from early in the establishment phase, with limited costs to initial development. The idea of ontogenetic contingency, as discussed by Mony *et al.* (2007), where plants respond differently to the same stimuli depending on developmental stage, is less distinct for *R. pseudofluitans* (although further examination will be required to observe growth into maturity). Typically, physiological changes are observed in response to factors such as nutrient availability, as this is deemed a lower cost and a more rapid response (Mony *et al.*, 2007). However, in the current study, it is difficult to determine whether there is more evidence for morphological or physiological plasticity, as both sets of plant traits contain responses that are hard to determine, alongside traits obviously influenced by particular factors. Responses to nutrient enrichment such as weakening of stems (Lamberti-Raverot & Puijalon, 2012), were not observed, although stem densities reduced in line with greater mechanical stimuli. This did not appear to increase breaking or washout risk either, however, which was related mainly to nutrient availability and algal growth.

The general responses of *R. pseudofluitans* in this study indicate that velocities, therefore mechanical stimuli, are the most influential factors for driving morphological and physiological plasticity. In particular, good indicators of overall development, such as

numbers of leaves and branches, branch lengths and increased root allocation, were all significantly increased in higher velocities, regardless of nutrient level or algal growth. Phosphate had less of a direct impact, and typically coincides with the influence of algae, or the interactive effect between them, tending to impose negative effects on elongation traits such as internode positions and lengths, adventitious root position, and resource controlling traits such as leaf pigments. Traits that cannot be linked to any particular combination of environmental factor (e.g. length, secondary branch length and relative growth rates) are not unaffected by differences in environmental variables, but it is probable that a combination of stimuli, and a necessity to reallocate resources in response, are minimising the impact of individual factors. Overall, morphological and physiological plasticity varies significantly in the face of multiple stimuli, but apparent costs to juvenile plants are minimal.

Several key considerations should be raised regarding conservation and management efforts where promotion of healthy *Ranunculus* communities is of importance. On the basis of the findings in this study, optimal flow conditions, as highlighted in Chapter 4, are still important for healthy juvenile development, but increasing phosphorus concentrations and algal growth impact on elongation and resource collection traits, and may be detrimental further when plants reach maturity. Whilst it appears that initial allofragment juveniles (<4 weeks old) are relatively robust during their development, older specimens may be weakened, growth may be stunted, and additional issues may occur as biomass is accrued, particularly regarding washouts and damage. The impact of algae is likely to be more problematic for macrophyte development, and this study has highlighted that abundant growth can occur at low levels of phosphorus, with flow being the principal controlling factor in those situations.

It should be mentioned that whilst the findings of this study relate to the important early stages of allofragment development post-colonisation, many other factors will determine the initial settling of a plant fragment, and also development into maturity. Further work should be undertaken to examine the colonisation potential under varying conditions, and also plant ontogenetic trait development. Moreover, increased frequency and magnitude of flow and nutrient-enrichment events may have considerably different effects on plant growth, requiring additional investigation.

5.5. CONCLUSION

Complex interactions between multiple environmental variables exist in riverine ecosystems, often to an extent where inference and identification of key driving parameters is significantly confounded by extraneous influential factors. Artificial stream mesocosms are useful, reliable tools for examining the responses of characteristic traits in keystone macrophytes under controlled experimental conditions with the ability to manipulate one or more physicochemical parameters. In this study, combinations of velocity (mechanical stimulus), phosphate (resource stimulus) and algal growth (competitive stimulus) were observed having a considerable influence on the early juvenile growth of the keystone chalk stream macrophyte *Ranunculus pseudofluitans*. Sets of particular traits responded differently to three forms of stimuli: 1) velocity was the principal driving factor for morphological and physiological traits related to lateral development, reconfiguration and functional responses to flow, 2) phosphorus influenced nutrient storage in plant tissues, and 3) algal interactive effects acted to confound some responses and influenced traits involved with photosynthesis and competitive form changes such as plant elongation. Filamentous algae were found to proliferate even in mesocosms that were not dosed with additional nutrients, and reductions in algae in

higher flowing channels highlight the role of flow as a removal mechanism. Regardless of the range of conditions specimens of *R. pseudofluitans* were exposed to, there did not appear to be any situation which caused serious senescence in plants, with even the densest of algal growth not being enough, although plants were weakened by the presence of algae. It is advised that riverine flow conditions are maintained or improved in order to reduce the impact of filamentous algal growth, which can proliferate regardless of soluble phosphate concentrations. Furthermore, consideration of plant ontogeny may be significant in defining the impact environmental stimuli can have on plant responses.

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Chapter VI

Macrophyte development varies throughout ontogeny and is dependent upon differences in environmental conditions

6.1. INTRODUCTION

Phenotypic plasticity of aquatic macrophytes allows for rapid examination of stress-induced responses in impacted river systems (Chapters 3-5; Idestam-Almquist & Kautsky, 1995; Garbey *et al.*, 2004; Puijalon *et al.*, 2008; Lamberti-Raverot & Puijalon, 2012), and enables their use as indicators of environmental variability and degradation (Crossley *et al.*, 2002; Garbey, Thiébaut, *et al.*, 2004; Mony *et al.*, 2007). Whilst the importance of plant ontogeny (life-stage or age; Coleman *et al.*, 1994) is frequently commented on (Watson *et al.*, 1995; Trémolières, 2004; Boege, 2005; Mony *et al.*, 2007; Puijalon *et al.*, 2008; Riis *et al.*, 2009), experimental studies often fail to consider responses due to ages or plant developmental stages (Idestam-Almquist & Kautsky, 1995; Crossley *et al.*, 2002; O'Hare *et al.*, 2007), with most results reported relative to specimen sizes, regardless of ontogeny. Often, observation of ontogeny is limited by experimental design (i.e. space, time), but studies of ontogenetic variation should be attempted more frequently, as the impact natural life-stage development has on plastic responses, alongside variable environmental conditions, is poorly researched. This may be particularly important where climate change and anthropogenic influences threaten to alter the distribution of water, causing disturbance to aquatic communities (Ledger *et al.*, 2012), as markedly different responses are observed in response to varying environmental stress (Chapters 4 &5).

Many macrophyte species have distinct stages of development (Westlake, 1969; Coleman *et al.*, 1994; Davis & McDonnell, 1997), but natural ontogeny can be partially determined by environmental conditions, such as temperature (e.g. Sand-Jensen, 1989). For most macrophyte species these stages are approximately determined by seasonal influences including light availability and temperature changes (Davis & McDonnell, 1997), but vary between taxa. Davis & McDonnell (1997) propose that stages are characterised by growth (development post- germination or dormancy), maturation (production of flowers/seeds

and minimal growth), senescence (dying and decay, post- maturation or environmental damage), and dormancy (overwintering of plant material).

Ontogeny in plants affects morphological and physiological traits, with plant age influencing features such as leaves (e.g. variation in form/production rates, greater nutrient storage capacity; Coleman *et al.*, 1994; Boege, 2005) and root architecture (e.g. reduction in root growth due to enhanced resource allocation efficiency; Boege, 2005). Typically, plants show greater resilience and recovery to environmental stressors in later stages of growth, as there are fewer resource constraints (acquisition and allocation) and larger resource storage capacity (Boege & Marquis, 2005). This change in traits with plant age is known as 'ontogenetic drift' (Evans, 1972; Coleman *et al.*, 1994), and is an important consideration when studying phenotypic trait responses in relation to environmental conditions.

Phenotypic plastic responses vary widely with environmental conditions (Chapters 4 & 5; Sultan, 1987; Garbey, Thiébaut, *et al.*, 2004; Puijalon *et al.*, 2008), but ontogenetic variation, considered a significant influence on intraspecific trait variability (Weiner, 2004), may constrain responses and inhibit interpretation of environmental influence (Fu *et al.*, 2013). For example, macrophytes growing under different flow conditions, may experience variation in root:shoot ratios that are both flow dependent and life-stage dependent: Early root biomass in some herbaceous plants is said to be higher in comparison to shoots due to initial establishment, but ratios reduce once developed (Coleman *et al.*, 1994); macrophytes in faster flows have greater root biomass as an adaptive response (Barrat-Segretain, 2001), so these specimens may maintain a higher root:shoot ratios with age compared to plants in slower flows. Additionally, observed responses may vary depending on whether age or size (stage) are being considered, with optimal partitioning models often using assessments of age (regardless of stage; Coleman *et al.*, 1994) whereas other studies may examine developmental stage (but not age; Garbey, Thiébaut, *et al.*, 2004). Biomass

allocation patterns related to different environmental conditions may highlight changes in traits that also affect growth rates (e.g. slower rate due to increased resource allocation to specific organs), meaning plants of the same age may be at different developmental stages or sizes (Mooney *et al.*, 1988; Coleman *et al.*, 1994). Nevertheless, differences in rates of growth and resource allocation according to phenotypic plasticity is an important consideration, as this may have a significant bearing on plant fitness (Coleman *et al.*, 1994); this is of particular importance for lotic macrophytes where time and efficiency of development into maturity may impact on washout or damage survivability from mechanical, competitive and other stimuli.

Many studies examining plant trait changes to contrasting environmental conditions provide limited consideration of ontogenetic influence (e.g. Idestam-Almquist & Kautsky, 1995; Crossley *et al.*, 2002; Puijalon *et al.*, 2005; O'Hare *et al.*, 2007; Sand-Jensen, 2008; Lamberti-Raverot & Puijalon, 2012), and where discussed (Mony *et al.*, 2007; Puijalon *et al.*, 2008), is often an afterthought or poorly integrated into study methods. Furthermore, several studies indicate developmental stage during harvesting and planting methodologies (e.g. Puijalon *et al.*, 2005, 2008; Riis *et al.*, 2009; Lamberti-Raverot & Puijalon, 2012), but lack suitable discussions of how this may affect measured responses in the study.

Artificial stream mesocosms are useful tools for lotic studies, allowing for a greater level of control on environmental factors, whilst also exposing study specimens to controlled treatments to determine cause and effect responses (Chapters 3-5; Stewart *et al.*, 2013). Stream mesocosms best represent patches of river habitat (Harris *et al.*, 2007), and are therefore typically size, volume and/or flow constrained. Consequently, the use of these systems to test flow-responses of macrophytes that can reach large sizes (sometimes several metres) when mature (Rich & Jermy, 1998), may be inadequate. In longer studies, where ontogenetic effects are examined, it may be necessary to conduct experiments in field-

based river reaches, where water volume is less of a concern, although physicochemical variability may be an issue; in chalk rivers, however, potential confounding effects of environmental variation are reduced due to characteristically stable hydrological regimes and physicochemical conditions (Heywood & Walling, 2003).

The aim of this chapter is to determine whether plant growth characteristics remain consistent as the keystone chalk stream macrophyte *Ranunculus penicillatus* ssp. *pseudofluitans* var. *pseudofluitans* (herein *R. pseudofluitans*) develops from juvenility into maturity, in velocity confined and unconfined conditions, within a semi-natural chalk stream reach. Hypotheses tested were: 1) macrophyte morphological and physiological traits vary considerably between juvenile and mature plants; 2) progression into maturity (determined by presence of flowers/buds) is inhibited by varying velocity conditions; 3) plant age remains consistent, but developmental stage differs depending on environmental influence; 4) chalk water physicochemistry does not fluctuate significantly and is less influential on macrophyte development than differences in velocity. This will be achieved by using a similar harvesting and planting method as in stream mesocosms, with mixed modelling to examine influential environmental variables.

6.2. METHOD

6.2.1. Study site and experimental design

The study was performed over a 70-day period between April and June 2012 in the River Itchen at Ovington, Hampshire (**Figure 6.1. & 6.3.**; 51°04'59"N, 1°11'27"W). The site was selected to represent a typical upstream section of the River Itchen with relatively flushing flows (average velocities 0.3-0.6+ m s^{-1}) and high water quality, yet it is also considered

unusual due to several years of complete absence of *R. pseudofluitans*, with taxa such as *Berula erecta* and *Callitriche* spp. being dominant in its place.

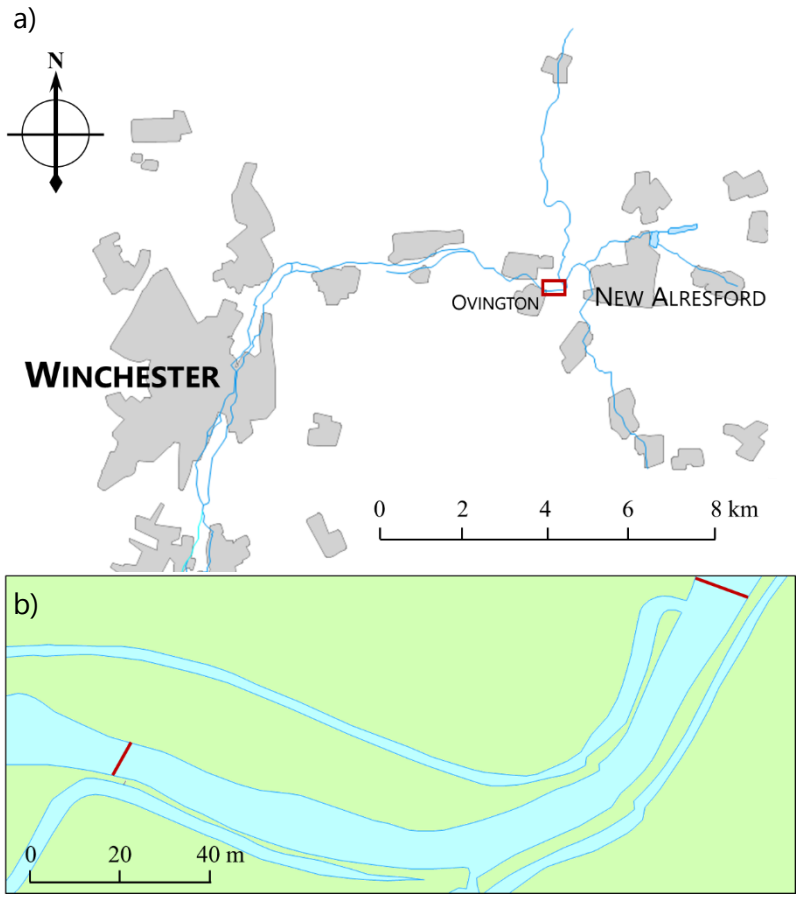

Figure 6.1.

Study site location on the River Itchen at Ovington, Hampshire. a) indicates the location of the study site in the upper Itchen catchment, b) details the study reach (red lines represent study boundary).

The field experiment was performed in a 100 m reach using a series of specimen planting plots, defined according to water velocities (**Figure 6.2.a**). Planting plots were arranged in 10 pairs (plot pairs A-J, downstream-upstream; each plot 2 m apart in cross-section) of velocity restricted (subplot 1; 0 – 0.1 m s^{-1}) and unrestricted (subplot 2; 0.3 – 0.6 m s^{-1}) locations, with 10 m between each pair. Each plot contained 5 replicate sample plant specimens, for a total of 100 plants. Plots were sited on comparable substrate (approx. particle sizes: 72 ± 1% 11-22 mm; 17 ± 1% 2-11 mm; 7 ± 1% 0.35-2 mm; 4 ± 1% <0.35 mm) with no riparian shading. Restricted velocity plots, used to imitate reductions in river flow, were located just downstream of flow deflector baffles (wooden barricades; **Figure 6.3.**),

positioned at 30° to the direction of flow, to divert flows and reduce velocities immediately behind. Baffles were monitored daily for one week prior to commencing the experiment, to determine variation in flow velocities and whether turbulent zones were created (**Figure 6.2.b**) and make sure light was not restricted by baffle positioning. Turbulent zones were small, not likely to interfere with planting plots, and velocities were consistently reduced downstream of the baffles. Planting plots were netted just above water surface to prevent swans and waterfowl grazing specimens.

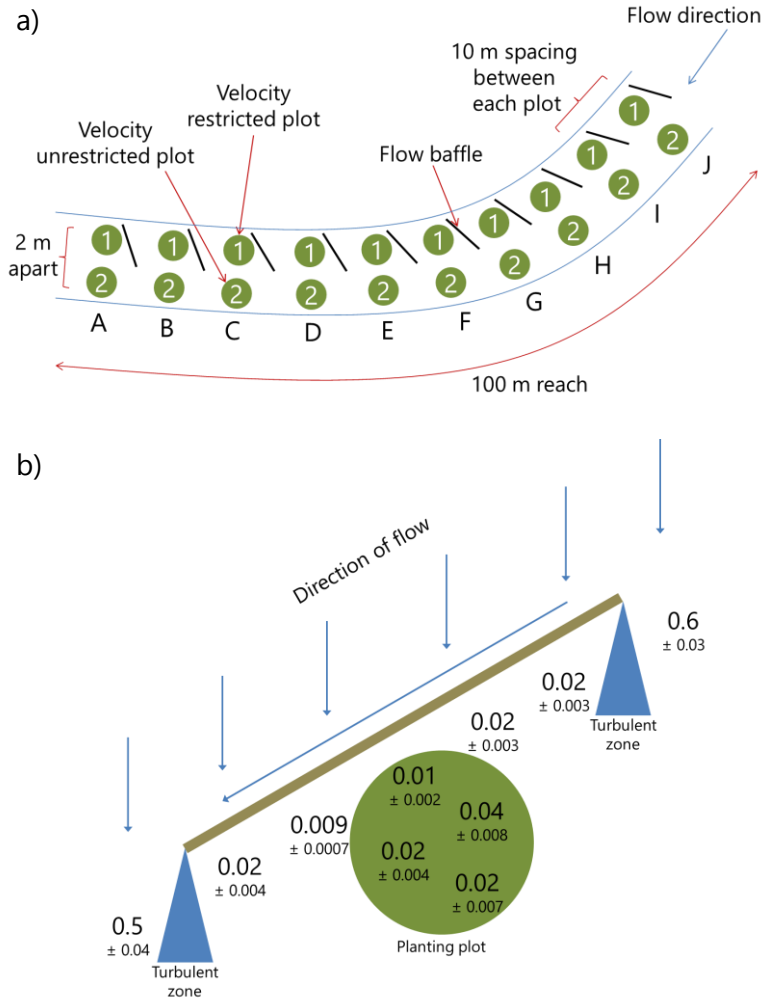

Figure 6.2.

Schematic diagrams of the experimental study site. a) shows planting plot locations (A-J, 1&2) within the study reach, and b) shows the flow deflector baffles with average velocity monitoring results (m s^{-1}) from all restricted plots.

Apart from velocity restrictions, no attempt was made to control riverine water physicochemistry, and specimens were subjected to the range of naturally occurring conditions during the study (**Figure 6.4.** & **Table 6.2.**).

Figure 6.3.

The study site, showing the wooden barricades used as flow baffles. Photograph taken at plot A at the downstream end of the study site.

6.2.2. Sampling process

6.2.2.1. Initial specimen harvesting and planting

As in mesocosm studies (Chapters 3-5), the keystone macrophyte *R. pseudofluitans* was used as the study taxon, and small fragments were used to simulate post-allofragmentation colonisation and development from a juvenile clonal fragment into maturity (defined here as the time taken to reach sexual reproductive capability; Davis & McDonnell, 1997).

100 genetically similar clonal specimen fragments were harvested at the start of the experiment (section 3.2.5.1., Chapter 3). Initial fragment lengths (437 ± 4 mm) and biomass measurements (6.64 ± 0.19 g f_w) were congruent. Specimens were longer and of greater mass than those used in mesocosms to allow for planting in the river bed substrate; approximately half of each specimen was buried in coarse gravel in a small pool made in the bed substrate. This additional buried plant material prevented initial washouts from occurring due to riverine debris, variations in flow etc.

6.2.2.2. Water physicochemistry

Riverine water physicochemistry was recorded weekly at each planting plot and analysed for velocity (m s^{-1}), water temperature (°C), pH, dissolved oxygen (%), conductivity (µS cm^{-1}),

nitrate (mg NO_3- l^{-1}) and soluble reactive phosphorus (mg PO_4- l^{-1}) – for methodological details, see section 3.2.5.2 (Chapter 3).

In addition, water depth (in cm; measured with a stainless-steel meter rule) was recorded on each sampling date, and fine sediment accumulation depth (in cm; measured by taking a core sample with a clear Perspex corer) was recorded at the beginning and end of the experimental period, with the difference representing accumulation rate over the study period. Suspended sediment was recorded, (section 3.2.5.2., Chapter 3), but was not detectable throughout the study. As in Chapters 4 and 5, discharge, turbulence (Reynolds numbers) and water residency were excluded due to collinearity.

6.2.2.3. Plant morphometry and physiology

Morphometric traits (Table 6.1.) - Macrophyte traits were recorded bi-weekly using the methods in section 3.2.5.4. (Chapter 3). In addition to those in Chapter 5, the following morphological traits were also included in this study: the number of flower buds and flowers. Relative rates of extension were then calculated, as per 4.2.3.2. (Chapter 4).

Biomass, biomechanical tissue properties, chlorophyll pigment concentrations and tissue nutrient concentrations (Table 6.1.) - Fresh/dry weights were used to calculate root:shoot allometric coefficients and relative growth rates (*RGR*), and stem and leaf tissue densities were all calculated as in 4.2.3.2. Leaf chlorophyll concentrations (Chlorophyll a, Chlorophyll b and Carotenoids) were determined as in 4.2.3.2. and total organic phosphate in plant tissues as in 5.2.3.3. (Chapter 5).

6.2.2.4. Algal biomass

Algal growth was not observed in any measurable quantities within planting plots during the experiment, and was therefore not used as an explanatory variable.

Table 6.1. **List of characteristic traits selected for sampling.** Updated from Chapter 5 – additions are highlighted in bold.

Identifier	Abbreviation	Plant characteristic trait	Measure of
i	L	Length of main stem	See 3.2.5.4. in Chapter 3.
ii	LL	Length of longest leaf (on main stem)	
iii	NL	Number of leaves	See 4.2.3.3. in Chapter 4.
iv	PI	Position of longest internode (on main stem)	See 3.2.5.4. in Chapter 3.
v	LI	Length of longest internode (on main stem)	
vi	SB	Length of longest secondary branch	
vii	TB	Length of longest tertiary branch	
viii	NB	Number of branches	See 4.2.3.3. in Chapter 4.
ix	AB	Angle of branches	See 3.2.5.4. in Chapter 3.
x	PR	Position of first adventitious roots	
xi	D	Percentage of damaged plants	See 5.2.3.3. in Chapter 5.
xii	W	Percentage of washouts	
xiii	**F**	**Number of buds and flowers**	Sexual reproductive capacity and maturation.
I	RGR	Relative growth rate	See 4.2.3.3. in Chapter 4.
II	R:S	Root:shoot allometric coefficient	See 3.2.5.4. in Chapter 3.
III	SD	Stem densities	See 4.2.3.3. in Chapter 4.
IV	LD	Leaf densities	
V	Ca	Chlorophyll a	
VI	Cb	Chlorophyll b	
VII	CAR	Carotenoids	
VIII	TP	Tissue phosphorus	See 5.2.3.3. in Chapter 5.

6.2.3. Data analysis

Analytical methods were comparable to those in 5.2.4. (Chapter 5). Data exploration was performed initially (Zuur, Ieno, & Elphick, 2009), and resulted in the removal of outliers; removal of collinear variables (VIFs > 3 (Zuur, Ieno, & Elphick, 2009) - sediment accumulation depth, temperature, pH, discharge, turbulence and water-residency); and selection of appropriate distributions. Zero-inflation was not observed. Sediment depth, a product of river flow and suspended sediments (Wood & Armitage, 1999), was minimal and found to be collinear with velocity (VIF >10), so excluded from analyses. Temperature and

pH showed slight collinearity with multiple explanatory variables, but varied little between planting plots; on this basis they were also excluded from analyses. Explanatory variables in final models were therefore: velocity, dissolved oxygen, conductivity, phosphate, nitrate, and water depth. Coplots of explanatory variables indicated no interactive effects were present between physicochemical variables, so interactions were excluded from models.

River water chemistry was analysed with repeated measures analysis of variance (RM ANOVA) to assess variation among planting plots. RM ANOVA was also used to assess differences between plant morphological trait RREs in restricted and unrestricted plots at mid-study (29 days) and end-study (71 days) sample dates to determine plant age effects.

Plant characteristics and environmental variable relationships were modelled using linear mixed-effects models with gaussian distributions (LMMs) in R (R Core Team, 2013), using the package *nlme* (Pinheiro *et al.*, 2013). Linear models were used because residuals showed linear patterns during data exploration (Zuur *et al.*, 2007) for all growth characteristics. Mixed-effects models were used as they allow the incorporation of variance structures and mixed-effects to account for nested experiments (Zuur, Ieno, Walker, *et al.*, 2009), which traditional statistical methods (e.g. ANOVA, basic regression) fail to account for. Models only included final sample data for plant traits, to prevent the need to incorporate temporal autocorrelation structures into models. Furthermore, as physicochemical temporal variation was largely consistent between plots (**Figure 6.4.**), influence of temporal variation on individual plots is minimal, so only end physicochemical data is used.

All plant morphological traits were used in both ANOVA tests and models, except numbers of damaged plants and washouts, which are only assessed qualitatively. Plant traits were modelled separately, and for each model, fixed structures, random structures, and optimal variance structures (**Table D.1.**, Appendix D) were first determined. Data transformations

were avoided (Zuur, Ieno, & Elphick, 2009). In all models, "planting plot" was defined as a random effect to account for nested measurements.

Best-fit models were determined using Akaike's Information Criterion *second-order* (AIC_C) using a top down stepwise selection approach, where model parameters were dropped depending on significance, and AIC_C was assessed until the best (lowest AIC_C) model with all significant parameters was determined (Burnham & Anderson, 2002; Zuur *et al.*, 2009b). AIC_C was used rather than AIC as it includes a small-sample bias correction (Burnham & Anderson, 2002). Coefficients of variation (R^2) were calculated using the mixed-effects models method by Nakagawa & Schielzeth (2013). This involves calculating two R^2 values: a marginal R^2 (R^2_{mar}) which shows variation explained by the fixed effects, and a conditional R^2 (R^2_{con}) which shows variation explained by fixed and random effects. In covariate selection, minimum significance was $p \leq 0.05$ for model parameters, although those close to the significance level should be treated with caution (Zuur, Ieno, Walker, *et al.*, 2009). Finally, model validation was performed on the best-fit model to determine if the model still adhered to model assumptions (Zuur, Ieno, Walker, *et al.*, 2009).

6.3. RESULTS

6.3.1. Water physicochemistry

River water physicochemistry varied between parameters (**Table 6.2.** & **Figure 6.4.**): velocity, conductivity, nitrate and water depth were all significantly different between planting plots, and temperature, pH, DO and SRP all had minimal variation between plots. Temporal variation was small for velocity, pH, nitrate and water depth (**Figure 6.4.**), but greater for temperature, DO, conductivity and SRP. All parameters experienced synchronous temporal changes among planting plots.

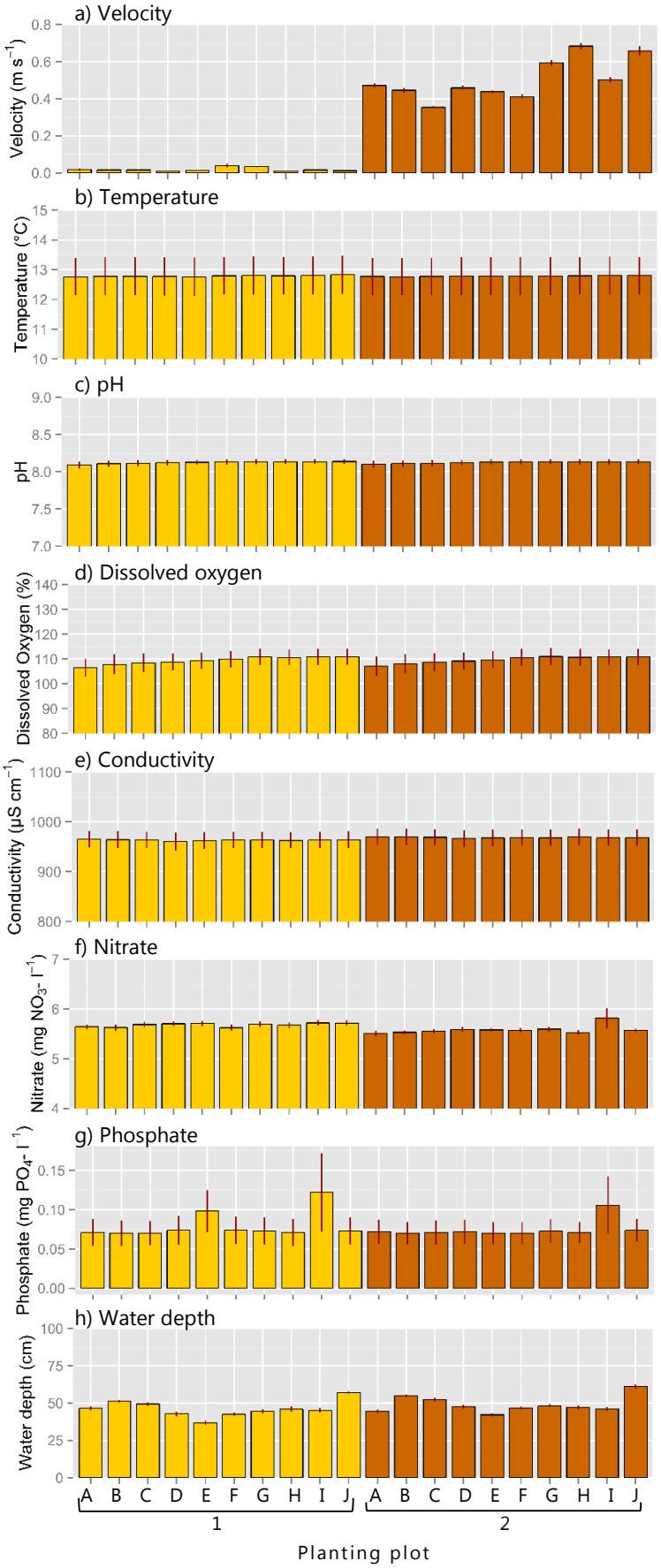

Figure 6.4.

Mean riverine planting plot physicochemistry throughout the study period. Light (left ten) bars represent restricted velocity plots, dark (right ten) bars represent unrestricted velocity plots. Parameters shown include a) velocity, b) temperature, c) pH, d) dissolved oxygen, e) conductivity, f) nitrate, g) phosphate, and h) water depth. Error bars represent temporal standard error (n = 11).

Table 6.2. **Mean, minimum and maximum physicochemistry and results of Repeated Measures Analysis of Variance on Ranks tests.** Performed between planting plots throughout the experimental period.

				RM ANOVA on Ranks Test Results		
	Mean	Min	Max	H	d.f.	*p*
Velocity (m s^{-1})	0.259	0.005	0.77	191.999	19	<0.001***
Restricted velocity plots	0.017	0.005	0.11	40.407	9	<0.001***
Unrestricted velocity plots	0.501	0.32	0.77	86.532	9	<0.001***
Temperature (°C)	12.8	9.6	17.1	24.367	19	0.182
pH	8.13	7.85	8.32	17.294	19	0.570
Dissolved oxygen (%)	109.5	75.2	135.9	7.025	19	0.994
Conductivity (µS cm^{-1})	966	865	1076	139.497	19	<0.001***
Nitrate (mg NO_3- l^{-1})	5.63	5.22	7.79	70.733	19	<0.001***
SRP (mg PO_4- l^{-1})	0.08	0.02	0.59	26.594	19	0.114
Water depth (cm)	48	31	68	189.433	19	<0.001***

*** p <0.001; ** p <0.01; * p <0.05

Flows in restricted plots were effectively stagnant (mean velocity 0.017 ± 0.002 m s^{-1}), and in unrestricted plots ranged from moderate flows (0.355 ± 0.005 m s^{-1} – plot C2) to brisk flushing flows (0.684 ± 0.016 m s^{-1} – plot H2). Stream temperature was considerably temporally variable, rising from its lowest 9.6°C on day 8 of the study, up to a relatively warm 17.1°C by day 36, before slowly decreasing again. Water chemistry at all planting plots throughout the study was characterised by slightly alkaline (pH 8.13 ± 0.01), highly oxygenated (109.53 ± 0.75 % DO) and relatively nutrient rich (0.08 ± 0.005 mg PO_4- l^{-1}, conductivity 966 ± 4 µS cm^{-1}) waters, although nitrate was lower than expected (5.63 ± 0.015 mg NO_3- l^{-1}).

6.3.2. Plant morphometry and physiology

There was a significant time effect relating to changes in morphological trait responses in both restricted and unrestricted flow plots (**Table 6.3.**, **Figure 6.5, 6.6. & 6.7.**). Mid-study trait RRE were found to be significantly different from trait RRE at the end of the study for all traits in unrestricted velocities and most traits in restricted velocity plots (**Table 6.3.**). As

physicochemistry was temporally low and synchronous among planting plots (see 6.3.1.), differences between sample dates can be attributed to plant ontogenetic influence.

Table 6.3. **Mean plant morphological trait RREs and results of Repeated Measures Analysis of Variance on Ranks tests.** Performed between mid-study (29 days) and end-study (71 days) samples in restricted and unrestricted velocity planting plots.

Morphological trait		Mean ± std. dev.		RM ANOVA on Ranks Test Results		
		29 days (mid-study)	71 days (end study)	H	d.f.	*p*
L	Restricted plots	35 ± 6	81 ± 88	7.338†	1	0.012*
	Unrestricted plots	12 ± 3	148 ± 91	104.959†	1	<0.001***
LL	Restricted plots	15 ± 17	2 ± 44	2.256†	1	0.145
	Unrestricted plots	2 ± 1	1 ± 1	37.964†	1	<0.001***
NL	Restricted plots	0.5 ± 0.5	2.5 ± 1.5	15.696	1	<0.001***
	Unrestricted plots	3.1 ± 1.4	16 ± 6.2	47.000	1	<0.001***
PI	Restricted plots	-3 ± 0.7	0.5 ± 0.5	27.000	1	<0.001***
	Unrestricted plots	-0.6 ± 0.6	2.5 ± 0.5	47.000	1	<0.001***
LI	Restricted plots	37 ± 1	58 ± 12	75.524†	1	<0.001***
	Unrestricted plots	2 ± 1	23 ± 15	35.766	1	<0.001***
SB	Restricted plots	1 ± 5	115 ± 39	235.534†	1	<0.001***
	Unrestricted plots	18 ± 11	591 ± 233	47.000	1	<0.001***
TB	Restricted plots	0 ± 0	25 ± 27	15.000	1	<0.001***
	Unrestricted plots	5 ± 12	294 ± 143	47.000	1	<0.001***
NB	Restricted plots	0 ± 0.2	1.6 ± 0.8	23.148	1	<0.001***
	Unrestricted plots	0.5 ± 0.5	4.5 ± 2	47.000	1	<0.001***
AB	Restricted plots	38 ± 14	36 ± 14	1.000	1	0.727
	Unrestricted plots	1.7 ± 13.4	-2.2 ± 2.7	4.087†	1	0.490*
PR	Restricted plots	0.7 ± 0.5	0.8 ± 1	0.088†	1	0.769
	Unrestricted plots	0.6 ± 0.5	-0.4 ± 1	19.600	1	<0.001***
F	Restricted plots	0 ± 0	0 ± 0	1.000	1	0.317
	Unrestricted plots	0 ± 0.2	7 ± 4	154.976†	1	<0.001***

*** p <0.001; ** p <0.01; * p <0.05

† Normality assumption passed and standard RM ANOVA run instead; 'H' represents F value in this case.

Generally, at 29 days plants in restricted velocities elongated more quickly, had longer but fewer leaves, longer internodes which were closer to the apex, and few secondary and tertiary branches which were smaller and at greater lateral angles, in comparison to plants in unrestricted velocities. All plants were observed to have first adventitious roots approximately 3-4 internodes away from the apex, and only one plant was observed to have a single flower in unrestricted velocities. Additionally, 5 plants were

damaged and 4 washouts occurred in restricted plots, compared to none in unrestricted plots. Significant reductions in leaf lengths in restricted plots are likely attributable to damage weakened plant form and damage from river debris.

At 71 days, some patterns between restricted and unrestricted plots had changed from those seen at the mid-study sample. Plants in unrestricted plots were elongating more quickly (although rates of elongation were beginning to slow compared with previous weeks), still had shorter but markedly more leaves, shorter internodes which remained further from the apex, and were considerably multi-branched with lengths better representing overall elongation, compared to restricted plots. Adventitious roots were also closer to the apex and most plants had multiple flowers and buds in unrestricted plots, suggesting these plants had reached maturation. Furthermore, in restricted plots, multiple plants appeared damaged (with stem breakages and leaf losses), and 17 additional plants had washed away by the end. For most morphological traits, plants in restricted plots had greater variation around the mean than those in unrestricted plots, suggesting greater unpredictability in trait responses.

At 71 days, most plant physiological traits were considerably different between restricted and unrestricted plots (**Figure 6.8.**). Plants in unrestricted plots had greater root mass allocation proportional to above substrate biomass, higher relative rates of biomass accumulation, higher leaf and stem tissue densities, higher concentrations of chlorophyll lower carotenoid concentration, and higher phosphorus concentrations in tissues when compared to plants in restricted plots.

In addition to pre-chosen traits, it was also noticed that there were considerable differences in the ratio of leaf lengths to internode lengths (the leaf:internode allometric coefficient) between velocity restricted and unrestricted plants depending on plant age. At mid- study,

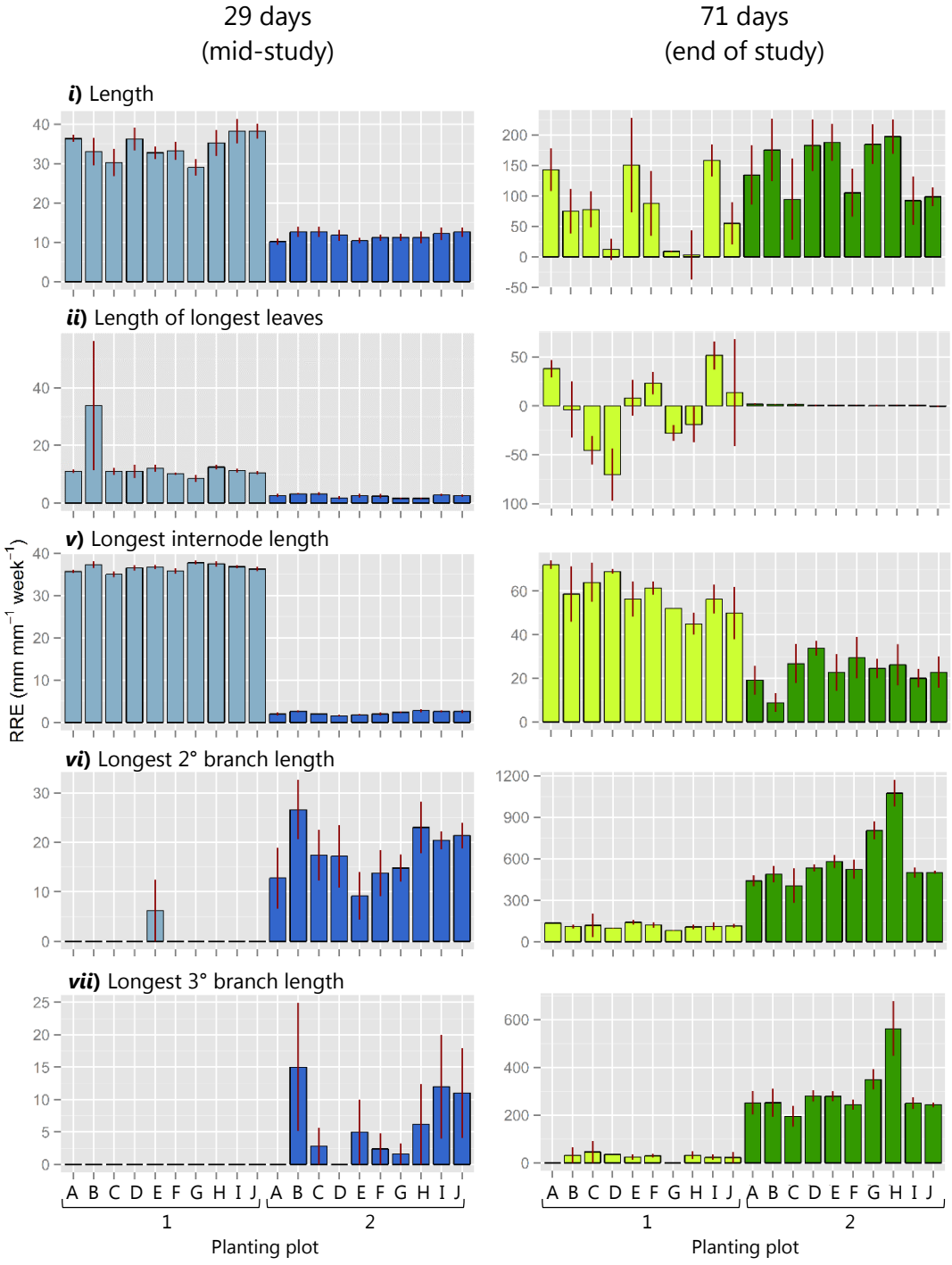

Figure 6.5. **Length-based plant morphological traits compared between planting plots and at mid-study (29 days) and end-study (71 days) sampling dates.** Left-hand plots represent mid-study samples and right-hand plots represent end-study samples. Light (left ten) bars represent velocity restricted planting plots and dark (right ten) bars represent velocity unrestricted planting plots. Length-measured morphological traits include *i*) length, *ii*) leaf length, *v*) length of longest internode, *vi*) length of longest secondary branch, *vii*) length of longest tertiary branch. Error bars represent standard error among replicates (n = ~5).

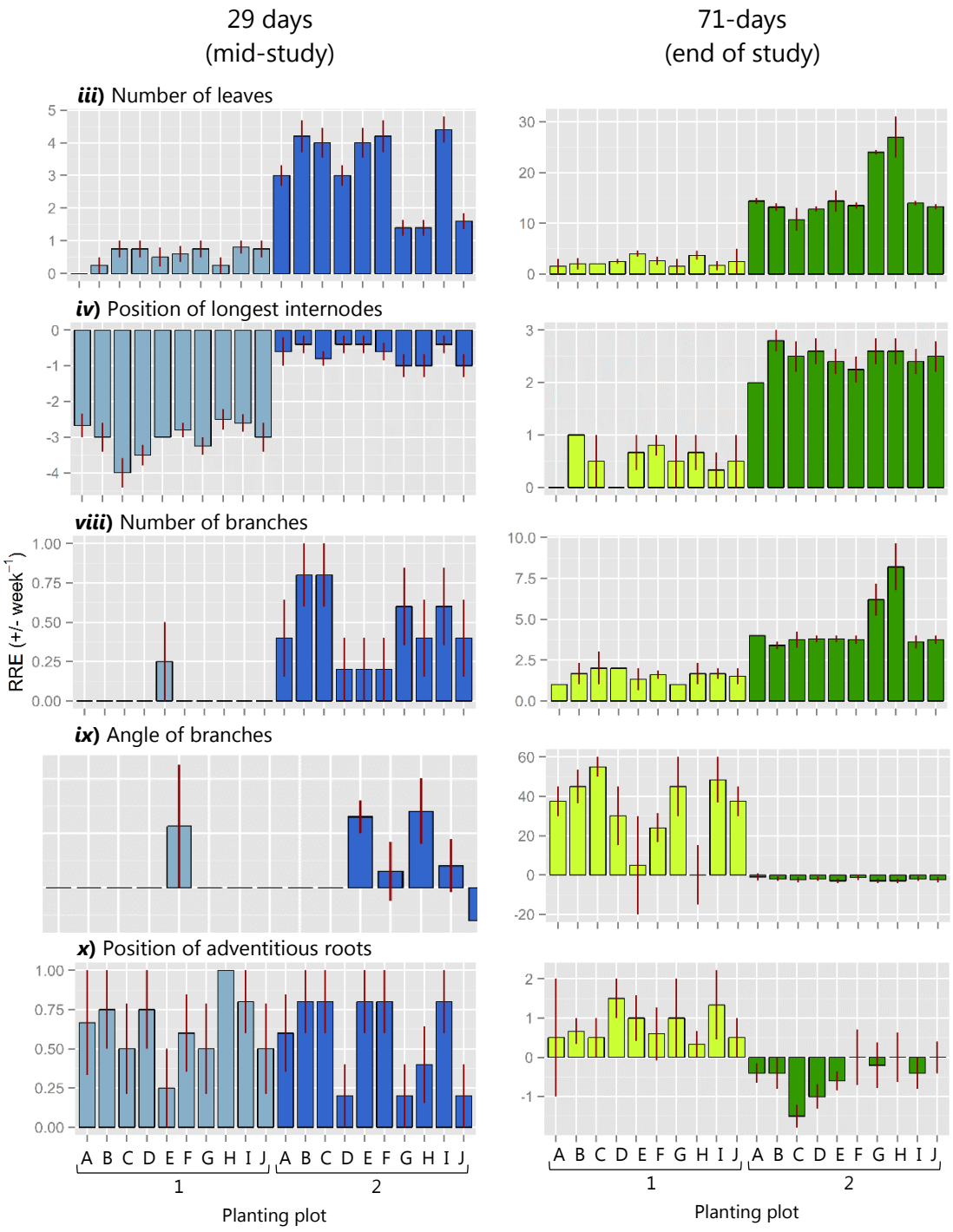

Figure 6.6. **Count-based plant morphological traits compared between planting plots and at mid-study (29 days) and end-study (71 days) sampling dates.** Left-hand plots represent mid-study samples and right-hand plots represent end-study samples. Light (left ten) bars represent velocity restricted planting plots and dark (right ten) bars represent velocity unrestricted planting plots. Morphological traits include *iii*) number of leaves, *iv*) position of longest internode, *viii*) number of branches, *ix*) branching angle and *x*) position of first adventitious roots. Error bars represent standard error among replicates (n = ~5).

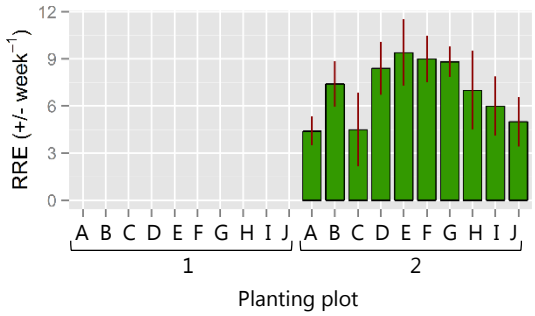

Figure 6.7. **Number of flowers and buds compared between planting plots at the end of the study (71 days).** Light (left ten) bars represent velocity restricted planting plots and dark (right ten) bars represent velocity unrestricted planting plots. Error bars represent standard error (*n* = ~5).

ratios for restricted plants were high (1.27 ± 0.04) and for unrestricted plants lower but closer to 1 (0.88 ± 0.03). At the end of the study, ratios were 0.74 ± 0.02 restricted, 0.95 ± 0.04 unrestricted, indicating greater leaf:internode changes for restricted plots and small changes to plants in unrestricted plots that meant leaf and internode lengths were highly comparable.

Best-fit model selection - The following linear mixed model was fitted to data for each plant trait to assess environmental influence:

$$T_{is} = \alpha + \beta_1 \times X_{1is} + a_i + \varepsilon_{is} \qquad 6.1.$$

$$\varepsilon_{is} \sim N(0, \sigma_i^2) \qquad 6.1.a.$$

where T_{is} is the plant trait for observation i in plot s, X_{1is} is the associated explanatory variable, α is the intercept, β is the slope, a_i is the random component, normally distributed with expectation 0 and variance σ_a^2, and ε_{is} represents residuals (6.1.a.), normally distributed with mean 0 and a variance defined by the associated structure in **Table D.1.** (Appendix D). Models with multiple explanatory variables were incorporated by adding additional covariate elements in as required after the first explanatory term in the form + $\theta_2 \times X_{2is}$ + ... + $\theta_M \times X_{Mis}$ (see **Table 6.4. & 6.5.** for included explanatory variables).

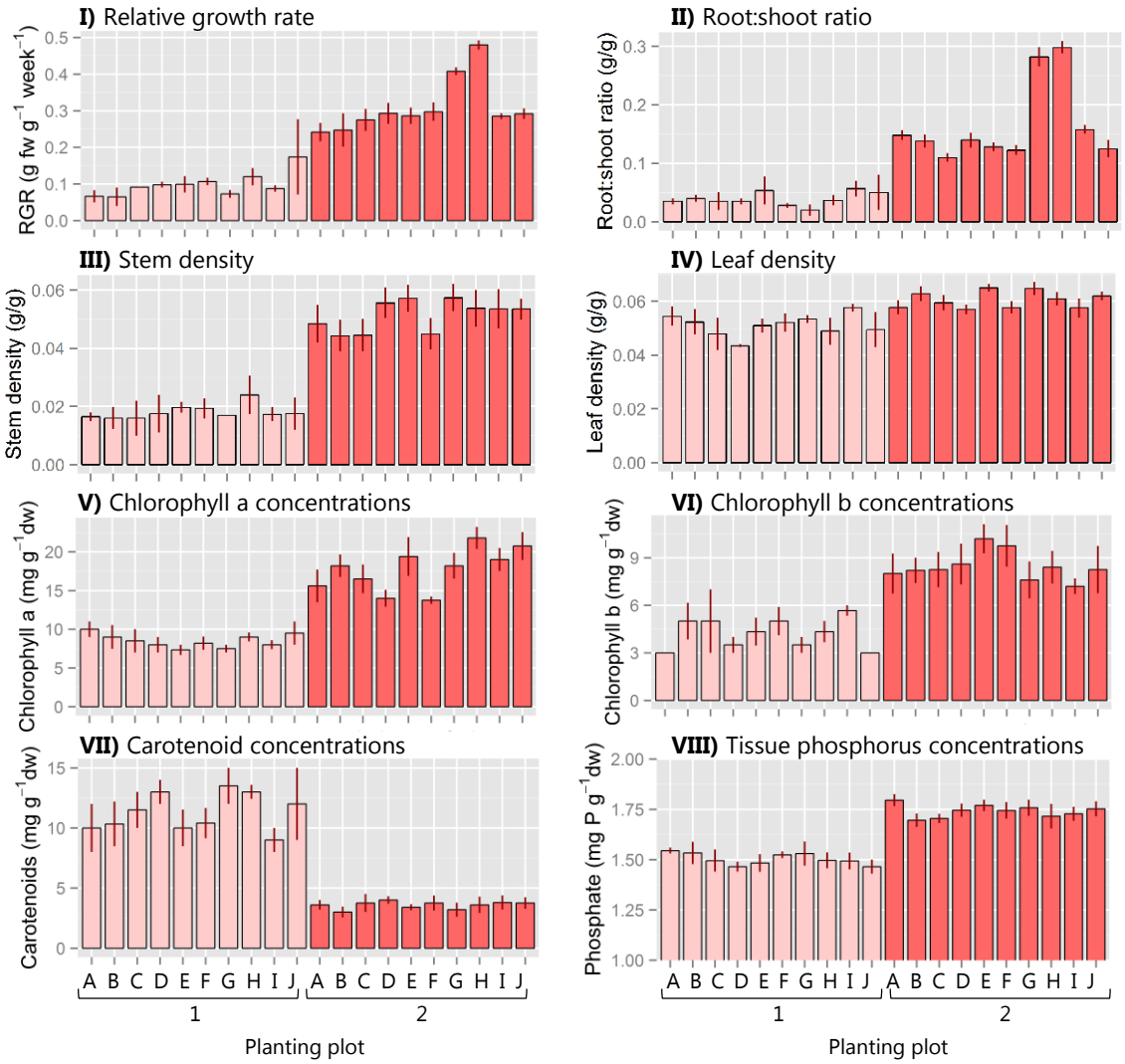

Figure 6.8. **Plant physiological traits compared between planting plots at the end of the study (71 days).** Light (left ten) bars represent velocity restricted planting plots and dark (right ten) bars represent velocity unrestricted planting plots. Physiological traits include I) Relative growth rate, II) root:shoot allometric coefficient , III) stem densities, IV) leaf densities, V) chlorophyll a concentrations, VI) chlorophyll b concentrations, VII) carotenoid concentrations, and VIII) tissue phosphorus concentrations. Error bars represent standard error (n = ~5).

Morphometric trait model outcomes - Best-fit models (**Table 6.4.**) for plant morphological traits agree with graphical observations of physicochemistry and plant morphology (**Figure 6.4., 6.5., 6.6. & 6.7.**).

Table 6.4. **Morphological trait vs. environmental variable best-fit model summaries.** Using linear mixed models. Model statistics: R^2_{mar} is the marginal R^2, R^2_{con} is the conditional R^2, and *p* signifies the *p*-values of the model components. "-" indicates no significant parameters were present in any model.

Response variable (morphological trait)	Best-fit model components	Model statistics		
		R^2_{mar}	R^2_{con}	*p*
L	Vel	0.158	0.158	0.002**
LL	Dep	0.191	0.191	0.04*
NL	Vel + Cond	0.900	0.978	<0.001*** + 0.026*
PI	Vel	0.750	0.776	<0.001***
LI	Vel	0.580	0.605	<0.001***
SB	Vel	0.999	0.999	<0.001***
TB	Vel	0.987	0.987	<0.001*** + (0.06)
NB	Vel	1	1	<0.001*** + (0.06)
AB	Vel	0.028	0.029	<0.001*** + (0.092) + (0.181)
PR	Vel	0.241	0.241	<0.001*** + (0.057)
F	Vel	0.999	0.999	<0.001*** + (0.144)

*** p <0.001; ** p <0.01; * p <0.05

All best fit models (excepting leaf length) signified velocity was the main explanatory variable driving trait responses, and therefore explains the differences observed in morphology between restricted and unrestricted plots. Water depth was considered the driver for leaf lengths according to the best-fit model, although variance explained was low (R^2_{mar} = 0.191) indicating only minor influence. In addition to velocity, NL was also positively associated with conductivity, which was unexpected, as conductivity varied very little between mesocosms during the study. Models typically had high coefficients of variation (R^2_{mar} > 0.5), which suggests environmental parameters explain much of the variation in plant form. In particular, variations in NL, SB, TB, NB and F were almost entirely explained by water velocities (R^2_{mar} > 0.9).

Physiologic trait model outcomes. As with morphology, best-fit models (**Table 6.5.**) for physiological traits agree with graphical observations of physicochemistry and plant physiology (**Figure 6.4. & 6.8.**).

Table 6.5. **Physiological trait vs. environmental variable best-fit model summaries.** Using linear mixed models. Model statistics: R^2_{mar} is the marginal R^2, R^2_{con} is the conditional R^2, and *p* signifies the *p*-values of the model components. "-" indicates no significant parameters were present in any model.

Response variable (physiological trait)	Best-fit model components	Model statistics		
		R^2_{mar}	R^2_{con}	*p*
RGR	Vel	0.787	0.992	<0.001***
R:S	Vel + Cond	0.888	0.943	<0.001*** + 0.02*
SD	Vel	0.968	0.968	<0.001***
LD	Vel	0.367	0.391	<0.001***
Ca	Vel	0.960	0.960	<0.001***
Cb	Vel	0.424	0.445	<0.001***
CAR	Vel	0.577	0.595	<0.001***
TP	Vel	0.848	0.848	<0.001***

*** p <0.001; ** p <0.01; * p <0.05

As with morphometric models, all physiological models indicate velocity was the main explanatory variable driving differences in plant functional responses. R:S ratios were also partially explained by an unexpected positive link with conductivity; as for NL, it is unknown why this was included in the best-fit model. Similarly high coefficients of variation ($R^2_{mar} > 0.5$), denote that much variation is attributable to environmental factors. In particular, SD, Ca were almost entirely explained by velocity ($R^2_{mar} > 0.9$), and R:S and TP were similarly mostly explained by velocity ($R^2_{mar} > 0.8$).

6.4. DISCUSSION

The prolonged timescale and experimental design in this study meant that ontogeny could be more easily assessed alongside the effects of water velocity. It was determined that both ontogenetic and water velocity effects were significant in affecting the characteristic traits of study specimens. Water velocity, as an environmental influence, drove differences in traits that produced optimum growth in faster flows, but the assessment of these differences were very much dependent on ontogeny. Ontogenetic influence showed that

plant age was important for the assessment of individual trait responses to environmental effects, and that plant developmental stage was dependent on a combination of age and environmental responses.

Riverine macrophytes are highly useful, sensitive indicators of environmental change (Onaindia *et al.*, 2005; Clayton & Edwards, 2006; Kopeć *et al.*, 2010; Sondergaard *et al.*, 2010) and plastic trait responses may be used as warning signs of degrading water quality. However, failure to consider effects of ontogeny on observed responses may result in skewed data and incorrect interpretation, which from a management perspective, may mean inappropriate actions taken for mitigation, remedial and conservation work.

This study attempted to determine whether significant differences in plant growth exist dependent upon age and environmental flow pressures, by cultivating juvenile specimens of the aquatic macrophyte *Ranunculus pseudofluitans* in a semi-natural river reach. Changes in developmental rates of characteristic morphological and physiological traits were examined during ontogeny in flow restricted and unrestricted planting plots that were exposed to a range of naturally variable physicochemical conditions.

River water physicochemistry was temporally variable throughout the study, but variation was consistent between planting plots for each sample date. Between-plot differences were greatest for velocity, conductivity, nitrate and water depth, and minimal for all other water chemistry. Most plant-environment relationships included some of these four explanatory parameters. Flow velocities were, by the nature of the experiment, markedly different between restricted and unrestricted velocity plots, but unrestricted plots also varied considerably from each other, providing a range of moderate to fast flows. Water temperatures were similar to other chalk rivers in April-June (Allen *et al.*, 2010), with temporal variation in line with prevailing weather conditions and air temperatures

experienced on each sample day. pH remained consistently slightly alkaline due to the presence of bicarbonate in chalk waters (Howden *et al.*, 2010), dissolved oxygen was typically saturated and well above 'high' quality standards (UKTAG, 2008; Howden *et al.*, 2010), and conductivity was significantly higher (~150 – 180% higher) than observed in other chalk rivers (Allen *et al.*, 2010). Nitrate remained close to 'very low' concentrations (Environment Agency, 2013) and approximated concentrations seen in other chalk rivers (Howden & Burt, 2008), but phosphates varied considerably, and whilst generally remained below 'moderate' quality thresholds (UKTAG, 2013), several peaks were observed substantially higher and close to 'poor' thresholds. It is unknown what caused these significant fluctuations, although there may be watercress/fish farms upstream from the site, and whilst no filamentous algae colonised plant specimens during the study, these high phosphate peaks may in part explain the presence of large quantities of algae observed in other areas of the study reach.

Overall plant form was significantly different both between mid-study (late-juvenile) and end of study (entering maturity) growth stages, but also between restricted and unrestricted velocity plots. Furthermore, the impact of velocity was determined as the main driving environmental parameter for differences in plant development. An unexpected finding was that higher flow velocities did not appear to restrict plant growth, as found in Chapter 4. It is possible this may be related to early-study upstream natural riverine vegetation providing shelter for juvenile plants and reducing velocities close to the river bed to below the 0.5 m s^{-1} 'optimum zone' in the swiftest flowing planting plots. This is an important finding, as mature plants exposed to the full force of 0.6-0.7 m s^{-1} velocities thrived in these conditions, suggesting optimum velocities determined in Chapter 4 may only be applicable to juvenile plant colonists.

Only specimens in the unrestricted plots displayed obviously different growth stages: An initial establishment stage occurred, involving slow but regular elongation, limited growth of adventitious roots and the development of lateral branches (weeks 0-5); following this, elongation, lateral expansion and leaf production occurred rapidly, then began to slow as adventitious root growth enhanced and flower/bud production began (weeks 5-11). Garbey, Thiébaut, *et al.* (2004) observed considerably different stages of growth for *R. peltatus*, with stages also occurring more slowly.

Mid-study morphological traits in restricted velocities differed from plants in unrestricted velocities by elongating more quickly, having longer and fewer leaves, longer internodes close to the apex, and minimal branching. Additionally, more plants were damaged and washed away in restricted velocities. Many of these traits suggest poor growth, and in particular lack of internodal development and lateral expansion indicate suboptimal growing conditions (Garbey, Thiébaut, *et al.*, 2004; Garbey *et al.*, 2006). At the end of the study, velocity restricted plants began to develop laterally, but traits remained slow growing, and increasing washouts and damaged plants further indicate suboptimal growth.

Physiological measurements also differed significantly between restricted and unrestricted plots. Root:shoot ratios were high, indicating greater root development, in plants growing in higher velocities. This agrees with previous findings (Chapters 4 & 5; Idestam-Almquist & Kautsky, 1995; Puijalon *et al.*, 2005), but plants in highest velocities (0.6+ m s^{-1}) had considerably higher root biomass relative to shoots. This was due to a large number of adventitious roots along stems closer to the river bed taking root in bed substrate, which may be a necessary adaptation as plants elongate, and demonstrates a form of ontogenetic drift from juvenile state into maturity (Evans, 1972; Coleman *et al.*, 1994). Relative growth rates were considerably higher for plants in faster flows, as expected in healthy developing plants (Halcrow Ltd., 2004). Stem densities were also slightly higher in faster flows,

contrary to previous findings (Chapters 4 & 5), although relatively reduced stem densities in flow restricted specimens accounts for much of the difference. Reduced stem density, associated with greater flexibility to enhance reconfiguration but reduced breakage resistance (Puijalon *et al.*, 2008), is likely related to greater biomass accrual, and substantiates the reason behind the greater numbers of damaged and washed away plants in lower flows. Chlorophyll was higher under faster flows, but of particular interest are the very high concentrations of carotenoids compared to chlorophyll in restricted velocity plots, which may indicate senescence (Sims & Gamon, 2002).

In contrast to mesocosm experiments (Chapter 5), phosphorus storage in plant tissues appeared unrelated to riverine SRP availability. The impact of water velocity on tissue phosphorus concentrations was more important, and with SRP concentrations consistent between planting plots, it would be unusual to expect differences related to riverine SRP. In this study, tissue phosphorus was positively correlated with increasing water velocities, suggesting reductions in diffusion boundary layers (Crossley *et al.*, 2002; Mommer & Visser, 2005). Additionally, tissue phosphorus concentrations in *R. pseudofluitans* specimens were noticeably lower than measured for related taxa in other studies (Spink *et al.*, 1997; Garbey, Murphy, *et al.*, 2004), despite being observed in similar physicochemical conditions. However, whilst there were no apparent correlations between riverine phosphate and tissue phosphorus, usually indicating P-limitation (Spink *et al.*, 1997), concentrations of phosphorus in *R. pseudofluitans* were still in excess of the critical concentrations (1.3 mg P g^{-1}) suggested to be the minimum required for maximum potential yield (Gerloff & Westlake, 1982), indicating that phosphorus was not likely to be limiting.

Findings relating to leaf:intenode allometry may have significant potential for assessing plant condition in future studies. The ratio of leaf length to internode length is commonly associated with taxonomic separation of *Ranunculus* spp. (Webster, 1988), but differences

here indicate an alternative use. Healthy plants growing in faster flows had ratios that were *c.* 1:1 (or with leaves parallel to, or slightly shorter than, corresponding internodes) and plants in lower flows had leaves either considerably longer or considerably shorter than internode lengths, and rarely had ratios of 1:1. It may therefore be possible to use this allometric trait as a field-tool for determining flow related stress; however, this will need additional examination first, particularly as confounding influences are possible.

Variations in developmental characteristics highlight plasticity in overall growth strategies to deal with environmental stress/stimuli (Grime, 1979), in addition to trait adaptive responses. Specimens grown in faster flows initially allocated more resources to functional traits, such as increased root production, reduced leaf and stem lengths, and increased lateral development and reduced branching angles, thereby enhancing anchorage (Idestam-Almquist & Kautsky, 1995; Puijalon *et al.*, 2005) and streamlining shape to reduce drag and lift forces (Sand-Jensen, 2008). This early development rewards specimens with enhanced growth leading into maturity; at this point elongation slows and production of reproductive organs occurs (Davis & McDonnell, 1997). In contrast, specimens in slower flowing conditions elongate significantly faster and produce larger leaves in an attempt to gain photosynthetic advantages (Hilton *et al.*, 2006), but appear more susceptible to damage or uprooting as a consequence. When reaching the equivalent age, where plants in faster flows have sexually matured, specimens in restricted velocities do not show signs of maturation, and seem to be present in a different developmental stage, only just beginning to produce lateral branches. This age-stage difference is commented on by Coleman *et al.* (1994), who warn of the difficulties of ontogenetic interpretation on the basis of only age or stage. In this study, with the consideration of both ontogenetic variation according to age and stage, environmental stimuli (in the form of mechanical resistance) causes plants to build supporting traits and enabling successful rapid transition into

maturity on the one hand, and poor adaptive development that creates weakened specimens that lag behind in developmental stage on the other (**Figure 6.9.**).

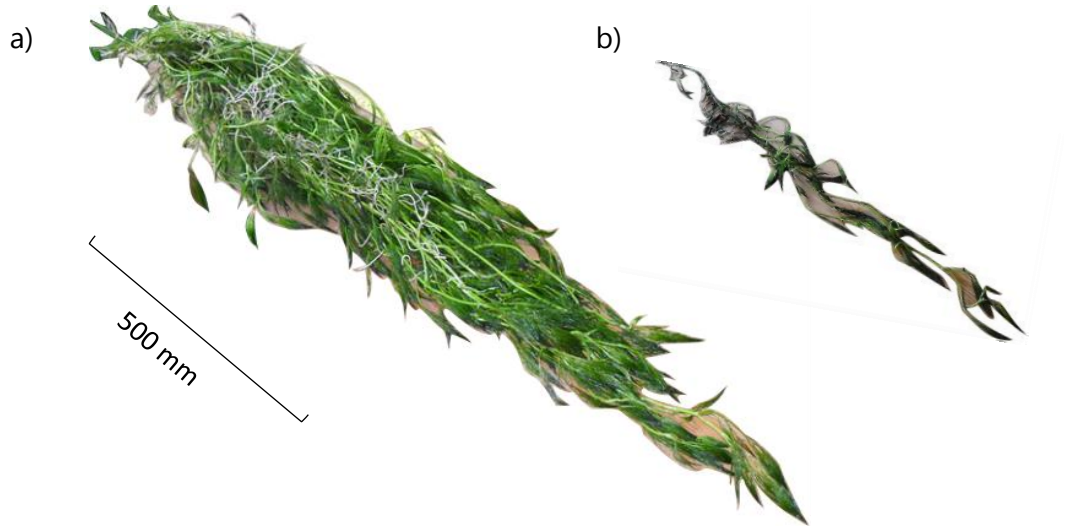

Figure 6.9. **Photographs of uprooted *R. pseudofluitans* specimens.** a) represents an average-sized specimen from an unrestricted velocity plot, and b) represents an average-sized specimen from a restricted velocity plot. Specimens are on a relative scale to each other.

The CSR (competition, stress tolerant, ruderal) model developed by Grime (1979), often encounters difficulties in its application to aquatic systems (Garbey, Thiébaut, *et al.*, 2004), but may fit with responses seen in *R. pseudofluitans* here. Typically, *R. pseudofluitans* is competitively dominant due to rapid growth and tolerance of a range of conditions (Franklin *et al.*, 2008). In low flow situations, developmental advantages appear to be reduced, and rapid biomass accumulation does not occur. It is possible that in these situations, taxon competitive advantage is also reduced, enabling other macrophyte species and herbivory pressures to outcompete and graze respectively. If plant specimens in slower flows therefore remain weaker for longer, there are greater opportunities for competitive exclusion to occur. This is a significant concern in the face of future reduced flows from climate change and abstraction, particularly as *R. pseudofluitans* is well known as a biological engineer that is able to promote healthy, heterogeneous flow conditions, and

enhance overall community diversity (Wharton *et al.*, 2006). Robust, effective future management of flow conditions in chalk rivers is required to promote the growth of this keystone macrophyte, which in return will enhance riverine biodiversity and water quality.

A further consideration, and limitation of this study, relates to flow frequency and magnitude changes. Flow conditions in this study had reasonably limited variation, with developmental plant responses relating to these. Future climate change is also suggested to bring more variability in flow conditions (Milly *et al.*, 2005), which may affect plant growth differently. Weaker specimens are likely to be more susceptible to this variation, but adaptive responses will probably be increasingly more complex. Additional work is necessary to determine the effect of this for future resilience.

6.5. CONCLUSION

Ontogenetic influence on macrophytic growth is, by its nature, critically important in the consideration of macrophytic development and success. However, many studies examining adaptive trait responses and growth strategies under varying environmental conditions often do not consider how growth stage or plant age might impact on the types of responses observed. In this study, both the effects of ontogeny (mainly as plant age) and water velocity (as a mechanical stimulus) were found to significantly affect plant characteristic trait responses of *Ranunculus pseudofluitans*. Ontogenetic effects were expressed as characteristic life-stage forms dependent on age, with plants in faster flows reaching sexual maturity relatively rapidly (~5/6 weeks) and plants in low flows lagging significantly behind in developmental stage. Faster flows produced stronger, more streamlined plants, typically with smaller features, but highly rooted and with significant lateral development, and therefore overall biomass, in contrast to lower flows. Heavy resource allocation to supporting structure (roots, lateral branching, adventitious roots)

early-on in fast-flow specimens plays an important function in establishment and overall success. The study also advocates several important considerations relating to plant ontogeny and/or plant surveys/studies: Firstly, that plant age or developmental stage should be a key part of any study involving highly plastic macrophytic vegetation, to avoid potentially inaccurate inference – as an example, higher flows in this study did not reduce growth and change plant form in mature plants, but may have done if exposed when in juvenile form; secondly, observations of key traits (such as the leaf:internode ratio) may be crucial in detecting early signs of environmental impacts, and warrants further investigation; and thirdly, establishing environmentally healthy flows to prevent weaker plant forms from developing should be a serious consideration for overall plant community success in chalk rivers. Finally, if weaker plant growth forms are possible in steady flows, understanding the effects of changing magnitude and frequency under different climate scenarios may be of paramount importance for future resilience in chalk macrophyte communities.

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CHAPTER VII

General Discussion

7.1. CHALK FLOW REGIMES AS REGULATORS OF RIVERINE WATER QUALITY AND MACROPHYTE COMMUNITY ASSEMBLAGE

The research project has taken the approach of assessing whole-river and river-reach scale macrophyte community dynamics to determine overall driving factors affecting community assemblage in a classic chalk stream, but then significantly scaling down to use experimental systems in order to test key environmental parameters and autecological responses of a keystone taxon that was still fundamentally under-studied in the literature. Examination of larger spatiotemporal scales enabled assessments of community distribution in accordance with downstream gradients of spatial change and temporal influences across distinct time-periods of different flow conditions. This has highlighted important patterns in general community turnover and key species linkages with environmental perturbations, and signifies the importance of river flow as a controlling mechanism. Furthermore, autecological investigations further highlight the significance of maintaining good flow conditions for healthy macrophyte growth throughout colonisation, establishment and maturation, and for the control of detrimental filamentous algae. Future management and conservation strategies are strongly recommended to consider, where possible, maintenance of good flow regimes to promote healthy macrophytic development.

Chalk macrophytes are so highly dependent on the characteristic stability of the chalk flow regime, and associated physicochemical conditions, that even with considerable evolutionary adaptive plasticity, changes in stream conditions due to anthropogenic influence and climate variability are typically greater than individual plant capacity to cope with multiple disturbances. Consequently, in chalk systems with multiple stressors, there is a high likelihood of macrophyte loss and subsequent changes in community structure.

By maintaining good river flow, the additional impacts of other stressors (e.g. nutrients, competition, siltation) are to some extent mitigated, improving conditions for colonisation of fragments and strong development of individual plants, which in turn promote ecological and hydrological heterogeneity, enhance floral diversity and provide support for riverine fauna.

The River Itchen is faced with increased frequencies of low-flow periods (Chapter 2; Cranston & Darby, 1992, 1995, 1997; Marsh & Turton, 1996), and in conjunction with greater nutrient and sediment input than experienced earlier in the last century (Butcher, 1927), pressures on the submerged aquatic macrophyte community will continue unless either, a) unrealistic rapid preventative actions are taken to reduce abstraction permits and minimise phosphorus and suspended sediment inputs, or b) mitigating methods are adopted to limit reductions in river flow and avoid limited dilution during periods of low-flow. Mitigating methods might include modification to river channels to allow deeper flowing sections during low-flow periods, or modification of river weed-cutting management regimes to raise river levels (Cox, 2008). More importantly, it may be necessary to take into consideration the historic management of the river, and appraise the removal of structures such as weirs and impoundments, which can impede river flows and act as storage reservoirs for particulate forms of phosphorus and algae (Withers & Jarvie, 2008). If this can be achieved, it may minimise the impact of low-flow periods, until longer-term changes can be made to reduce abstractions and minimise phosphate and sediment inputs into the river. If not, it is likely greater numbers of river reaches will be affected by macrophyte degradation, with eventual patterns of marginal/emergent encroachment into river channels and flow-impacted reaches resembling profiles of headwater streams, including submergent taxa dominated by *Callitrche* spp., *B. erecta* and *C. glomerata*.

Determination of the level of degradation is important on a reach-by-reach basis, but also in the context of wider catchment-level effects. In particular river reaches, regular observation may give an indication of the level of impacts, i.e. presence/absence of key taxa, channel parameter influence on flow, water clarity, sedimentation of the river bed, presence of filamentous algae etc, but in reality, more thorough assessments of river physicochemistry and macrophyte status are required to gain more detailed understanding of processes and interactions. However, with powerful tools such as artificial stream mesocosms, plant-environment causality can be determined in easy to monitor and maintain systems that are both realistic analogues of natural chalk streams and considerably replicable under the right conditions. The experimental mesocosm studies performed in this research project highlight the particular usefulness of outdoor once-through mesocosms fed directly from aquifer water. In these systems, velocity tolerance thresholds and interactive effects between multiple plant stimuli revealed considerable changes to plant form and function by assessing morphological and physiological characteristic traits of the keystone macrophyte *Ranunculus pseudofluitans.*

Under varying velocities, *R. pseudofluitans* post-allofragmentation development exhibited distinct adaptive advantages to growing in moderate-fast flow velocities compared with much slower flow. This not only demonstrated marked levels of phenotypic plasticity to changing environmental conditions, but also suggests potential evolved polymorphism (Puijalon & Bornette, 2004; Lamberti-Raverot & Puijalon, 2012), which may explain why different clonal forms have been observed in the River Itchen (Lansdown, 2007), and quite possibly between and within other chalk rivers. Plasticity was also high with multiple treatments of flow, nutrient and algal growth, with flow once again driving many of the responses, although nutrient and algal effects also existed. The mesocosm experiments illustrate the capability of *Ranunculus* spp. to adapt to a variety of different environmental

conditions, and in part highlight the success of the taxon in chalk rivers. In addition, it should be noted that although pronounced differences were observed in plant form, even under extreme low flows (with hyporheic zone and substrate still wetted) or faced with excessive filamentous algal growth, there were no cases where conditions were fatal for *R. pseudofluitans*. Nevertheless, specimens were noticeably weaker in such situations, with some washouts or damage to plant tissues occurring, and should flow improve or further environmental fluctuations occur, these weakened forms may not have the capacity to cope.

Plant ontogenetic influence was also deemed important for macrophyte post-colonisation developmental success. Specimens were observed having different rates of trait development depending on plant age, reflecting differences in plant form between juvenile and mature plants. However, plant developmental stage was driven by prevailing environmental conditions, but dependant on plant age; overall specimens growing in faster velocities experienced more rapid development of traits leading into maturity. This is an important discovery, as higher river flows appear to directly enhance trait development and increase plant survivability; in the face of additional environmental stress, good flow may enable plants to adapt and cope with the impact of these additional stressors, where otherwise they may fail.

The findings from the stream mesocosms and the in-river study, whilst autecological and small in scale, can easily be translated into reach and catchment scale impacts, and assist with the interpretation of processes affecting distributions, assemblages and species turnover between and within river reaches. Velocity-driven successful growth, as demonstrated in this study, is important for the development and fecundity of individual plants, which in turn enhances stand biomass (O'Hare *et al.*, 2010), colonisation and fragmentation potential (Franklin *et al.*, 2008; Riis *et al.*, 2009), and modifies river flow

patterns to create flow and habitat heterogeneity (Green, 2005; Gurnell *et al.*, 2006), and improve stream bed conditions (Biggs *et al.*, 2005). Under such conditions, promotion of higher velocity areas and flushing events would contribute to the control of filamentous algal growth (Mainstone & Parr, 2002) and reduce inter-stand sediment deposition (Sand-Jensen, 1998), keeping chalk gravels clear for plant colonisation and spawning fish (Acornley & Sear, 1999). Whilst all of these processes are determined by local flow velocities, the significant link between river discharge regimes, reach-scale velocities and therefore micro-scale velocities at plant stand levels (Franklin *et al.*, 2008) suggest that catchment-scale considerations of discharge regimes are wholly important for determining reach scale velocities, which in turn drive stand-scale velocity profiles. With enhanced flows at plant stand-scales, the stronger induced growth-forms of keystone species such as *R. pseudofluitans* will further promote healthy stand development, improving the patchy nature of chalk macrophyte assemblages, maintaining a pseudo-braided effect (Dawson & Robinson, 1984) and driving flow heterogeneity.

Findings relating to environmental controls on macrophyte development from the community and experimental studies, with some influence from established literature concepts, have been combined to form a conceptualisation of reach-scale macrophytic assemblage changes under varying environmental conditions (**Figure 7.1.**). This conceptual model further emphasises the need to promote sustained reach and catchment scale flow regimes, conducive of healthy macrophytic development, individually, as stands, and as cohesive functional pseudo-braided patchy reach-scale assemblages.

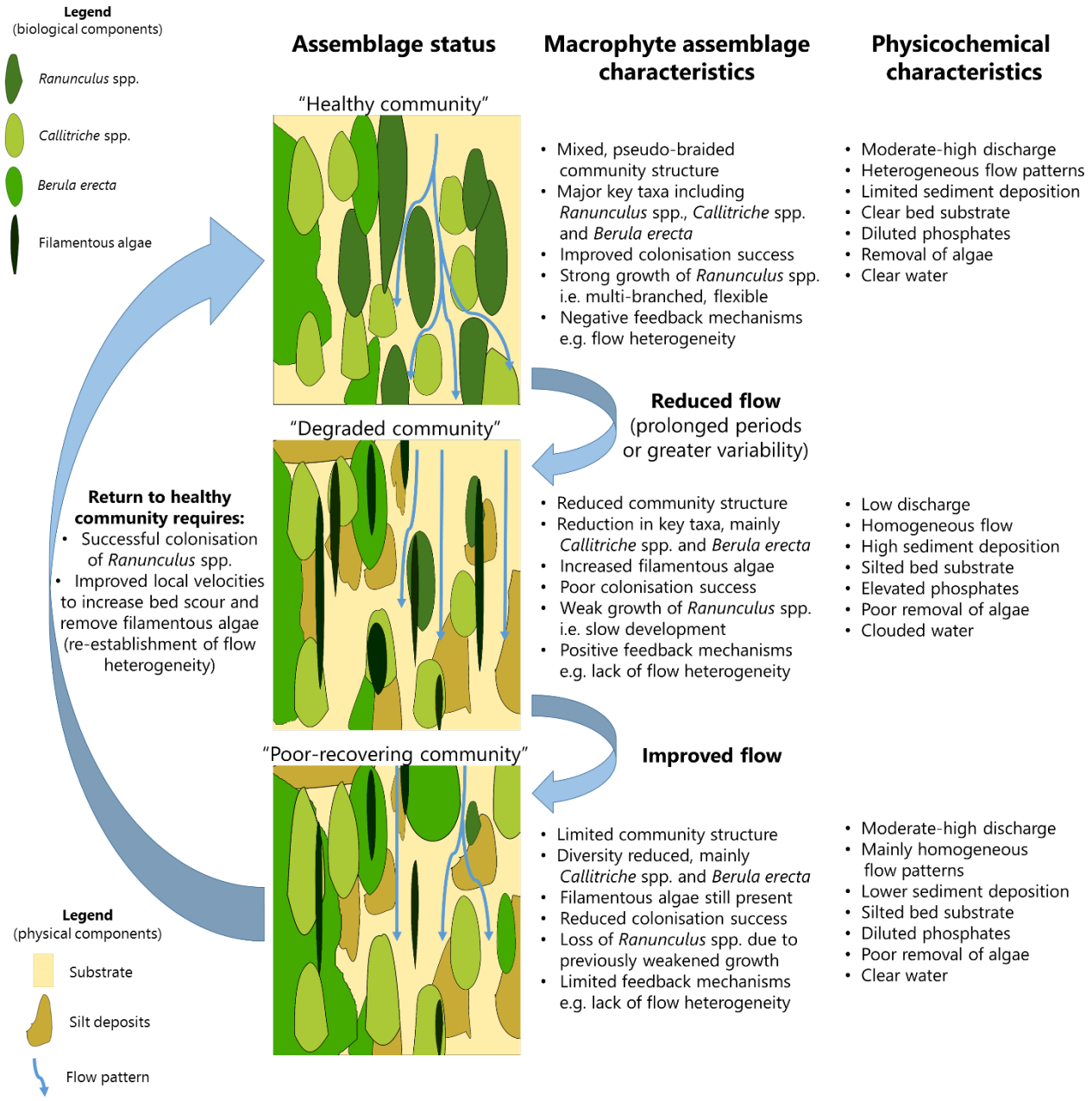

Figure 7.1. **Conceptual model of the processes involved in macrophyte community degradation in chalk streams.** The conceptualisation demonstrates the interactive complexity between multiple environmental parameters, signifies the importance of maintaining heterogeneous flows to promote healthy macrophyte assemblages, and suggests potential dangers involving positive feedback mechanisms under low flows.

Although the conceptual model provides a reliable general overview of macrophyte community degradation in relation to river flows, it must be noted that this may not be applicable in all cases. Many river reaches are unique in space and time, and may show particular symptoms of degradation without the presence of other typically linked environmental factors. This further demonstrates the complexity of chalk ecosystems, and without thorough examination at reach scales, alongside the consideration of wider catchment impacts, it can still be difficult to determine cause-and-effect under certain circumstances. Nevertheless, the findings of this research project contribute considerably to our understanding of chalk macrophyte responses to key environmental stressors, and with application into management situations, should go some way towards alleviating uncertain cases of environmental degradation, along with more obvious cases.

7.2. LIMITATIONS OF THE RESEARCH PROJECT

The research project has established the roles of flow as a controlling mechanism on individual plant-scale development, reach-scale assemblages and catchment wide interactions, and provides an enhanced understanding of macrophyte responses to key environmental stimuli. However, a number of limitations were identified from within the research that must be considered relative to further research or practical applications of study findings:

1) Limitations relating to the community study – Whilst a number of key limitations of the community study were signified in Chapter 2, a number of general observations were made with regard to study methodologies. These were:

- Limited spatial correlation between water quality sites, flow gauging stations and macrophyte survey sites meant some macrophyte data were paired with equivalent

hydrological and chemical data from river reaches not in close proximity. Whilst not thought to have been considerably influential, this may have affected statistical relationships and to a certain extent inference.

- Environmental variables were not entirely comprehensive, with several important influential factors for macrophytes (Lacoul & Freedman, 2006; Franklin *et al.*, 2008) either absent or having questionable recording/sampling procedures. As a consequence, correlations between macrophytes and these variables, and their interactive effects, with other environmental parameters could not be determined. In addition, poor sampling frequency of water quality variables means one-off events may have been missed. Both of these constraints were not thought to have affected overall findings, but may have divulged further information regarding macrophyte distribution if included.
- Macrophyte survey techniques based on the Mean Trophic Rank (MTR) methodology (Holmes *et al.*, 1999) may have influenced recording in two main ways: Firstly, as the MTR method requires cover scores to be converted into 'scores' of macrophyte presence, percent cover was often in categorical form, or rounded up/down, potentially causing valuable information to be missing; secondly, there have been debates regarding the most effective way to record plants in surveys, with percent cover being suggested as a weaker measure, compared to information from biomass or cross-sectional areas (O'Hare *et al.*, 2010).

2) Limitations relating to experimental mesocosms.

- Scale and water resource availability were key issues with the experimental mesocosms. Limitations to aquifer water volume at the experimental site meant smaller mesocosm channels had to be constructed than originally intended. Whilst macrophyte growth did not seem to be affected by these reduced-size channels, it

may have accounted for differences in growth rates between channels and natural river reaches. Furthermore, filamentous algal growth may have been enhanced due to the small-volume capacities of channels. Additionally, competitive interactive effects between multiple plant taxa may have been possible in larger mesocosm units.

- The use of outdoor systems and key taxa with capillary leaves (as opposed to laminar forms) also meant restrictions with the selection of physiological measurements. For example, in-situ chlorophyll measurements and photosynthetic/respiration rates would have been a useful addition to the physiological measurements taken, but incredibly difficult to achieve with the study setup. It may be possible to modify future mesocosms to accommodate this.

3) Limitations relating to the in-stream experiment.

- The main limitation with the in-stream experiment was with planting/sampling difficulties. Planting was more difficult than in mesocosms due to firmer substrate and more variable conditions. Whilst every attempt was made to make sure specimens were planted methodically, random difficulties at certain sites may have caused disturbance that influenced plant growth. Sampling was made difficult due to the increased water depths and more variable conditions, which may have caused minor errors in measurement. Neither of these effects are thought to have influenced final results.
- Removal of plant specimens by swan grazing and/or in-stream debris may have affected the results of damaged or washout plants. Whilst every attempt was made to prevent grazing of plant specimens, on several occasions persistent swans were observed to evade the preventative netting placed over plant specimens. It is not thought any specimens were eaten by swans, however, as none showed signs of

having been grazed (O'Hare *et al.*, 2007). In-stream debris (e.g. woody debris, gravel, plant fragments) could not be controlled, so the impact this might have had on specimen development is unknown, but was again thought to be relatively low impact.

7.3. SUGGESTIONS FOR FURTHER RESEARCH

Future research themes can be suggested on the basis of study findings and still-existing gaps in our understanding of chalk macrophytes. These are mainly related to enhancing the complexity of experimental systems, providing greater understanding of macrophytic distribution and the development of novel methods for determining sub-lethal indicators of environmental stress.

- **Increased experimental complexity** – Future climate change scenarios suggest increased variability of frequency and magnitude rainfall patterns (Matear *et al.*, 2012) which will cause chalk rivers to have greater flow regime variability (Milly *et al.*, 2005). As a result, macrophyte assemblages will be required to tolerate this greater variation, but the impacts on form, function and successful development are still little known. Mesocosm experiments could therefore be extended to incorporate fluctuations of conditions to test responses under particular scenarios.
- **Competitive interactions under environmental stress** – If mesocosm scale and water-provision limitations could be overcome, there is significant value in the assessment of competitive interactions between key macrophyte taxa (e.g. *Ranunculus* spp., *Berula erecta*, *Calitriche* spp. and filamentous algae) under different environmental conditions.
- **Examination of additional potential stressors** – Additional environmental factors (e.g. sediments/siltation, grazing, response to cutting) which may have confounding

influences on macrophytic development (Franklin *et al.*, 2008), or may have important individual and/or combined effects on macrophyte growth should be assessed in controlled experimental situations. Appropriate stream mesocosm setups may be ideal for this.

- **Determination of tools for macrophyte monitoring** – This study has shown that certain form traits (e.g. easy to measure leaf:internode allometric coefficients) may be useful tools for the monitoring of *Ranunculus* spp. to examine environmental degradation. While typically used for assessments of taxonomic identification, separation between environmental-triggered traits and taxonomic traits is important before examining what traits might prove useful.
- **Assessments of status and distribution** – Although Spink *et al.* (1997) comprehensively studied the distribution of aquatic *Ranunculus* taxa, there is still particular need to clarify ranges with regards to environmental tolerance. In particular, close attention should be given to potential variation in morphotypes both between and within river catchments (Lansdown, 2007).
- **Novel determination of sub-lethal metabolite responses –** Recent advancements in the use of environmental metabolomics for assessing stressors in plant-environment interactions (Gong *et al.*, 2007; Fiehn *et al.*, 2008) indicate the potential for using sub-lethal changes in plant metabolites to signify environmental degradation. Preliminary studies alongside this research have shown that aquatic macrophytes may be useful as indicators of environmental stress via metabolite responses. Additional research in this novel field is required to determine if detected variation in metabolites are related to environmental stress.

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APPENDICES

APPENDIX A

Chapter 2 supplementary information

Table A.1. **Names and locations of survey sites, water quality monitoring sites and flow gauging stations throughout the length of the River Itchen.** For mapped locations see Figure 2.2., Chapter 2.

Macrophyte survey sites

No.	Site name	OS Grid Ref. (SU)
1	New Cheriton	588 278
2	u/s A31 Bridge	573 315
3	d/s Drove Lane	574 325
4	Abbotstone	563 344
5	u/s disused railway	567 326
6	d/s B3047 Bridge	569 321
7	Itchen Stoke	561 319
8	d/s Yavington Farm	546 323
9	Itchen Abbas	537 328
10	Chilland	522 324
11	Easton south channel, carrier	514 325
12	Easton north channel	514 326
13	Abbots Worthy, north channel	498 324
14	Abbots Worthy, south channel	497 321
15	Water Lane	486 295
16	Winchester College Fields	482 285
17	Between M3 and Tumbling Bay	478 264
18	Between Tumbling Bay and Hockley Cottages	478 259
19	d/s Norris' Bridge	476 244
20	d/s Shawford House	472 242
21	u/s Highbridge	465 215
22	d/s Highbridge	468 210
23	Stoke Common	460 205
24	Canal, Eastleigh, loop west of railway	457 206
25	Bishopstoke	466 186
26	Chickenhall, u/s railway	467 177
27	Chickenhall d/s railway	463 172
28	Gator's Mill	453 156

Water quality monitoring sites

No.	Site name	OS Grid Ref. (SU)
1	Itchen d/s Alresford bypass	574 317
2	u/s Itchen valley fish farm 1	574 318
3	R. Arle Drove Lane	574 325
4	u/s Abbotstone Road bridge	563 345
5	Candover Borough bridge	568 323
6	u/s Itchen valley fish farm 2	574 319
7	R. Itchen at Itchen Stoke	558 322
8	Itchen Abbas trout farm inlet	539 329
9	R. Itchen Easton	511 324
10	d/s Harestock WWTW	486 295
11	R. Itchen St. Cross bridge	476 270
12	Otterbourne pumping station	470 232
13	R. Itchen at Highbridge	467 214
14	R. Itchen at Bishopstoke	464 194
15	R. Itchen d/s Eastleigh STW	468 178
16	R. Itchen Gaters Mill	453 156

Flow gauging stations

No.	Site name	OS Grid Ref. (SU)
1	Sewards Bridge	573 322
2	Drove Lane Total	574 326
3	Borough Bridge	568 323
4	Easton EM	510 323
5	Highbridge	467 214
6	Allbrook	461 211
7	Allbrook & Higbridge	461 211
8	Riverside Park	444 153

Table A.2. **List of surveyed macrophyte taxa for the study period (2004-2009).** PTA = proportion of total abundance. Abbreviated names highlighted in bold indicate taxa included in ordination analyses. Taxa which are only named by genus and denoted by "sp." were unidentifiable to species level, and may represent any of the species listed for that genus, unless otherwise denoted.

Macrophyte taxon	Abbreviation	PTA (%)
Achillea millefolium	***Achi mil***	<0.01
Agrostis stolonifera	***Agro sto***	0.45
Alchemilla mollis	*Alch mol*	<0.01
Alisma plantago-aquatica	*Alis pla*	<0.01
Alnus glutinosa	*Alnu glu*	<0.01
Alopecurus geniculatus	*Alop gen*	<0.01
Amblystegium fluviatile	*Ambl flu*	<0.01
Amblystegium humile	***Ambl hum***	0.01
Amblystegium serpens var. *serpens*	***Ambl ser***	0.01
Amblystegium tenax	***Ambl ten***	0.01
Amblystegium varium	*Ambl var*	<0.01
Angelica sylvestris	***Ange syl***	0.01
Apium nodiflorum	***Apiu nod***	1.86
Aquilegia vulgaris	*Aqui vul*	<0.01
Arrhenatherum elatius	*Arrh ela*	<0.01
Aster sp.	*Aste* sp.	<0.01
Atriplex patula	*Atri pat*	<0.01
Azolla filiculoides	***Azol fil***	0.03
Auduinella sp.	***Audu* sp.**	0.01
Bangia atropurpurea	*Bang atr*	<0.01
Barbula sardoa	*Barb sar*	<0.01
Barbula unguiculata	*Barb ung*	<0.01
Batrachospermum sp.	***Batr* sp.**	0.08
Berula erecta	***Beru ere***	13.04
Bidens cernua	*Bide cer*	<0.01
Blue-green algae	**Blue alg**	0.02
Brachythecium mildeanum	*Brac mil*	<0.01
Brachythecium rivulare	***Brac riv***	0.04
Brachythecium rutabulum	***Brac rut***	0.02
Branched alga	**Bran alg**	0.01
Bryum argenteum	*Bryu arg*	<0.01
Bryum bicolor	*Bryu bic*	<0.01
Bryum caespiticium	*Bryu cae*	<0.01
Bryum capillare	*Bryu cap*	<0.01
Bryum laevifilum	*Bryu lae*	<0.01
Bryum pseudotriquetrum	*Bryu pse*	<0.01
Calliergonella cuspidata	***Call cus***	0.01
Callitriche obtusangula	***Call obt***	2.32
Callitriche platycarpa	***Call pla***	0.76
Callitriche sp. (obt./pla.)	***Call* sp.**	12.35
Callitriche stagnalis	***Call sta***	0.06
Caltha palustris	*Calt pal*	<0.01
Calystegia sepium	***Caly sep***	0.01
Cardamine flexuosa	***Card fle***	0.02
Cardamine pratensis	*Card pra*	<0.01
Carex acuta	***Care acu***	0.42
Carex acutiformis	***Care act***	0.02
Carex hirta	*Care hir*	<0.01
Carex nigra	*Care nig*	<0.01
Carex paniculata	***Care pan***	0.16
Carex pendula	***Care pen***	0.01
Carex riparia	***Care rip***	0.23
Carex sp.	***Care* sp.**	0.48
Catabrosa aquatica	***Cata aqu***	0.31
Cerastium fontanum	*Cera fon*	<0.01
Cerastium glomeratum	*Cera glo*	<0.01
Chaetophora elegans	*Chae ele*	<0.01
Chaetophora incrassata	*Chae inc*	<0.01
Chantransia	*Chantran*	<0.01
Chenopodium album	*Chen alb*	<0.01
Chenopodium rubrum	*Chen rub*	<0.01
Chiloscyphus polyanthos	*Chil pol*	<0.01
Cirsium arvense	***Cirs arv***	0.01
Cirsium palustre	***Cirs pal***	0.01
Cirsium vulgare	*Cirs vul*	<0.01
Cladophora glomerata	***Clad glo***	12.06
Conocephalum conicum	***Cono con***	0.02
Cotoneaster sp.	***Coto* sp.**	0.01
Cratoneuron filicinum	***Crat fil***	0.18
Cymbalaria muralis	*Cymb mur*	<0.01
Deschampsia cespitosa	*Desc ces*	<0.01
Diatom scum	**Diatom s**	2.20
Dicranella schreberiana	*Dicr sch*	<0.01
Dicranella varia	*Dicr var*	<0.01
Didymodon luridus	*Didy lur*	<0.01
Didymodon rigidulus	*Didy rig*	<0.01
Drepanocladus aduncus	***Drep adu***	0.03
Eleocharis palustris	*Eleo pal*	<0.01
Elodea canadensis	***Elod can***	0.13
Elodea nuttallii	*Elod nut*	<0.01
Enteromorpha flexuosa	***Ente fle***	<0.01
Epiloibium ciliatum	***Epil cil***	<0.01
Epilobium hirsutum	***Epil hir***	0.31
Epilobium parviflorum	***Epil par***	0.01
Epilobium sp.	***Epil* sp.**	0.01
Epilobium x erroneum	*Epil x e*	<0.01
Epilobium x subhirsutum	*Epil x s*	<0.01
Equisetum arvense	***Equi arv***	<0.01
Equisetum fluviatile	*Equi flu*	<0.01
Equisetum palustre	***Equi pal***	<0.01
Eucladium verticillatum	*Eucl ver*	<0.01
Eupatorium cannabinum	***Eupa can***	0.04
Eurhynchium crassinervium	*Eurh cra*	<0.01
Eurhynchium praelongum	***Eurh pra***	0.01
Eurhynchium hians	***Eurh hia***	0.01
Fallopia japonica	*Fall jap*	<0.01
Filamentous green algae	**Fila alg**	0.02
Filipendula ulmaria	***Fili ulm***	0.07
Fissidens adianthoides	*Fiss adi*	<0.01
Fissidens bryoides	*Fiss bry*	<0.01
Fissidens crassipes	***Fiss cra***	0.18
Fissidens taxifolius	*Fiss tax*	<0.01
Fontinalis antipyretica	***Font ant***	0.73
Fontinalis antipyretica var. *gigantea*	*Font gig*	<0.01

Funaria hygrometrica	***Funa hyg***	<0.01
Galium aparine	***Gali apa***	0.01
Galium palustre	***Gali pal***	0.04
Galium uliginosum	*Gali uli*	<0.01
Geranium robertianum	*Gera rob*	<0.01
Geum rivale	***Geum riv***	<0.01
Geum urbanum	*Geum urb*	<0.01
Glechoma hederacea	*Glec hed*	<0.01
Glyceria declinata	***Glyc dec***	0.08
Glyceria fluitans	***Glyc flu***	0.03
Glyceria maxima	***Glyc max***	2.58
Glyceria notata	***Glyc not***	0.19
Glyceria notata/fluitans/declinata	***Glyc* sp.**	0.46
Green encrusting algae	**Gree alg**	0.43
Hedera helix	***Hede hel***	<0.01
Heribaudiella fluviatilis	***Heri flu***	0.06
Hildenbrandia rivularis	***Hild riv***	0.69
Hippurus vulgaris	***Hipp vul***	1.49
Holcus lanatus	*Holc lan*	<0.01
Hygrohypnum luridum	***Hygr lur***	0.01
Hypericum perforatum	*Hype per*	<0.01
Hypericum sp.	*Hype* sp.	<0.01
Hypericum tetrapterum	***Hype tet***	0.01
Hypericum x desetangsii	*Hype x d*	<0.01
Impatiens capensis	***Impa cap***	0.19
Impatiens glandulifera	***Impa gla***	0.02
Iris pseudacorus	***Iris pse***	0.28
Juncus articulatus	*Junc art*	<0.01
Juncus bufonius	*Junc buf*	<0.01
Juncus effusus	*Junc eff*	<0.01
Juncus inflexus	***Junc inf***	0.03
Juncus x surrejanus	***Junc x s***	<0.01
Lagarosiphon major	*Laga maj*	<0.01
Lemanea fluviatilis	*Lema flu*	<0.01
Lemna gibba	***Lemn gib***	0.26
Lemna minor	***Lemn min***	0.27
Lemna minor/gibba	***Lemn m/g***	0.29
Lemna minuta	***Lemn mia***	0.02
Lemna trisulca	***Lemn tri***	0.20
Leptodictyon riparium	***Lept rip***	0.20
Leskea polycarpa	*Lesk pol*	<0.01
Ligustrum vulgare	*Ligu vul*	<0.01
Lonicera nitida	***Loni nit***	0.01
Lophocolea bidentata	*Loph bid*	<0.01
Lophocolea heterophylla	*Loph het*	<0.01
Lotus pedunculatus	*Lotu ped*	<0.01
Lunularia cruciata	***Lunu cru***	0.01
Lycopus europaeus	***Lyco eur***	0.04
Lysimachia nummularia	*Lysi num*	<0.01
Lythrum salicaria	***Lyth sal***	0.02
Marchantia polymorpha	***Marc pol***	<0.01
Mentha aquatica	***Ment aqu***	0.29
Mentha spicata	*Ment spi*	<0.01
Mentha x piperata	***Ment x p***	0.03
Mentha x verticillata	***Ment x v***	0.01
Metzgeria fruticulosa	***Metz fru***	<0.01
Mimulus guttatus	***Mimu gut***	0.07
Mimulus sp.	***Mimu* sp.**	0.02
Mimulus x robertsii	***Mimu x r***	0.03
Montia fontana	*Mont fon*	<0.01
Myosotis laxa	*Myos lax*	<0.01
Myosotis scorpioides	***Myos sco***	<0.01
Myosotis sp.	***Myos* sp.**	0.30
Myosotis x suzae	***Myos x s***	0.11
Myosoton aquaticum	*Myos aqu*	0.01
Narcissus pseudonarcissus	*Narc pse*	<0.01
Nymphaea alba	*Nymp alb*	<0.01
Oenanthe crocata	***Oena cro***	0.19
Oenanthe fluviatilis	***Oena flu***	1.89
Oxyrhynchium speciosum	*Oxyr spe*	<0.01
Pellia endiviifolia	***Pell end***	0.09
Pellia sp.	***Pell* sp.**	0.04
Persicaria amphibia	***Pers amp***	0.01
Persicaria hydropiper	*Pers hyd*	<0.01
Persicaria maculosa	***Pers mac***	0.01
Petasites hybridus	***Peta hyb***	0.07
Phalaris arundinacea	***Phal aru***	1.72
Phragmites australis	***Phra aus***	0.25
Physcomitrium pyriforme	***Phys pyr***	0.01
Plagiomnium ellipticum	*Plag ell*	<0.01
Plagiomnium rostratum	*Plag ros*	<0.01
Plagiomnium undulatum	*Plag und*	<0.01
Plantago lanceolata	*Plan lan*	<0.01
Plantago major	*Plan maj*	<0.01
Poa trivialis	***Poa triv***	0.01
Pohlia lescuriana	*Pohl les*	<0.01
Pohlia melanodon	***Pohl mel***	0.01
Pohlia nutans	*Pohl nut*	<0.01
Polygonum aviculare	*Poly avi*	<0.01
Populus x canadensis	*Popu x c*	<0.01
Potamogeton crispus	***Pota cri***	0.25
Potamogeton lucens	***Pota luc***	0.14
Potentilla anserina	*Pote ans*	<0.01
Potentilla reptans	*Pote rep*	<0.01
Pseudotaxiphyllum elegans	*Pseu ele*	<0.01
Pulicaria dysenterica	***Puli dys***	0.03
Ranunculus aquatilis	***Ranu aqu***	0.32
Ranunculus Batrachian sp.	*Ranu* Bat	0.04
Ranunculus ficaria	***Ranu fic***	<0.01
Ranunculus flammula	*Ranu fla*	<0.01
Ranunculus penicillatus subsp. *pseudofluitans* (var. *pseudofluitans/vertumnus*)	***Ranu pse***	19.90
Ranunculus repens	***Ranu rep***	0.05
Rhynchostegiella sp.	*Rhyn* sp.	0.03
Rhynchostegium riparioides	***Rhyn rip***	<0.01
Riccia fluitans	*Ricc flu*	<0.01
Rorippa amphibia	*Rori amp*	<0.01
Rorippa nasturtium-aquaticum agg.	***Rori* agg**	0.89
Rorippa nasturtium-aquaticum *Rorippa x sterilis*	***Rori nas***	0.66
Rubus fruticosus agg.	***Rori x s***	0.10
Rumex acetosa	***Rubu fru***	0.02
Rumex conglomeratus	*Rume ace*	<0.01
Rumex hydrolapathum	***Rume con***	0.03
Rumex obtusifolius	***Rume hyd***	0.06
Rumex x schultzei	***Rume obt***	<0.01
Salix cinerea	*Rume x s*	<0.01
Salix fragilis	***Sali cin***	0.53
Salix viminalis	***Sali fra***	<0.01
Salix x sepulchralis nothovar. chrysocoma	***Sali vim***	0.02
Schoenoplectus lacustris	***Sali x s***	0.12
Scrophularia auriculata	***Scho lac***	2.72

Scutellaria galericulata	***Scro aur***	0.08
Senecio aquaticus	*Scut gal*	<0.01
Senecio jacobaea	***Sene aqu***	0.08
Solanum dulcamara	*Sene jac*	<0.01
Solanum nigrum	***Sola dul***	0.43
Soleirolia soleirolii	***Sola nig***	<0.01
Sonchus asper	***Sole sole***	0.20
Sparganium emersum	*Sonc asp*	0.00
Sparganium erectum	***Spar eme***	0.48
Sparganium erectum subsp.	***Spar ere***	1.10
erectum	***Spar sub***	0.03
Sparganium erectum subsp. *microcarpum*	***Spar mic***	0.04
Sparganium erectum subsp. *neglectum*	***Spar neg***	0.50
Sparganium erectum subsp. *oocarpum*	***Spar ooc***	0.03
Stachys palustris		
Stachys x ambigua	***Stac pal***	0.01
Stigeoclonium sp.	*Stac x a*	<0.01
Symphytum officinale	***Stig* sp.**	0.06
Tetraspora gelatinosa	***Symp off***	0.09
Thallose liverwort	***Tetr gel***	0.02
Thlaspi arvensis	***Thal liv***	<0.01

Tortula acaulon	*Thla arv*	<0.01
Tortula muralis	*Tort aca*	<0.01
Trifolium repens	*Tort mur*	<0.01
Tussilago farfara	*Trif rep*	<0.01
Typha latifolia	*Tuss far*	<0.01
Urtica dioica	***Typh lat***	0.09
Valeriana officinalis	***Urti dio***	0.26
Vaucheria sp.	***Vale off***	<0.01
Veronica anagallis-aquatica	***Vauc* sp.**	2.76
Veronica beccabunga	***Vero ana***	0.07
Veronica catenata	***Vero bec***	0.19
Veronica anagallis-aquatica/catenata / x lackschewitzii	***Vero cat***	0.01
	***Vero* sp.**	0.16
Veronica x lackschewitzii		
Verrucaria aethiobola	***Vero x l***	0.34
Verrucaria aquatilis	***Verr aet***	0.21
Verrucaria rheitrophila	***Verr aqu***	<0.01
Verrucaria sp.	*Verr rhe*	<0.01
*Zannichellia palustris (*subsp. *palustris)*	***Verr* sp.**	0.35
	Zann pal	2.87
Zygnematalean algae		
	Zygn alg	0.01

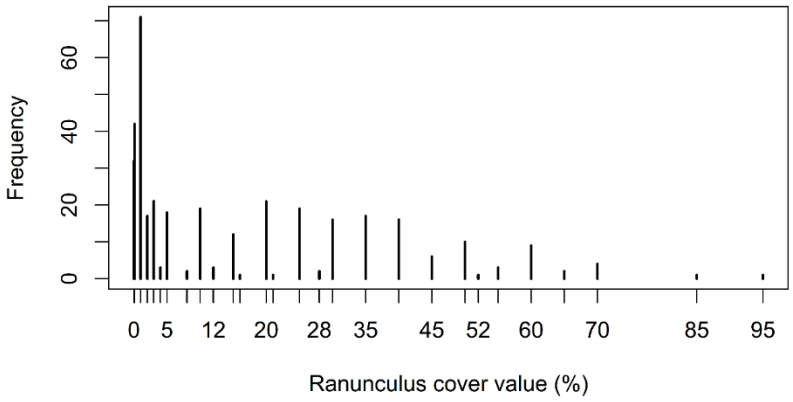

Figure A.1. **Frequency plot of *Ranunculus* cover data assessing zero-inflation.**

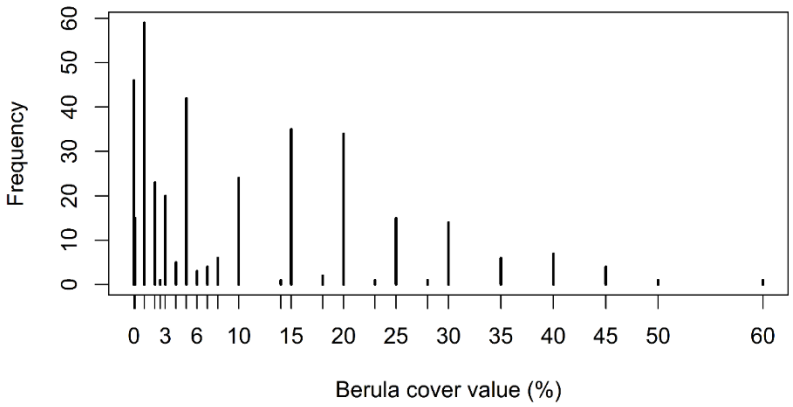

Figure A.2. **Frequency plot of *Berula* cover data assessing zero-inflation.**

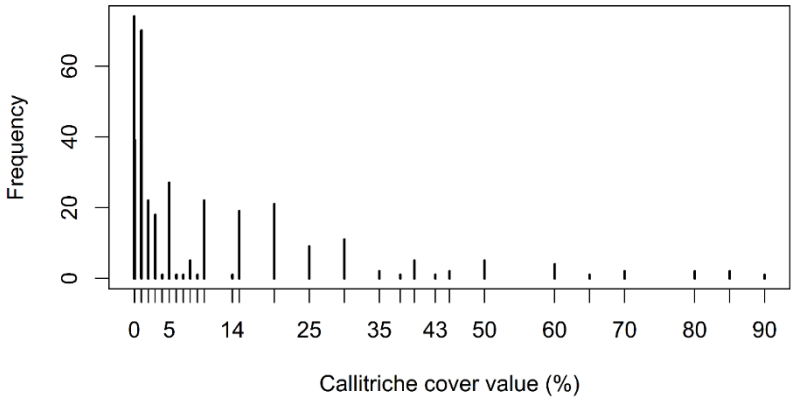

Figure A.3. **Frequency plot of *Callitriche* cover data assessing zero-inflation.**

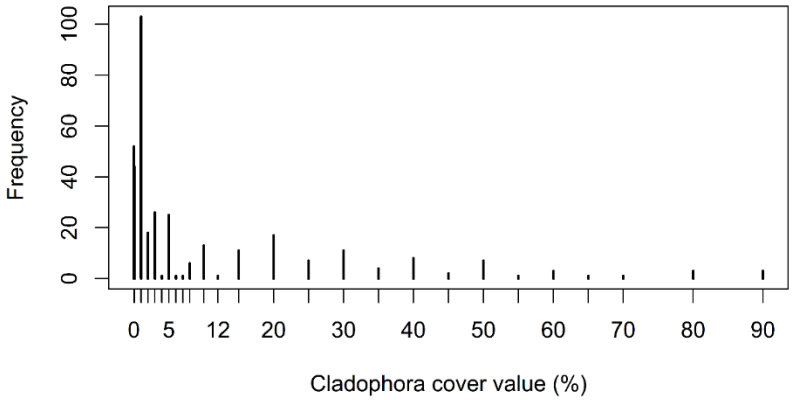

Figure A.4. **Frequency plot of *Cladophora* cover data assessing zero-inflation.**

Table A.3. **Selection table of most influential discharge parameters affecting distribution of *Ranunculus***. Model selection performed using AICc ranking.

Q	AICc	Delta_AICc
pwq25	168.14	0
p2wq25	168.14	0
pwq10	168.25	0.12
p2wq10	168.25	0.12
pwq50	168.47	0.33
p2wq50	168.47	0.33
pwm	168.54	0.41
p2wm	168.54	0.41
pwq70	168.58	0.44
p2wq70	168.58	0.44
pwq90	169.02	0.88
p2wq90	169.02	0.88
pwq95	169.6	1.47
p2wq95	169.6	1.47
pwq99	169.64	1.5
p2wq99	169.64	1.5
pwmin	169.66	1.52
p2wmin	169.66	1.52
yq25	170.39	2.25
w20q10	170.72	2.58
yq10	170.92	2.78
yq50	171.2	3.06
w32q50	171.44	3.3
w32m	171.46	3.33
ym	171.5	3.36
pwmax	171.65	3.52
p2wmax	171.65	3.52
w20min	171.72	3.58
w20q99	171.82	3.69
w32q70	171.83	3.69
w20q95	171.87	3.73
w20q90	171.91	3.78
w24q10	172.08	3.94
w32q95	172.09	3.95
w32q90	172.18	4.04
w32q99	172.28	4.14
w32min	172.35	4.21
w24max	172.41	4.28
w32q10	172.47	4.33
w32q25	172.47	4.34
w24q25	172.89	4.76
w20max	172.94	4.81
w20m	173.17	5.03
w24m	173.4	5.27
w20q25	173.54	5.4
w16min	173.91	5.78
yq70	174.1	5.97
ymax	174.22	6.08
w16q99	174.45	6.31
w24q90	174.46	6.32

w24q95	174.58	6.44
w24min	174.6	6.46
w24q99	174.65	6.51
w24q70	174.97	6.83
w20q70	175.06	6.92
w20q50	175.22	7.09
w32max	175.52	7.38
w24q50	175.76	7.62
w16q70	176.2	8.06
psq10	176.21	8.08
w16q10	176.23	8.09
psq25	176.24	8.1
yq95	176.28	8.15
w16q95	176.3	8.16
w16q90	176.35	8.21
psmax	176.35	8.21
wmax	176.36	8.23
psq50	176.38	8.24
ymin	176.39	8.25
psm	176.41	8.27
psmin	176.41	8.27
yq99	176.42	8.28
psq99	176.42	8.28
w16m	176.46	8.32
psq95	176.46	8.33
w16q25	176.48	8.34
psq90	176.52	8.38
wq10	176.56	8.42
yq90	176.79	8.65
psq70	176.79	8.65
wq25	176.85	8.71
w12min	176.89	8.75
w12q99	176.9	8.76
w16q50	176.94	8.8
w16max	177	8.86
w12q95	177.26	9.12
w12q90	177.27	9.13
w2max	177.52	9.38
w4q95	177.63	9.49
wm	177.71	9.57
w4q90	177.72	9.58
w3max	177.73	9.59
wq50	177.81	9.68
Q	177.84	9.7
w4max	177.86	9.72
w12q50	177.89	9.75
w4q99	177.92	9.78
w2q10	177.94	9.8
w12m	177.94	9.8
w3q95	177.99	9.86
w12q70	178.03	9.89
w4min	178.04	9.91
w4m	178.05	9.92
w3q99	178.06	9.93
wq70	178.07	9.93
w3min	178.08	9.94
w3q90	178.09	9.95
w4q50	178.09	9.95
w4q70	178.13	9.99
w4q10	178.14	10
w3q70	178.2	10.06
w12q25	178.21	10.07
w2q90	178.22	10.08
w3q10	178.22	10.08
w12max	178.22	10.08
w3q50	178.3	10.17
w2q25	178.34	10.2
w2q95	178.34	10.21
w8q25	178.34	10.21
w3m	178.35	10.22
w8q50	178.4	10.26
w2m	178.46	10.33
w8max	178.47	10.33
p2sq10	178.49	10.35
w8q10	178.51	10.37
p2sq25	178.51	10.38
w2q99	178.53	10.39
w8m	178.55	10.41
w8min	178.55	10.42
w8q99	178.56	10.42
w2min	178.58	10.44
w4q25	178.59	10.45
w12q10	178.59	10.46
p2sm	178.67	10.53
w8q95	178.75	10.61
p2sq50	178.78	10.65
w8q70	178.8	10.66
w3q25	178.82	10.69
w2q70	178.89	10.75
w8q90	178.98	10.84
p2sq70	178.98	10.84
p2smax	179.04	10.9
wq90	179.21	11.07
p2sq90	179.21	11.08
w2q50	179.25	11.12
wq95	179.35	11.21
wq99	179.46	11.32
wmin	179.48	11.35
p2sq95	179.58	11.44
p2smin	180.47	12.34
p2sq99	180.47	12.34

Table A.4. **Selection table of most influential discharge parameters affecting distribution of *Berula***. Model selection performed using AICc ranking.

Q	AICc	Delta_AICc
Q	77.73	0
w24max	77.75	0.02
w24q10	77.75	0.02
w24q25	77.75	0.02
w32q10	77.75	0.02
w32q25	77.75	0.02
w24m	77.76	0.02
w24q50	77.76	0.02
w32m	77.76	0.03
w32q50	77.76	0.03
w24q70	77.77	0.04
w2max	77.78	0.04
w2q10	77.78	0.05
w4max	77.78	0.05
w4q10	77.78	0.05
w12max	77.78	0.05
w16max	77.78	0.05
w20max	77.78	0.05
w24q90	77.78	0.05
w32max	77.78	0.05
w32q70	77.78	0.05
pwmax	77.78	0.05
p2wmax	77.78	0.05
wm	77.79	0.05
wmax	77.79	0.05
wmin	77.79	0.05
wq10	77.79	0.05
wq25	77.79	0.05
wq50	77.79	0.05
wq70	77.79	0.05
wq90	77.79	0.05
wq95	77.79	0.05
wq99	77.79	0.05
w2m	77.79	0.05
w2q25	77.79	0.05
w2q50	77.79	0.05
w2q70	77.79	0.05
w2q90	77.79	0.05
w3m	77.79	0.05
w3max	77.79	0.05
w3q10	77.79	0.05
w3q25	77.79	0.05
w3q50	77.79	0.06
w4m	77.79	0.06
w4q25	77.79	0.06
w4q50	77.79	0.06
w8max	77.79	0.06
w8q10	77.79	0.06
w8q25	77.79	0.06
w12q10	77.79	0.06
w12q25	77.79	0.06
w16q10	77.79	0.06
w16q25	77.79	0.06
w20q10	77.79	0.06
w24q95	77.79	0.06
yq10	77.79	0.06

yq25	77.79	0.06
p2smax	77.79	0.06
w2min	77.8	0.06
w2q95	77.8	0.06
w2q99	77.8	0.06
w3q70	77.8	0.06
w3q90	77.8	0.06
w3q95	77.8	0.06
w4q70	77.8	0.06
w4q90	77.8	0.06
w4q95	77.8	0.06
w8m	77.8	0.06
w8q50	77.8	0.06
w8q70	77.8	0.06
w8q90	77.8	0.06
w12m	77.8	0.07
w12q50	77.8	0.07
w12q70	77.8	0.07
w16m	77.8	0.07
w16q50	77.8	0.07
w16q70	77.8	0.07
w20m	77.8	0.07
w20q25	77.8	0.07
w20q50	77.8	0.07
w20q70	77.8	0.07
w24min	77.8	0.07
w24q99	77.8	0.07
w32q90	77.8	0.07
w32q95	77.8	0.07
ym	77.8	0.07
ymax	77.8	0.07
p2sq10	77.8	0.07
pwm	77.8	0.07
pwmin	77.8	0.07
pwq10	77.8	0.07
pwq95	77.8	0.07
pwq99	77.8	0.07
p2wm	77.8	0.07
p2wmin	77.8	0.07
p2wq10	77.8	0.07
p2wq95	77.8	0.07
p2wq99	77.8	0.07
w3min	77.81	0.07
w3q99	77.81	0.07
w4min	77.81	0.07
w4q99	77.81	0.07
w8min	77.81	0.07
w8q95	77.81	0.07
w8q99	77.81	0.07
w12q90	77.81	0.07
w16q90	77.81	0.07
yq50	77.81	0.08
p2sq25	77.81	0.08
pwq25	77.81	0.08
pwq50	77.81	0.08
pwq70	77.81	0.08
pwq90	77.81	0.08
p2wq25	77.81	0.08
p2wq50	77.81	0.08
p2wq70	77.81	0.08
p2wq90	77.81	0.08
w12min	77.82	0.08
w12q95	77.82	0.08
w12q99	77.82	0.08
w16q95	77.82	0.08
w32min	77.82	0.08
w32q99	77.82	0.08
yq70	77.82	0.09
psmax	77.82	0.09
p2sm	77.82	0.09
p2sq50	77.82	0.09
p2sq70	77.82	0.09
psq10	77.83	0.09
p2sq90	77.83	0.1
w16q99	77.84	0.11
w20q90	77.84	0.11
psq25	77.84	0.11
p2sq95	77.84	0.11
w16min	77.85	0.12
w20q95	77.85	0.12
yq90	77.85	0.12
psm	77.85	0.12
psq50	77.86	0.12
psq70	77.86	0.12
w20q99	77.87	0.14
yq95	77.87	0.14
psq90	77.87	0.14
w20min	77.88	0.14
yq99	77.88	0.14
ymin	77.89	0.15
psq95	77.9	0.16
p2sq99	77.92	0.18
psmin	77.93	0.19
psq99	77.93	0.19
p2smin	77.93	0.2

Table A.5. **Selection table of most influential discharge parameters affecting distribution of *Callitriche*** Model selection performed using AICc ranking.

Q	AICc	Delta_AICc
w12max	118.08	0
w8q10	118.24	0.16
w12q10	118.42	0.34
w8max	118.45	0.38
w8q25	118.59	0.52
w16max	118.6	0.53
w12q25	118.62	0.54
w16q50	118.62	0.55
w12q50	118.65	0.57
w4max	118.71	0.64
w4q10	118.79	0.72
w12m	118.8	0.72
w2q10	118.92	0.84
w3q10	118.93	0.85
w3q25	118.95	0.88
w2max	118.96	0.88
w3max	118.96	0.89
w16q70	118.96	0.89
w8m	118.97	0.9
w16m	118.98	0.9
w20q70	118.98	0.91
w2q25	118.99	0.91
w20max	119.02	0.94
w8q50	119.07	0.99
w4q25	119.1	1.02
w20q50	119.1	1.03
w16q25	119.12	1.05
w16q10	119.14	1.06
w12q70	119.16	1.08
w24max	119.16	1.09
w2q50	119.2	1.13
w2m	119.21	1.14
w4m	119.24	1.17
w3m	119.26	1.19
w3q50	119.28	1.21
w4q50	119.28	1.21
wq25	119.35	1.27
wq10	119.37	1.3
w2q70	119.37	1.3
w4q70	119.38	1.31
w20m	119.41	1.34
wq50	119.43	1.36
wmax	119.44	1.37
wm	119.46	1.38
w8q70	119.46	1.39
ymax	119.47	1.4
Q	119.48	1.4
w3q70	119.49	1.41
wq70	119.53	1.46
w16q90	119.6	1.52
w32max	119.61	1.53
w12q90	119.62	1.54
w24q70	119.64	1.57
wq90	119.65	1.58
w24q50	119.66	1.59
w2q90	119.69	1.61
wq95	119.7	1.63
w20q10	119.7	1.63
w8q90	119.71	1.64
w20q25	119.72	1.64
wq99	119.75	1.67
w24m	119.75	1.68
wmin	119.76	1.69
w4q90	119.79	1.72
w3q90	119.8	1.72

w2q95	119.81	1.73
w16q95	119.83	1.75
w2q99	119.89	1.81
w2min	119.91	1.83
w4q95	119.91	1.84
w24q25	119.91	1.84
w12q95	119.96	1.89
w3q95	119.97	1.89
w24q10	119.98	1.9
w4q99	120.07	2
w8q95	120.08	2.01
w3q99	120.12	2.04
w4min	120.12	2.05
w3min	120.16	2.09
w8q99	120.29	2.21
w12q99	120.33	2.26
w8min	120.34	2.26
w12min	120.36	2.29
yq10	120.49	2.41
p2sq10	120.55	2.48
w32q25	120.56	2.48
p2smax	120.6	2.52
w20q90	120.66	2.59
w32m	120.7	2.62
w16q99	120.74	2.67
ym	120.76	2.68
yq25	120.79	2.72
w32q10	120.84	2.77
yq50	120.85	2.77
w16min	120.89	2.82
psmax	120.97	2.9
psq10	120.99	2.91
w20q95	121	2.92
w32q50	121	2.93
p2sq25	121.03	2.96
w24q90	121.12	3.05
w24q95	121.23	3.15
w32q70	121.32	3.25
yq70	121.33	3.26
pwmax	121.37	3.29
p2wmax	121.37	3.29
psq25	121.5	3.42
pwq99	121.5	3.42
p2wq99	121.5	3.42
p2sq50	121.51	3.44
p2sm	121.53	3.45
w32q90	121.54	3.47
w20q99	121.6	3.52
pwmin	121.61	3.53
p2wmin	121.61	3.53
w32q95	121.69	3.62
w20min	121.71	3.63
pwq95	121.73	3.65
p2wq95	121.73	3.65
yq90	121.75	3.68
w24q99	121.89	3.82
psm	121.89	3.82
pwq50	121.95	3.87
p2wq50	121.95	3.87
yq95	121.99	3.91
psq50	121.99	3.92
w24min	122	3.93
pwm	122	3.93
p2wm	122	3.93
pwq10	122.08	4.01
p2wq10	122.08	4.01
pwq70	122.14	4.07
p2wq70	122.14	4.07
pwq25	122.17	4.09
p2wq25	122.17	4.09
psq70	122.2	4.12
pwq90	122.22	4.15
p2wq90	122.22	4.15
w32q99	122.24	4.16
w32min	122.39	4.32
yq99	122.45	4.37
p2sq70	122.46	4.39
psq90	122.74	4.67
ymin	123	4.93
p2sq90	123.02	4.94
psq95	123.55	5.47
p2sq95	123.77	5.7
psq99	124.53	6.45
psmin	124.72	6.64
p2sq99	125.75	7.68
p2smin	126.11	8.04

Table A.6. **Selection table of most influential discharge parameters affecting distribution of *Cladophora*** Model selection performed using AICc ranking.

Q	AICc	Delta_AICc
p2smax	116.83	0
p2sq10	117.16	0.34
p2sq25	117.91	1.08
p2sq50	118.15	1.32
p2sm	118.34	1.52
p2sq70	118.75	1.92
p2sq90	119.94	3.11
p2sq95	120.49	3.67
psq10	120.95	4.12
yq50	120.97	4.15
psq25	121.01	4.18
ymax	121.15	4.32
yq70	121.16	4.34
psmax	121.21	4.38
w16max	121.34	4.52
psm	121.4	4.57
ym	121.43	4.61
w32q70	121.49	4.67
psq50	121.51	4.69
w32q50	121.53	4.7
psq70	121.53	4.71
yq90	121.56	4.74
yq25	121.59	4.77
Q	121.61	4.78
w20max	121.62	4.79
pwq90	121.62	4.79
p2wq90	121.62	4.8
w16q10	121.63	4.8
pwq99	121.63	4.8
p2wq99	121.63	4.81
yq95	121.65	4.82
p2sq99	121.66	4.83
pwmin	121.66	4.83
p2wmin	121.66	4.83
w20q25	121.67	4.84
w20q50	121.67	4.85
yq99	121.69	4.86
pwq70	121.7	4.87
p2wq70	121.7	4.87
w16q25	121.71	4.88
yq10	121.71	4.88
pwq95	121.71	4.88
p2wq95	121.71	4.89
p2smin	121.72	4.89
w20m	121.73	4.9
w32m	121.73	4.91
psq90	121.73	4.91
ymin	121.74	4.91
w24max	121.75	4.92
pwq50	121.75	4.92
p2wq50	121.75	4.93
w20q90	121.76	4.93
w16m	121.77	4.94
w32max	121.77	4.95
w2max	121.78	4.95
w4max	121.78	4.95
w8min	121.78	4.95
w20q10	121.78	4.96
w24q50	121.78	4.96
w3max	121.79	4.96
w32q25	121.79	4.97
wmax	121.8	4.97
w2q10	121.8	4.97
w4q25	121.8	4.97
w20q70	121.8	4.97
pwm	121.8	4.98
p2wm	121.8	4.98
wmin	121.81	4.98
wq10	121.81	4.98
wq25	121.81	4.98
wq99	121.81	4.98
w2q25	121.81	4.98
w2q50	121.81	4.98
w3q25	121.81	4.99

w4q10	121.81	4.99
w8max	121.81	4.99
w8q99	121.81	4.99
w12q10	121.81	4.99
wm	121.82	4.99
wq50	121.82	4.99
wq70	121.82	4.99
wq90	121.82	4.99
wq95	121.82	4.99
w2m	121.82	4.99
w3q10	121.82	5
w8q10	121.82	5
w16q50	121.82	5
pwmax	121.82	5
p2wmax	121.82	5
w2q70	121.83	5
w12max	121.83	5
w12q25	121.83	5.01
w16q70	121.83	5.01
w3m	121.84	5.01
w3q95	121.84	5.01
w4m	121.84	5.01
w8q25	121.84	5.01
w8q95	121.84	5.02
w20q95	121.84	5.02
w32q90	121.84	5.02
w2q90	121.85	5.02
w2q95	121.85	5.02
w3q99	121.85	5.02
w4min	121.85	5.02
w4q99	121.85	5.02
w8m	121.85	5.02
w8q50	121.85	5.02
w8q90	121.85	5.03
w32q95	121.85	5.03
w2min	121.86	5.03
w2q99	121.86	5.03
w3min	121.86	5.03
w3q70	121.86	5.03
w3q90	121.86	5.03
w4q50	121.86	5.03
w12m	121.86	5.03
w12q50	121.86	5.03
w3q50	121.87	5.04
w4q70	121.87	5.04
w4q95	121.87	5.04
w8q70	121.87	5.04
w12q70	121.87	5.04
w24m	121.87	5.04
w24q90	121.87	5.05
w4q90	121.88	5.05
w16q90	121.89	5.06
w24q25	121.89	5.06
w24q95	121.89	5.06
w32q10	121.89	5.06
pwq25	121.89	5.07
p2wq25	121.89	5.07
w12q90	121.91	5.09
w12min	121.92	5.09
w24q70	121.92	5.09
w16q95	121.93	5.1
w24q10	121.93	5.1
w12q95	121.94	5.11
w12q99	121.94	5.11
psq95	121.95	5.13
w20q99	121.99	5.16
pwq10	121.99	5.16
p2wq10	121.99	5.17
w32q99	122.01	5.19
w16q99	122.04	5.21
w20min	122.04	5.21
w24q99	122.04	5.22
w16min	122.06	5.23
w32min	122.06	5.24
psq99	122.07	5.25
psmin	122.08	5.25
w24min	122.09	5.26

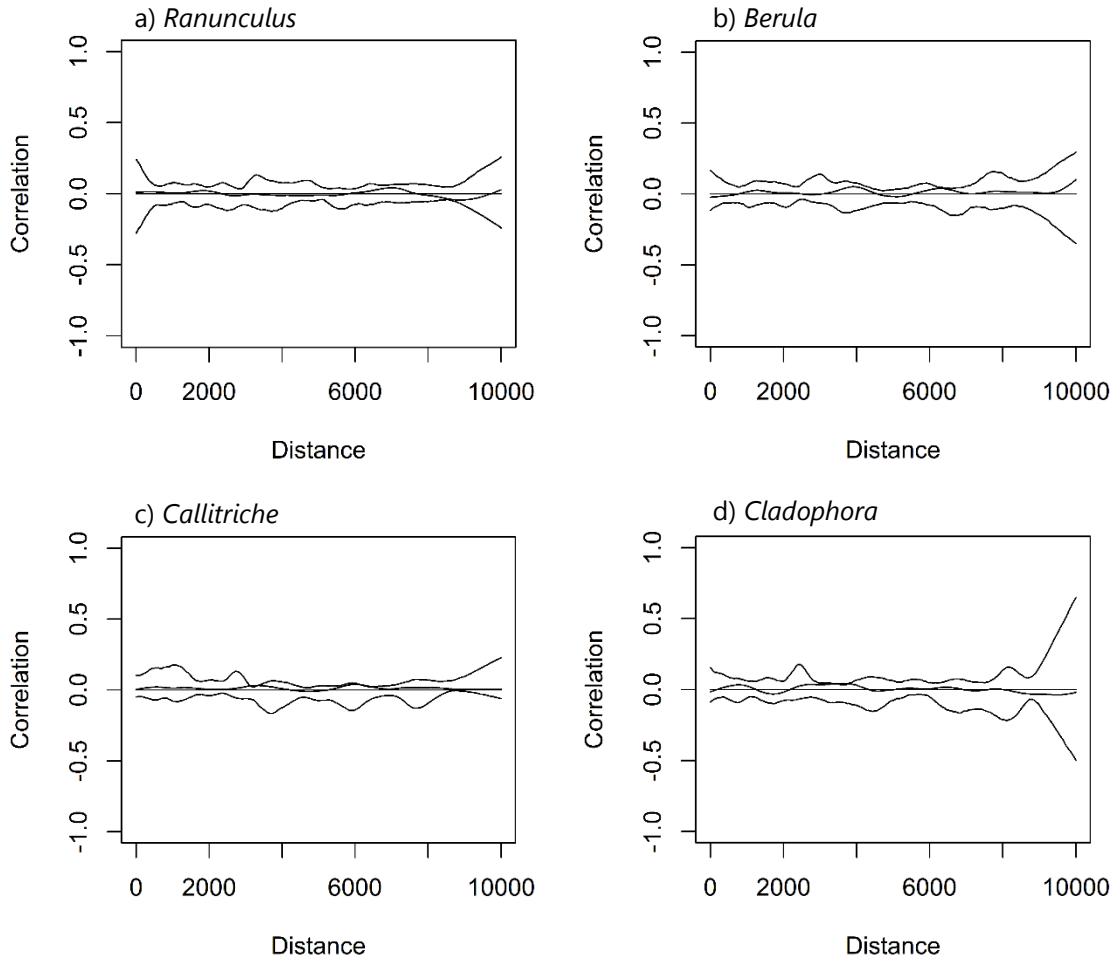

Figure A.5. **Spline correlograms of Pearson residuals for key macrophyte taxa** demonstrating lack of spatial auto-correlation. Correlograms were generated from linear mixed models including all explanatory variables and random effects, and are displayed with 95% pointwise bootstrap confidence intervals. Key taxa are a) *Ranunculus*, b) *Berula*, c) *Callitriche*, d) *Cladophora*.

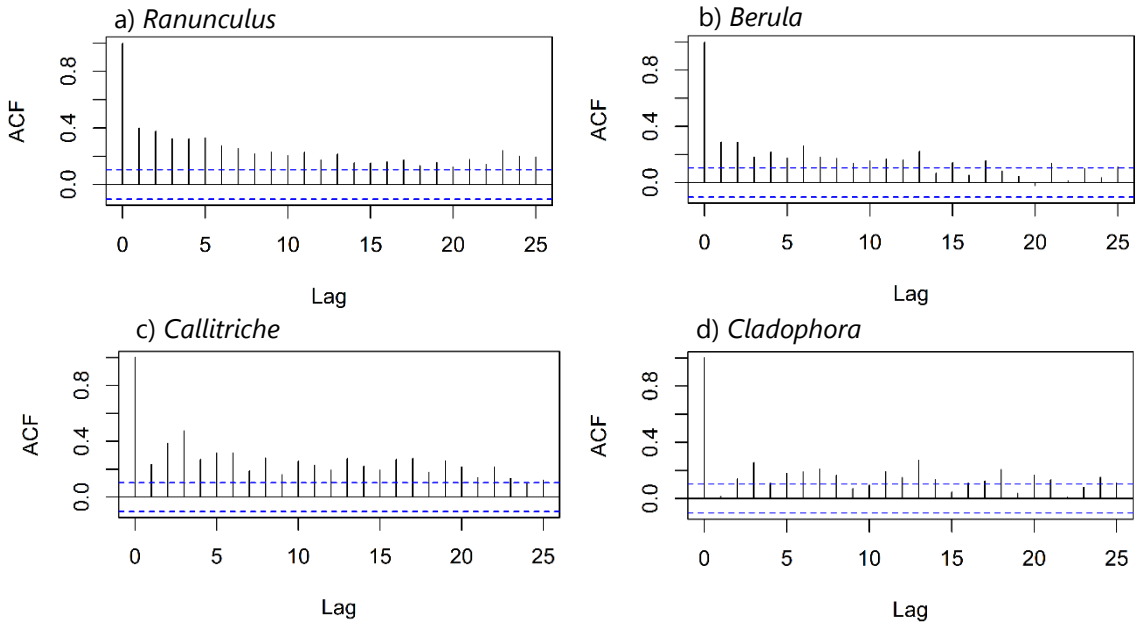

Figure A.6. **Auto-correlation function (ACF) plots of residuals for key macrophyte taxa** demonstrating temporal auto-correlation, particularly at short lag-distances. ACFs were generated from mixed models including spatial (sampling site) variables. Key taxa are a) *Ranunculus*, b) *Berula*, c) *Callitriche*, d) *Cladophora*.

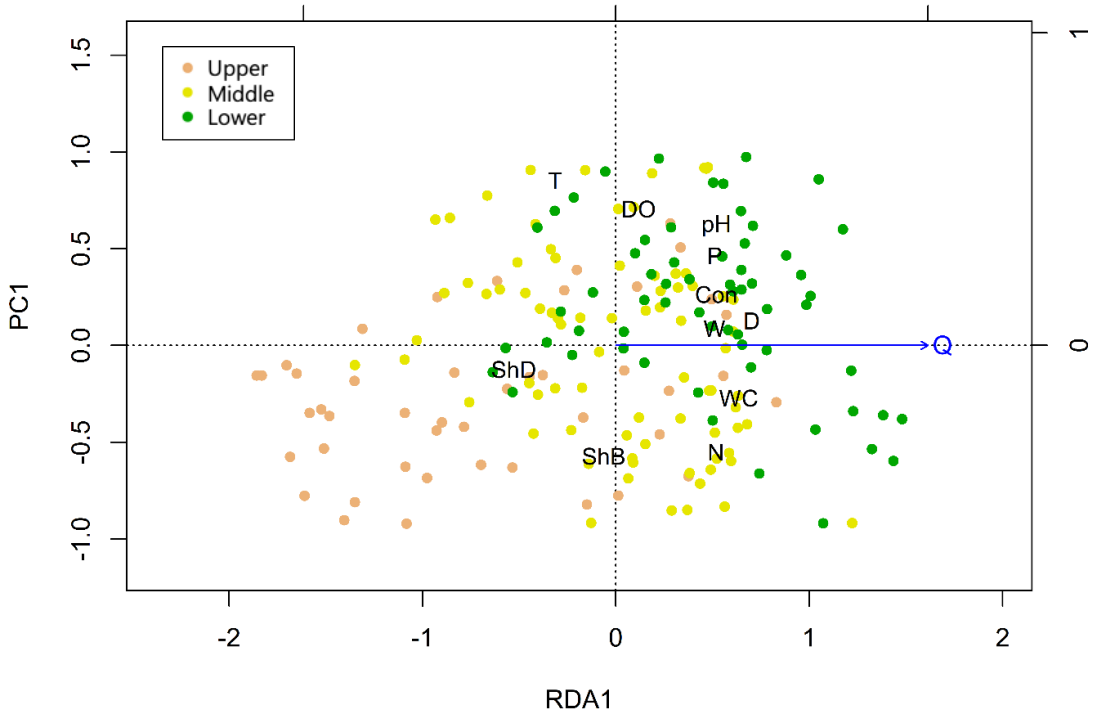

Figure A.7. **Partial redundancy analysis (pRDA) illustrating spatial differences in riverine physicochemistry constrained to river discharge (Q).** Influence of temporal, spatial and all other environmental variables partialled out. Blue arrow represents direction and significance of Q to axis 1 (RDA1), Abbreviated names represent water physicochemical parameters and coloured dots are samples. Con – conductivity, DO – dissolved oxygen, N – nitrate, P – phosphate, pH – water pH, ShB – broken shade, ShD – dense shade, T – temperature, D – channel depth, W – channel width, WC – water clarity, Q – discharge.

Table A.7. **Key macrophyte model summaries of optimal variance structures.** Statistics represent comparisons with no variance structure: *L* is the log likelihood ratio statistic, *df* is degrees of freedom, and *p* is the *p*-value. Where "*none* " is stated for the optimal variance structure, data did not show heterogeneity and therefore no structure was used.

Key macrophyte	Optimal variance structure	Variance structure statistics		
		L	*df*	*p*
R. pseudofluitans	varIdent (Site)	78.4637	27	<0.001
B. erecta	varIdent (Site)	82.0642	27	<0.001
Callitriche spp.	varIdent (Site)	105.1124	27	<0.001
C. glomerata	varIdent (Site)	64.2123	27	<0.001

Table A.8. **Summary of mixed model selection statistics for *Ranunculus*.** Only models in the 95% candidate set ($\sum w_i > 0.95$) are displayed in the summary table. Models are ranked in order of highest AICc. Explanatory variables displayed are those present in the top models and represent a sub-selection of full suite of model parameters. Con = conductivity, D = water depth, N = nitrate, P = orthophosphate, DO = dissolved oxygen pwq25 = Q_{25} since the previous winter, ShD = dense shade, ShB = broken shade, T = temperature, W = river channel width, WC = water clarity.

Rank	Explanatory variables (fixed components)*											K	AIC_C	Δ_i	w_i
	Con	D	N	P	DO	pwq 25	ShD	ShB	T	W	WC				
1	1	1				1						7	651.5	0	0.13
2	1	1		1		1						8	651.9	0.35	0.109
3	1	1				1			1			8	653	1.47	0.063
4	1	1				1				1		8	653.4	1.9	0.05
5	1	1		1		1				1		9	653.7	2.22	0.043
6	1	1		1		1			1			9	654	2.46	0.038
7	1	1				1						8	654.1	2.61	0.035
8	1	1	1			1						8	654.2	2.66	0.034
9	1	1		1		1						9	654.5	2.95	0.03
10	1	1	1	1		1						9	654.5	3.02	0.029
11	1	1				1			1	1		9	654.8	3.28	0.025
12	1			1		1						7	655	3.53	0.022
13	1					1						6	655.3	3.79	0.02
14	1	1				1			1			9	655.4	3.94	0.018
15	1	1				1				1		9	656	4.51	0.014
16	1	1	1			1				1		9	656.1	4.59	0.013
17	1	1		1		1			1	1		10	656.1	4.62	0.013
18	1				1							6	656.2	4.67	0.013
19	1	1			1	1						9	656.2	4.68	0.013
20	1	1		1		1				1		10	656.3	4.81	0.012
21	1	1		1	1	1						10	656.4	4.91	0.011
22	1	1	1	1		1				1		10	656.4	4.95	0.011
23	1	1				1		1				8	656.6	5.11	0.01
24	1	1		1		1		1				9	656.9	5.44	0.009
25	1	1	1			1						9	657	5.48	0.008
26	1		1	1		1						8	657.2	5.71	0.008
27	1	1				1			1	1		10	657.3	5.77	0.007
28	1	1	1	1		1						10	657.3	5.8	0.007
29	1	1		1		1			1			10	657.3	5.83	0.007
30	1					1			1			7	657.4	5.94	0.007
31	1		1		1	1			1			8	657.7	6.17	0.006
32	1			1		1				1		8	657.8	6.26	0.006

33	1		1				1						7	657.8	6.32	0.006
34	1						1					1	7	657.8	6.32	0.006
35	1				1		1				1		8	657.8	6.35	0.005
36	1	1					1	1					8	658.3	6.79	0.004
37	1				1	1							7	658.3	6.83	0.004
38	1	1					1		1			1	9	658.4	6.9	0.004
39	1	1			1		1	1					9	658.4	6.92	0.004
40	1	1				1	1					1	10	658.4	6.93	0.004
41	1	1	1			1					1		9	658.5	7.02	0.004
42	1					1							7	658.5	7.04	0.004
43	1	1	1				1		1				9	658.6	7.07	0.004
44	1					1						1	7	658.6	7.08	0.004
45	1	1	1			1	1						10	658.6	7.14	0.004
46	1	1			1	1	1					1	11	658.7	7.17	0.004
47	1	1	1		1	1	1						11	658.7	7.25	0.003
48	1	1			1		1		1			1	10	658.8	7.3	0.003
49	1	1				1	1				1		10	658.8	7.3	0.003
50	1	1				1							7	658.8	7.31	0.003
51	1	1				1	1						10	658.8	7.33	0.003
52	1	1			1		1	1			1		10	658.9	7.39	0.003
53	1	1	1				1					1	10	658.9	7.39	0.003
54	1	1	1	1			1		1				10	658.9	7.42	0.003
55	1	1		1	1		1						11	658.9	7.45	0.003
56	1			1			1						8	659	7.52	0.003
57	1	1					1	1			1		9	659.1	7.59	0.003
58	1	1	1	1			1					1	11	659.2	7.7	0.003
59	1						1						7	659.4	7.87	0.003
60	1	1					1		1		1		9	659.4	7.91	0.002
61	1		1			1					1	1	9	659.5	7.99	0.002
62	1	1			1		1				1	1	11	659.5	8.04	0.002
63	1				1	1	1						9	659.6	8.09	0.002
64	1	1	1			1					1	1	10	659.6	8.12	0.002
65	1					1		1					7	659.7	8.21	0.002
66	1		1			1							7	659.7	8.22	0.002
67	1		1	1		1					1		9	659.8	8.27	0.002
68	1					1	1						8	659.9	8.38	0.002
69	1		1	1			1					1	9	659.9	8.41	0.002
70	1	1		1	1	1	1				1		11	660	8.46	0.002
71	1					1					1		7	660	8.49	0.002
72	1	1		1			1		1		1		10	660.2	8.69	0.002
73	1					1			1				7	660.3	8.76	0.002
74	1						1				1	1	8	660.3	8.82	0.002
75	1		1				1					1	8	660.3	8.82	0.002
76	1	1	1				1	1					9	660.4	8.92	0.002
77	1	1					1	1				1	9	660.6	9.06	0.001
78	1	1					1		1				9	660.6	9.07	0.001
79	1	1	1				1		1			1	10	660.6	9.14	0.001
80	1	1		1			1	1				1	10	660.6	9.15	0.001
81	1	1	1	1			1	1					10	660.7	9.17	0.001
82	1	1	1	1	1						1		10	660.7	9.19	0.001
83	1			1	1							1	8	660.7	9.24	0.001
84	1			1	1								8	660.8	9.25	0.001
85	1	1				1							8	660.8	9.26	0.001
86	1	1	1			1	1					1	11	660.8	9.27	0.001
87	1	1				1	1		1				10	660.8	9.32	0.001
88	1	1				1	1				1	1	11	660.8	9.34	0.001
89	1	1		1		1							8	660.9	9.42	0.001
90	1	1					1		1	1	1		10	660.9	9.44	0.001
91	1	1		1			1		1				10	661	9.48	0.001
92	1	1	1	1	1		1					1	12	661	9.52	0.001

93	1					1						1	8	661	9.53	0.001
94	1	1				1	1					1	11	661	9.53	0.001
95	1				1		1	1					8	661.1	9.56	0.001
96	1	1			1	1	1					1	12	661.1	9.59	0.001
97	1	1	1		1		1			1		1	11	661.1	9.59	0.001
98	1	1			1	1	1			1			11	661.1	9.62	0.001
99	1		1		1	1	1						10	661.2	9.73	0.001
100	1	1					1	1			1	1	10	661.3	9.8	0.001
101	1	1				1				1			8	661.3	9.83	0.001
102	1	1			1		1	1			1	1	11	661.4	9.86	0.001
103	1		1			1					1		9	661.4	9.86	0.001
104	1						1				1		8	661.4	9.92	0.001
105	1	1	1		1	1	1						12	661.5	9.95	0.001
106	1					1					1	1	8	661.5	9.98	0.001
107	1	1	1			1					1		10	661.5	9.98	0.001
108	1	1	1			1	1						11	661.5	9.98	0.001
109	1		1		1	1					1	1	10	661.5	10.02	0.001
110	1	1				1	1				1		11	661.6	10.1	0.001
111	1		1			1		1			1		9	661.6	10.12	0.001
112	1						1	1					7	661.7	10.18	0.001
113	1				1		1					1	9	661.7	10.22	0.001
114	1		1		1		1						9	661.8	10.26	0.001
115	1						1					1	8	661.8	10.31	0.001
116	1	1				1						1	8	661.8	10.32	0.001
117	1					1		1				1	8	661.8	10.32	0.001
118	1	1			1	1	1				1	1	12	661.8	10.34	0.001
119	1	1	1		1	1					1	1	11	661.9	10.37	0.001
120	1	1				1					1		8	661.9	10.37	0.001

Table A.9. **Summary of mixed model selection statistics for *Berula*.** Only models in the 95% candidate set ($\sum w_i > 0.95$) are displayed in the summary table. Models are ranked in order of highest AICc. Explanatory variables displayed are those present in the top models and represent a sub-selection of full suite of model parameters. D = water depth, DO = dissolved oxygen, N = nitrate, Con = conductivity, P = orthophosphate, Q = sample day Q, ShB = broken shade, ShD = dense shade, T = temperature, W = river channel width, WC = water clarity

Rank	Explanatory variables (fixed components)*											K	AIC_c	Δ_i	w_i
	D	DO	N	Con	P	Q	ShB	ShD	T	W	WC				
1				1		1		1			1	9	531.6	0	0.087
2				1		1	1	1			1	11	532.5	0.84	0.057
3	1			1		1		1			1	10	532.6	1	0.053
4				1		1					1	7	533.7	2.02	0.032
5		1		1		1		1			1	10	533.7	2.05	0.031
6			1	1		1		1			1	10	533.7	2.07	0.031
7	1			1		1	1	1			1	12	533.9	2.22	0.029
8				1		1		1			1	10	534	2.39	0.026
9				1		1		1			1	10	534.4	2.73	0.022
10				1		1	1	1	1		1	12	534.7	3.07	0.019
11	1		1	1		1		1			1	11	534.8	3.12	0.018
12		1		1		1	1	1			1	12	534.8	3.17	0.018
13	1			1		1		1	1		1	11	535	3.42	0.016
14			1	1		1					1	8	535.1	3.43	0.016
15	1		1	1	1	1			1		1	13	535.1	3.46	0.015
16	1			1		1		1			1	11	535.1	3.51	0.015
17				1		1					1	8	535.2	3.59	0.015
18	1			1		1		1			1	11	535.4	3.72	0.014
19			1	1		1	1	1			1	12	535.5	3.84	0.013

20		1	1	1		1		1			1	11	535.5	3.87	0.013
21		1		1		1					1	8	535.8	4.15	0.011
22				1		1					1	8	535.9	4.3	0.01
23		1		1		1		1			1	11	535.9	4.31	0.01
24	1			1		1	1	1	1		1	13	536	4.41	0.01
25	1			1		1					1	8	536.1	4.44	0.01
26				1		1	1	1			1	12	536.2	4.52	0.009
27			1	1		1		1			1	11	536.2	4.55	0.009
28	1	1		1		1	1	1			1	13	536.2	4.56	0.009
29				1	1	1		1			1	11	536.2	4.56	0.009
30				1		1			1		1	8	536.4	4.78	0.008
31	1	1	1	1		1		1			1	12	536.4	4.8	0.008
32	1		1	1		1	1	1			1	13	536.4	4.81	0.008
33		1		1		1		1			1	11	536.4	4.81	0.008
34			1	1		1		1	1		1	11	536.4	4.82	0.008
35			1	1		1		1			1	11	536.6	4.94	0.007
36		1		1		1		1	1		1	11	536.7	5.03	0.007
37				1		1		1			1	11	536.7	5.07	0.007
38			1	1		1	1	1	1		1	13	536.8	5.16	0.007
39		1	1	1		1					1	9	536.8	5.21	0.006
40	1			1	1	1		1			1	12	537	5.33	0.006
41				1		1		1	1		1	11	537	5.34	0.006
42			1	1		1					1	9	537	5.36	0.006
43			1	1	1	1					1	10	537.1	5.45	0.006
44	1		1	1		1		1	1		1	12	537.2	5.53	0.006
45		1		1		1					1	9	537.3	5.64	0.005
46			1	1		1			1		1	9	537.3	5.65	0.005
47		1		1		1	1	1	1		1	13	537.3	5.69	0.005
48	1		1	1		1					1	9	537.3	5.7	0.005
49	1	1		1		1		1			1	12	537.3	5.7	0.005
50	1		1	1		1		1			1	12	537.3	5.72	0.005
51	1			1		1	1	1			1	13	537.5	5.83	0.005
52	1	1		1		1		1	1		1	12	537.5	5.86	0.005
53	1			1		1					1	9	537.5	5.89	0.005
54	1		1	1		1		1			1	12	537.6	5.96	0.004
55				1		1					1	9	537.6	6.01	0.004
56		1	1	1	1	1		1			1	13	537.8	6.15	0.004
57	1			1		1		1			1	12	537.8	6.17	0.004
58	1			1		1		1	1		1	12	537.8	6.19	0.004
59		1	1	1	1	1					1	11	537.9	6.23	0.004
60		1		1	1	1		1			1	12	537.9	6.29	0.004
61			1	1		1					1	9	537.9	6.29	0.004
62		1	1	1		1		1			1	12	537.9	6.3	0.004
63	1			1		1		1	1		1	12	537.9	6.32	0.004
64				1		1	1				1	9	538.1	6.47	0.003
65		1		1		1					1	9	538.1	6.47	0.003
66				1	1	1					1	9	538.1	6.51	0.003
67	1		1	1		1	1	1	1		1	14	538.2	6.54	0.003
68	1	1		1		1					1	9	538.2	6.57	0.003
69	1			1		1	1	1			1	13	538.3	6.71	0.003
70	1			1		1					1	9	538.4	6.73	0.003
71				1		1	1	1	1		1	13	538.4	6.75	0.003
72	1	1	1	1	1	1		1			1	14	538.4	6.77	0.003
73		1	1	1		1		1			1	12	538.4	6.78	0.003
74				1		1			1		1	9	538.4	6.8	0.003
75		1		1		1	1	1			1	13	538.5	6.84	0.003
76	1		1	1	1	1					1	11	538.5	6.86	0.003
77	1			1		1			1		1	9	538.5	6.9	0.003
78			1	1		1	1				1	10	538.5	6.91	0.003
79			1	1	1	1					1	11	538.6	6.93	0.003

80	1			1	1	1		1			1	13	538.6	7.02	0.003
81		1		1		1		1			1	12	538.7	7.03	0.003
82	1	1	1	1		1	1	1			1	14	538.7	7.04	0.003
83	1	1		1		1	1	1	1		1	14	538.7	7.05	0.003
84	1	1		1	1	1		1			1	13	538.7	7.06	0.003
85		1	1	1		1					1	10	538.7	7.09	0.003
86		1		1		1			1		1	9	538.8	7.13	0.002
87				1		1			1		1	9	538.8	7.13	0.002
88	1	1	1	1	1	1					1	12	538.8	7.15	0.002
89				1	1	1		1			1	12	538.8	7.16	0.002
90	1		1	1		1			1		1	10	538.9	7.23	0.002
91	1		1	1	1	1			1		1	14	538.9	7.24	0.002
92		1	1	1		1		1	1		1	12	538.9	7.25	0.002
93			1	1		1		1			1	12	539	7.32	0.002
94	1	1	1	1		1		1			1	13	539	7.34	0.002
95	1	1	1	1		1					1	10	539	7.41	0.002
96				1	1	1					1	10	539.1	7.5	0.002
97	1		1	1		1					1	10	539.2	7.58	0.002
98			1	1		1	1	1			1	13	539.2	7.58	0.002
99		1		1	1	1		1			1	13	539.2	7.61	0.002
100	1	1	1	1		1		1			1	13	539.3	7.65	0.002
101		1	1	1	1	1					1	12	539.3	7.66	0.002
102		1	1	1	1	1		1			1	14	539.3	7.69	0.002
103		1		1		1		1	1		1	12	539.4	7.79	0.002
104			1	1		1		1	1		1	12	539.5	7.84	0.002
105	1	1		1		1					1	10	539.6	7.93	0.002
106	1	1	1	1		1		1	1		1	13	539.6	7.94	0.002
107	1			1	1	1		1			1	13	539.6	7.95	0.002
108	1	1		1		1		1			1	13	539.6	8.01	0.002
109			1	1		1		1	1		1	12	539.6	8.02	0.002
110			1	1		1	1	1			1	13	539.7	8.06	0.002
111		1	1	1		1			1		1	10	539.7	8.07	0.002
112				1		1		1	1		1	12	539.7	8.09	0.002
113		1		1		1					1	10	539.7	8.11	0.002
114	1	1		1		1	1	1			1	14	539.8	8.14	0.001
115			1	1	1	1					1	11	539.8	8.15	0.001
116				1		1	1	1	1		1	13	539.8	8.16	0.001
117		1	1	1		1					1	10	539.8	8.16	0.001
118				1		1	1				1	10	539.8	8.18	0.001
119			1	1		1					1	10	539.8	8.2	0.001
120		1		1		1	1	1			1	13	539.9	8.24	0.001
121	1			1	1	1					1	10	539.9	8.29	0.001
122			1	1	1	1	1	1			1	14	539.9	8.29	0.001
123	1			1		1					1	10	540	8.32	0.001
124	1		1	1	1	1					1	12	540	8.36	0.001
125	1	1	1	1	1	1		1			1	15	540.1	8.43	0.001
126			1	1		1			1		1	10	540.1	8.45	0.001
127	1		1	1		1		1			1	13	540.1	8.45	0.001
128	1	1		1	1	1		1			1	14	540.1	8.51	0.001
129	1			1		1	1				1	10	540.2	8.53	0.001
130	1		1	1		1					1	10	540.2	8.54	0.001
131			1	1		1	1		1		1	11	540.2	8.55	0.001
132		1	1	1	1	1	1	1			1	15	540.2	8.55	0.001
133		1		1	1	1		1			1	13	540.2	8.57	0.001
134		1	1	1	1	1		1			1	14	540.2	8.59	0.001
135	1	1		1		1		1	1		1	13	540.2	8.6	0.001
136	1	1	1	1	1	1					1	13	540.3	8.63	0.001
137	1		1	1	1	1	1	1			1	15	540.3	8.63	0.001
138		1		1	1	1					1	10	540.3	8.64	0.001
139			1	1	1	1		1			1	13	540.3	8.64	0.001

140	1		1	1		1		1	1		1	13	540.3	8.67	0.001
141				1	1	1					1	10	540.3	8.69	0.001
142	1		1	1		1	1	1			1	14	540.3	8.7	0.001
143		1	1	1		1	1	1	1		1	14	540.3	8.7	0.001
144	1			1		1			1		1	10	540.4	8.75	0.001
145	1	1		1		1		1	1		1	13	540.4	8.76	0.001
146	1		1	1		1		1	1		1	13	540.4	8.76	0.001
147	1		1	1		1	1				1	11	540.4	8.78	0.001
148		1		1		1	1				1	10	540.5	8.88	0.001
149	1	1		1		1					1	10	540.5	8.91	0.001
150	1		1	1		1	1	1			1	14	540.5	8.91	0.001
151				1	1	1	1	1			1	14	540.6	9.02	0.001
152	1	1	1	1	1	1	1	1			1	16	540.7	9.04	0.001
153	1			1		1		1	1		1	13	540.7	9.05	0.001
154		1		1	1	1	1	1			1	14	540.7	9.05	0.001
155				1		1	1		1		1	10	540.7	9.06	0.001
156		1	1	1		1		1			1	13	540.7	9.1	0.001
157		1	1	1	1	1					1	12	540.7	9.12	0.001
158	1			1		1	1	1	1		1	14	540.8	9.13	0.001
159			1	1		1			1		1	10	540.8	9.14	0.001
160		1	1	1		1	1				1	11	540.8	9.19	0.001
161		1		1		1			1		1	10	540.8	9.21	0.001
162	1	1	1	1	1	1		1			1	15	540.9	9.23	0.001
163	1	1		1		1	1	1			1	14	540.9	9.24	0.001
164	1	1	1	1		1					1	11	540.9	9.25	0.001
165	1			1	1	1					1	11	540.9	9.26	0.001
166	1			1		1			1		1	10	540.9	9.27	0.001
167	1	1		1		1			1		1	10	540.9	9.29	0.001
168			1	1		1	1				1	11	540.9	9.3	0.001
169		1		1		1	1	1	1		1	14	540.9	9.3	0.001
170	1		1	1	1	1		1			1	14	541	9.33	0.001
171				1		1			1		1	10	541	9.34	0.001
172			1	1	1	1	1				1	12	541	9.36	0.001
173				1	1	1		1	1		1	12	541.1	9.44	0.001
174	1	1		1	1	1		1			1	14	541.1	9.46	0.001
175		1		1		1			1		1	10	541.1	9.47	0.001

Table A.10. **Summary of mixed model selection statistics for *Callitriche*.** Only models in the 95% candidate set ($\sum w_i > 0.95$) are displayed in the summary table. Models are ranked in order of highest AICc. Explanatory variables displayed are those present in the top models and represent a sub-selection of full suite of model parameters. Con = conductivity, D = water depth, DO = dissolved oxygen, N = nitrate, P = orthophosphate, ShB = broken shade, ShD = dense shade, w12max = Q_{max} over the preceding 12 weeks, T = temperature, W = river channel width, WC = water clarity

	Explanatory variables (fixed components)*														
Rank	**Con**	**D**	**DO**	**N**	**P**	**ShB**	**ShD**	**W12 max**	**T**	**W**	**WC**	**K**	**AICc**	**Δ_i**	**w_i**
1	1			1			1	1	1	1	1	11	670.4	0	0.288
2	1			1		1	1	1	1	1	1	13	671.1	0.69	0.205
3	1	1		1			1	1	1	1	1	12	672.2	1.73	0.121
4	1	1		1		1	1	1	1	1	1	14	672.6	2.15	0.098
5	1		1	1			1	1	1	1	1	12	673.7	3.29	0.056
6	1		1	1		1	1	1	1	1	1	14	674	3.56	0.049
7	1			1			1	1	1	1	1	12	674.3	3.82	0.043
8	1	1	1	1		1	1	1	1	1	1	15	675.5	5.08	0.023
9	1	1	1	1			1	1	1	1	1	13	675.8	5.34	0.02
10	1	1		1			1	1	1	1	1	13	676	5.58	0.018

11	1			1	1		1	1	1	1	1	13	676.1	5.61	0.017
12	1			1	1	1	1	1	1	1	1	15	676.8	6.39	0.012
13	1	1		1		1	1	1	1		1	13	676.8	6.4	0.012
14	1		1	1			1	1	1	1	1	13	677.6	7.17	0.008
15	1			1		1	1	1	1	1	1	14	677.6	7.19	0.008
16	1		1	1		1	1	1	1		1	13	677.9	7.42	0.007
17	1	1		1	1		1	1	1	1	1	14	678.1	7.68	0.006
18	1	1		1	1	1	1	1	1	1	1	16	678.6	8.12	0.005
19	1	1		1		1	1	1	1	1	1	15	678.7	8.28	0.005

Table A.11. **Summary of mixed model selection statistics for *Cladophora*.** Only models in the 95% candidate set ($\sum w_i > 0.95$) are displayed in the summary table. Models are ranked in order of highest AICc. Explanatory variables displayed are those present in the top models and represent a sub-selection of full suite of model parameters. Con = conductivity, D = water depth, DO = dissolved oxygen, N = nitrate, P = orthophosphate, p2sqmax = prev 2 summers Q_{max}, ShB = broken shade, ShD = dense shade, T = temperature, W = river channel width, WC = water clarity

Rank	Explanatory variables (fixed components)*											K	AIC_C	Δ_i	w_i
	Con	D	DO	N	P	p2sqmax	ShB	W	ShD	T	WC				
1	1		1		1	1	1					11	740.3	0	0.248
2	1	1	1		1	1	1					12	742.3	2.03	0.09
3	1		1		1	1	1			1		12	742.9	2.6	0.068
4	1		1			1	1		1			10	743.1	2.89	0.058
5	1					1	1		1			9	743.7	3.49	0.043
6	1		1		1	1						9	744.4	4.19	0.031
7	1		1			1	1		1			11	744.9	4.62	0.025
8	1	1	1		1	1	1			1		13	744.9	4.64	0.024
9	1	1	1			1	1		1			11	745.4	5.15	0.019
10	1		1		1	1	1		1			12	745.4	5.17	0.019
11	1				1	1	1			1		11	745.5	5.2	0.018
12	1		1			1						7	745.6	5.32	0.017
13	1					1						6	745.6	5.36	0.017
14	1		1		1	1	1					12	745.7	5.43	0.016
15	1				1	1	1		1			11	745.9	5.62	0.015
16	1					1	1	1				10	745.9	5.69	0.014
17	1	1				1	1		1			10	746	5.7	0.014
18	1		1			1	1	1				11	746.1	5.82	0.014
19	1				1	1						8	746.3	6.02	0.012
20	1	1	1		1	1						10	746.4	6.11	0.012
21	1					1	1		1			10	746.4	6.17	0.011
22	1		1	1	1	1	1					12	746.7	6.49	0.01
23	1		1		1	1				1		10	746.9	6.64	0.009
24	1		1			1	1			1		10	747.2	6.97	0.008
25	1		1			1	1			1		11	747.2	6.98	0.008
26	1	1	1			1	1		1			12	747.2	6.98	0.008
27	1					1	1			1		10	747.3	7.05	0.007
28	1					1	1			1		9	747.3	7.09	0.007
29	1	1	1		1	1	1					13	747.5	7.25	0.007
30	1	1				1	1	1				11	747.8	7.52	0.006
31	1	1				1						7	747.8	7.54	0.006
32	1		1		1	1	1		1	1		13	747.8	7.54	0.006
33	1	1	1		1	1	1		1			13	747.8	7.56	0.006
34	1	1	1			1	1	1				12	747.9	7.61	0.006
35	1		1		1	1	1		1			13	748.2	7.94	0.005
36	1				1	1				1		9	748.2	7.98	0.005

37	1		1				1						8	748.2	7.98	0.005
38	1		1			1	1	1				1	13	748.3	8	0.005
39	1		1		1	1	1	1				1	13	748.3	8.07	0.004
40	1		1			1	1				1		10	748.4	8.15	0.004
41	1						1	1		1			11	748.4	8.18	0.004
42	1	1				1	1	1				1	12	748.6	8.34	0.004
43	1						1						7	748.7	8.42	0.004
44	1	1					1	1			1		11	748.7	8.42	0.004
45	1	1	1				1						8	748.7	8.46	0.004
46	1		1				1	1		1			12	748.7	8.46	0.004
47	1					1	1	1			1	1	12	748.7	8.5	0.004
48	1		1				1			1			9	748.8	8.52	0.003
49	1						1				1		7	748.8	8.54	0.003
50	1	1	1			1	1					1	11	748.9	8.6	0.003
51	1	1	1	1		1	1	1					13	748.9	8.65	0.003
52	1					1	1				1		9	749.1	8.89	0.003
53	1	1	1				1	1				1	11	749.3	9.07	0.003
54	1	1				1	1						9	749.4	9.12	0.003
55	1	1	1				1	1				1	12	749.5	9.25	0.002
56	1	1					1	1				1	10	749.5	9.26	0.002
57	1	1					1	1				1	11	749.6	9.34	0.002
58	1	1				1	1	1			1		12	749.7	9.41	0.002
59	1						1					1	7	749.7	9.42	0.002
60	1						1			1			8	749.7	9.45	0.002
61	1					1	1	1				1	12	749.8	9.5	0.002
62	1		1				1				1		8	749.8	9.53	0.002
63	1		1				1					1	8	749.9	9.62	0.002
64	1									1			6	750	9.71	0.002
65	1					1	1	1			1		12	750	9.76	0.002
66	1	1	1			1	1	1				1	14	750.2	9.92	0.002
67	1	1	1			1	1	1			1	1	14	750.2	9.94	0.002
68	1						1	1			1	1	11	750.3	10.05	0.002
69	1					1	1	1					10	750.4	10.14	0.002
70	1	1	1			1	1	1			1		14	750.4	10.17	0.002
71	1		1				1	1			1	1	12	750.4	10.17	0.002
72	1	1	1				1	1		1			13	750.4	10.19	0.002
73	1						1	1			1	1	10	750.4	10.2	0.002
74	1	1	1			1	1				1		11	750.5	10.2	0.002
75	1		1			1	1				1	1	11	750.5	10.25	0.001
76	1	1	1	1		1	1	1				1	14	750.5	10.28	0.001
77	1		1	1		1	1						10	750.5	10.29	0.001
78	1		1			1	1						10	750.6	10.31	0.001
79	1		1				1	1			1	1	11	750.6	10.35	0.001
80	1	1	1				1						9	750.7	10.45	0.001

APPENDIX B

Chapter 4 supplementary information

Table B.1. **Growth trait model summaries of optimal variance structures.** Statistics represent comparisons with no variance structure: *L* is the log likelihood ratio statistic, *df* is degrees of freedom, and *p* is the *p*-value. Where "*none* " is stated for the optimal variance structure, data did not show heterogeneity and therefore no structure was used.

Response variable (plant growth trait)	**Optimal variance structure**	**Variance structure statistics**		
		L	*df*	*p*
i	varIdent (Channel)	139.6695	11	<0.001
ii	varPower (PlantPos | Channel)	73.0057	12	<0.001
iii	varPower (Channel)	10.2242	2	0.006
iv	*none*	-	-	-
v	varIdent (Channel)	73.5604	11	<0.001
vi	varFixed (Vel)	21.9096	1	<0.001
vii	varFixed (Vel)	58.2810	1	<0.001
viii	varFixed (Vel)	45.6734	1	<0.001
ix	varConstPower (Channel)	31.8379	3	<0.001
x	*none*	-	-	-
I	varPower (PlantPos | Channel)	39.1195	13	<0.001
II	varPower (Channel)	76.7380	2	<0.001
III	varIdent (Channel)	77.9184	12	<0.001
IV	varPower (PlantPos | Channel)	42.9821	13	<0.001
V	varConstPower (Channel)	14.9491	3	<0.001
VI	varPower (Channel)	22.1865	2	<0.001
VII	varFixed (Channel)	14.7458	1	<0.001

APPENDIX C

Chapter 5 supplementary information

Table C.1. **Growth trait model summaries of optimal variance structures.** Statistics represent comparisons with no variance structure: *L* is the log likelihood ratio statistic, *df* is degrees of freedom, and *p* is the *p*-value. Where "*none* " is stated for the optimal variance structure, data did not show heterogeneity and therefore no structure was used.

Response variable (plant growth trait)	Optimal variance structure	Variance structure statistics		
		L	*df*	*p*
i	varConstPower(PlantPos)	9.1817	11	0.027
ii	*none*	-	-	-
iii	varFixed(PlantPos)	5.7154	9	0.017
iv	*none*	-	-	-
v	varPower (Channel)	4.7582	10	0.09†
vi	varPower(PlantPos | Channel)	44.6350	25	<0.001
vii	*none*	-	-	-
viii	*none*	-	-	-
ix	varIdent(Channel)	40.6407	24	<0.001
x	*none*	-	-	-
I	*none*	-	-	-
II	varIdent(Channel)	48.5977	24	<0.001
III	varIdent (Channel)	57.3652	24	<0.001
IV	varPower (PlantPos | Channel)	54.3137	25	<0.001
V	varIdent(Channel)	44.8055	24	<0.001
VI	varIdent(Channel)	44.4589	24	<0.001
VII	varComb(varIdent(PlantPos) + varPower(Channel))	31.2807	14	<0.001
VII	varPower(Channel)	14.1485	10	<0.001

Note: *xi* (damaged plants) and *xii* (washouts) were not modelled due to limited response variables (Figure 5.4.).
† Although likelihood ratio test suggest a non-significant *p* value, the data were visibly improved by this structure.

APPENDIX D

Chapter 6 supplementary information

Table D.1. **Growth trait model summaries of optimal variance structures.** Statistics represent comparisons with no variance structure: *L* is the log likelihood ratio statistic, *df* is degrees of freedom, and *p* is the *p*-value. Where "*none* " is stated for the optimal variance structure, data did not show heterogeneity and therefore no structure was used.

Response variable (plant growth trait)	Optimal variance structure	Variance structure statistics		
		L	*df*	*p*
i	*none*	-	-	-
ii	varExp (Plot)	131.7816	10	<0.001
iii	varComb(varIdent (PlantPos) + varPower (Plot))	59.8639	14	<0.001
iv	*none*	-	-	-
v	*none*	-	-	-
vi	varPower (Plot)	22.20649	10	<0.001
vii	*none*	-	-	-
viii	varConstPower (Plot)	32.6544	11	<0.001
ix	varExp (Plot)	83.4703	10	<0.001
x	*none*	-	-	-
xiii	varConstPower (Plot)	53.1259	11	<0.001
I	varIdent (Plot)	72.0258	28	<0.001
II	varPower (Plot)	9.3051	10	0.009
III	varFixed (Plot)	6.7696	9	0.009
IV	*none*	-	-	-
V	varFixed (Plot)	5.3015	9	0.021
VI	*none*	-	-	-
VII	varExp (Plot)	11.5892	10	<0.001
VIII	varExp (Plot)	3.7447	10	0.05

Note: *xi* (damaged plants) and *xii* (washouts) were not modelled.