Fennel pondweed: a world citizen

Geographic variation in life-cycle characteristics
of the clonal water plant *Potamogeton pectinatus* L.

Jörn Pilon

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Abstract

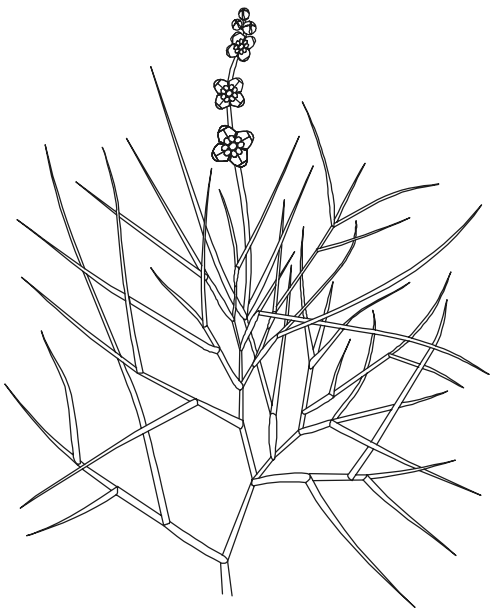
The performance of broadly distributed plants is potentially constrained by geographic variation in climatic factors. Several patterns of response have been proposed that can be considered of adaptive value if variation in abiotic conditions is pronounced. Firstly, species may possess a high capacity for phenotypic plasticity that results in increased tolerance to variation in abiotic factors. Secondly, locally adapted genotypes may evolve that show phenotypic characteristics that are only suited for a restricted set of environmental conditions. Thirdly, populations may avoid unfavourable conditions by showing changes in phenology that result in a compression of the life cycle.

As compared to terrestrial species, many aquatic plants are widespread and have the ability to thrive in different climatic regions. To evaluate to what extent phenotypic plasticity, local adaptation and differences in phenology might contribute to the globally wide distribution of the aquatic macrophyte fennel pondweed (*Potamogeton pectinatus* L.), a series of experiments were performed that focused on geographic variation in life-history traits. For this purpose we used up to 15 clones obtained from a gradient in latitude (24-68°N) and studied their performance in dependence of variation in abiotic factors that relate to climate. We thereby focussed at various stages of the life cycle, such as tuber sprouting, vegetative growth and asexual reproduction.

At northern localities, where the length of the growth season is restricted, genetically fixed changes in phenology result for *P. pectinatus* in a compression of the life cycle. To prevent young plants to be damaged from low spring temperatures, tubers of higher-latitude clones possess a higher thermal threshold for sprouting. In addition, northern clones show early reproduction, which constrains the size of the tubers. Furthermore, adaptive phenotypic plasticity allows *P. pectinatus* to grow at contrasting environmental conditions. Although thermal acclimation in gas-exchange is constrained, plastic changes in morphology are of special importance to attain a comparable biomass between 15/20 and 30°C. *P. pectinatus* can also cope with considerable differences in the light climate. Increased light capture through canopy formation and acclimative changes in photosynthesis result in a relatively high biomass yield at low irradiance (i.e. 2.5% of full sunlight in temperate regions). Similarly, photoperiods varying between 13 and 22 h did result in plastic changes in morphology and physiology that limited the loss of biomass productivity at shorter days. Despite the fact that changes in phenology and high phenotypic plasticity allowed *P. pectinatus* to grow and reproduce in different climatic regions, the Mediterranean clones showed local adaptation resulting from increased perenniality and the absence of asexual reproduction.

In conclusion, this thesis has shown that not a single evolutionary mechanism is responsible for the ability of *P. pectinatus* to thrive in different climatic regions, but that at different stages of the plant's life cycle, phenotypic plasticity, local adaptation and changes in phenology play an important role in maintaining the observed distributional pattern.

Dit proefschrift draag ik op aan
mijn vader Jan J. Pilon, voor wie
het vergaren van kennis en het
anderen laten delen in kennis een
levensdoel was, en mijn moeder
Ingrid Pilon-Meyer die dit op
bijzonder zorgzame wijze
heeft ondersteund.



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'With respect to plants, it has long been known what enormous ranges many fresh-water and even marsh-species have, both over continents and to the most remote oceanic islands.'

Charles Darwin (1859)

CHAPTER 1

General introduction

The distribution of aquatic vascular plants

Differences and similarities between terrestrial floras have led to the recognition of major vegetation types (Eyre, 1971). Many studies have shown that the presence or absence of plant species in particular regions is strongly correlated with seasonal extremes in climatic factors (e.g. Woodward, 1988; Woodward & Williams, 1987). Biogeographers predominantly focussed on terrestrial vegetations, while the distribution of aquatic plants received much less attention. Although aquatic plants only represent a small proportion of all vascular plant species (i.e. 1-2%; Cook, 1990), the available information on their distribution draws an intriguing picture. In comparison with terrestrial plants, many aquatic plants show broad distributional ranges, which makes them true 'citizens of the world'.

Aquatic plants

Aquatic plants may be simply defined by their most eye-catching feature: the permanent or temporal, occurrence in wet environments. Moreover they often possess distinctive ecological characteristics (e.g. high phenotypic plasticity, extensive clonal propagation, reduced sexual reproduction, hydrophilous pollination and water-dispersed diaspores) that clearly set them apart from species native to the terrestrial environment (Barret et al., 1993). Aquatic plants have various growth forms, that range from partially submerged (i.e. emergent, floating leaved, free floating) to completely submerged life cycles (Duarte et al., 1994). In addition, one may discriminate between salt tolerant plants that inhabit marine waters (e.g. oceans, coastal wetlands, salt marshes) and those that are intolerant to high salinity and occur in freshwater ecosystems (e.g. lakes, ponds, rivers, bogs, swamps) (Begon et al., 1996). In this introductory essay the term 'aquatic plant' will refer to all possible growth forms, although the emphasis will be floating-leaved and submerged freshwater species.

Distributional ranges and historic effects

Although rivers flow over large geographic distances and contribute to habitat connectivity, tracts of land that are inhospitable for aquatic plant establishment frequently separate freshwater ecosystems (i.e. lakes and ponds). As aquatic species thus occur in island-like habitats, one might expect them to

show limited spatial distribution. However, already since Darwin (1859), many botanists (e.g. Arber, 1920; Ridley, 1930; Sculthorpe, 1967) have noted the broad distribution of aquatic plants. Sculthorpe (1967) estimated that about 60% of all species show extensive distributional ranges and organised them into three different groups: cosmopolitans, north-temperate species and pan-tropical plants. The complementary proportion of the aquatic flora (40%) has smaller ranges, confined to the limits of a single continent. In addition, a recent study on the biogeography of the freshwater plants of Australasia also indicated a major disjunction between temperate and tropical aquatic floras (Jacobs & Wilson, 1996). Altogether it seems clear that the distribution of aquatic macrophytes reflects the zonation described for terrestrial species, although the pattern is simplified and restricted to broad climatic regions (Santamaría, 2002).

The current distributional patterns of aquatic plants are not only the consequence of ongoing evolutionary processes, but also the result of historical factors. Over the past 150 million years the tectonic plates of the earth's crust have moved in such manner that the continents of Pangea have drifted apart. As a consequence, existing populations were split and moved across broad climatic regions (Begon et al., 1996). In more recent times, the aquatic flora was largely influenced by the glacial cycles of the Pleistocene. While the Arcto-Tertiary flora of the Northern Hemisphere consisted of many species with relatively broad (northern and circumpolar) distributions, glaciation resulted in spatial disruption and only those species with southern affinities could survive near ice margins or in isolated refugia. Some 11.000 years ago, when global temperatures began to rise and the ice age came to an end, meltwater collected in numerous streams and runoff was trapped in ponds and lakes. This resulted in a perfect avenue along which aquatic pioneer species that had survived glaciation (i.e. Arcto-Tertiary flora remnants) could migrate and colonise newly available habitats (Stuckey, 1993).

Dispersal and establishment

The fact that many aquatic vascular plants show broad distributional ranges, suggests that they have the ability to disperse propagules over relatively long distances (Barret et al., 1993). Although seeds of emergent species can be dispersed by wind (e.g. *Typha* and *Phragmites*), most aquatic plant propagules are spread by water or biotic agents (Cook, 1987). Nevertheless, wind can be an important secondary force that blows floating seeds or detached plant parts across lakes. While propagules dispersed by water are transported over relatively short distances (within the limits of catchment areas), migratory waterfowl is probably responsible for long-distance dispersal (Figuerola & Green, 2002). Although ducks and other waterbirds may carry seeds attached to various body parts (ectozoochory), more evidence exists for the dispersal of ingested propagules (endozoochory). After seeds have been swallowed accidentally (i.e. seeds attached to consumed plant parts) or on purpose (i.e. seeds are a food item), the effectiveness of dispersal heavily relies on the propagules' ability to survive gut passage. Moreover, the maximum distance of dispersal is limited by how long propagules remain in the gut of a waterbird (i.e. their retention time) and the range over which the particular migrant can fly during that period of time (Figuerola & Green, 2002; Charalambidou & Santamaría, 2002).

Whether aquatic plants can occupy new habitats, largely depends on the interaction between the founding genotype(s) and the ecological characteristics of a newly adopted homes (Barret et al., 1993). Most importantly, the encountered abiotic conditions should not critically constrain processes like germination, growth and reproduction. Moreover, a high competitive ability would be advantageous in areas already occupied by others. One possibility by which colonisers could persist in

novel environments is that they have the ability to adapt their responses to changes in abiotic conditions (Barret et al., 1993). In this context, genetic recombination through sexual reproduction is of particular importance. Although aquatic plants show a wide diversity of sexual mating systems, clonal propagation is probably the predominant mode of multiplication (Les, 1988). This may ensure rapid colonisation (even in the absence of compatible mating types), but it constrains genetic recombination. As a consequence, the occurrence of locally adapted phenotypes may be less frequent in recent populations. Under these circumstances pronounced phenotypic plasticity (as observed in many broadly distributed aquatic plants) may play an alternative role in facilitating the occupation of new habitats.

Climatic factors and their influence on aquatic plant performance

From a global perspective some abiotic factors that influence aquatic plant performance vary stochastically and show heterogeneity on relatively small spatial scales (i.e. nutrient availability, salinity and sediment anoxia). Other determinants of growth are related to climate and vary in a more predictable manner across latitude. Both the intensity of light and the temperature it creates on the earth's surface are dependent of solar elevation (β). This variable that oscillates between a minimal value at midnight and a maximum value at noon, also determines the length of the photoperiod (i.e. $\beta > 0$; Fig. 1A). The amplitude of β and its variation with time of the day depends on the angle through which the hemispheres are tilted towards the sun (i.e. solar declination, δ) and the degree of latitude (Kirk, 1994). Solar declination varies seasonally and is positive during summer (maximally +23°27'), negative during winter (maximally –23°27') and zero during the spring and autumn equinoxes (Fig. 1B). As a consequence, light intensity and temperature decrease with increasing latitude, while during spring (which coincides with the first half of the growth season) the length of the photoperiod increases (Fig. 2A to C).

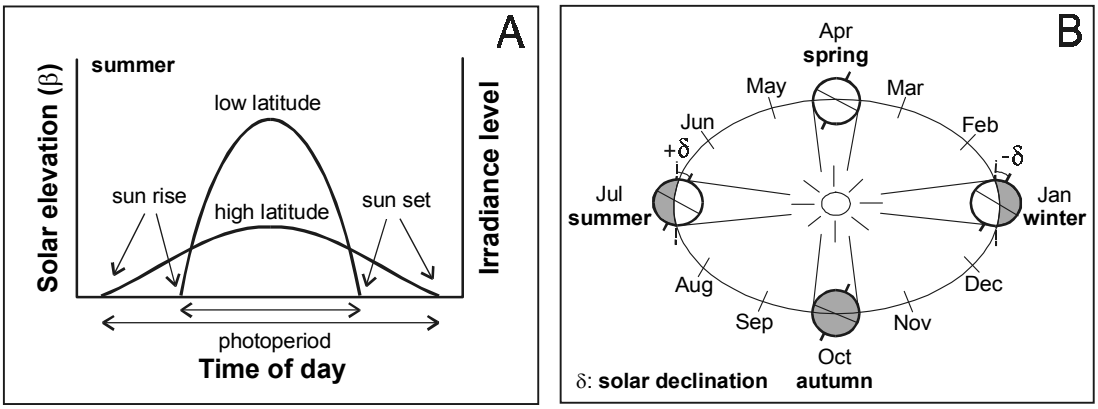

Figure 1. Schematic representation of diurnal changes in solar elevation and irradiance at contrasting latitudes (**A**) and seasonal variation in solar declination (**B**; modified from Kirk, 1994). Note that the presented data relate to the Northern Hemisphere.

Several authors have attributed the wide distribution of aquatic vascular plants to the relative uniformity of the aquatic environment (Sculthorpe, 1967; Les, 1988). Although the exact nature of this uniformity is seldom specified, it probably refers to the fact that the water column has the capacity to moderate the climatic extremes that are thought to determine terrestrial plant distribution. However, other authors have argued that the alleged uniformity is misleading and that variation in climatic

factors across latitude may constrain growth and reproduction of aquatic plants (Bowes, 1987; Santamaría, 2002). In the following sections, a brief overview will be given on how climatic variables are influenced by the optical and physical properties of the water column and what their effect may be on the functioning and distribution of aquatic vascular plants.

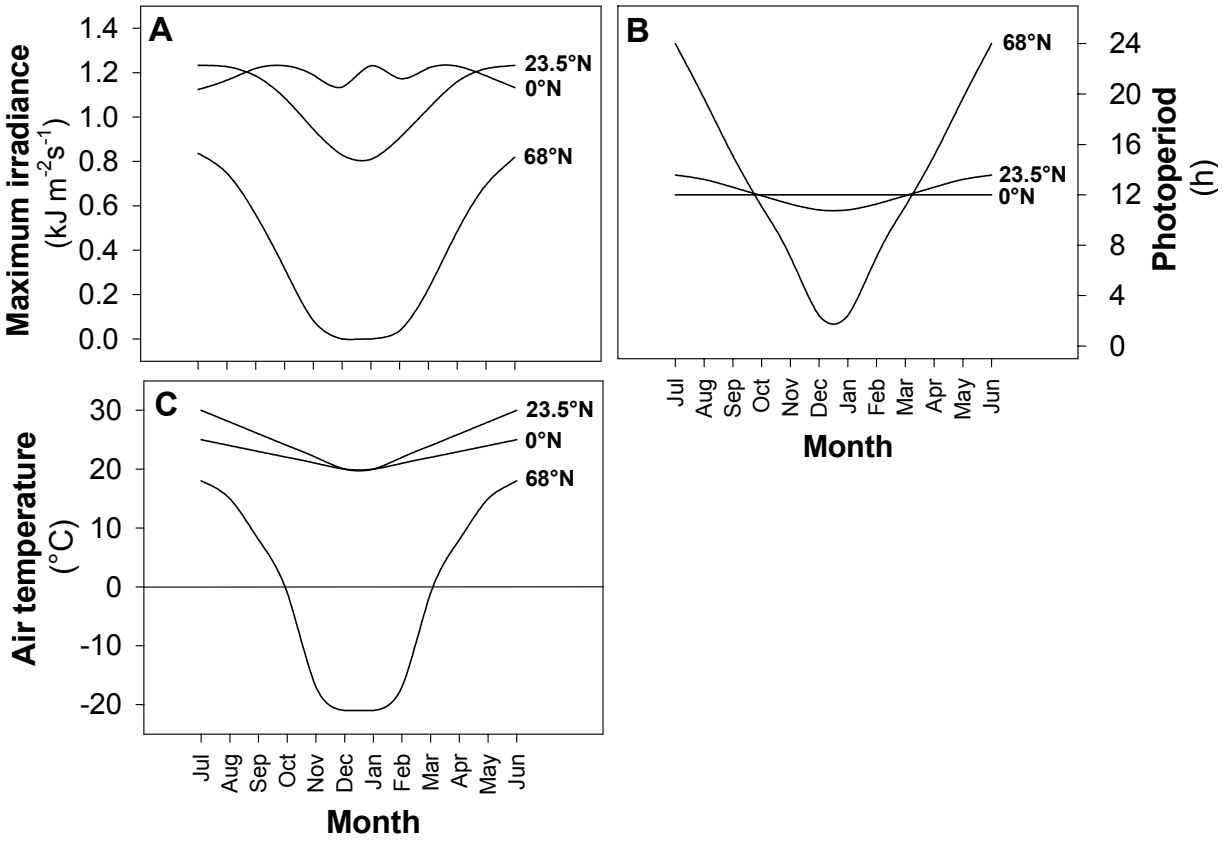

Figure 2. Changes in maximum solar irradiance (**A**), photoperiod (**B**) and air temperature (**C**) throughout the year at different latitudes. Maximum solar irradiance and photoperiod were calculated by making use of the online calculator developed by Lammi (1996-2001), while air temperatures were plotted from data presented in The Times Atlas of the World (1997). Note that 68°N represents the northern distributional limit of *P. pectinatus* and that during summer highest air temperatures are recorded near the tropic of cancer (23.5°N).

Light intensity

Aquatic plants are exposed to a wider range of irradiance levels than most species native to the terrestrial environment (Jeffrey, 1981). While emergent species and plants that float on the water surface receive approximately the same quantum flux than species that grow in open terrestrial habitats, the irradiance level experienced by submerged plants is generally much lower. The latter is a consequence of the light-absorbing properties of the water column itself, suspended particles (e.g. phytoplankton, bottom sediment, detritus) and/or dissolved substances (e.g. peat-derived colorants) (Morgan & Smith, 1981). Since differences in trophic status (Jeppesen et al., 1994) and the amount of sediment resuspension (Blom et al., 1994) may introduce large differences in water transparency

among neighbouring lakes, latitudinal gradients in solar irradiance may be modified by local conditions.

Since aquatic plants show morphological and physiological characteristics that promote growth under low-light conditions, they invariably have been categorised as shade plants (Bowes, 1987; Bowes & Salvucci, 1989). While emergent species minimise self-shading by the vertical placement of linear-shaped leaves; submerged plants frequently increase light capture by pronounced stem elongation that concentrates their leaves near the water surface (Spence, 1975; Barko & Smart, 1981; Barko et al., 1982; Wetzel, 1988). Also the gas-exchange characteristics of aquatic plants are adapted to low irradiance, since they typically show low photosynthetic rates, low light-compensation points (LCP) and reduced rates of dark respiration (Bowes & Salvucci, 1989; Duarte et al., 1994). Finally, species-specific variation in morphological and physiological flexibility may determine the growth potential of aquatic plants under various light conditions. For example, introduced *Hydrilla verticillata* successfully invaded Florida due to a high competitive ability related to extensive foliar canopy formation (Haller & Sutton, 1975).

Temperature

The high specific heat of standing waters buffers aquatic plants against rapid fluctuations in air temperature (Wetzel, 1988). Nevertheless, seasonal extremes may vary between 0 and 40°C, while diurnal fluctuations in temperature can also be substantial (Bowes & Salvucci, 1989). In addition, aquatic plants may be exposed to high within-plant thermal variation. Besides strong vertical temperature gradients that occur in densely vegetated waterbodies, the amount of thermal variation that exists among various plant tissues also depends on the growth form. For example, shoots of emergent species are exposed to the same temperature regime as neighbouring terrestrial plants, while roots growing in water-saturated sediments may experience cooler or warmer conditions depending on the season (Wetzel, 1988). Finally, aquatic macrophytes lack insulating or heat dissipating mechanisms that give terrestrial species native to arctic or desert regions the opportunity to regulate tissue temperatures so that they become less harmful for physiological functioning (Santamaría, 2002).

In both terrestrial and aquatic plants, changes in temperature show their most prominent effect on the biochemical conversions that relate to photosynthesis and growth (Berry & Raison, 1981; Bowes & Salvucci, 1989). In submerged plants the thermal optima for net photosynthesis are generally high (i.e. between 20 and 35°C) and outside the range that is found for terrestrial C_3-species (Bowes, 1987; Bulthuis, 1987; Santamaría & Van Vierssen, 1997). Since in temperate regions actual water temperatures are frequently lower than the observed thermal optima, it is likely that photosynthesis is predetermined to profit from high summer temperatures, or that other abiotic factors (e.g. low carbon availability) constrain the photosynthetic reactions from being thermally optimal. In agreement with the thermal response of photosynthesis, biomass productivity of aquatic plants normally increases with increasing temperature, while growth becomes arrested between 10 and 15°C (Barko & Smart, 1981). Though frequently disputed (e.g. Sculthorpe, 1967; Pip, 1989), temperature is probably the most important climatic factor that affects the global distribution of aquatic plants. For example, the extensive latitudinal range of *Myriophyllum spicatum* in North America could be related to broad thermal tolerance that was found in this species (Barko & Smart, 1981).

Photoperiod

Water depth and turbidity have a large impact on the instantaneous light level, but the length of the photoperiod is hardly affected (Van Vierssen & Hootsmans, 1994). As a result, aquatic plants are exposed to photoperiods that are largely comparable to those experienced by terrestrial species. The water column has, however, a large impact on the spectral quality of light, which affects the mechanism by which aquatic plants can detect differences in photoperiod. While in terrestrial species, the red (R) to far-red (FR) photoreversible pigment phytochrome acts as a sensor of photoperiod (Salisbury, 1981; Thomas & Vince-Prue, 1994); in aquatic plants the involvement of phytochrome in timekeeping is doubted, because diurnal shifts in underwater R:FR are more pronounced than those above the water surface. Moreover, they often fall outside the range that is physiologically active in terrestrial plants (Chambers & Spence, 1984). Alternatively, aquatic plants may perceive photoperiod in a more direct manner (i.e. as a change in photosynthetically active radiation), while also the involvement of other photoreceptors (e.g. cryptochrome) has been suggested (Spence et al., 1987).

In terrestrial plants, the induction of flowering is the most widely studied photoperiodic response (Thomas & Vince-Prue, 1994), while in aquatic species research on photoperiodism predominantly focussed on the initiation of asexual reproduction (e.g. Chambers et al., 1985; Van Vierssen & Hootsmans, 1994). Other aquatic plant processes that are affected by photoperiod include propagule germination (Flint & Madsen, 1995; Weber & Nooden, 1976), photosynthesis (Van Vierssen & Hootsmans, 1994), growth (Spencer & Ksander, 1995) and leaf-pigment composition (Spencer & Anderson, 1987). Despite this multiplicity of investigations, the influence of photoperiod on aquatic plant distribution received virtually no scientific attention. However, from studies on broadly distributed terrestrial species it is known that across latitude, physiological ecotypes based on varying responses to photoperiod may develop (Salisbury, 1981). The only aquatic study that relates to this, focussed on geographically distant populations of *Zostera noltii* and revealed that morphological characteristics of low-latitude plants are less sensitive to photoperiod seasonality as compared to plants native to higher latitude (Vermaat et al., 2000).

How to cope with latitudinal variation in climatic conditions

To understand the widespread distribution of aquatic plants, it is of importance to investigate responses to differences in climatic factors. For this purpose, the construction of reaction norm diagrams is an important method to evaluate genotype-environment interactions (Schlichting, 1986). A reaction norm can be defined as a set of phenotypic character states that is produced when a genotype is exposed to different intensities of a particular condition (Via et al., 1995). From an evolutionary perspective it is desirable to determine reaction norms for plant variables that are directly related to fitness (Begon et al., 1996). Various types of generalised reaction norms have been described, but the specific shape appears to be critically dependent on the character and environment under study (Fig. 3A).

To prevent plant performance to be constrained by latitudinal variation in climatic conditions, several evolutionary solutions can be proposed. Firstly, plants could possess a high potential for adaptive phenotypic plasticity. In such case, an integrated change in character states that depends on the intensity of a condition can provide an increased range of tolerance and a relatively high fitness in multiple environments (Fig. 3B) (Via et al., 1995). Although changes in growth and photosynthesis in

response to light and temperature have been reported for a number of aquatic plant species (Barko & Smart, 1981; Barko et al., 1982; Hootsmans & Vermaat, 1994; Vermaat & Hootsmans, 1994), reaction norms of different genotypes were never reported. In addition, no information exits on the plasticity ranges of plants that have different latitudinal origins.

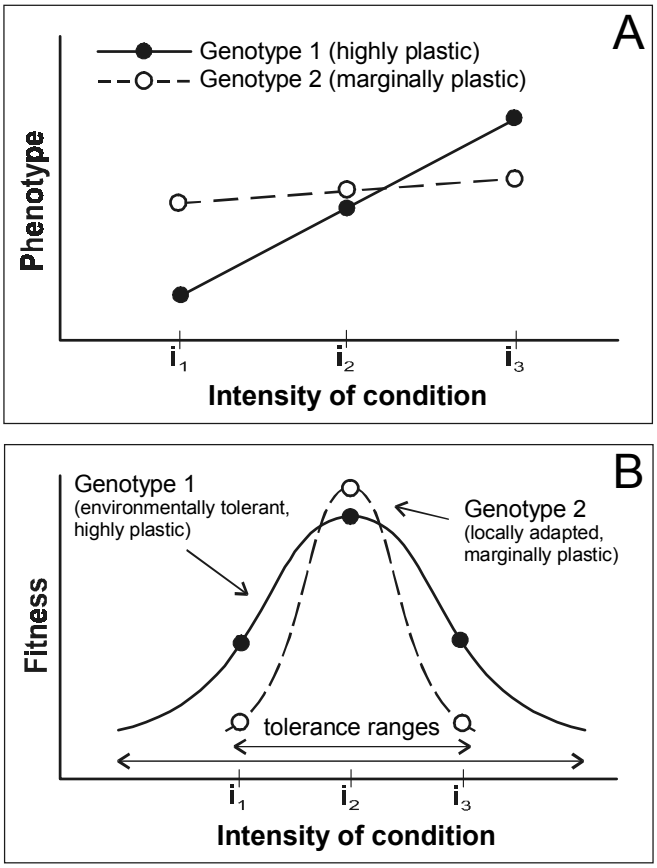

Figure 3. Schematic representation of phenotype (**A**) and fitness (**B**) in dependence of the intensity of condition (i_{1-3}) for two hypothetical genotypes that differ in plasticity.

Although adaptive phenotypic plasticity can be adopted as a mechanism to explain the distribution of aquatic plants across climatic regions, the ability to express optimal traits in many different environments is likely to be constrained. Several authors have argued that it is costly to maintain sensory systems that monitor the environment, while also the establishment of alternative character sets may involve expenditures (e.g. Van Tienderen, 1991; Huber, 1996; but see Sultan, 1992). In addition, genetic processes may limit the adaptiveness of plasticity, since genes that confer beneficial changes in one trait may furnish negative effects on others (Dewitt et al., 1998). If plasticity is advantageous, but constrained, locally adapted genotypes may evolve that show phenotypic traits that are only suited for certain conditions (Fig. 3B) (Van Tienderen, 1991). In broadly distributed terrestrial and emergent plants, local adaptation through natural selection was evidenced by reciprocal transplant experiments (e.g. Chapin III & Chapin, 1981; Joshi et al., 2001), while no such experiments exist for submerged aquatic species that are widely distributed.

A third possibility by which geographically distant populations of broadly distributed plants may respond to differences in climate is a change in phenology or life-cycle strategy. For example, in

terrestrial (Criddle et al., 1994), emergent (Clevering et al., 2001) and submerged plants (Phillips, 1983) it was shown that higher-latitude populations could escape from low temperatures by growing later in the season or postponing their reproductive phase. Such shift in phenology may become so pronounced that within-species differences in life-cycle strategy may arise. For instance, in Southern Europe submerged plants may show a perennial or winter-annual life cycle, while at higher latitudes populations are most frequently summer annuals (Van Vierssen, 1982; Van Wijk, 1988). The capacity of plants to avoid unfavourable climatic conditions by a shift in seasonal growth may reduce the requirements for high plasticity or strong local adaptation.

The model species *Potamogeton pectinatus* L.

Fennel pondweed (*Potamogeton pectinatus* L.) is a submerged aquatic macrophyte that was scientifically named and described by Linneaeus (1707-1778) in his *Species plantarum* of 1753. The name *Potamogeton* is derived from the Greek for 'river-neighbour', which refers to the emergent growth form of *Polygonum amphibium* (water smartweed) that in former times was classified among the pondweeds (Weeda et al., 1991). The epithet *pectinatus* ('comb-like') refers to the dense 'brushes' of narrow leaves that radiate out over the water surface (Kantrud, 1990). *P. pectinatus* belongs to the family of the Potamogetonaceae, among which two different genera have been recognised: *Groenlandia* that includes only one species (i.e. *G. densa*) and *Potamogeton* that includes about 70 species worldwide (Wiegleb & Kaplan, 1998). On the basis of leaf shape two *Potamogeton* species-types have been recognised: the magno-potamids that have broad submerged or floating leaves and the parvo-potamids that produce submerged, narrow and linear leaves that are typical of *P. pectinatus*.

Figure 4. Distribution of *P. pectinatus* throughout the world (shaded area) (partially based on maps presented in Hultén & Fries, 1986 and Meusel et al., 1965). Dotted lines mark the latitudinal range (24-68°N) from where *P. pectinatus* clones studied in this thesis were obtained.

The genus *Potamogeton* is present in nearly all parts of the world. Highest species densities (20-35) are found in North America, Western Europe and Asia, while less species (5-15) occur in South America, Africa, New Zealand and Australia (Wiegleb & Kaplan, 1998). Many *Potamogeton* species

are interior or northern in their distribution, while among the widespread species (i.e. *P. crispus*, *P. nodosus*, *P. perfoliatus*), *P. pectinatus* is the only true cosmopolitan. In the Northern Hemisphere, fennel pondweed occurs circumboreally to about 70°N, and it is also present in South America, South Eurasia, Africa, Australia and New Zealand (see Fig. 4) (Casper & Krausch, 1980). From the previous it is clear that *P. pectinatus* can persist in nearly all climatic zones, from the sub-arctic to the tropics.

P. pectinatus is most commonly found in standing waters (i.e. lakes, ponds, ditches), but the species also thrives in running waters (rivers, streams, irrigation channels) characterised by low-velocity currents. In addition, the plant frequently inhabits coastal ponds and estuarine wetlands (Van Wijk, 1988; Menendez & Sanchez, 1998), as it can cope with moderate salinity and fluctuating water tables. Luxurious growth has been reported on a variety of substrates (from coarse sand to fine clay) that may largely vary in the content of organic carbon, phosphorus and other nutrients (Casper & Krausch, 1980). *P. pectinatus* shows a wide tolerance to differences in nutrient status and has the ability to grow in oligotrophic to eutrophic and even in polluted waters. Water transparency frequently limits the depth distribution of *P. pectinatus* and under conditions of high turbidity its presence is restricted to shallow habitats (<1.5 m). However, in (crystal) clear waters the species may occur to a maximum depth of about 10 m, although this seems to be an exception (Kantrud, 1990).

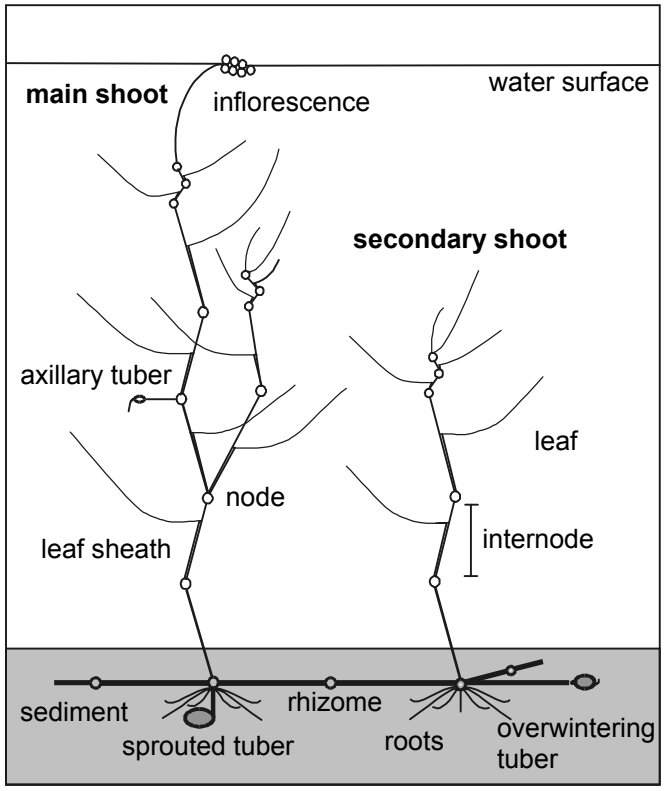

Figure 5. Schematic representation of a *P. pectinatus* shoot complex, showing morphological features of importance for this study.

The life cycle of *P. pectinatus* can be deducted from the plants growth habit (Fig. 5). Most frequently, seasonal growth starts with the sprouting of subterranean tubers (Fig. 6A), although seeds, axillary tubers and rhizome parts may also give rise to new plants. After the formation of a main shoot (with long narrow leaves), a constant production of secondary shoots that sprout from (branched)

rhizomes can be observed (Fig. 6B). In summer, monoecious flowers arise on the leafy tips of sexual shoots. Following hydrophilous pollination at the water surface, a maximum of 10-15 buoyant seeds can be formed on each peduncle (Fig. 6C). Although the importance of seed production for yearly survival is probably limited (Van Wijk, 1989), waterfowl-mediated long distance dispersal may depend on it. Asexual reproduction is completed in late summer or early fall, when maximum tuber density is reached (Fig. 6C and D). At higher latitudes, most *P. pectinatus* populations show a pseudo-annual life cycle, which means that during autumn the vegetative plant parts (i.e. shoots, rhizomes, roots) die-off and only seeds and (subterranean) tubers survive the winter (Fig. 6D). However, under mild climatic conditions fennel pondweed may show a perennial life cycle, with only partial decomposition of the aboveground biomass (Spence et al., 1979; Van Wijk, 1988).

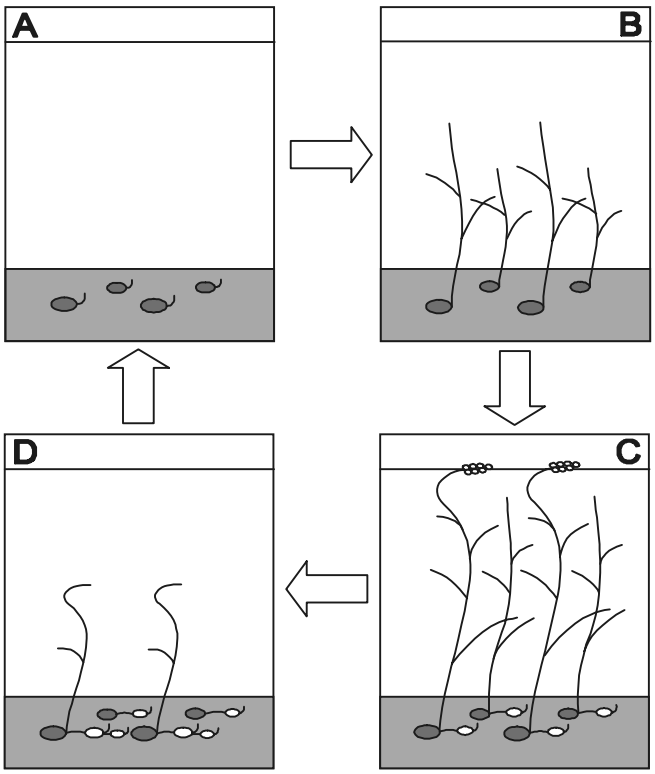

Figure 6. Schematic representation of the pseudo-annual life cycle of *P. pectinatus* as it can be observed in Central to Northern Europe.

For the genus *Potamogeton*, more than 70 different hybrids have been proposed, of which 50 are relatively well established. At higher latitudes, *P. pectinatus* frequently crosses with both *P. filiformis* and *P. vaginatus*, and the resulting hybrids (*P. x suecicus* and *P. x bottnicus*, respectively) are morphologically very similar to both parents (Preston, 1995; Wiegleb & Kaplan, 1998). Chromosome counts were previously used to discriminate between hybrids and their parental species. However, for *P. pectinatus* and the two species it hybridises with (i.e. *P. filiformis*, *P. vaginatus*), the same average chromosome number of 2n=78 was found (Hollingworth et al., 1998). As a consequence, cytological recognition of hybrids is impossible. Recently, however, molecular tools have become available that provide a reliable method for the identification of *P. pectinatu*s hybrids on the basis of comparisons of nuclear and chloroplast DNA (King et al., 2001).

P. pectinatus is an important food source for many species of herbivorous waterfowl. During summer, large flocks of coots (*Fulica atra*) and ducks (*Anas spp.*) may feed on the aboveground shoots and ripening seeds (Fig. 7A) (Anderson & Low, 1976; Søndergaard et al., 1996), while in autumn migrating Bewick's swans (*Cygnus colombianus bewickii*) depend nearly exclusively on the starch-rich subterranean tubers (Fig. 7B) (Beekman et al., 1991; Nolet et al., 2001). As a consequence, peak standing crop of *P. pectinatus* may be reduced by 17 to 40% (Anderson & Low, 1976; Van Wijk, 1988), while 48% of the tuber biomass may be lost due to extensive swan feeding (Santamaría & Rodríguez-Gironés, 2002).

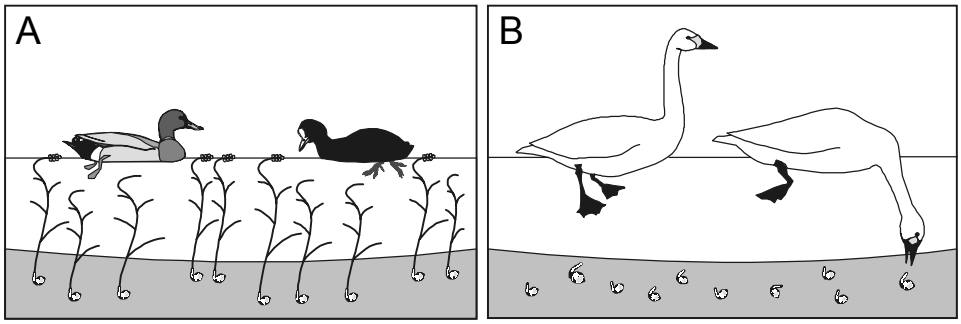

Figure 7. Grazing of coots (*Fulica atra*) and ducks (*Anas spp.*) on the aboveground plant parts of *P. pectinatus* (**A**) and foraging of Bewick's swans (*Cygnus colombianus bewickii*) on subterranean tubers (**B**). Note that the size of the tubers is exaggerated.

Outline of the thesis

The primary aim of this thesis was to evaluate the contribution of phenotypic plasticity, local adaptation and changes in life-cycle strategy (phenology) to the globally wide distribution of *P. pectinatus*. For this purpose we used up to 15 clones obtained from a gradient in latitude (24-68°N) and studied their performance in dependence of variation in climatic factors. We thereby focussed at various stages of the life cycle, such as tuber sprouting *(Chapter 6)*, vegetative growth *(Chapter 2-6)* and asexual reproduction *(Chapter 2)*. In addition, a transplantation experiment was used to test the effect of *in situ* variation in climate on the performance of *P. pectinatus* in Western Europe *(Chapter 7)*.

Several investigators have studied *P. pectinatus* at various locations in Western Europe. Although population-specific differences in biomass productivity and reproductive output were found, the detection of clear latitudinal trends was hampered by variation in habitat conditions (Ozimek et al., 1986; Kautsky, 1987; Van Wijk, 1988). We therefore performed two laboratory experiments (under standardised conditions) that focussed on the existence of genetically fixed and latitude-correlated differences in growth and (asexual) reproduction for *P. pectinatus* clones obtained from 24 to 68°N. The results of these experiments are described in *Chapter 2*.

Seasonal differences in temperature vary across latitude and plants may respond to this by acclimative changes in physiological processes (Berry & Björkman, 1981). While for terrestrial and marine plants seasonal acclimation in photosynthesis and respiration received some attention (e.g. Drew, 1978; Singh et al., 1996), no such studies were available for species native to freshwater ecosystems. *Chapter 3* reports on an experiment in which seasonal acclimation in gas-exchange was

examined for three freshwater macrophyte species, among which *P. pectinatus*. Photosynthetic and respiratory temperature-response curves were measured for shoots collected from the field at monthly intervals. In addition, a simple model was used to evaluate species-specific differences in acclimation capacity.

Besides seasonal variation in the thermal regime, cosmopolitan plants have to cope with pronounced latitudinal variation in the prevailing environmental temperatures. For *P. pectinatus*, some aspects of the thermal response have been studied previously (e.g. Spencer, 1986; Madsen & Adams, 1989; Vermaat & Hootsmans, 1994), although ecotypic variation across latitude was never considered. We therefore characterised the thermal response (i.e. morphology, growth and photosynthesis) of five *P. pectinatus* clones obtained from 42 to 68°N and evaluated if thermal differentiation and/or acclimation could contribute to the species broad distribution *(Chapter 4)*

Previous research suggested that distant populations of *P. pectinatus* might vary in their response to irradiance (Hootsmans & Vermaat, 1994; Hootsmans et al., 1996), although this was not tested experimentally. In addition, no information existed on geographic variation in vegetative growth responses of *P. pectinatus* to differences in photoperiod. Therefore an experiment was performed in which three clones obtained from contrasting latitudes (42.5-68°N) were grown at a factorial combination of two irradiances and three photoperiods *(Chapter 5)*. Differences in morphology, growth and photosynthesis in response to variation in the light climate were characterised. In addition, the contribution of local adaptation and/or acclimation to the species broad distribution was evaluated.

For broadly distributed plants native to the terrestrial environment, latitude-correlated differences in the thermal requirements for propagule germination were found (e.g. Sawada et al., 1994; Minggang et al., 2000). We investigated if such differences also existed for *P. pectinatus* and characterised tuber sprouting at a range of different water temperatures for six clones obtained from 49-68°N. Sprouted tubers were also used to study the combined effect of temperature and photoperiod on subsequent growth and biomass allocation. We hypothesised that clones from higher latitudes would have the capacity to make efficient use of a long photoperiod, thus compensating for the growth limiting effect of low water temperatures *(Chapter 6)*.

Climate is one of the largest sources of environmental variation that has a large impact on plant functioning (Woodward & Williams, 1987). To assess the contribution of plasticity and local adaptation to the performance of *P. pectinatus* at contrasting climates, a transplantation experiment was performed. For this purpose, 54 clones collected from 14 geographically distant populations (situated within four climatic regions) were grown in Norway (Oslo), The Netherlands (Heteren) and Spain (Doñana) *(Chapter 7)*.

Finally, the results presented in all previous chapters are summarised and discussed in *Chapter 8*. In particular the contribution of local adaptation, adaptive phenotypic plasticity and phenological changes was evaluated in relation to the capacity to grow at different latitudes. Moreover, the environmental variables and plant traits of major importance for the problem under analysis are discussed.

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CHAPTER 2

Latitudinal variation in life-cycle characteristics of *Potamogeton pectinatus* L.: vegetative growth and asexual reproduction

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Plant Ecology, in press

Summary

Across latitudinal gradients, environmental conditions that influence plant growth and reproduction largely change. Here we study clonal variation in life-cycle characteristics of the cosmopolitan water plant *Potamogeton pectinatus* L. across a broad latitudinal range.

Two consecutive experiments were performed under standardised laboratory conditions (photoperiod, irradiance and temperature). In the first experiment we investigated asexual reproduction among fifteen clones, obtained from latitudes ranging from 24 to 68°N. After 90 days of growth, high-latitude clones produced more but smaller tubers, while the aboveground biomass was lower as compared to the clones obtained from low latitudes.

In a second experiment we studied inherent differences in early growth, morphology and photosynthesis for eleven clones (obtained from the same latitudinal range as in experiment I). We found high among clonal variation for most measured variables, but the number of latitude-correlated traits was limited. The only trait that correlated with latitude was the number of leaves per plant, which increased in clones from higher latitudes.

Our results agree with the hypothesis of a latitude-correlated switch in life-cycle strategy for this species. For northern clones this results in a short life cycle, with an early and high investment in tuber biomass, while for low latitude clones the length of the life cycle is prolonged, with a delayed reproduction and increased total plant biomass.

Introduction

Environmental factors that vary with latitude, such as temperature, irradiance and photoperiod influence plant morphology, growth and reproduction (Boardman, 1977; Berry & Raison, 1981; Salisbury, 1981). Therefore, cosmopolitan species might be expected to show phenotypic variability across latitude. Several studies on terrestrial plant populations have shown the existence of latitudinal patterns in phenology (Potvin, 1986) and traits related to growth (Li et al., 1998), morphology (Chapin

III, 1974) and sexual reproduction (Aizen & Woodcock, 1992; Winn & Gross, 1993). Reciprocal transplant and laboratory experiments have revealed that the variation observed in the field was partly genetically based. In general, ecotypes from higher latitudes were inherently smaller due to lower growth rates or short development cycles. Simultaneously, mean flowering date for plants grown in common environments was successively later from high to low latitudes, while seed production increased (Chapin III & Chapin, 1981; Winn & Gross, 1993; Li et al., 1998).

Although the aquatic environment can affect latitudinal gradients by buffering temperature fluctuations, changing light intensities and influencing phytochrome-mediated detection of photoperiod (Chambers & Spence, 1984), several studies have reported latitudinal variation in aquatic species, similar to those of terrestrial plants. However, previous studies concentrated mainly on marine (macro) algae and seagrasses (Phillips et al., 1983; Strömgren, 1986; Peters & Breeman 1993; Molenaar & Breeman, 1994), while information about latitudinal responses in freshwater angiosperms is lacking. This is surprising because the latter are considered to be among the most widely dispersed (Sculthorpe, 1967), and are excellent tools to study local differentiation induced by environmental factors that vary with latitude.

In the present study we focus on inherent phenotypic variation among clones of fennel pondweed (*Potamogeton pectinatus* L.), collected along a broad latitudinal range. *P. pectinatus* is a submerged aquatic macrophyte with a predominant pseudo-annual life cycle. Unlike most other *Potamogeton* species, it has a cosmopolitan distribution and occurs in nearly all climatic zones (Casper & Krausch, 1980; Wiegleb & Kaplan, 1998). At sub-arctic latitudes *P. pectinatus* has to cope with short summer seasons, followed by long winters with extensive ice-coverage. At temperate latitudes, strong life-cycle seasonality becomes less important due to extended periods suitable for plant growth. Under mild climatic conditions, *P. pectinatus* is reported to be perennial (Spence et al., 1979; Van Wijk, 1988).

Although *P. pectinatus* can flower abundantly, the significance of seed production for yearly survival is reported to be very limited (Van Wijk, 1989). As in many other aquatic macrophyte species, asexual reproduction is achieved through subterranean tubers that play an important role in annual regeneration. Several authors showed that the production of tubers in *P. pectinatus* is induced by short photoperiods (Spencer & Anderson, 1987; Spencer et al., 1993), though Van Vierssen & Hootsmans (1994) have shown that the interaction between photoperiod and photosynthetic period has a stronger effect on tuber formation.

Some studies have focussed on the phenology, biomass production and reproductive allocation of *P. pectinatus* at different locations in Western Europe. These studies revealed that in Southern Europe, peak biomass takes place earlier (May-June in Southern Spain [personal observation] and France [Van Wijk, 1988]), than in Central to Northern Europe (July in the Netherlands [Van Wijk, 1988], Poland [Ozimek et al., 1986], Sweden [Kautsky, 1987] and Finland [Van Wijk, 1988]; August in Northern Russia [personal observation]). Furthermore, maximum plant biomass and the number of tubers per unit area seemed to be higher for lower latitude populations. Because specific habitat conditions largely influenced phenotypic variation in life-history traits, the cited field studies made it possible to only recognise very general latitudinal trends. To discriminate between genetic effects and the effect of varying environmental conditions, we performed two laboratory experiments with *P. pectinatus* clones from a wide latitudinal range (24 to 68°N).

In the first experiment we studied tuber formation in fifteen clones of *P. pectinatus*, grown under standardised conditions. We hypothesised that the clones would show genetically based and latitude-correlated variation in traits related to asexual reproduction. This hypothesis was based on the assumption that reproduction of individual clones should be adapted to the length of the growing

season. For clones obtained from higher latitudes, we therefore expected early formation of tubers and subsequently smaller tuber-sizes (e.g. Aizen & Woodcock, 1992).

In a second experiment we concentrated on differences in morphology, growth and photosynthesis for eleven clones of *P. pectinatus*. Since terrestrial plants from high latitudes, normally produce smaller ecotypes (Chapin III & Chapin, 1981; Reinartz, 1984) with inherently lower growth rates (Li et al., 1998), we expected a similar pattern for *P. pectinatus*. Simultaneously we explored, if clonal differences in growth could be attributed to correlated changes in morphology and photosynthesis.

Material and methods

Origin, propagation and storage of plant material

Tubers of *P. pectinatus* were collected at 18 locations in Europe, North Africa and North America (see Table 1). Since *P. pectinatus* reproduces predominantly by means of tubers (Van Wijk, 1988), populations are largely clonal. Sampling of several individuals per population therefore involves a high risk of pseudo-replication (Hurlbert, 1984). Hence, we selected a single clone per sampling site.

Table 1. Localities of origin of the clonal lines of *P. pectinatus* used in experiment I and/or II

Clone	Country	Location of origin	Climatic region	Latitude*	Longitude*	Experiment	
1	Russia	Korovinskaya Bay, Kashin Island	Sub-arctic	68.24°N	53.88°E	I	II
2	Russia	Sukhoye Bay, Mudyug Island	Sub-arctic	64.92°N	40.30°E		II
3	Russia	St. Petersburg, pond in city park	Sub-arctic	59.95°N	30.36°E	I	II
4	Denmark	Lake Stigsholm	Mild-coastal	55.98°N	9.50°E	I	II
5	Scotland	River Kelvin	Mild-coastal	55.88°N	4.37°E	I	
6	Poland	Lake Mikolajskie	Mild-coastal	53.80°N	21.57°E	I	II
7	Netherlands	Lake Lauwersmeer, Babbelaar	Mild-coastal	53.34°N	6.22°E	I	II
8	Netherlands	Lake Lauwersmeer, Zoutkamperril	Mild-coastal	53.34°N	6.23°E	I	
9	Netherlands	Lake IJsselmeer, near Lemmer	Mild-coastal	52.84°N	5.71°E		II
10	Netherlands	Lake Nuldernauw	Mild-coastal	52.29°N	5.55°E	I	
11	France	River Orne, near La Forêt-Auvray	Mild-coastal	48.82°N	0.33°E	I	II
12	France	Drainage canal along river Rhine	Mild-coastal	48.53°N	7.80°E	I	
13	Italy	Pond near Desana	Mild-coastal	45.27°N	8.39°E	I	II
14	Spain	Lake Carucedo	Mild-coastal	42.48°N	6.77°E		II
15	USA (Illinois)	Illinois River	Continental	39.95°N	90.53°E	I	
16	USA (California)	Imperial irrigation district	Desert	32.85°N	115.57°E	I	
17	Egypt	Lake Nasser, near Aswan Dam	Desert	23.97°N	32.87°E	I	
18	Egypt	Lake Nasser, near Aswan Dam	Desert	23.97°N	32.87°E	I	II

I: asexual reproduction experiment, II: vegetative growth experiment, * geographical coordinates in decimal degrees

At higher latitudes, *P. pectinatus* hybridises with *P. filiformis* and *P. vaginatus* (Preston, 1995; Preston et al., 1998, 1999). The hybrids (*P.* x *suecicus* and *P.* x *bottnicus*, respectively) can easily be confused with *P. pectinatus*. All clones used in this experiment have been analysed by using molecular tools (restriction fragment length polymorphism [RFLP] on nuclear and chloroplast DNA extracted from freshly grown material; King et al., 2001) to ensure that only *P. pectinatus*, and no *P.* x *suecicus* or *P.* x *bottnicus* hybrids were included.

Tubers of all clones were propagated once a year in the outdoor plant growth facilities of the Centre for Limnology (May-September in Nieuwersluis, The Netherlands [52.20°N, 5.01°E]) and the Plant Protection Research Institute (November-March in Pretoria, South Africa [25.75°S, 28.20°E]). This enabled us to complete two growth cycles per year and thereby maximise tuber production. At both locations, *P. pectinatus* plants were grown in polyethylene tanks (1m^3), filled with a mixture of sand and clay (3:1 by dry mass [DM]) and supplemented with tap water.

To remove harmful micro-organisms, all harvested tubers were surface sterilised with 5% NAOCl (Madsen, 1985). Subsequently, tubers of each clone were wrapped in wet tissue paper and stored at 4°C until further use.

Plant cultivation and characterisation

Experiment I: latitudinal variation in asexual reproduction

For each clone, 8-9 tubers of a standard size-class (50-100 mg fresh mass, FM) were planted individually in disposable coffee beakers (150 cm^3). Each beaker contained a sediment mixture of sand and potting clay (3:1 by DM) and was covered with 1 cm washed aquarium sand. All beakers were randomly distributed over eight aquaria (96 l; 60x40x40 cm) and placed in two phytotrons. The plants were grown in tap water, without additional nutrients. As shown by Van Vierssen & Hootsmans (1994), tuber production in *P. pectinatus* is promoted by a short photosynthetic period. To enhance tuber formation we used the following light regime: 3 h dim light (< 5 µmol m^{-2} s^{-1}), 10 h full light (200 µmol m^{-2} s^{-1}), 3 h dim light (< 5 µmol m^{-2} s^{-1}), 8 h darkness. Irradiance was provided by 17 fluorescent light tubes (TLD 36W/85o, Philips Nederland B.V., Eindhoven, The Netherlands) per phytotron, and was measured with an underwater quantum sensor (LI-192SA, LICOR, Lincoln, NE, USA) 1 cm below the water surface. During the experiment, temperature was kept constant at 20 ± 1°C. Whenever necessary, tap water was used to replenish the evaporated water.

After 90 days of growth, all plants were washed free of sediment and the length of the longest shoot (leaves included) was measured. Thereafter, the DM (after 24 h at 70°C) and ash free dry mass (AFDM, after 3 h at 520°C) of the above- (shoots) and belowground (roots+rhizomes, tubers) plant fractions were determined. In addition, we recorded the number and individual size (DM and AFDM) of the tubers produced per plant.

Experiment II: latitudinal variation in vegetative growth, morphology and photosynthesis

Tubers of 50-150 mg FM were pre-sprouted during one week in plastic trays filled with washed aquarium sand. All trays were completely submerged in aquaria filled with tap water and exposed to the following conditions: a photoperiod of 16/8 h day/night, an irradiance of 133 ± 1.5 µmol m^{-2} s^{-1} (TLD 50W/83o HF, Philips Nederland B.V., Eindhoven, The Netherlands) and a water temperature of 22 ± 1.5 °C. Sprouted tubers were planted individually in disposable coffee beakers (150 cm^3) filled with a sediment mixture of sand and clay (3:1 by DM) and covered with a layer of washed aquarium sand. All beakers were randomly distributed over fifteen aquaria (96 l; 60x40x40 cm). Growing conditions were the same as described for the pre-sprouting of tubers. The pH of the water was adjusted weekly to 7.3 with 3 M H_2SO_4.

Per clone, 6-9 randomly selected plants were carefully washed free of sediment on each of days 9, 17, 31, 39, 46 and 53 (counted from the day when pre-sprouted tubers were planted in the beakers). At all harvests, plants were separated into leaves, stems and belowground parts (roots, rhizomes and old and new tubers). Subsequently, DM (70°C for 48 h) and AFDM (520°C for 3h) were determined for the separated fractions. The following morphometric variables were also recorded for each individual plant: total number of shoots, length of the longest stem and number of nodes of the longest stem. After 31 days of growth, 9 plants per clone were collected for photosynthesis measurements and subsequent biomass determination (as before). The same plants were also used to measure leaf characteristics (leaf length, leaf mass) and determine the mean number of leaves per plant. For this purpose a sub-sample of 60 leaves per clone was collected. Finally, the collected leaves were weighed (FM), pooled in groups of three plants per clone, and stored temporarily at -20°C. At the end of the experiment, leaf samples were used to determine the chlorophyll a and b concentrations according to the method of Porra et al. (1989).

Oxygen-exchange measurements

All measurements were performed in three closed replicate systems, with a volume of 422 ml each. Individual systems were assembled by interconnecting a Perspex cuvette (L: 14 cm, ∅: 5 cm) and an electrode chamber (D201, WTW, Weilheim, Germany) with different types of tubing (PVC/Tygon®). All oxygen-exchange systems were placed in two aquaria (96 l; 60x40x40 cm) filled with tap water. A thermoregulation system (heating and cooling) connected to the aquaria, maintained the water temperature at 20 ± 0.1°C. Within the systems, water was circulated with a peristaltic pump (Masterflex pump drive 7520-45, pump head 7519-05; Cole Parmer Instrument Co., Vernon Hills, IL, USA) at a flow rate of 750 ml min^{-1}. This flow rate was expected to have no limiting effect on the photosynthesis rate of submerged macrophyte species (Westlake, 1967). The cuvettes were submerged 2 cm below the water surface and held horizontally by a Perspex frame. The dissolved oxygen concentration was measured with Cellox 325 oxygen probes (WTW, Weilheim, Germany) and recorded (every 10 s) with a micrologger (21X, Campbell Scientific Ltd, Leicestershire, UK).

A SON-T AGRO lamp (Philips Nederland B.V., Eindhoven, The Netherlands) provided photosynthetically active radiation (PAR). Different irradiances were created by varying the distance between the lamp and the water surface and by using neutral density shading nets. As it was difficult to maintain a homogeneous light field at high light intensities, we measured the irradiance at three points along the inside of each cuvette and used the mean irradiance per cuvet for further calculations. For the three cuvettes we aimed at mean irradiances of approximately 25, 50, 75, 100, 150, 200, 300, 400 and 500 µmol m^2 s^{-1} (PAR). Prior to the oxygen-exchange measurements, both aquaria were filled with tap water and a single addition of 20 g $NaHCO_3$ was made. This produced a dissolved inorganic carbon concentration (ΣDIC) of 4.6 mM; high enough to saturate net photosynthesis of *P. pectinatus* (Sand-Jensen, 1983). After addition of bicarbonate, pH of the water was 7.7, corresponding with a distribution of 95% HCO_3^- and 5% CO_2 (Prins & Elzenga, 1989).

Three intact plants per cuvette were used to derive light-response curves. After covering the aquarium with black plastic foil to prevent light penetration, dark respiration measurements were started at an initial oxygen concentration of 9-10 mg l^{-1} (the normal concentration in tap water at 20°C). Subsequently, the plants were exposed to the different irradiances, starting with the lowest intensity and ending with the highest. Prior to the photosynthesis measurements, water was bubbled with nitrogen gas to reduce the dissolved oxygen concentration to 3-4 mg l^{-1}. All oxygen-exchange

measurements were performed during 30–45 min, until the oxygen concentration had increased or decreased by at least 0.5 mg l^{-1}. Between measurements, the systems were opened and the medium inside was completely replenished with surrounding water.

Calculations and statistical analysis

All statistical analyses were performed with the STATISTICA statistical package (Statsoft Inc., 1999). Variables related to morphology and biomass accumulation were tested for normality and homogeneity of variances. Log-transformations were carried out whenever necessary (Sokal & Rohlf, 1995). Differences between the clones were tested by using one-way analyses of variance (ANOVAs). To allow for (unplanned) pairwise comparisons between clonal lines, the minimum significant range (MSR; based on the studentised range distribution) was calculated for each variable (i.e. according to the Tukey-method; Sokal & Rohlf, 1995). In the case of unequal sample sizes, the critical values and the standard errors were modified according to the Tukey-method (Sokal & Rohlf, 1995). The relationship between plant variables and latitude (experiment I) were tested by using linear regressions. The correlation structure of traits was analysed by using multiple correlations (experiment I and II). The outcome of all pairwise Spearman correlations was summarised in correlation matrices. Initial growth rates (RGR; experiment II) were calculated from exponential regressions of total biomass data.

In experiment II, 33 light-response curves were obtained (11 clones x 3 replicate curves). From each data set, in which linear increases and decreases in oxygen concentration were related to time, initial lag phases were excluded. Linear regressions were used to calculate oxygen-exchange rates per unit time and biomass. The parameters of the light-response curves were calculated by fitting these gas-exchange data to the following equation describing a rectangular hyperbola (e.g. Santamaría et al., 1994):

$$P = \frac{Pm \times I}{K_{0.5} + I} - R_d \qquad (1)$$

where **P** (in µg O_2 g^{-1} AFDM min^{-1}) is the rate of net photosynthesis, **Pm** (in µg O_2 g^{-1} AFDM min^{-1}) is the light saturated rate of gross photosynthesis, **I** (in µmol m^{-2} s^{-1}) is the independent variable irradiance, $\mathbf{K_{0.5}}$ (in µmol m^{-2} s^{-1}) the half-saturation constant and $\mathbf{R_d}$ (in µg O_2 g^{-1} AFDM min^{-1}) is the dark respiration. The apparent quantum yield (α in µg O_2 m^2 s g^{-1} AFDM min^{-1}) was calculated as Pm divided by $K_{0.5}$ (e.g. Hootsmans & Vermaat, 1994), while the light compensation point (LCP in µmol m^{-2} s^{-1}) was estimated by using equation (1) at P=0. Fitted and calculated parameters from the three replicate curves per clone were compared by one-way analyses of variance (ANOVAs). Data were log-transformed if needed for normality and homogeneity of variances and MSR-values were computed according to the Tukey-method to allow for unplanned pairwise comparisons between means (Sokal & Rohlf, 1995).

Results

Experiment I: latitudinal variation in asexual reproduction

All variables related to morphology, biomass production and asexual reproduction varied significantly among clones (Table 2). Latitude was positively correlated with the absolute and proportional allocation of biomass invested in tubers (Fig. 1C and F, Fig. 2). Clones originating from higher latitudes produced more, but smaller tubers (Fig. 1G and H, Fig. 2).

Table 2. $F_{14,104}$-values and significance levels (***P < 0.001 for all variables) for one-way ANOVAs on various plant variables recorded during the reproductive part of the life cycle (experiment I). The effect of the independent variable clone is shown. AFDM: ash free dry mass.

variable	clone
length of longest shoot (cm)	13.6***
allocation to aboveground (%)	26.8***
allocation to roots + rhizomes (%)	5.5***
allocation to tubers (%)	21.9***
aboveground biomass (mg AFDM)	15.4***
root + rhizome biomass (mg AFDM)	9.3***
total tuber biomass (mg AFDM)	10.4***
total plant biomass (mg AFDM)	6.5***
tubers per plant	13.6***

The absolute and proportional investment of dry matter in aboveground plant tissue and the length of the longest shoot decreased with latitude (Fig. 1A and E, Fig. 2). This suggests that higher latitude clones had less photosynthetic tissue available to produce a higher total tuber biomass. The number of tubers per plant and the total tuber biomass correlated positively with the proportion of biomass allocated to tubers (Fig. 2). The absolute and proportional allocation of biomass in aboveground plant organs was positively correlated with the investment of biomass in roots and rhizomes (both DM and DM%), while these increases correlated negatively with the proportional allocation of resources to tubers (Fig. 2).

Experiment II: latitudinal variation in vegetative growth, morphology and photosynthesis

Biomass allocation and productivity

Biomass production and the allocation of biomass into different plant parts (leaves, stems, roots+rhizomes) varied significantly among clones (Table 3). RGR varied between 27 (clone 18) and 52 mg AFDM g^{-1} day^{-1} (clone 4) and was positively correlated with total plant AFDM (Table 4, Fig. 3). However, neither the RGR, the total plant biomass, nor the absolute investment of biomass in above- and belowground parts correlated significantly with latitude (Fig. 3). Proportional biomass allocation into leaves significantly increased with latitude, while proportional allocation into stems and belowground parts peaked at intermediate or low latitudes respectively (Fig. 4). However, those relationships relied heavily on one single point (clone 18). After excluding this clone from the data set, no significant correlations between latitude and the proportional allocation of dry matter into leaves

and belowground parts existed, while latitude correlated negatively with the proportional allocation of biomass to stems (Fig. 4). Still, proportional allocation of dry matter to leaves and stems correlated negatively with the allocation to belowground biomass (Fig. 3).

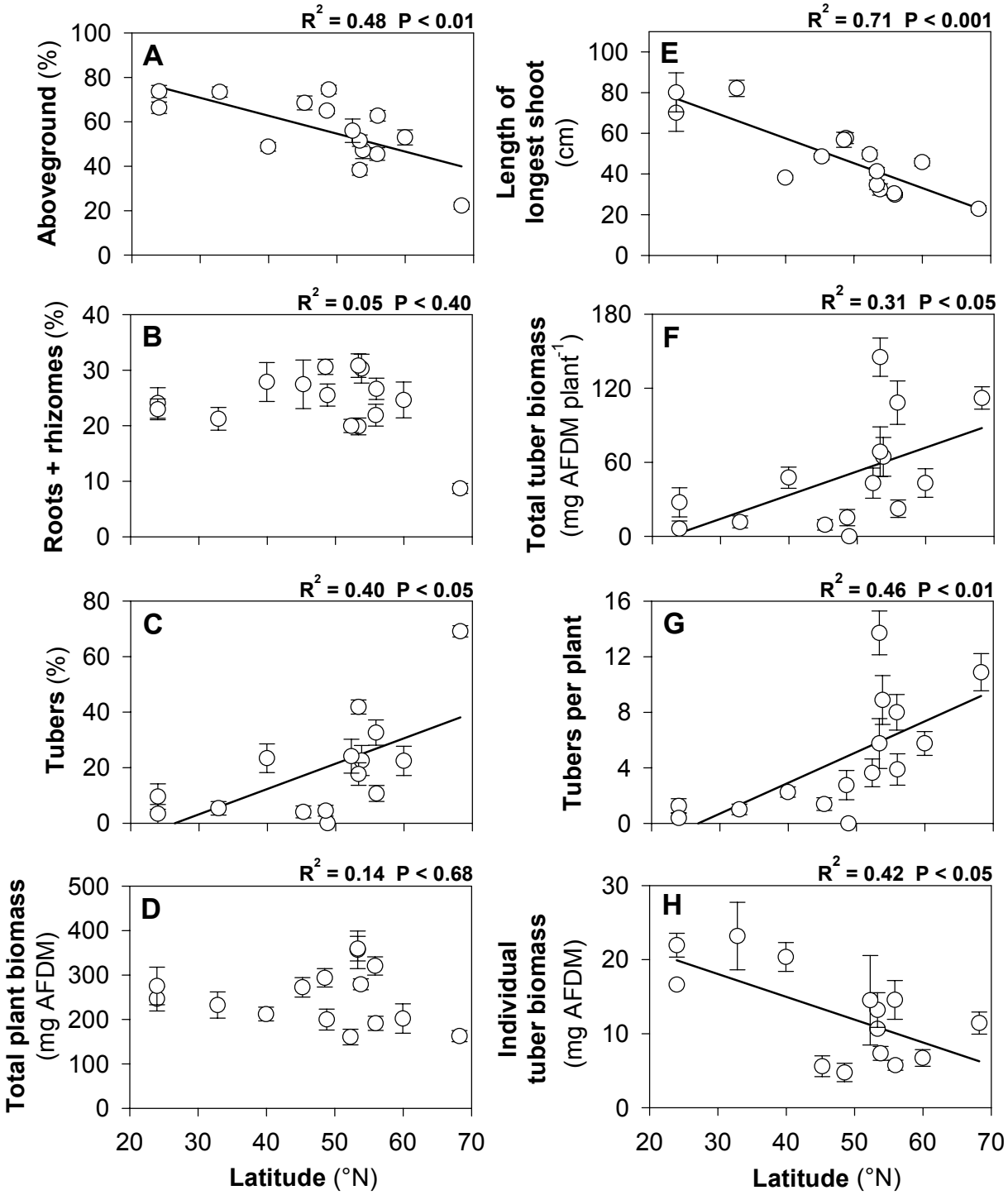

Figure 1. Linear regressions, describing the relationship between latitude and plant characteristics related to growth (A, B, C, D, E) and asexual reproduction (F, G, H) for fifteen clones of *P. pectinatus*, grown under standardised conditions (experiment I). Indicated are means ± standard errors (n = 7-8). Standard errors can be obscured due to the size of the data points. Lines represent significant regressions. R^2-values and the significance level of the regressions are shown in the figures. Biomass is expressed as ash free dry mass (AFDM).

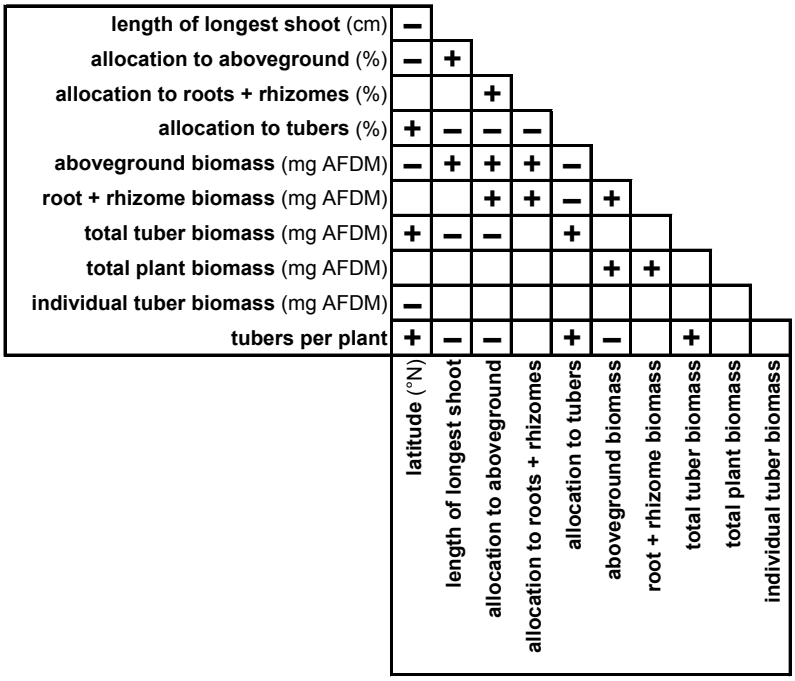

Figure 2. Correlation matrix, representing multiple correlations among characteristics recorded during the reproductive part of the life cycle for fifteen clones of *P. pectinatus*, grown under standardised conditions (experiment I). Significant positive (**+**) and negative (**-**) Pearson correlations ($P < 0.05$) between the actual or log-transformed variables are indicated. Blank cells indicate non-significant correlations.

Table 3. F-values and significance levels (NS: non-significant, *P < 0.05, **P < 0.01, ***P < 0.001) for one-way ANOVAs on various plant variables recorded during vegetative growth of eleven clones of *P. pectinatus* (experiment II). The effect of the independent variable clone is shown. Pm: light saturated rate of photosynthesis, $K_{0.5}$: half saturation constant, R_d: rate of dark respiration, α: quantum yield, LCP: light compensation point, a+b: total chlorophyll, a/b: chlorophyll a/b-ratio, AFDM: ash free dry mass, FM: fresh mass.

df (effect)	10	df (error)	**clone**
morphology	length of longest stem (cm)	88	17.39***
	nodes on longest stem	88	17.64***
	internode length (cm)	88	17.62***
	shoots per plant	88	4.86***
	leaves per plant	88	23.07***
	leaf length (cm)	22	20.93***
	individual leaf biomass (µg AFDM)	22	26.49***
biomass	leaf biomass (mg AFDM)	53	4.38***
	stem biomass (mg AFDM)	53	5.69***
	root + rhizome biomass (mg AFDM)	53	4.51***
	total plant biomass (mg AFDM)	53	4.44***
	allocation to leaves (%)	418	31.33***
	allocation to stems (%)	418	51.97***
	allocation to belowground (%)	418	20.61***
photosynthesis	Pm (µg O_2 g^{-1} AFDM min^{-1})	22	4.60**
	$K_{0.5}$ (µmol m^{-2} s^{-1})	22	2.88*
	R_d (µg O_2 g^{-1} AFDM min^{-1})	22	1.44^{NS}
	α (µg O_2 m^2 s µmol g^{-1} AFDM min^{-1})	22	1.93^{NS}
	LCP (µmol m^{-2} s^{-1})	22	2.82^{NS}
chlorophyll	a+b (µg g^{-1} FM)	22	11.13***
	a/b (µg $µg^{-1}$ FM)	22	0.74^{NS}

Table 4. Vegetative growth characteristics of eleven clones of *P. pectinatus* recorded after 31 or 53 days (RGR and plant biomass) of development under standardised conditions (experiment II). Shown are means ± standard errors. Differences between the clones were tested by one-way ANOVA. The minimum significant range (MSR; based on the studentised range distribution) indicates the minimum difference between any pair of means that is significant at $P < 0.05$. Clone numbers increase with decreasing latitude. AFDM: ash free dry mass, RGR: relative growth rate. *Due to missing values, pairwise comparisons involving clone 3 (n=4) have the following MRS: 180.

Clone	Leaves per plant	Leaf length	Individual leaf biomass	Length of longest stem	Nodes per shoot	Internode length	Shoots per plant	RGR	Total plant biomass
		(cm)	(µg AFDM)	(cm)		(cm)		(mg AFDM g^{-1} day^{-1})	(mg AFDM)
	(n=9)	(n=60)	(n=3)	(n=9)	(n=9)	(n=9)	(n=9)		(n=6)
1	34 ± 2	9.0 ± 0.5	537 ± 41	19 ± 1	8.0 ± 0.2	2.4 ± 0.1	2.9 ± 0.6	36	96 ± 10
2	50 ± 4	5.9 ± 0.3	335 ± 33	29 ± 2	9.0 ± 0.2	3.2 ± 0.3	4.3 ± 0.3	45	235 ± 30
3	29 ± 4	8.0 ± 0.4	549 ± 35	42 ± 4	11.3 ± 0.4	3.7 ± 0.2	3.1 ± 0.4	29	94 ± 17*
4	54 ± 5	6.1 ± 0.2	274 ± 4	22 ± 2	10.0 ± 0.5	2.1 ± 0.1	4.2 ± 0.3	52	179 ± 43
6	49 ± 3	5.0 ± 0.3	294 ± 19	34 ± 3	11.8 ± 0.3	2.8 ± 0.2	5.0 ± 0.3	43	115 ± 9
7	42 ± 4	7.3 ± 0.4	380 ± 26	38 ± 3	11.4 ± 0.5	3.3 ± 0.2	4.3 ± 0.4	39	112 ± 12
9	51 ± 3	6.6 ± 0.3	385 ± 15	47 ± 3	11.2 ± 0.2	4.1 ± 0.3	3.2 ± 0.1	38	122 ± 11
11	19 ± 1	8.4 ± 0.5	809 ± 32	47 ± 1	11.9 ± 0.3	3.9 ± 0.1	3.1 ± 0.3	41	126 ± 10
13	45 ± 6	7.3 ± 0.4	538 ± 61	56 ± 2	11.3 ± 0.3	5.0 ± 0.1	3.6 ± 0.3	50	268 ± 81
14	37 ± 3	6.5 ± 0.3	365 ± 18	41 ± 4	11.1 ± 0.4	3.6 ± 0.2	3.9 ± 0.2	31	93 ± 5
18	13 ± 1	11.5 ± 0.4	719 ± 52	32 ± 4	7.8 ± 0.5	4.0 ± 0.3	2.7 ± 0.3	27	129 ± 15
MSR	17	1.7	174	13	1.7	0.9	1.6	-	147

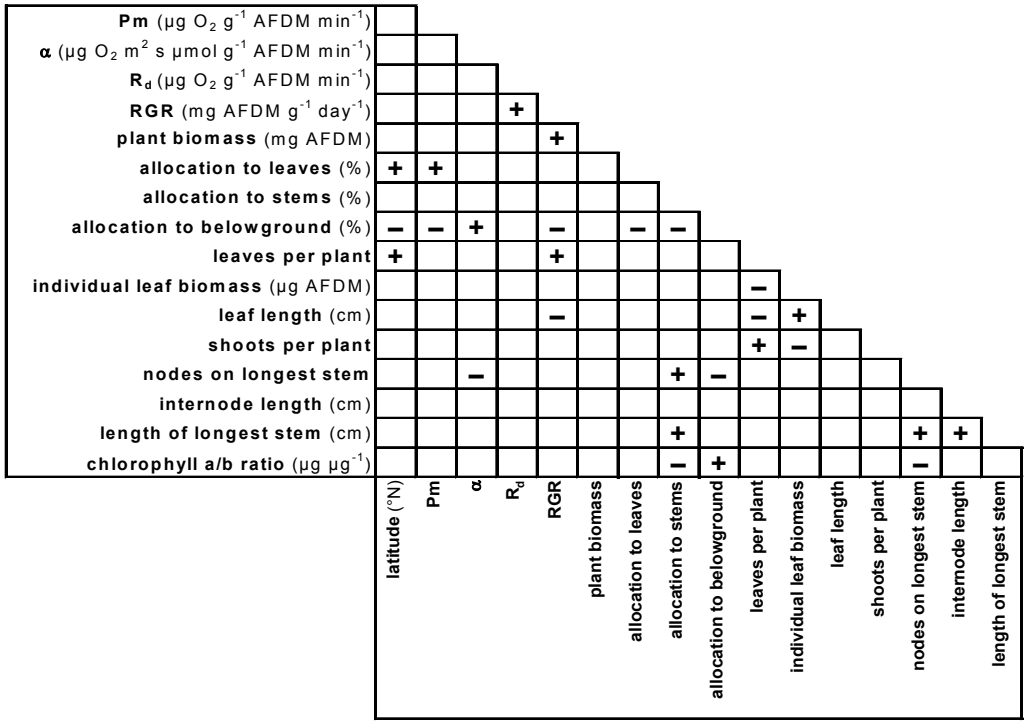

Figure 3. Correlation matrix, representing multiple correlations among vegetative growth characteristics of eleven clones of *P. pectinatus*, grown under standardised conditions (experiment II). Significant positive (**+**) and negative (**-**) Pearson correlations ($P < 0.05$) are indicated for actual or log-transformed variables and photosynthetic parameter estimates. Blank cells indicate non-significant correlations. Pm: light-saturated rate of photosynthesis, α: apparent quantum yield, R_d: rate of dark respiration, RGR: relative growth rate.

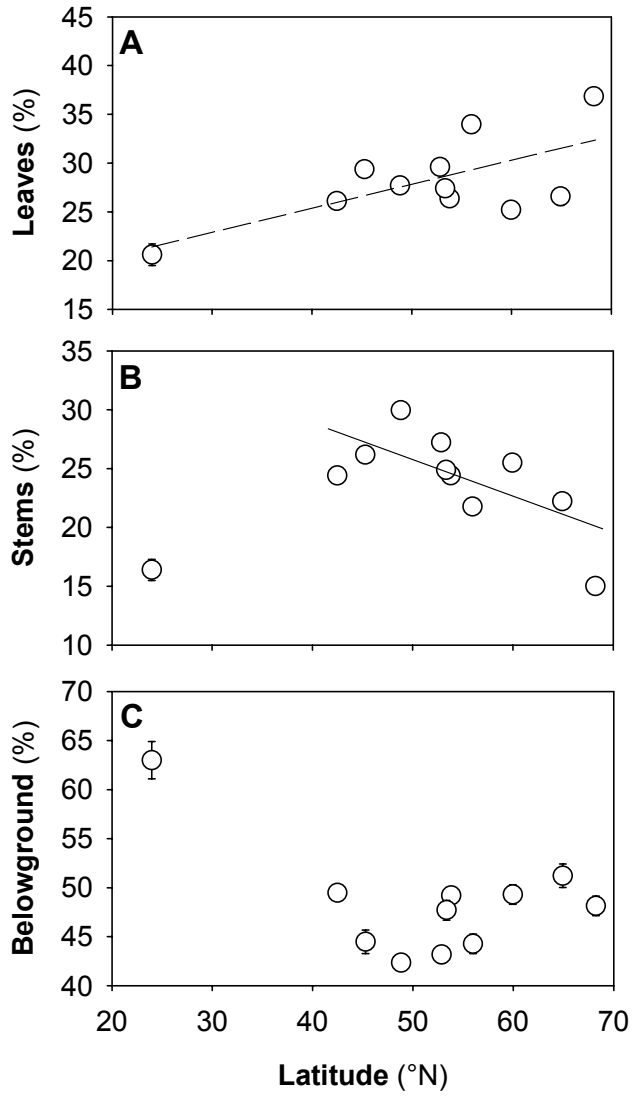

Figure 4. The relationship between latitude and proportional biomass allocation during the vegetative part of the life cycle for eleven clones of *P. pectinatus*. Indicated are means ± standard errors (n = 39). Standard errors can be obscured due to the size of the data points. (**A**) Allocation to leaves (clone 18 included: $P < 0.05$, R^2: 0.43 [broken line]; clone 18 excluded: not significant, R^2: 0.16), (**B**) allocation to stems (clone 18 included: not significant, R^2: 0.0006; clone 18 excluded: $P < 0.05$, R^2: 0.48 [solid line]), (**C**) allocation to belowground (clone 18 included: not significant, R^2: 0.27; clone 18 excluded: not significant, R^2: 0.17).

Leaf and stem morphology

All morphometric variables varied significantly among clones (Table 3). While we found several correlated responses between morphometric variables, only a single morphometric variable varied significantly with latitude. Clones from higher latitudes produced more leaves than their southern counterparts (Fig. 3).

The number of leaves per plant was negatively correlated with both leaf length and leaf biomass, which suggests a trade-off between leaf number and size (Fig. 3). The correlation matrix also shows that an increase in the number of leaves per plant is correlated with an increased number of shoots and a higher RGR (Fig. 3).

Oxygen-exchange measurements and chlorophyll concentration

All light-response curves were fitted in an acceptable way by the rectangular hyperbola (R^2 was always above 0.7 and in most cases above 0.9, Fig. 5). Statistical analysis of the photosynthesis parameters obtained from fits of individual curves showed no significant differences between the clones for R_d, α and LCP (Table 3 and 5). The rate of light-saturated photosynthesis (Pm) and the half-saturation constant ($K_{0.5}$) were the only parameters that varied significantly among clones (Table 3; multiple comparisons only significant for Pm, Table 5).

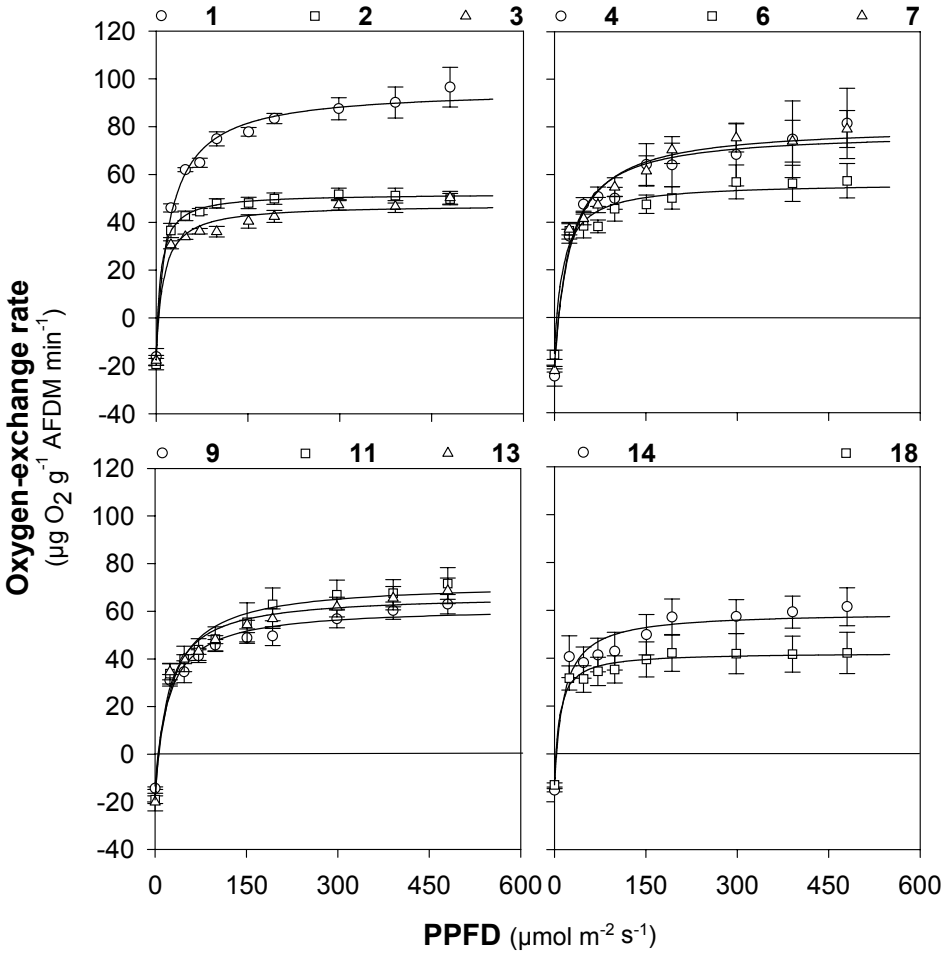

Figure 5. Fitted light-response curves (rectangular hyperbola) for eleven clones of *P. pectinatus* recorded during the vegetative part of the life cycle. Individual data points represent mean oxygen-exchange rates (± standard errors, n = 3) at different photosynthetic photon flux densities (PPFD). Please note that parameter estimates used for statistical analysis (see Table 5) were derived from each replicate curve separately (not shown). Clone numbers increase with decreasing latitude. The locality of origin of the clonal lines is indicated in Table 1.

None of the fitted photosynthesis parameters listed in Table 5 was significantly correlated with latitude (Fig. 3). The multiple-correlation matrix revealed however that light saturated photosynthesis (Pm) was positively correlated with the proportional allocation of biomass into leaves and negatively correlated with the proportional allocation into belowground parts (Fig. 3).

Table 5. Photosynthesis parameters and data related to the chlorophyll concentration for eleven clones of *P. pectinatus*, after 31 days of vegetative growth under standardised conditions (experiment II). Pm: light-saturated photosynthetic rate (µg O_2 g^{-1} AFDM min^{-1}), $K_{0.5}$: half saturation constant (µmol $m^{-2}s^{-1}$), R_d: rate of dark respiration (µg O_2 g^{-1}AFDM min^{-1}), α: quantum yield (µg O_2 m^2 s µmol g^{-1}AFDM min^{-1}), LCP: light compensation point (µmol $m^{-2}s^{-1}$), a+b: total chlorophyll concentration (µg g^{-1}FM), a/b: chlorophyll a/b-ratio (µg $µg^{-1}$FM). Shown are means ± standard errors (n = 3). Differences between the clones were tested by one-way ANOVA. The minimum significant range (MSR; based on the studentised range distribution) indicates the minimum difference between any pair of means that is significant at $P < 0.05$. NS: non-significant differences between any pair of means. Clone numbers increase with decreasing latitude.

clone	Pm	$K_{0.5}$	R_d	α	LCP	a+b	a/b
1	112 ± 10	23 ± 4	15.4 ± 3.0	5.1 ± 0.4	3.7 ± 1.1	443 ± 38	3.97 ± 0.07
2	71 ± 5	7± 1	19.3 ± 2.4	10.5 ± 1.8	2.6 ± 0.1	755 ± 30	2.93 ± 0.08
3	65 ± 4	11 ± 1	17.4 ± 2.0	5.7 ± 0.5	4.1± 0.3	362 ± 24	2.69 ± 0.04
4	104 ± 15	28 ± 14	22.8 ± 5.7	5.3 ± 1.5	5.9 ± 0.2	600 ± 26	2.77 ± 0.11
6	72 ± 8	14 ± 2	15.2 ± 1.8	5.2 ± 0.5	3.7 ± 0.3	524 ± 9	2.96 ± 0.10
7	102 ± 9	27 ± 5	20.7 ± 0.2	3.9 ± 0.4	6.8 ± 0.7	620 ± 4	2.88 ± 0.08
9	76 ± 5	25 ± 7	13.2 ± 0.3	3.4 ± 0.6	5.1 ± 1.0	595 ± 31	2.71 ± 0.19
11	91 ± 9	28 ± 8	17.8 ± 2.0	4.1 ± 1.4	6.3 ± 1.2	473 ± 23	2.82 ± 0.09
13	87 ± 6	22 ± 6	19.1 ± 3.9	4.8 ± 1.4	5.7 ± 1.4	483 ± 27	2.77 ± 0.14
14	74 ± 6	16 ± 4	14.3 ± 0.8	5.6 ± 2.0	4.0 ± 1.1	506 ± 28	2.81 ± 0.24
18	55 ± 9	8 ± 2	12.9 ± 0.9	8.6 ± 2.9	2.5 ± 0.8	605 ± 68	3.01 ± 0.11
MSR	42.2	NS	NS	NS	NS	162	NS

Total chlorophyll concentration varied significantly among clones (Table 3 and 5), while there where no significant differences in chlorophyll a/b ratio (Table 3 and 5). The total chlorophyll content was not significantly correlated with latitude, or with any other variable measured (not shown).

Discussion

Both experiments were designed to study latitudinal variation in growth and asexual reproduction for clones of *P. pectinatus*. As hypothesised, we found latitude-correlated responses in traits related to asexual reproduction. Most interestingly, *P. pectinatus* clones from higher latitudes produced more but smaller tubers. In addition, clones from higher-latitude regions were smaller and invested less in aboveground tissue, which is similar to the pattern of variation observed for terrestrial plant species (e.g. Chapin III & Chapin, 1981; Reinartz, 1984), However, the previous was found only after the onset of asexual reproduction. For the non-reproductive part of the life cycle we were unable to demonstrate latitudinal differences in morphology, photosynthetic parameters, RGR or biomass yield.

Asexual reproduction

Under standardised conditions, higher latitude clones produced more, but smaller tubers. Differences in tuber production might be explained by the existence of ecotypes based on varying responses to photoperiod (Salisbury, 1981). Alternatively, they might be the result of intrinsic differences in the timing of tuber formation. In either case, clonal differences in tuber production suggest the existence of

a genetically based phenological response to the varying length of the growing season. As *P. pectinatus* plants from northern habitats are exposed to a short growing season, there is a selective advantage to clones that produce tubers early in the growing season. Early tuber formation also means that less photosynthetic tissue is available to provide the developing tubers with carbohydrates and this limitation in resources could lead to a decrease in tuber size. Similar positive relationships between the length of the growing season and propagule size have been described for several terrestrial species along latitudinal, altitudinal and snow melting gradients (Baker, 1972; McNaughton, 1975; Galen & Stanton, 1991; Aizen & Woodcock, 1992).

During the reproductive part of the life cycle the aboveground biomass decreased in plants from higher latitudes, while the total tuber biomass increased. As leaf to stem ratios were comparable for most of the clones (see experiment II), we conclude that higher latitude clones produced a higher total tuber biomass with less photosynthetic tissue. The higher demand of carbohydrates per unit leaf biomass could be achieved in several ways. First, higher latitude clones could have a higher photosynthetic rate (Orians & Solbrig, 1977). However, we did not find a correlation between latitude and Pm (experiment II). Second, low latitude clones could have reduced respiration rates, but we found similar rates of dark respiration for all clones (experiment II). Third, differences in the timing of reproduction could explain why lower latitude clones produced less tuber biomass per unit leaf biomass. Since we harvested all plants after 90 days of growth, we did not fully account for differences in the length of the reproductive period. For higher latitude clones, tuber formation was probably completed at the end of the experimental period, while lower latitude clones might have produced more tubers had the experimental period been longer. The higher shoot biomass of the lower latitude clones suggests that they would produce more tuber biomass should they have a sufficiently long growth season.

Vegetative growth

Although during the vegetative part of the life cycle we found considerable clonal variation in relative growth rate (i.e. RGR: 27-52 mg g^{-1} day^{-1}), no correlation with latitude was apparent. This is in contrast with the results obtained for 40 ecotypes of *Arabidopsis thaliana* grown under standardised conditions (Li et al., 1998). In this herbaceous species, RGR was negatively correlated with latitude. The fact that we did not find such a relationship could be due to the limited number of clones, or the restricted latitudinal range. Furthermore, in *Arabidopsis* large variation in RGR was also found among clones obtained from 35 to 63°N, but a clear latitudinal trend only emerged when ecotypes from 16 to 35°N were included in the analysis (Li et al., 1998). We therefore conclude that further studies are needed to explore the existence of latitudinal trends in RGR, both in aquatic and terrestrial plant species.

Morphology

During vegetative growth, we found a trade-off between the number of leaves and the leaf mass and length. This suggests that the clones have different strategies of leaf production that could be related to differences in leaf life-span. When leaf loss rates are low (long life-span) fewer and bigger leaves can be produced that are more costly in terms of construction and maintenance. For the evergreen shrub, *Ledum palustre* ssp. *decumbens*, leaf traits at three sites along a latitudinal gradient (43-71°N) were correlated with the length of the growing season (Kudo, 1995). At higher latitudes *L. palustre* produced fewer leaves with a higher nitrogen content and a longer life-span. Kudo (1992) argued that

the extension of leaf longevity is adaptive, because it maintains the carbon balance under a short growing season. For *P. pectinatus* the number of leaves increased with latitude, which is opposite to the trend found for *L. palustre*. Furthermore, leaf length or mass did not vary systematically with latitude. It is therefore unclear whether leaf size variation in *P. pectinatus* is related to the length of the growing season. Alternatively, mechanical leaf damage due to wave action could also explain the observed latitudinal variation. At high latitudes, harsh weather conditions represent a greater risk of leaf damage. Under these conditions it is efficient to invest in more, but less costly leaves.

Photosynthesis

During vegetative growth we observed significant clonal differences in light-saturated photosynthesis (Pm), although these were not related to latitude. On average our Pm-values are within the range of those found by others working with *P. pectinatus* (Sand-Jensen, 1983; Hootsmans & Vermaat, 1994). For dark respiration (R_d), apparent quantum yield (α) and light compensation point (LCP) we did not find significant clonal differences. The obtained values for dark respiration (mean 17.1 µg O_2 g^{-1}AFDM min^{-1}) are comparable with the data from Madsen and Adams (1989), but slightly lower than the values from Hootsmans and Vermaat (1994). Measured apparent quantum yield values (mean 5.7 µg O_2 m^2 s µmol g^{-1}AFDM min^{-1}) were higher than reported by Hootsmans and Vermaat (1994), but in the range of those found by Van der Bijl et al. (1989). As a consequence of the relatively high apparent quantum yields, we also found light compensation points (mean: 5.5 µmol m^{-2} s^{-1}) that were lower than the LCPs of several other macrophyte species (Van et al., 1976; Pokorný et al., 1984; Orr et al., 1988). However, for some *Potamogeton* species LCP-values (1-8 µmol m^{-2} s^{-1}) were comparable with the values we found (Spence & Chrystal, 1970).

None of the photosynthesis parameters was correlated with RGR or plant biomass. Pm did however show significant relationships with proportional biomass allocation. A higher investment in leaf biomass is reflected by a higher rate of saturated photosynthesis, while the opposite is true for a higher investment in roots and rhizomes. Dark respiration was positively correlated with RGR, but not with the proportional biomass allocation. The higher respiration rates of fast growing *P. pectinatus* clones is probably due to their increased metabolic activity, as similar results were also found for terrestrial plant species (Poorter et al., 1990).

Conclusions

Although we found clonal variation in traits recorded during the vegetative part of the life cycle (experiment II), we did not find many correlations with latitude. We therefore suggest that abiotic conditions at the original growing site (e.g. water depth, flow regime, salinity, trophic status etc.) interact with the conditions that vary with latitude. As a consequence, small-scale adaptation could cause the detected differences in morphology, growth and photosynthesis.

The observed latitude-correlated responses for the reproductive part of the life cycle clearly point to a switch in life-cycle strategy. For higher latitude clones this results in a short life cycle, with an early and high investment in tuber biomass, while for the lower latitude clones the length of the life cycle is prolonged, with a delayed reproduction and a subsequent high biomass yield.

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CHAPTER 3

Seasonal acclimation in the photosynthetic and respiratory temperature responses of three submerged freshwater macrophyte species

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Summary

Investigations of seasonal temperature acclimation in gas-exchange are few and only exist for terrestrial and marine plants. Here we report on results obtained for three freshwater macrophyte species (*Callitriche obtusangula*, *Potamogeton pectinatus* and *Potamogeton perfoliatus*).

We collected plants from the field at monthly intervals and measured photosynthetic and respiratory temperature-response curves. Fitted and calculated parameters were derived from the curves and a simple model was used to evaluate the acclimative capacity to seasonal variation in water temperature.

For all species, optimal temperatures for gross photosynthesis showed little temporal variation. In addition, the shape of the temperature-response curves at sub-optimal temperature was not optimised to temporal differences in water temperature. The only consistent seasonal trend in gas-exchange was a gradual decrease in photosynthetic and respiratory capacity over time.

Our measurements and model predictions did not point to an acclimative seasonal response in the thermal dependence of oxygen exchange. Hence, we conclude that either other processes constrain the plants' response, or temporal variation in water temperature is less important than seasonal loss of photosynthetic capacity.

Introduction

In many habitats, individual plants are exposed to considerable seasonal variation in the prevailing environmental temperatures. Since photosynthesis and respiration are strongly affected by temperature, a high acclimative capacity to temporal changes in temperature might enhance plant productivity (Berry & Björkman, 1980). Studies on seasonal temperature acclimation in gas-exchange are few and mainly concentrate on terrestrial plants from temperate and arctic climates, where variation in environmental temperatures is most pronounced (Berry & Björkman, 1980). For a wide

diversity of species from different habitats, seasonal differences in the thermal regime resulted in a shift in optimal temperature for net photosynthesis (e.g. Lange, 1974; Oechel, 1976; Singh et al., 1996). In general, the observed shifts were in the expected direction, as higher environmental temperatures generally led to higher optimal temperatures and *vice versa*. However, in many cases acclimative adjustments in the photosynthetic response seemed to be less than perfect, as for the majority of species the recorded optima were substantially higher than predominant air-temperatures (e.g. Oechel, 1976; Slatyer & Morrow, 1977). Acclimative changes in the thermal response of net photosynthesis are often paralleled by similar changes in respiration. However, for most terrestrial plant species the respiratory pathway is more tolerant to higher temperatures, as compared to the photosynthetic reactions (Berry & Raison, 1981).

Marine autotrophs like seagrasses (Drew, 1978), macro-algae (Terrados & Ros, 1992) and phytoplankton species (Blanchard et al., 1997) have also been subject of studies on seasonal temperature acclimation. Despite the relative thermal homogeneity of the marine environment, temporal and regional variation in water temperature can be considerable (Bulthuis, 1987). However, a seasonal increase in photosynthetic optimal temperature, as observed for many terrestrial species, is less common in marine plants (Gacia et al., 1996; Terrados & Ros, 1992). In addition, the presence of an acclimative response in net photosynthesis seemed to be highly species dependent, since some marine angiosperms from the same habitat and exposed to comparable thermal variation showed significant seasonal acclimation, while others did not vary their optimal temperature (Drew, 1978).

Although seasonal acclimation in the photosynthetic and respiratory response of terrestrial and marine plants has received some attention, similar studies on freshwater macrophytes are lacking. The only study we are aware of is a re-analysis of Maberly's (1985) data, presented in Santamaría & Van Vierssen's (1997) review on the temperature response of aquatic plants, where a seasonal increase in the photosynthetic optimal temperature of *Fontinalis antipyretica* was suggested. However, in this re-analysis light acclimation and plant age could not be excluded as explanatory factors for the observed response.

The objective of this study was to examine seasonal acclimation in the photosynthetic and respiratory temperature response of three freshwater macrophyte species with a different temporal growth pattern. Similar investigations on both terrestrial and marine plants have mainly focused on the temperature dependence of net photosynthesis (e.g. Lange et al., 1974; Gacia et al., 1996). Other researchers concentrated on the thermal response of gross photosynthesis, which they calculated from the summation of net light fixation and dark respiratory loss (Santamaría & Hootsmans, 1998; Fair & Meeke, 1983; Drew, 1978). The latter approach might overestimate the actual rate of gross photosynthesis, since respiration in the light can be lower than in darkness (Hough, 1974; Søndergaard, 1979) and may vary with temperature (Atkin et al., 2000). To describe the maximal range of seasonal variation in photosynthetic rate (i.e. potential carbon gain) we included both gross and net photosynthesis.

Three submerged macrophytes, two with a pseudo-annual (*Potamogeton pectinatus* L. and *Potamogeton perfoliatus* L.) and one with a perennial life cycle (*Callitriche obtusangula* Le Gall) were selected for this study. We will test five hypotheses on the occurrence of seasonal temperature acclimation.

I. Seasonal acclimation may occur in two possible ways:

(a) A seasonal shift in T_{opt} (for net and/or gross photosynthesis), that is in correspondence with the expected changes in environmental temperatures (Fig. 1A). (b) Changes in the photosynthetic performance (net and/or gross) at sub- and/or supra-optimal temperatures (Fig. 1B and C).

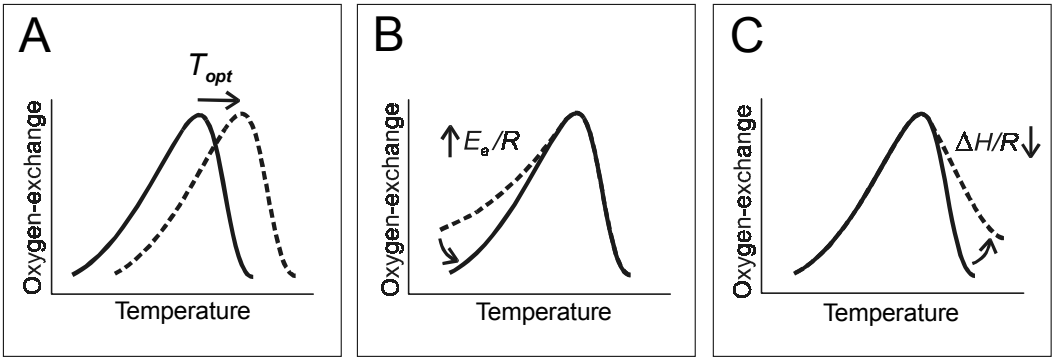

Figure 1. Three possible ways for photosynthetic acclimation in response to seasonal changes in environmental temperatures: (A) an upward shift of the temperature response curve (seasonal increase in optimal temperature [Topt]); (B) changes in the sensitivity to sub-optimal temperatures (seasonal increase in Ea/R; see eq. [1]); (C) changes in the sensitivity to supra-optimal temperatures (seasonal decrease in ΔH/R; see eq. [2]). Please note that combinations of (A), (B) and (C) may also occur.

II. Seasonal acclimation should lead to:
(a) An enhancement of the standardised rate of gross photosynthesis (SRP), i.e. highest at each month for its corresponding temperature response and lower if calculated with previous or ulterior temperature responses. Here, SRP represents the average rate of gross photosynthesis achieved when thermal variation over the day is taken into account and temperature is the only factor that varies during the season (i.e. SRP is standardised for seasonal variation in daylength and photosynthetic capacity). (b) An enhancement of the daily (gross) photosynthetic gain (DGP; dependent of temperature, daylength and photosynthetic capacity), i.e. highest at each month for its corresponding temperature response and lower if calculated with previous or ulterior temperature responses.
III. Species-specific differences in seasonal acclimation.
Due to its longer life cycle, we expect the perennial *C. obtusangula*, to show a more pronounced acclimative response than both *Potamogeton* species that grow later on in the season.

Material and methods

Site description and plant material

P. pectinatus and *P. perfoliatus* were sampled from a shallow (45-75 cm depth) mixed stand (approx. 150 m^2 of area) in the north-west part of Lake IJsselmeer (near the village of Lemmer, 52.85°N, 5.72°E), The Netherlands. Complete shoots were collected once a month between May and August 1998. Both *Potamogeton* species were pseudo-annuals that started to grow from tubers (*P. pectinatus*) or rhizome-buds (*P. perfoliatus*) at the beginning of May. The aboveground plant parts were in good condition until the beginning of September, when stems became fragile and leaves started to lose their green colour. All plant material was stored in plastic containers filled with water from the collection site. Until the start of oxygen-exchange measurements (in most cases less than 8 hours after collection, and always less than 32 hours), the containers were placed in a refrigerator at 4°C.

Plant samples of the perennial angiosperm *C. obtusangula* were collected from a ditch near Breukelen (52.11°N, 5.00°E), The Netherlands. This small (30 m^2 of area) and shallow (30-85 cm

depth) water body was dominated by *C. obtusangula* throughout the year. Apical shoots were collected once a month between March and July 1998. Prior to the experimental period (i.e. from October to February), the plants had a low biomass, developed slowly and were completely submerged. In early spring (March), new shoots developed and by the end of May a dense meadow (with floating rosettes) filled the whole water column. The plants were in good condition until July 1998 when leaves on the basal stems started to turn yellow after a period of high temperature. All oxygen-exchange measurements were performed on the day of collection.

Oxygen-exchange measurements

For each species and collection time, light-saturated net photosynthesis and dark respiration were measured in triplicate at the following water temperatures: 6, 12, 19, 26, 33, 40°C. When the photosynthetic rates did not decrease at 40°C (as compared with 33°C), we performed an additional measurement at 42-45°C. Dark respiration and net photosynthesis were measured successively, starting at the lowest and ending at the highest water temperature. Before oxygen-exchange rates were recorded, plants were acclimated to each incubation temperature for about 15 minutes. Dark respiration measurements were started at an initial oxygen concentration of 6-11 mg l^{-1} (representing oxygen saturation at the different incubation temperatures). For the measurement of net photosynthesis, an initial oxygen concentration of 3-4 mg l^{-1} was used (to minimise photorespiration). Oxygen-exchange rates were registered for 30-45 minutes, after which the oxygen concentration had increased or decreased with at least 0.5 mg l^{-1}. Between measurements, the gas-exchange systems were opened and the medium inside was completely replenished with surrounding water from the aquaria.

The experimental set-up and procedure to measure oxygen-exchange rates was largely comparable to the one described in Santamaría & Hootsmans (1998). All measurements were performed in three closed replicate systems that were operated simultaneously. By interconnecting a Perspex cuvette and an electrode chamber with different types of tubing (PVC/Tygon®) individual systems were assembled. All oxygen-exchange systems were completely submerged in a set of two glass aquaria filled with tap water (96 l each). Within the systems water was circulated with a peristaltic pump (Masterflex pump drive 7520-45, pump heads 7519-05, Cole Parmer Instrument Co., Vernon Hills, IL, USA) at a flow rate of 750 ml min^{-1}. The dissolved oxygen concentration was measured with Cellox 325 oxygen probes (WTW, Weilheim, Germany) and recorded (every 10 s) with a micrologger (Campbell Scientific Ltd, Leicestershire, UK) connected to a personal computer. A SON-T AGRO lamp (Philips Nederland B.V., Eindhoven, The Netherlands) provided a photon flux density of 500 µmol m^{-2} s^{-1} (PAR), which was sufficient to ensure irradiance-saturated photosynthesis. Prior to the oxygen-exchange measurements, 20 g $NaHCO_3$ was added to the water of each aquarium. This produced a dissolved inorganic carbon concentration (ΣDIC) of 4.6 mM; high enough to saturate net photosynthesis of several aquatic macrophyte species (including *P. pectinatus* and *Callitriche* spp.; Bodner, 1994; Sand-Jensen, 1983). After the addition of bicarbonate, the pH of the water was 7.7, corresponding with a relative distribution of 95% HCO_3^- and 5% CO_2 (Prins & Elzenga, 1989).

Cuvette size and shoot biomass was varied to minimise internal shading. For oxygen-exchange measurements on *P. pectinatus* and *C. obtusangula*, 4-5 g fresh weight (FW) was inserted in medium sized cuvettes (L: 14 cm, ∅: 5 cm), while for *P. perfoliatus* the measurements were performed on 8-9 g FW, that was inserted in large cuvettes (L: 20 cm ∅: 5 cm).

At the end of the oxygen-exchange measurements, shoots were separated into leaves and stems. Per cuvette, a leaf sub-sample of 100-140 mg FW (i.e. 9-12 leaves) was collected to extract chlorophyll with 100% methanol on ice. The concentration chlorophyll a and b was determined spectrophotometrically, by making use of the extinction coefficients and equations of Porra et al. (1989). Subsequently, FW and dry weight (DW; 48 h at 70°C) of the stems and remaining leaf material was determined. To estimate the DW of the leaf samples collected for chlorophyll, a FW to DW linear regression, based on the remaining leaf material was used.

Curve fitting and parameter estimation

For all experimental water temperatures, oxygen-exchange rates per unit time were calculated by means of linear regression (initial lag-phases were excluded) and expressed per unit DW. For each series of measurements, the resulting data sets (6-7 datapairs, relating oxygen-exchange rates to incubation temperature) were used to fit the photosynthetic and respiratory temperature responses.

The temperature responses of both net and gross photosynthesis were fitted to the following equations (after Johnson et al., 1974). The formulae are based on the assumption that a single temperature sensitive enzyme, with active and inactive conformations, controls the metabolic process to be fitted (for its application to plant photosynthesis, see also Santamaría & Van Vierssen, 1997):

$$Pm = A\frac{1}{1+\kappa}e^{-Ea/(RT)} \quad (1)$$

where **Pm** is the rate of irradiance-saturated photosynthesis (in µg O_2 g^{-1}DW min^{-1}), **A** is an integration constant (in µg O_2 g^{-1}DW min^{-1}), $\mathbf{E_a}$ is the activation energy for the enzyme's conformational change (in J), **R** is the gas constant (8.314 J mol^{-1} K^{-1}), **T** is the absolute temperature (in °C + 273) and κ is the equilibrium-constant of the reaction responsible for the enzyme's conformational change (in mol), depending on the absolute temperature according to the following expression:

$$\kappa = e^{-(\Delta H - T\Delta S)/(RT)} \quad (2)$$

where Δ**H** and Δ**S** are the increases in enthalpy (in J) and entropy (in J mol^{-1} K^{-1}), involved in the enzyme's conformational change. E_a/R and $\Delta H/R$ (both in mol K) indicate the sensitivity of the photosynthetic reactions to sub- and supra-optimal temperatures respectively (i.e. high E_a/R value: sharp increase of Pm with temperature; high $\Delta H/R$ value: sharp drop in Pm at temperatures above T_{opt}). For each species and collection time, the highest rate of irradiance-saturated photosynthesis (Pm at T_{opt}) was calculated by using equations (1) and (2).

The thermal response of dark respiration was fitted to a linear equation, since the expected exponential relationship did not result in a significant better fit. When the respiration rate decreased above a certain thermal optimum, we used equations (1) and (2). For each species and collection time, highest rate of dark respiration (R_d at T_{opt}) was calculated from equations (1) and (2), or by using the linear regression at the highest experimental water temperature (40 or 42°C). The increase of respiration with temperature (Slope) was calculated by linear regression. For the non-linear respiration data, slopes were calculated from linear regressions on oxygen-exchange values for $T < T_{opt}$.

Seasonal variation in SRP and DGP

For the different species, we investigated whether seasonal variation in photosynthetic temperature-response results in a comparable photosynthetic performance at the combination of daylength and water temperature (hereafter referred to as environment) of the month they were collected. For this purpose we used a simple model that calculates the standardised rate of (gross) photosynthesis (SRP: average gross photosynthetic rate divided by photosynthetic capacity) and the daily gross photosynthesis (DGP; in mg O_2 g^{-1}DW day^{-1}). SRP represents the average rate of gross photosynthesis achieved when thermal variation over the day is taken into account and temperature is the only factor that varies along the season. To calculate SRP, we divided the average gross photosynthetic rate (daily gross photosynthesis / daylength) by the photosynthetic capacity (Pm at T_{opt}; similar to Johnson et al., 1974). Hence SRP only depends on temperature but not on photosynthetic capacity or daylength, whereas DGP depends on temperature, photosynthetic capacity and daylength.

Calculating SRP and DGP

For the period between March and August 1998, we obtained daily minimum and maximum air temperatures, as recorded by a nearby weather station (Royal Dutch Institute of Meteorology, De Bilt, The Netherlands). From these, daily minimum and maximum water temperatures were calculated, using the equation developed by Mooij & Van Tongeren (1990) for shallow Dutch lakes:

$$T_a - T_w = \frac{0.036 + 0.062 \times \sin(2\pi \times (D_{nr} - 80.8)/365.25)}{0.034} \quad (3)$$

where $\mathbf{T_a}$ is air temperature (this and all temperatures hereafter in °C), $\mathbf{T_w}$ is water temperature and $\mathbf{D_{nr}}$ is day number.

The daytime changes in water temperature (averaged for each month) were then calculated according to Goudriaan & Van Laar (1994):

$$T_a = T_{min} + (T_{max} - T_{min}) \times \sin\frac{\pi(t_h - 12 + d/2)}{d + 2p} \quad (4)$$

where $\mathbf{T_a}$ is the water temperature, calculated at time t_h (in h), $\mathbf{T_{min}}$ and $\mathbf{T_{max}}$ respectively are the daily minimum and maximum temperature for that particular month (from Eq. 3), **d** is the mean daylength for that particular month (in h; calculated after Kirk [1983]), $\mathbf{t_h}$ is the solar time (in h) and **p** is a fixed time lag between solar noon and maximum temperature (1.5 h). The daytime changes in water temperature were calculated using a time-step of half an hour.

The realised gross photosynthetic gain was estimated for every half-hour, by combining equations (1) and (2) with equation (4). Subsequently, the obtained values were summated for the light period to obtain the DGP (in mg O_2 g^{-1}DW day^{-1}). Thereafter, SRP (in %) was computed by dividing DGP by the daylength and the photosynthetic capacity (Pm at T_{opt}). Both DGP and SRG were estimated for all available combinations of temperature response and environment.

Statistical analysis

The parameter estimates of the fitted temperature-response curves were compared by means of two-way ANOVAs, with species and collection time (a restricted data set, including only May to July) as main effects. Tukey a posteriori tests were used to identify specific differences between the species across collection times (May to July). In addition, the effect of collection time within each species (the complete data set, including May to August for both *P. pectinatus* and *P. perfoliatus* and March to July, for *C. obtusangula*) was tested by means of one-way ANOVAs, followed by Tukey a posteriori tests. SRP- and DGP-values (May to July) were compared by means of three-way ANOVAs with species, temperature-response (May to July) and environment (May to July) as main effects.

Prior to all statistical analyses, data were log-transformed if required to ensure normality and homogeneity of variances (Sokal & Rohlf, 1995). In heteroscedastic datasets, if means and variances were not correlated ($R^2 > 0.5$), ANOVAs were still performed. Occasionally, homogeneity of variances did not improve after transformation of the data. In those cases multiple comparisons were used on separate t-tests with case-specific variance estimates. Comparisonwise error rates for each comparison were adjusted to maintain an experimentwise error rate (EER) of 0.05. All data were analysed using the STATISTICA statistical package (Statsoft Inc., 1999).

Results

Temperature-response curves

The temperature-response model as proposed by Johnson et al. (1974), described photosynthesis and part of the respiration data with high accuracy (R^2 was always above 0.90 and in most cases above 0.95; Figs. 2-4). However, when maximum respiration rates were measured at the highest experimental water temperature, respiration was best fitted by linear regression (R^2 was always above 0.97). Two-way ANOVAs revealed significant differences among the species for nearly all fitted and calculated oxygen-exchange parameters (except E_a/R for gross photosynthesis; Table 1A). The species also differed in total chlorophyll concentration, while the chlorophyll a/b-ratio did not vary (Table 1A). Seasonal variation in photosynthesis was only found for a single variable (E_a/R for gross photosynthesis). In addition, maximum dark respiration, its increase-rate over temperature (i.e. the slope of the linear fits) and total chlorophyll concentration varied over time (Table 1A). Significant interactions (species $\times$ time) for practically all variables (except E_a/R for net photosynthesis and chlorophyll a/b-ratio) indicated that the investigated species differed in their acclimative response over time (Table 1A).

Net photosynthesis

For net photosynthesis, only some of the temperature-response curves showed a maximum at a particular optimal temperature (Figs. 2-4). In all other cases, when Pm slightly decreased over the whole experimental temperature-range, photosynthesis at 6°C was taken as a maximum. C. *obtusangula* had the highest Pm at T_{opt}, while lower values were observed for both *Potamogeton* species (Table 1A and 2). All species showed seasonal variation in the maximum rate of light-saturated photosynthesis (Table 1B). For *C. obtusangula*, highest values for Pm at T_{opt} were found at

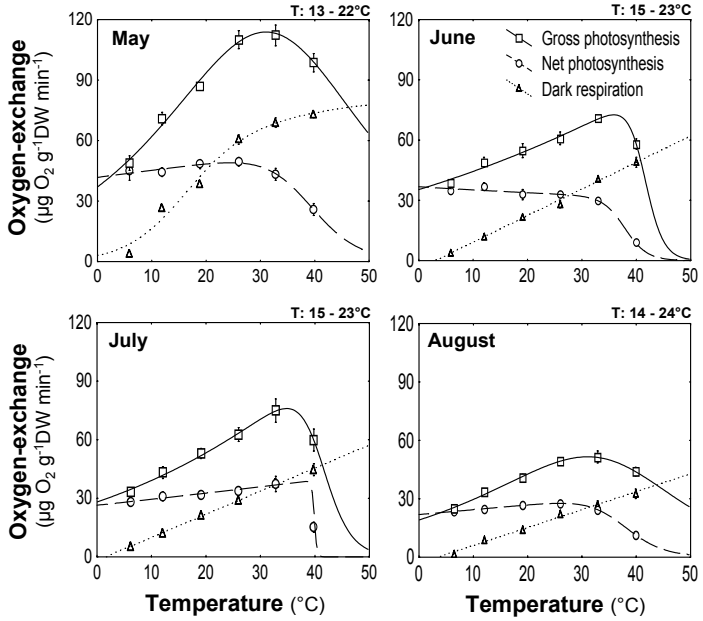

Figure 2. Effect of temperature on the irradiance–saturated (gross and net) photosynthesis and dark respiration for complete shoots of *Potamogeton pectinatus*, collected between May and August. Photosynthesis data were fitted by non-linear estimation (see eq. [1] and [2]; after Johnson et al., 1974). Dark respiration values for the plants collected in May were fitted with the same equations, while the respiration values for the shoots collected from June to August were fitted by linear regression. Data points are means ± SE (n=3). Please note that the curves are based on average oxygen-exchange rates, while parameter estimates were obtained from separate fits for each of the three replicate datasets. Monthly averages for the minimum and maximum daytime water temperature (as modelled from air temperatures by using eq. [3]) are indicated in the graphs.

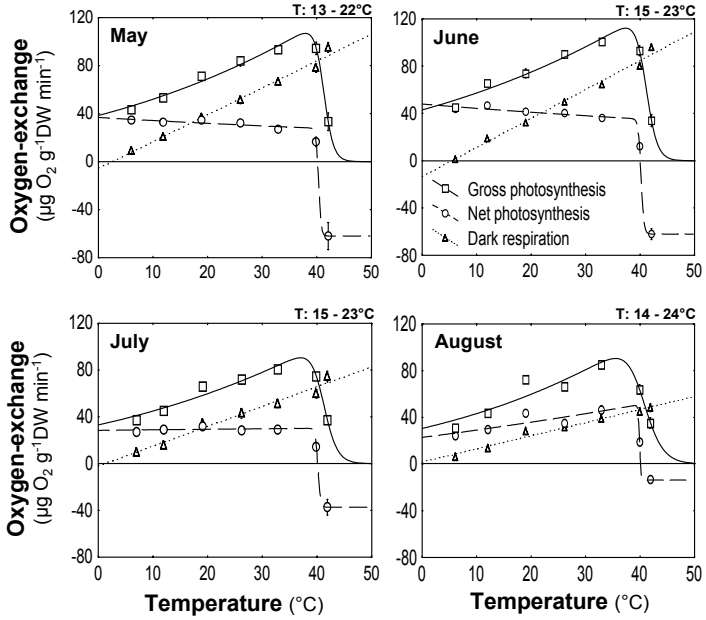

Figure 3. Effect of temperature on the irradiance–saturated (gross and net) photosynthesis and dark respiration for complete shoots of *Potamogeton perfoliatus*, collected between May and August. Photosynthesis data were fitted by non-linear estimation (see eq. [1] and [2]; after Johnson et al., 1974). Dark respiration values were fitted by linear regression. Data points are means ± SE (n=3). Please note that the curves are based on average oxygen-exchange rates, while parameter estimates were obtained from separate fits for each of the three replicate datasets. Monthly averages for the minimum and maximum daytime water temperature (as modelled from air temperatures by using eq. [3]) are indicated in the graphs.

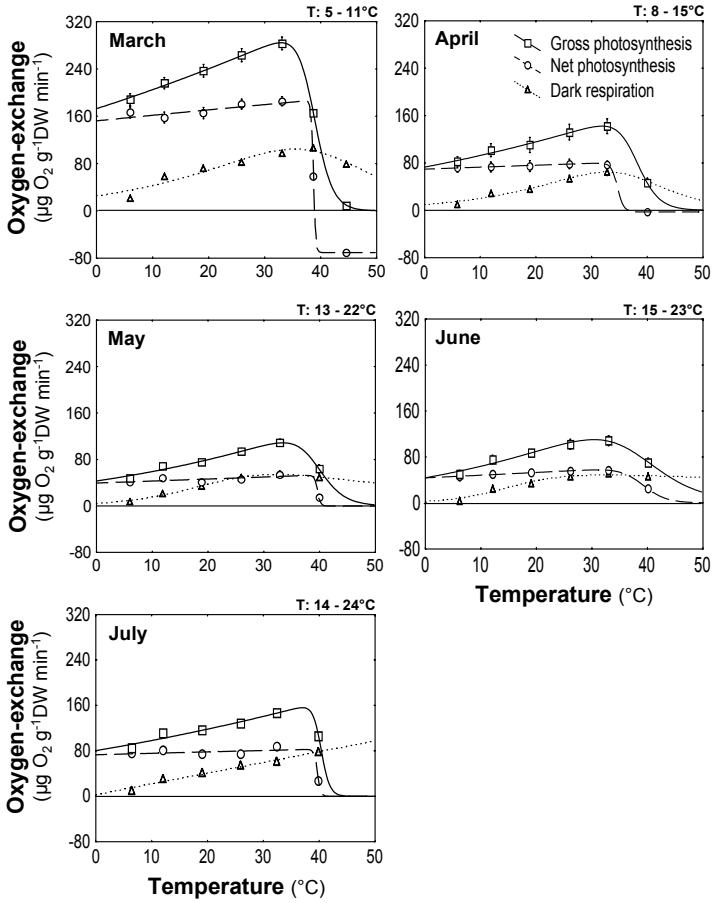

Figure 4. Effect of temperature on the irradiance–saturated (gross and net) photosynthesis and dark respiration for apical plant parts of *Callitriche obtusangula*, collected between March and July. Photosynthesis data were fitted by non-linear estimation (see eq. [1] and [2]; after Johnson et al., 1974). Dark respiration values for the plants collected between March and June were fitted with the same equation, while the respiration values for the shoots collected in July were fitted by linear regression. Data points are means ± SE (n=3). Please note that the curves are based on average oxygen-exchange rates, while parameter estimates were obtained from separate fits for each of the three replicate datasets. Monthly averages for the minimum and maximum daytime water temperature (as modelled from air temperatures by using eq. [3]) are indicated in the graphs.

the beginning and end of the season, while in May and June photosynthetic rates were comparable (Table 2). For *P. pectinatus*, a 44% seasonal decrease in Pm at T_{opt} was found, while small fluctuations but no marked seasonal trend was observed for *P. perfoliatus* (Table 2).

For all species, net photosynthesis showed very limited variation between 6 and 30°C (Figs. 2-4); hence the fitted E_a/R-values were rather small and occasionally negative (Table 2). At temperatures above 33°C, a rapid decrease in net photosynthesis was observed for the investigated species (Figs. 2-4), resulting in high ΔH/R-values (Table 2). Despite some temporal variation, the parameters describing the shape of the temperature-response curves (e.g. E_a/R and ΔH/R) did not show a seasonal trend (Table 2).

Table 1. **(A)** Two-way ANOVAs on oxygen-exchange parameters and chlorophyll concentration, with species (S) and time (T; May to July) as main factors. **(B)** One-way ANOVAs on oxygen-exchange parameters and chlorophyll concentration with time (T) as main factor (analysed for individual species and for the complete period on which observations were made). All variables as described in tables 1-5. **(C)** Three-way ANOVAs on modelled standardised rate of gross production (SRP) and daily gross photosynthesis (DGP) with species (S), environment (E) and temperature response (T) as main factors. NS: not significant, * P < 0.05, ** P < 0.01, *** P < 0.001, –: no ANOVA results available, as variances were not homogeneous.

	A				**B**			
	Variable	**S**	**T**	**SxT**	**Variable**	***Potamogeton pectinatus***	***Potamogeton perfoliatus***	***Callitriche obtusangula***
Net Photosynthesis	Pm at T_{opt}	***	NS	***	Pm at T_{opt}	**	**	***
	E_a/R	*	NS	NS	E_a/R	NS	–	NS
	$\Delta H/R$	***	NS	**	$\Delta H/R$	*	NS	NS
Gross Photosynthesis	Pm at T_{opt}	***	NS	**	Pm at T_{opt}	NS	***	*
	T_{opt}	***	NS	***	T_{opt}	***	**	***
	E_a/R	NS	**	***	E_a/R	**	–	***
	$\Delta H/R$	**	NS	*	$\Delta H/R$	–	*	*
Dark Respiration	R_d at T_{opt}	***	**	***	R_d at T_{opt}	***	***	***
	slope	***	***	***	slope	***	***	NS
Chlorophyll	a+b	***	***	***	a+b	NS	NS	***
	a/b	NS	NS	NS	a/b	NS	*	NS

	C						
	Variable	**S**	**E**	**T**	**SxE**	**SxT**	**ExT**	**SxExT**
Gross Photosynthesis	SRP	***	NS	*	NS	***	NS	NS
	DGP	***	**	***	NS	***	NS	NS

Pm at T_{opt}: light-saturated photosynthesis at the optimal temperature, **E_a/R** and **$\Delta H/R$**: coefficients in the temperature-response formula proposed by Johnson et al. (1974); see eq. (1) and (2), **T_{opt}**: optimal temperature, **R_d at T_{opt}**: maximum rate of dark respiration, **slope**: the increase-rate of dark respiration with temperature, **a+b**: total chlorophyll concentration, **a/b**: chlorophyll a/b-ratio.

Dark respiration

For all species, respiration rates at 6°C were very low, but rapidly increased with increasing temperature (Figs. 2-4). During spring and early summer, the dark respiration of *C. obtusangula* decreased at temperatures between 31 and 35°C (Fig. 4), while for *P. pectinatus* and *P. perfoliatus* a decrease in dark respiration was not observed at the maximum incubation temperatures (Fig. 2 and 3).

Between May and July, maximum respiration rates (R_d at T_{opt}) and the slopes (S) of the regression lines were higher for *P. perfoliatus* than for the other species, that did not differ significantly (Table 1A and 2). Seasonal variation in both R_d at T_{opt} and S differed among the species (significant species × time interaction; Table 1A). For *P. pectinatus*, R_d at T_{opt} and S decreased from May to August, while for *P. perfoliatus* a decrease was found from May/June to August (Table 1B and 2). While *C. obtusangula* showed significant seasonal variation in the maximum respiration rate, the slopes did not vary significantly (Table 1B and 2). Maximum respiration decreased from March until June, and increased again in July (Table 1B and 2).

Table 2. Parameter estimates, derived from fitted temperature-response curves (net photosynthesis and respiration) of three aquatic macrophyte species, collected in different months during the growth season. Shown values are means ± SE (n=3). The effect of species and month (May-July) was tested by two-way ANOVA. Significant differences between the species are indicated with different capital letters (Tukey a posteriori test, $P < 0.05$). Within a species, different lower case letters stand for significant differences between the months (one-way ANOVA, Tukey a posteriori test, $P < 0.05$). If the lower case letters are in italics, homogeneity of variances did not improve after transformation of the data. In those cases we performed multiple pairwise comparisons using separate t-tests with case-specific variance estimates (EER < 0.05).

Species	Month	Net photosynthesis					
		Pm at T_{opt} ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)		**E_a/R** (mol K)		**$\Delta H/R$** (mol K)	
P. pectinatus	May	B	49.3 ± 2.3^{a}	AB	1063 ± 911^{a}	B	27587 ± 8966^{b}
	June		35.6 ± 1.8^{bc}		-326 ± 50^{a}		53188 ± 7426^{ab}
	July		38.0 ± 4.0^{ab}		902 ± 157^{a}		251604 ± 101419^{a}
	August		27.8 ± 0.3^{c}		908 ± 211^{a}		28804 ± 6531^{b}
P. perfoliatus	May	B	35.1 ± 2.1^{b}	B	-193 ± 58^{b}	A	673079 ± 91830^{a}
	June		46.0 ± 1.0^{a}		-266 ± 56^{b}		596652 ± 42394^{a}
	July		30.9 ± 0.2^{b}		66 ± 101^{b}		574916 ± 15756^{a}
	August		50.7 ± 4.4^{a}		1245 ± 306^{a}		494507 ± 18213^{a}
C. obtusangula	March	A	185.6 ± 7.1^{a}	A	334 ± 60^{a}	B	207228 ± 64227^{a}
	April		80.0 ± 8.0^{b}		390 ± 149^{a}		212990 ± 44572^{a}
	May		52.0 ± 1.6^{c}		649 ± 73^{a}		243102 ± 13335^{a}
	June		58.6 ± 5.4^{c}		911 ± 258^{a}		103198 ± 64471^{a}
	July		81.7 ± 1.0^{b}		296 ± 75^{a}		112151 ± 50646^{a}
		Dark respiration					
		T_{opt} (°C)		**R_d at T_{opt}** ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)		**slope** ($\mu g\ O_2\ g^{-1}DW\ min^{-1}\ °C^{-1}$)	
P. pectinatus	May	40.0^{1}		B	73.4 ± 1.4^{a3}	B	2.41 ± 0.12^{a2}
	June	40.0^{1}			48.9 ± 2.2^{b3}		1.32 ± 0.06^{b}
	July	40.0^{1}			45.5 ± 3.0^{b3}		1.18 ± 0.09^{bc}
	August	40.0^{1}			33.6 ± 2.3^{c3}		0.92 ± 0.06^{c}
P. perfoliatus	May	42.0^{1}		A	83.8 ± 3.7^{a3}	A	2.24 ± 0.10^{a}
	June	42.0^{1}			84.7 ± 2.01^{a3}		2.45 ± 0.07^{a}
	July	42.0^{1}			65.7 ± 4.3^{b3}		1.69 ± 0.09^{b}
	August	42.0^{1}			46.7 ± 2.7^{b3}		1.12 ± 0.07^{c}
C. obtusangula	March	35.1 ± 0.5		B	103.7 ± 4.7^{a}	B	2.38 ± 0.11^{a2}
	April	33.1 ± 1.1			66.2 ± 6.9^{bc}		1.97 ± 0.19^{a2}
	May	32.7 ± 0.5			54.3 ± 4.7^{c}		1.81 ± 0.22^{a2}
	June	31.6 ± 0.5			49.5 ± 3.4^{c}		1.70 ± 0.10^{a2}
	July	40.0^{1}			78.5 ± 2.1^{b3}		1.89 ± 0.06^{a}

Pm at T_{opt}: maximum light-saturated net photosynthesis at the optimal temperature, **E_a/R** (activation energy/gas constant) and **$\Delta H/R$** (increase in entropy/gas constant) are coefficients in the temperature-response formula proposed by Johnson et al. (1974); see eq. (1) and (2), **T_{opt}**: temperature at which maximum respiration was measured, **R_d at T_{opt}**: highest fitted rate of dark respiration, **slope**: the increase of respiration rate with increasing temperature. **1**: Optimal temperature for dark respiration above the highest given experimental temperature, **2**: slopes based on linear regressions on respiration values for $T < T_{opt}$, **3**: maximum respiration rates at the highest experimental temperature (40 or 42°C).

Gross photosynthesis

Between May and July, the highest temperature optima were recorded for *P. perfoliatus*, while significantly lower values were found for both other species (Table 1A and 3). Seasonal variation in T_{opt} was significant for *P. perfoliatus* and *C. obtusangula* (Table 1B), but the range of variation was small (≤ 5.4°C) and not directed towards higher thermal optima at the end of the growing season (Table 3).

Table 3. Parameter estimates, derived from individual temperature-response curves (gross photosynthesis) of three aquatic plant species, collected in different months during the growth season. Shown values are means ± SE (n=3). The effect of species and month (May-July) was tested by two-way ANOVA. Significant differences between the species are indicated with different capital letters (Tukey a posteriori test, $P < 0.05$). Within a species, different lower case letters stand for significant differences between the months (one-way ANOVA, Tukey a posteriori test, $P < 0.05$). If the lower case letters are in italics, homogeneity of variances did not improve after transformation of the data. In those cases we performed multiple pairwise comparisons using separate t-tests with case-specific variance estimates (EER < 0.05).

Species	**Month**	**Gross Photosynthesis**							
		T_{opt} (°C)		**Pm at T_{opt}** (µg O_2 g^{-1}DW min^{-1})		**E_a/R** (mol K)		**$\Delta H/R$** (mol K)	
P. pectinatus	May	B	31.0 ± 0.6^{a}	C	113.3 ± 5.1^{a}	A	4741 ± 1260^{a}	B	12880 ± 1506^{a}
	June		35.0 ± 1.5^{a}		72.0 ± 0.4^{b}		1908 ± 84^{b}		71657 ± 43498^{a}
	July		35.1 ± 1.3^{a}		78.4 ± 9.2^{b}		2598 ± 76^{ab}		66948 ± 34654^{a}
	August		30.9 ± 0.9^{a}		51.8 ± 2.8^{c}		3619 ± 280^{a}		13208 ± 2046^{a}
P. perfoliatus	May	A	37.9 ± 0.2^{a}	B	106.8 ± 4.7^{a}	A	2337 ± 78^{a}	A	115108 ± 12787^{a}
	June		37.4 ± 0.2^{a}		112.5 ± 1.9^{a}		2231 ± 37^{a}		99802 ± 5336^{a}
	July		37.0 ± 0.1^{a}		90.1 ± 3.9^{b}		2373 ± 166^{a}		81839 ± 6613^{ab}
	August		35.5 ± 0.4^{b}		90.0 ± 2.4^{b}		2704 ± 231^{a}		59371 ± 6621^{b}
C. obtusangula	March	B	32.9 ± 0.3^{ab}	A	284.0 ± 10.2^{a}	A	1328 ± 71^{c}	B	61457 ± 3573^{ab}
	April		32.1 ± 0.8^{ab}		143.3 ± 13.7^{b}		1847 ± 12^{bc}		53840 ± 6887^{ab}
	May		33.9 ± 0.9^{ab}		110.0 ± 6.3^{b}		2465 ± 225^{ab}		52859 ± 13286^{ab}
	June		30.1 ± 0.7^{b}		109.6 ± 8.8^{b}		3155 ± 450^{a}		20780 ± 3151^{b}
	July		35.5 ± 1.7^{a}		152.9 ± 6.3^{b}		1602 ± 66^{c}		106571 ± 37735^{a}

T_{opt}: fitted optimal temperature for light-saturated gross photosynthesis, **Pm at T_{opt}**: rate of light-saturated gross photosynthesis at T_{opt}, **E_a/R** (activation energy/gas constant) and **$\Delta H/R$** (increase in entropy/gas constant) are coefficients in the temperature-response formula proposed by Johnson et al. (1974); see eq. (1) and (2).

The highest rates of irradiance-saturated photosynthesis at T_{opt} (for the period between May and July) were recorded for *C. obtusangula*, while intermediate and lowest values were found for *P. perfoliatus* and *P. pectinatus* respectively (Table 1A and 3). All species showed seasonal variation in Pm at T_{opt} (Table 1B). For *P. pectinatus*, the highest value of Pm at T_{opt} was recorded in May, while

during the rest of the season it gradually decreased to a final 46% of the initial rate of irradiance-saturated photosynthesis at T_{opt}. (Table 3). Photosynthetic activity of *P. perfoliatus* was also highest in early summer (June), but it only slightly decreased during the growth season (less than 20%; Table 3). For *C. obtusangula*, the irradiance-saturated rate of photosynthesis at T_{opt} decreased sharply from March to May (to 39% of the initial value), but at the end of the experimental period (July) it increased again (Table 3).

For the period between May and July, we only found species-specific differences in the shape of the curves at supra-optimal temperatures (i.e. significant species x time interaction for $\Delta H/R$; Table 1A). In general, *P. perfoliatus* showed a sharper drop in Pm above the optimal temperature (i.e. higher $\Delta H/R$; Table 3) as compared with both other species. For the complete data set, significant seasonal variation in the shape of the temperature-response curves at sub-optimal temperatures (E_a/R) was found for *P. pectinatus* and *C. obtusangula* (Table 1B and 3). We also found seasonal variation in the response at supra-optimal temperatures ($\Delta H/R$) for both *P. perfoliatus* and *C. obtusangula* (Table 1B and 3). Despite these differences, we do not see systematic variation in the shape of the photosynthetic temperature response at either sub- and/or supra-optimal temperatures (i.e. a continuous increase or decrease of E_a/R or $\Delta H/R$ from spring to summer; Table 3).

Chlorophyll concentration

Total chlorophyll concentration varied significantly among the species (Table 1A). Between May and July, *P. perfoliatus* showed the highest total chlorophyll concentration, while *P. pectinatus* and *C. obtusangula* displayed intermediate and lower concentrations respectively (Table 4).

Table 4. Total chlorophyll concentration (**a+b**) and chlorophyll a/b-ratios (**a/b**) for three aquatic macrophyte species, collected in different months during the growth season. Shown values are means ± SE (n=3). The effect of species and month (May-July) was tested by two-way ANOVA. Significant differences between the species are indicated with different capital letters (Tukey a posteriori test, $P < 0.05$). Within species, different lower case letters stand for significant differences between the months (one-way ANOVA, Tukey a posteriori test, $P < 0.05$).

Species	**Month**	**Chlorophyll**			
		a+b ($\mu g\ g^{-1}$ FW)		**a/b** ($\mu g\ \mu g^{-1}$)	
P. pectinatus	May	B	903 ± 33^{a}	A	3.37 ± 0.06^{a}
	June		1283 ± 169^{a}		3.48 ± 0.12^{a}
	July		1161 ± 55^{a}		3.48 ± 0.12^{a}
	August		876 ± 82^{a}		3.30 ± 0.21^{a}
P. perfoliatus	May	A	2868 ± 91^{a}	A	3.46 ± 0.03^{ab}
	June		2597 ± 218^{a}		3.26 ± 0.10^{b}
	July		2852 ± 73^{a}		3.45 ± 0.04^{ab}
	August		2731 ± 249^{a}		3.58 ± 0.02^{a}
C. obtusangula	March	C	1105 ± 17^{a}	A	3.64 ± 0.14^{a}
	April		826 ± 18^{b}		3.68 ± 0.05^{a}
	May		573 ± 45^{c}		3.46 ± 0.04^{a}
	June		743 ± 81^{bc}		3.37 ± 0.11^{a}
	July		1161 ± 19^{a}		3.60 ± 0.07^{a}

C. obtusangula was the only species for which we found seasonal variation in total chlorophyll concentration (Table 1B). Highest concentrations were found in March and July and lowest in May (Table 4).

Between May and July, chlorophyll a/b-ratios did not vary significant among species, collection times or their interaction (Table 1A). *P. perfoliatus* was the only species showing temporal variation in chlorophyll a/b-ratio, although no marked seasonal trend was observed (Table 1A and 4).

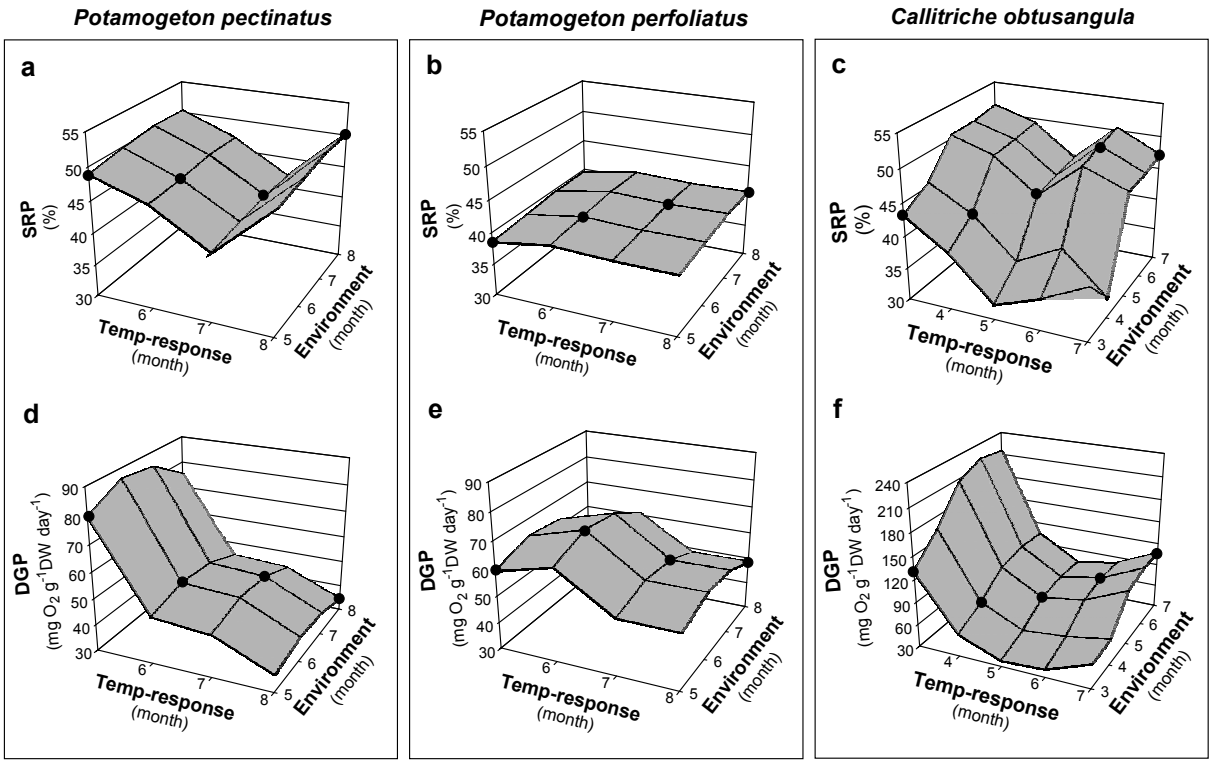

Figure 5. Three-dimensional plots for the standardised rates of gross photosynthesis (SRP; a, b and c) and daily gross photosynthetic gain (DGP; d, e and f) of three submerged freshwater macrophyte species. SRP- and DGP-values were calculated for all available combinations of 'photosynthetic temperature response' and 'environment' (daylength and water temperature). Monthly combinations corresponding to the measured values are marked with black dots. Numbers on the x- and y-axis represent the different months (3: March, 4: April, 5: May, etc.).

Seasonal variation in SRP and DGP

The standardised rate of gross photosynthesis (SRP) and the daily gross photosynthesis (DGP) varied significantly among species (Table 1C). The shape of the photosynthesis curves significantly affected both SRP and DGP, while the environment (daylength and temperature regime) only had a significant effect on the DGP (Table 1C). For both SRP and DGP, a three-way ANOVA revealed no significant temperature response × environment interactions, which means that no changes in seasonal production pattern due to photosynthetic acclimation were found (Table 1C).

For *P. pectinatus* and *C. obtusangula*, changes in the shape of temperature-response curves caused monthly variation in SRP, with highest values at the beginning and end of the growing season (Fig. 5A, B and C). In *P. perfoliatus*, however, changes in the thermal response only introduced minor

variation in SRP (Fig. 5A, B and C). For both *Potamogeton* species, changes in the environment did not affect SRP, while for *C. obtusangula* the SRP increased from April to July (Fig. 5A, B and C).

For all three species, variation in the temperature response (mainly in the maximum rate of gross photosynthesis, i.e. Pm at T_{opt}) leads to a seasonal decrease in DGP, although for *C. obtusangula* a slight increase at the end of the season is observed (Fig. 5D, E and F). Variation in DGP due to changes in the environment is negligible in both *Potamogeton* species, but fairly high in *C. obtusangula* (Fig. 5D, E and F).

Discussion

In contrast with the results for other aquatic macrophyte species (e.g. Titus & Adams, 1979; Menendez & Peñuelas, 1993), the oxygen-exchange rates for net photosynthesis showed little change over a large range of experimental temperatures (Figs. 2-4). We therefore could not determine a distinct maximum at a particular optimal temperature. For gross photosynthesis however, the expected asymmetric optimum-response curve was found. The recorded optimal temperatures for *P. pectinatus* (31-35°C) were somewhat higher than the one reported by Madsen & Adams (1989).

As in many other studies, we used the optimal temperature for gross photosynthesis as an indicator for the degree of thermal acclimation (e.g. Berry & Björkman, 1980; Santamaría & Van Vierssen, 1997). All species showed significant temporal variation in T_{opt}. Nevertheless, the changes were small ($\leq$ 5.4°C; Table 3) and not directed towards higher values at the end of the growing season (i.e. rejection of hypothesis Ia). The relative stability of T_{opt} for gross photosynthesis suggests that the temperature response of freshwater macrophytes is comparable to the response of marine autotrophs, in which a seasonal shift in optimal temperature seems to be less common than in terrestrial plants (e.g. Drew, 1978; Gacia et al., 1996).

As an alternative to seasonal changes in T_{opt}, plants could also show acclimative variation in the shape of the thermal-response curves (e.g. Santamaría & Van Vierssen, 1997). From this perspective, and assuming limited variation in T_{opt} due to physiological constraints, we hypothesised that early in the season, E_a/R-values for both net and gross photosynthesis would be reduced. For net photosynthesis, temporal variation in E_a/R was negligible in all investigated species (Table 2). While, *C. obtusangula* showed an increase in E_a/R for gross photosynthesis (from May to June), both *P. pectinatus* and *P. perfoliatus*, showed no clear trend of variation in E_a/R over time (Table 3). This indicates that the perennial *C. obtusangula* is the only species that showed (limited) seasonal acclimation to sub-optimal temperatures (i.e. confirmation of hypothesis Ib). However, the acclimative significance of temporal variation in the shape of the (gross) photosynthesis curves can only be evaluated when combined with actual changes in water temperature and daylength. For this purpose we calculated standardised rates of gross photosynthesis (SRP), which are independent of seasonal differences in photosynthetic capacity. If individual species would show acclimative variation in the shape of the response curves, we expected enhanced SRP-values for corresponding combinations of thermal response and environment and lower values for all other combinations. *P. perfoliatus* showed little variation in SRP-values, and for both other species the interplay between corresponding combinations of temperatures response and environment did not lead to enhanced SRP-values (Fig. 5A, B and C). Consequently, we conclude that for the investigated species, the observed changes in the shape of the thermal response curves do not represent an acclimative response (i.e. rejection of hypothesis IIa).

For all investigated species, Pm at T_{opt} for both net and gross photosynthesis decreased from spring to summer (Table 2 and 3). Other researchers studying seasonal variation in oxygen-exchange for *P. pectinatus* and *P. perfoliatus* also observed high photosynthetic rates in spring, followed by a progressive decrease during summer (Van der Bijl et al., 1989; Caffrey & Kemp, 1991). In terrestrial plants a similar pattern of variation has been attributed to senescence, since it was accompanied by decreased levels of chlorophyll and loss of enzymatic activity (DePuit & Caldwell, 1973; Slatyer & Morrow, 1977). On the other hand, an unbalanced growth potential that leads to a dilution of the available nutrients might also explain the seasonal loss of photosynthetic capacity (Lugg & Sinclair, 1981).

While in *C. obtusangula* changes in Pm at T_{opt} are paralleled by changes in total chlorophyll concentration (Table 4), this was not the case for either *Potamogeton* species. Similar to the pattern of temporal variation in photosynthetic capacity, maximum respiration rates also showed a seasonal decrease (Table 2). High respiration rates early in the season might reflect a higher metabolic activity related to rapid growth (Man & Lieffers, 1997), hence a reduction in dark respiration could also be related to plant senescence or to reduced growth due to nutrient dilution.

While temporal differences in T_{opt} and the influence of E_a/R on SRP-values describe acclimative changes in the thermal response, the daily gross photosynthesis (DGP) is also determined by seasonal variation in the photosynthetic capacity and respiration. Since for all species Pm (for gross photosynthesis) decreased during the season, the DGP-values also decreased (Table 3; Fig. 5D, E and F). Similar to the findings for seasonal variation in SRP, we conclude that (small) temporal differences in the shape of the photosynthesis curves are of minor importance for the daily gross photosynthetic gain (i.e. rejection of hypothesis IIb). The decrease in DGP also indicates that the extended photosynthetic period (i.e. longer days) at the end of the growth season cannot compensate for the loss of photosynthetic capacity.

Finally, it is of interest to view the obtained results on seasonal temperature acclimation from the perspective of species-specific differences in seasonal growth. As *C. obtusangula* starts to grow two months earlier than both *Potamogeton* species, we expected *C. obtusangula* to show a stronger acclimative response. In spite of the short days during early spring and the absence of seasonal temperature acclimation, *C. obtusangula* is still able to reach the highest daily gross photosynthetic gain in March (Fig. 5 D, E and F). These results indicate that early seasonal growth is not determined by the degree of thermal acclimation (i.e. rejection of hypothesis III), but rather results from a high photosynthetic capacity.

From the collected data it seems clear that the investigated species did not show a thermal response that is tuned to the predicted seasonal changes in water temperature. To explain these results we could think of physiological constraints that prevent the plants from having an optimal response. In this respect, thermal optimisation of the photosynthetic apparatus might result in high metabolic costs that are not in proportion with the acclimative benefits. As an alternative hypothesis, seasonal changes in both water temperature and daylength might be of minor importance when compared with differences in daily gross photosynthetic gain, caused by differences in photosynthetic capacity due to plant ageing.

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CHAPTER 4

Clonal variation in the thermal response of the submerged aquatic macrophyte *Potamogeton pectinatus* L.

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Summary

Broadly distributed plants have to cope with dramatic differences across latitude in the prevailing environmental temperatures. We investigated the effect of water temperature on plant morphology, biomass accumulation and oxygen-exchange for five clones of the submerged aquatic macrophyte *Potamogeton pectinatus* L., originating from 42 to 68°N.

We tested whether *P. pectinatus* clones show local adaptation to the prevailing environmental temperatures (in which case high-latitude clones would perform better at lower temperatures and *vice versa*). Alternatively, pronounced phenotypic plasticity in the response to temperature could enable individual clones to perform well over a wide range of environmental temperatures (i.e. high degree of thermal tolerance).

The overall pattern of thermal response was similar for all clones. In addition, we detected acclimative phenotypic plasticity in both physiological and morphological plant parameters. The optimal temperatures for neither gross nor net photosynthesis varied with growth temperature, but morphological acclimation partly compensated for the loss of photosynthetic capacity at higher temperatures, enabling comparable rates of ambient gross photosynthesis. Respiratory reactions also showed some degree of thermal acclimation to higher temperatures.

As a result of the combined effects of changes in morphology and physiology, all clones produced similar amounts of plant biomass over a relatively wide range of water temperatures. We therefore conclude that *P. pectinatus* is thermally tolerant and not locally adapted.

Introduction

Temperature is one of the most important environmental factors that influence plant performance. Small thermal oscillations occur on a diurnal basis, whilst there is more pronounced variation across seasons and, particularly dramatic across latitude between the extremes of arctic and desert climates (Berry & Björkman, 1980; Berry & Raison, 1981).

Plant species native to different climatic regions may be genetically adapted to prevailing temperatures (Björkman et al., 1974; Björkman et al., 1975) and, as a result, physiological function may be limited in a way that restricts the species' distribution (Woodward & Williams, 1987; Woodward, 1988). In addition, phenotypic modifications may allow individuals to adapt to local or temporal variation in temperature (i.e. acclimation, Berry & Björkman, 1980). Widely distributed plants however have to cope with a broad range of thermal regimes, and overcoming limiting effects in their distributional range depends on either acclimation or strong genetic differentiation among populations (Bradshaw, 1965).

The ability to thrive and survive under diverse thermal conditions involves many integrated plant processes, but the effect of temperature on photosynthesis is crucial and may exhibit both population-specific differences and acclimation. For example, substantial variation in optimal temperature (T_{opt}) for light-saturated photosynthesis and the degree of thermo-tolerance was found for clones of *Camellia sinensis*, obtained from different agro-climatic zones in India (Joshi & Palni, 1998). Population-specific differences may also exist in the overall photosynthetic or respiratory capacity, as in enhanced photosynthesis at low temperature in the arctic species *Oxyria digyna*, which enables a more rapid growth under adverse thermal conditions (Billings et al., 1971). Similarly, more pronounced respiration in a high-latitude population of *Lathyrus japonicus*, may reflect a higher growth rate which is advantageous during short growing seasons (Lechowicz et al., 1980).

Morphological adjustments or changes in the pattern of biomass allocation can also improve plant performance under contrasting thermal regimes, especially when they lead to increased growth or competitive ability. In *Dactylis glomerata* for example, the relative growth rate and the rate of root-cell division showed an increased tolerance to lower temperatures in high-latitude populations (Eagles, 1967; Creber et al., 1993). In addition, the diversion of assimilates into the base and roots of individual plants provide storage reserves, which can be quickly mobilised to produce photosynthetic tissue in spring (Eagles, 1967). However, populations may differ in the timing of growth and this, combined with preferences for particular micro-sites, may minimise differences in tissue temperature between environments and thus reduce the need for adaptation or acclimation (Berry & Raison, 1981).

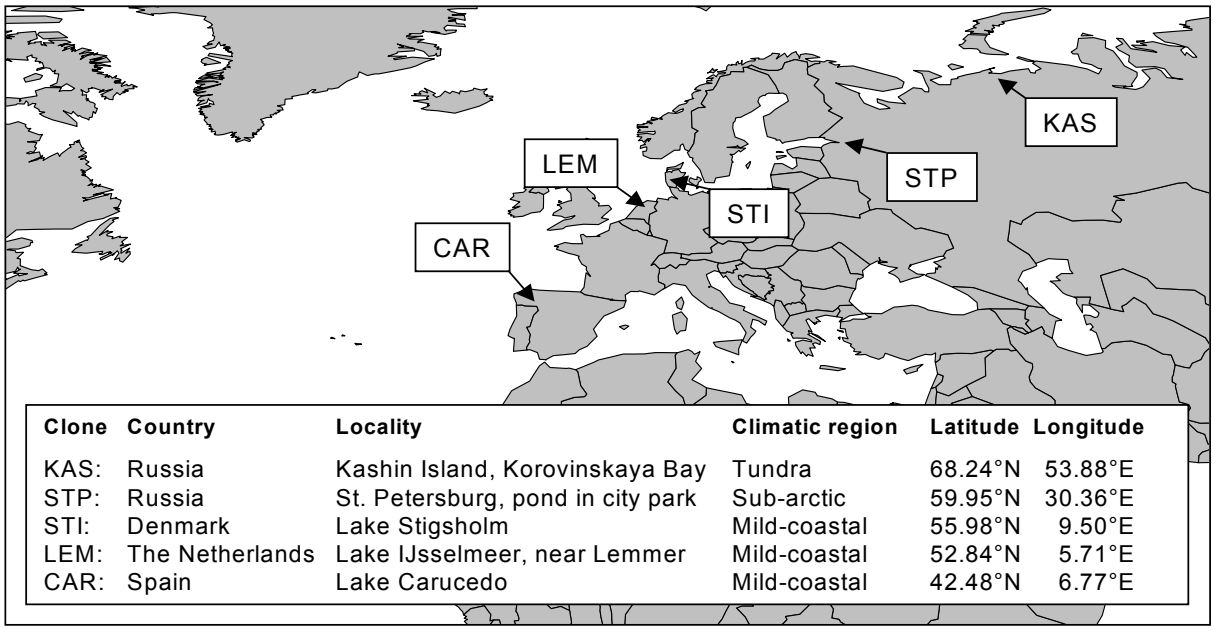
Little is known about ecotypic variation across latitude in the thermal response of growth and photosynthesis in terrestrial plants. Although patterns of adaptation to environmental conditions could emerge from similar studies on submerged aquatic macrophytes (Madsen & Adams, 1988), to the best of our knowledge none have been reported. Some aspects of the (short-term) thermal response of fennel pondweed (*Potamogeton pectinatus* L.) have been studied at locations that differed in latitude (Spencer, 1986; Madsen & Adams, 1988; Madsen & Adams, 1989; Van Wijk, 1989; Vermaat & Hootsmans, 1994a; Menendez & Sanchez, 1998), but experimental conditions were rather diverse and acclimation was rarely considered. We therefore characterised variation in the response to temperatures between 10 and 30°C among five clones of *P. pectinatus* from contrasting latitudes. We consider (1) thermal differentiation (i.e. plants are adapted to the prevailing temperature) and/or acclimation (thermal tolerance), enabling individual clones of this species to perform well over a wide range of temperature, (2) the extent to which morphology and biomass allocation rather than photosynthesis and respiration contribute to thermal differentiation and/or acclimation and (3) how the clones differ in these responses.

Material and methods

Description, origin and propagation of plant material

P. pectinatus is a submerged aquatic macrophyte with a nearly cosmopolitan distribution (Casper & Krausch, 1980; Wiegleb & Kaplan, 1998), encompassing great variation in water depth, flow regime, trophic status and salinity (Van Wijk, 1988). Its morphology is extremely plastic, and a number of local growth forms have been identified (Van Wijk et al., 1988; Vermaat & Hootsmans, 1994b). *P. pectinatus* has a pseudo-annual life cycle (Van Wijk, 1988), which means that it is a perennial clonal plant behaving as a vegetatively reproducing annual (Verburg & During, 1998).

We selected five clonal lines of *P. pectinatus*, each originating from a single tuber. The lines whose sources ranged from 42-68°N (Fig. 1) were propagated once a year at the Centre for Limnology (Nieuwersluis, The Netherlands [52.20°N, 5.01°E]; growing season from May to September) and at the Plant Protection Research Institute (Pretoria, South Africa [25.75°S, 28.20°E]; growing season from November to March). Plants were grown outdoors in polyethylene tanks ($1m^3$) filled with tap water over a mixture of sand and clay (3:1 by dry weight). Following collection from the tanks, all tubers were surface-sterilised with 5% sodium hypochlorite (e.g. Madsen, 1985) before storage at 4°C until March 1998.



Clone	Country	Locality	Climatic region	Latitude	Longitude
KAS:	Russia	Kashin Island, Korovinskaya Bay	Tundra	68.24°N	53.88°E
STP:	Russia	St. Petersburg, pond in city park	Sub-arctic	59.95°N	30.36°E
STI:	Denmark	Lake Stigsholm	Mild-coastal	55.98°N	9.50°E
LEM:	The Netherlands	Lake IJsselmeer, near Lemmer	Mild-coastal	52.84°N	5.71°E
CAR:	Spain	Lake Carucedo	Mild-coastal	42.48°N	6.77°E

Figure 1. Localities from where the investigated clonal lines of *P. pectinatus* were obtained.

Growth conditions

Tuber sprouting and subsequent growth of all clones was investigated at five different water temperatures (10, 15, 20, 25 or 30°C) in glass aquaria (30 x 40 x 40 cm) filled with tap water, whose temperature was maintained by separate heating and cooling units. The aquaria (two per temperature) were distributed between three phytotrons, each of which imposed a 16 hours photoperiod with an irradiance of 157 ± 0.8 µmol m^{-2} s^{-1} (Philips TLD 50W/85o HF, combined with neutral density shading nets). Tubers (200 per clone, each with a fresh weight [FW] of 50-150 mg) were randomly selected

from the stock and planted in plastic trays filled with washed aquarium sand, which were then distributed between the five temperature treatments. After one week, sprouted tubers (30 per clone x temperature combination) were transferred individually to plastic beakers (volume 150 cm^3) that had been filled with a mixture of potting clay and aquarium sand (1:3 by dry weight [DW]). Tubers were then covered with a layer of washed aquarium sand, before being returned to one of the two aquaria per treatment. Aquarium pH was adjusted weekly to 7.3 with H_2SO_4, and water levels were maintained by adding tap water as necessary.

Experimental design

After 21, 35 and 49 days in the beakers, 5 plants per clone and treatment were selected at random and carefully washed free of sediment. Dry weights of leaves, stems and root/rhizomes were determined and (at day 49 only) stem length (of the longest shoot), number of nodes (on the longest shoot) and the total number of shoots per plant were measured.

For three out of five clones (Kashin, Lemmer and Carucedo; see Fig. 1), we measured light-saturated net photosynthesis and dark respiration at a range of different water temperatures. Growth rates differed between treatments, and in order to have plants of comparable size, individuals were sampled on the day 35 for the 15°C treatment, on day 98 for the 10°C treatment and on day 21 for all other treatments. For accurate recording of oxygen-exchange rates, a total of 9 plants per clone and per treatment were needed and the harvested plants (used before fractionation and drying at 70°C for 48 h) were supplemented as necessary. A video image processing system (COMEF 2.1, OEG GmbH, Frankfurt a/d Oder, Germany) was used for leaf area determination on a sub-sample of 12 leaves of each plant used for oxygen-exchange measurements and of equivalent plants from the remaining clones (St. Petersburg and Stigsholm). The collected leaves were stored at –20°C until the end of the experiment, when chlorophyll a and b concentrations were measured according to Porra et al. (1989). Dry weight of the leaf sub-samples was estimated from a linear regression of FW on DW, determined using the remaining leaf material.

Oxygen-exchange measurements

Measurement techniques (see Fig. 2) were adapted from Santamaría & Hootsmans (1998). Measurements were performed simultaneously in three closed replicate systems (each consisting of a Perspex cuvette and an electrode chamber connected with PVC and Tygon® tubing to give a total volume of 422 ml) that were completely submerged in a set of two glass aquaria filled with tap water (96 l each). Each system included a peristaltic pump (Masterflex pump drive 7520-45, pump heads 7519-05, Cole Parmer Instrument Co., Vernon Hills, IL, USA) working at a flow rate of 750 ml min^{-1} and a Cellox 325 oxygen probe (WTW, Weilheim, Germany) from which values were recorded (every 10 s) with a micrologger (Campbell Scientific Ltd, Leicestershire, UK) connected to a personal computer. A SON-T AGRO lamp (Philips Nederland B.V., Eindhoven, The Netherlands) provided photosynthetically active radiation (PAR) of 500 µmol m^{-2} s^{-1}, which was sufficient to ensure irradiance-saturated photosynthesis in *P. pectinatus* (Hootsmans & Vermaat, 1994). Prior to the oxygen-exchange measurements a single addition of 20 g $NaHCO_3$ was made to each aquarium. The resulting dissolved inorganic carbon concentration (ΣDIC, 4.6 mM) was high enough to saturate net photosynthesis (Sand-Jensen, 1983) and the pH (7.7) indicated that 95% of the carbon was present as HCO_3^- and 5% as CO_2 (Prins & Elzenga, 1989).

Three intact plants were used in each system and gas-exchange was measured at approximately 6, 12, 19, 26, 33 and 40°C (actual temperatures were recorded by a sensor integrated in the oxygen probes). When photosynthetic rates had not decreased by 40°C, we performed an additional measurement at 42°C. At each temperature, starting with the lowest and ending with the highest, we first measured dark respiration (with the medium initially saturated with 6-11 mg l^{-1} oxygen depending on temperature) and then quantified net photosynthesis (N_2 was used to reduce the initial oxygen concentration to 3-4 mg l^{-1} thus suppressing photorespiration). Between measurements, the systems were opened and the medium inside was completely replenished with the surrounding water from the aquaria. All oxygen-exchange measurements were performed for 30–45 minutes, during which time the oxygen concentration increased or decreased by at least 0.5 mg l^{-1}.

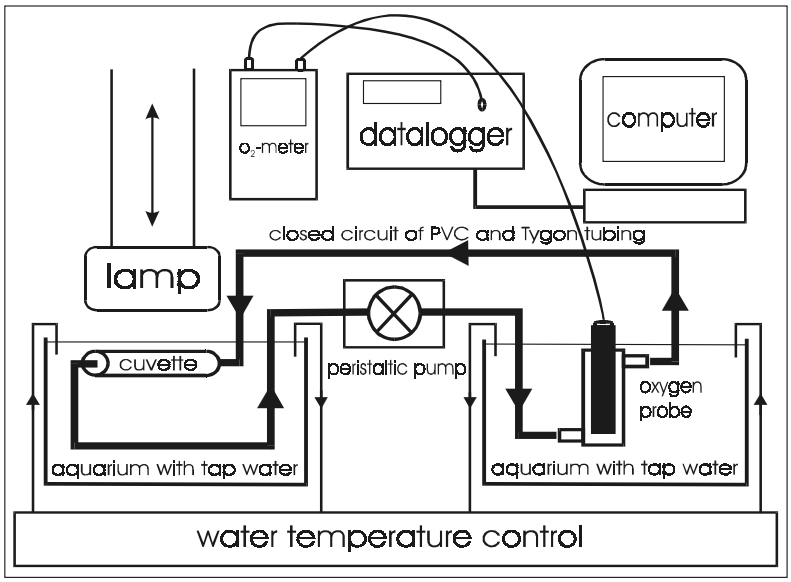


Figure 2. Schematic illustration of the experimental set-up used to measure oxygen-exchange in *P. pectinatus*.

Curve fitting and parameter estimation

Linear regression was used to calculate oxygen-exchange rates per unit time and biomass (initial lag-phases were excluded) and gross photosynthesis was assumed to be the sum of net photosynthesis and dark respiration (e.g. Sand-Jensen, 1997). Each series of measurements gave 6-7 datapairs, relating oxygen-exchange and incubation temperature, and these were used to describe the thermal response of photosynthesis (net and gross) and dark respiration.

The temperature response of both gross and net photosynthesis was fitted to equations derived from a conceptual model in which the proportions of an essential enzyme are temperature dependent (after Johnson et al., 1974; for its application to aquatic plant photosynthesis, see Santamaría & Van Vierssen, 1997):

$$Pm = A\frac{1}{1+\kappa}e^{-E_a/(RT)} \qquad (1)$$

where **Pm** is the rate of irradiance-saturated photosynthesis (in μg O_2 g^{-1}DW min^{-1}), **A** is an integration constant (in μg O_2 g^{-1}DW min^{-1}), $\mathbf{E_a}$ is the activation energy (in J), **R** is the gas constant (8.314 J mol^{-1} K^{-1}), **T** is the absolute temperature (i.e. °C + 273) and κ is the equilibrium constant of the reaction responsible for the conformational change, which depends on the absolute temperature according to the following expression:

$$\kappa = e^{-(\Delta H - T\Delta S)/(RT)} \quad (2)$$

where Δ**H** and Δ**S** are the increases in enthalpy (in J) and entropy (in J mol^{-1} K^{-1}). After parameter estimation (A, E_a, ΔH and ΔS) we calculated the highest rate of irradiance-saturated photosynthesis (**Pm at T_{opt}**) by maximising equation (1). We also used equation (1) to calculate the light-saturated rate of ambient photosynthesis (i.e. during incubation at the temperature the plants were grown; **Pm amb**). All parameters were calculated for both gross and net photosynthesis.

Each set of data points relating dark respiration to water temperature was fitted to a hyperbolic function, since exponential and linear regressions did not represent a better fit. In addition, we calculated the maximum fitted rate of dark respiration (**R_d max**) at the highest incubation temperature used in all measurements (i.e. 40°C) and the ambient rate of dark respiration (**R_d amb**) for each treatment (i.e. at the incubation temperature the plants were grown).

Statistical analysis

All statistical analyses was performed in STATISTICA (Statsoft Inc. 1999). Prior to the analysis, data were log-transformed if required to ensure normality and homogeneity of variances (Sokal & Rohlf, 1995). For all variables (including parameters related to the oxygen-exchange measurements), differences between the clones, the treatments and their interaction were analysed by means of two-way ANOVAs. Specific contrasts were explored by the use of Tukey a posteriori tests. Owing to the lack of plant growth at 10°C (Kashin only) and technical problems during photosynthesis measurements (Lemmer, grown at 30°C), some ANOVAs had missing treatment levels. Hence, we analysed clonal differences using only three or four out of five temperature levels.

Within each clone, the significance of the temperature effect was tested by means of one-way ANOVAs (not shown), followed by Tukey a posteriori comparisons. Occasionally, when homogeneity of variances did not improve after transformation of the data, we performed multiple pairwise comparisons, using separate t-tests with case-specific variance estimates. Comparisonwise error rates for each comparison were adjusted to maintain an experimentwise error rate (EER) of 0.05.

Results

Morphology and growth

At least 80% of the tubers sprouted for all clones and temperature treatments, except for that from Kashin at 10°C. This combination of clone and temperature was removed from the experiment because very few plants were produced. Clone, temperature and their interaction significantly affected all variables relating to morphology, leaf area production, biomass yield and allocation (Table 1).

Table 1. F-values and significance levels (*P < 0.05, **P < 0.01, ***P < 0.001) for factorial ANOVAs of the effect of the independent variables clone (C), temperature (T) and their interaction (C x T) on growth of *P. pectinatus*. Please note that only four temperature treatments (i.e. 15, 20, 25 and 30°C) were included in the ANOVAs.

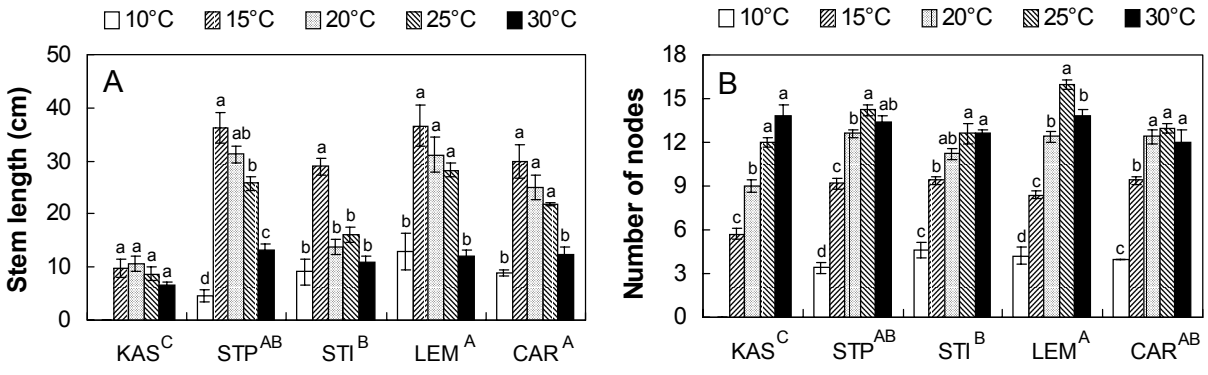
		df (error)	C	T	C x T
	df (effect)		4	3	12
Morphology	stem length	82	76.5 ***	64.1 ***	3.7 ***
	number of nodes	82	25.0 ***	152.7 ***	8.9 ***
	internode length	82	61.3 ***	215.8 ***	4.0 ***
	number of shoots	82	16.0 ***	61.7 ***	2.4 *
Leaf area	total	158	9.9 ***	87.3 ***	4.2 ***
	LAR	158	11.7 ***	123.8 ***	4.7 ***
	SLA	158	23.4 ***	152.1 ***	6.0 ***
Biomass allocation	LMR	357	65.5 ***	15.8 ***	6.2 ***
	SMR	357	162.6 ***	75.3 ***	6.2 ***
	BMR	357	31.1 ***	11.3 ***	6.3 ***
Biomass	total	82	4.3 **	21.9 ***	3.1 **

LAR: leaf area ratio, **SLA**: specific leaf area, **LMR**: leaf mass ratio, **SMR**: stem mass ratio, **BMR**: belowground mass ratio.

Shoot morphology

Stem length and mean internode length increased strongly between 10 and 15°C, and decreased again at higher growth temperatures (Fig. 3a and c), whereas the number of nodes per shoot and the total number of shoots per plant continued to increase up to at least 25°C (Fig. 3b and d).

Shoot morphology of the two lower-latitude clones (Lemmer and Carucedo) differed significantly in almost all cases from Kashin, while Stigsholm and St. Petersburg tended to show intermediate values (Fig. 3). Thus Kashin produced shorter shoots and internodes, fewer nodes per shoot and fewer shoots per plant and Lemmer and Carucedo had longer shoots and internodes and more nodes per shoot than the other clones (Fig. 3).



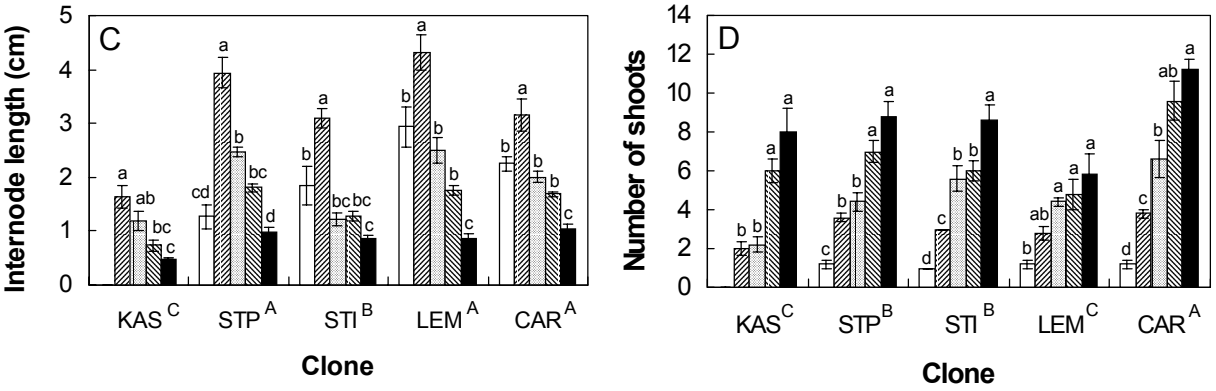


Figure 3. Shoot morphology for clones (abbreviations as in Fig. 1) of *P. pectinatus*, grown at a range of water temperatures. (**A**): Stem length, (**B**): Number of nodes, (**C**): Internode length, (**D**): Number of shoots. Values are means ± SE (n = 5), after 49 days growth. The effect of clone and temperature (for T: 15-30°C) was tested by two-way ANOVA. Different capital letters indicate significant differences between clones and different lower case letters significant treatment effects within clones (Tukey a posteriori tests, $P < 0.05$).

Leaf area

Not only the total leaf area per plant, but also the leaf area ratio (LAR; leaf area per unit plant mass) and the specific leaf area (SLA; leaf area per unit leaf mass) increased with increasing temperature (Fig. 4).

Total leaf area was similar for all clones except for Stigsholm, which had significantly lower values (Fig. 4a). Clonal differences in LAR and SLA were also small, although Kashin showed significantly higher values than most other clones (Fig. 4b and c).

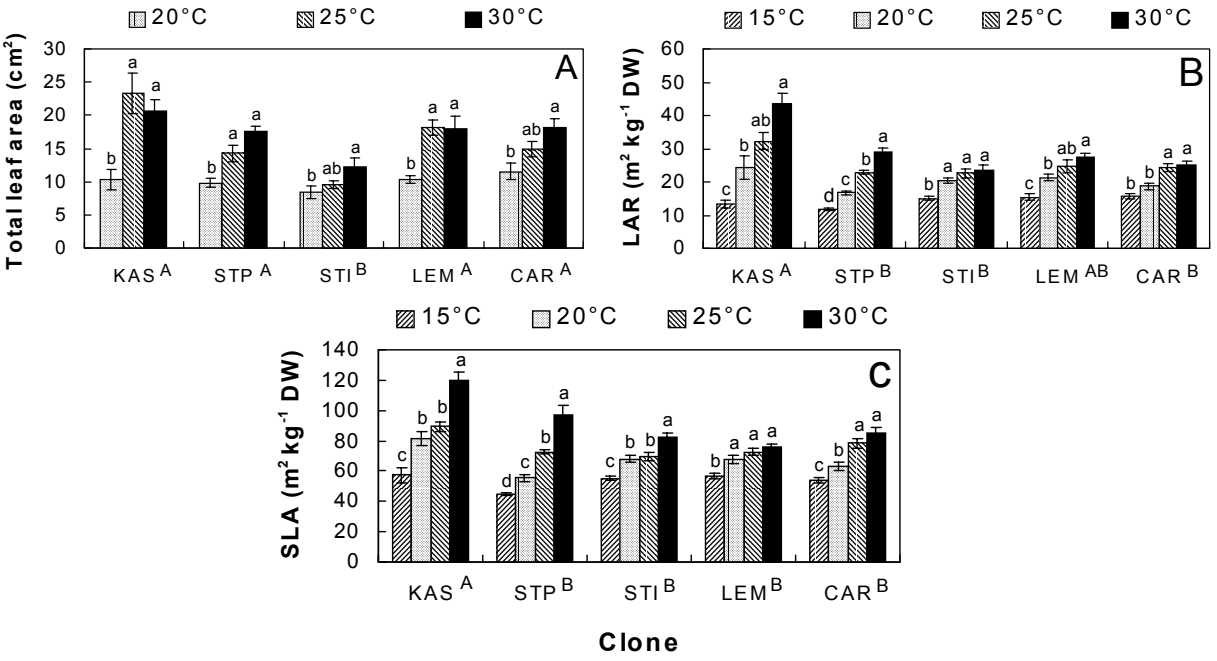


Figure 4. Effect of temperature on leaf area production in clones of *P. pectinatus*. (**A**): total leaf area, (**B**): leaf area ratio (LAR), (**C**): specific leaf area (SLA). Values are means ± SE (n=9), recorded after 21 or 35 (15°C treatment) days. Statistical conventions as in Fig. 3.

Biomass allocation

The stem mass ratio (SMR) of all clones decreased with increasing temperature, particularly between 10 and 15°C, although this was only significant for St. Petersburg and Lemmer (Fig. 5b). Leaf mass ratio (LMR) was largely independent of temperature, except for Kashin and Lemmer, which allocated more biomass to the leaves at higher temperatures (Fig. 5a). The opposite pattern of variation was shown in belowground mass ratio (BMR); Kashin and Lemmer did not vary their response, while the others showed an increase of BMR with temperature (Fig. 5c).

Since Kashin produced only a few short shoots (Fig. 3a and d), this clone invested a relatively lower percentage of its total biomass in stems (Fig. 5b) and the highest proportion in leaves (Fig. 5a). In contrast, the clone from the most southern locality (Carucedo) produced the highest percentage of belowground biomass, at the expense of both leaf and stem biomass (Fig. 5a, b and c).

Biomass yield

Irrespective of the origin of the clones, final biomass (i.e. total after 49 days) strongly increased between 10 and 15°C, and levelled off at higher growth temperatures (20-25°C, Fig. 5d). Although differences between clones were rather small, those from lower latitudes (Lemmer and Carucedo) had the highest productivity and Stigsholm the lowest (Fig. 5d).

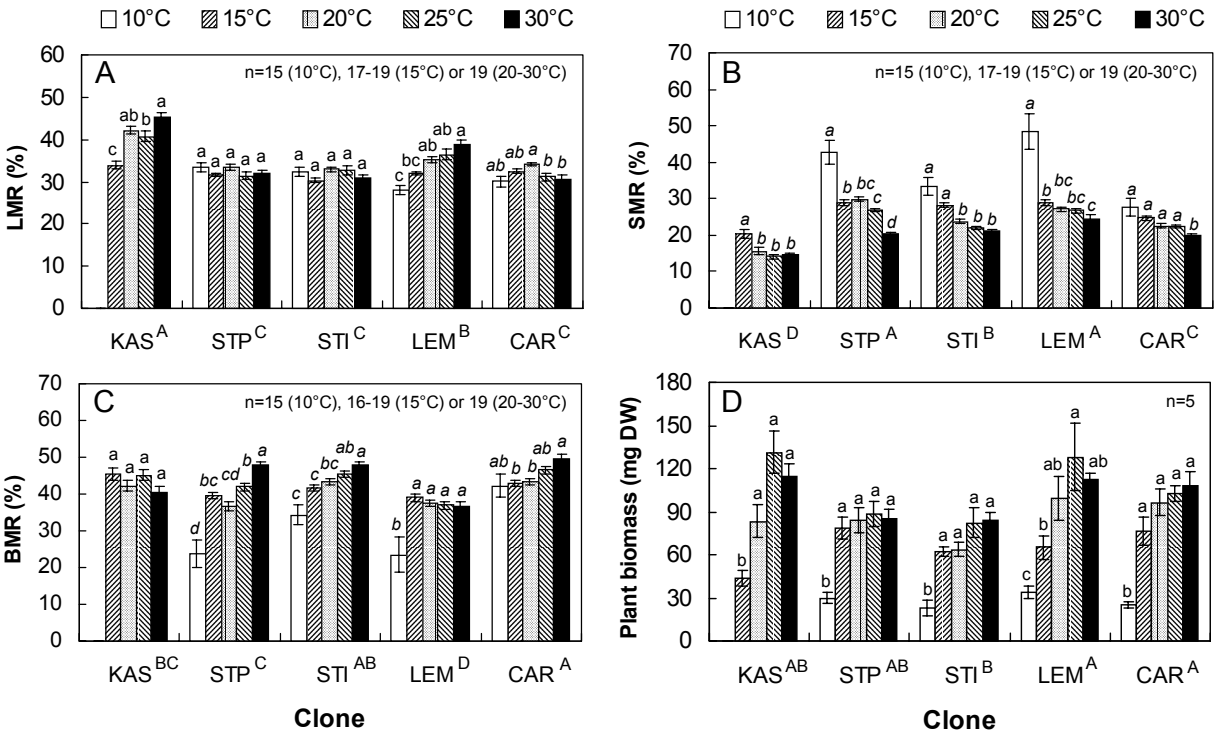


Figure 5. Proportional biomass allocation (averaged for the harvests between day 21 and 49) and final plant biomass (at day 49) for clones of *P. pectinatus*, grown at a range of different water temperatures. (**A**): leaf mass ratio (LMR), (**B**): stem mass ratio (SMR), (**C**): belowground mass ratio (BMR), (**D**): final plant biomass. Values are means ± SE. Statistical conventions as in Fig. 2, except where lower case letters are in italics (transformation of the data did not improve homogeneity of variances and multiple pairwise comparisons were made using separate t-tests with case-specific variance estimates, EER < 0.05).

Oxygen-exchange and chlorophyll measurements

Clone and temperature significantly affected all parameter estimates derived from the fitted temperature-response curves (i.e. photosynthesis and dark respiration; Table 2). While both factors also showed a significant influence on the total chlorophyll content, the chlorophyll a/b-ratio was only affected by temperature. A significant clone x temperature interaction for nearly all photosynthesis variables (except Pm amb for net photosynthesis), but not for dark respiration (Table 2), indicated that thermal responses varied among clones only for the former process.

Table 2. F-values and significance levels (NS: not significant, *P < 0.05, **P < 0.01, ***P < 0.001) for factorial ANOVAs of the effect of the independent variables clone (C), temperature (T) and their interaction (C x T) on oxygen-exchange and chlorophyll. Please note that only three temperature treatments (i.e. 15, 20 and 25°C) were included in the ANOVAs.

		df (error)	**C**	**T**	**C x T**
	df (effect)		2	2	4
Gross photosynthesis	T_{opt}	18	22.8 ***	24.4 ***	14.2 ***
	Pm at T_{opt}	18	58.3 ***	30.1 ***	7.8 ***
	Pm amb	18	17.3 ***	35.0 ***	2.9 *
Net photosynthesis	T_{opt}	18	6.94 **	39.4 ***	7.6 ***
	Pm at T_{opt}	18	158.2 ***	72.5 ***	7.4 *
	Pm amb	18	51.5 ***	45.7 ***	2.3 NS
Respiration	R_d max	18	5.7 *	7.6 **	1.4 NS
	R_d amb	18	4.5 *	62.1 ***	2.9 NS
Chlorophyll	a+b	18	7.2 **	4.5 *	3.6 *
	a/b	18	1.8 NS	11.5 ***	3.1 *

T_{opt}: optimal temperature, **Pm at T_{opt}**: maximum light-saturated photosynthetic rate, **Pm amb**: ambient rate of light-saturated photosynthesis, **R_d max**: dark respiration at 40°C, **R_d amb**: ambient rate of dark respiration, **a+b**: total chlorophyll concentration, **a/b**: chlorophyll a/b-ratio.

Photosynthesis

The temperature-response model, as proposed by Johnson et al. (1974), described the photosynthesis data with high accuracy (Fig. 6; R^2 always above 0.90). Although the expected optimum curve for the temperature dependence of photosynthesis was found in all cases, net photosynthesis showed a rather flat response (especially for Lemmer and Carucedo; Fig. 6). Both gross and net photosynthesis were maximal at high incubation temperatures (T_{opt} between 27.0 and 39.6°C; Table 3). Differences in T_{opt} between growth temperatures and clones were small and unpredictable, although Carucedo showed consistently lower values (Table 3).

The rate of irradiance-saturated photosynthesis (Pm at T_{opt}) for both gross and net photosynthesis tended to be highest in plants grown at 20°C (but significant only for Kashin and Lemmer). Interestingly, this temperature tended to differ from that which gave maximum biomass yield (Table 3, Fig. 5d). Kashin displayed the highest Pm at T_{opt} (for both gross and net photosynthesis), while the lowest value was found for the clone from Carucedo (Table 3).

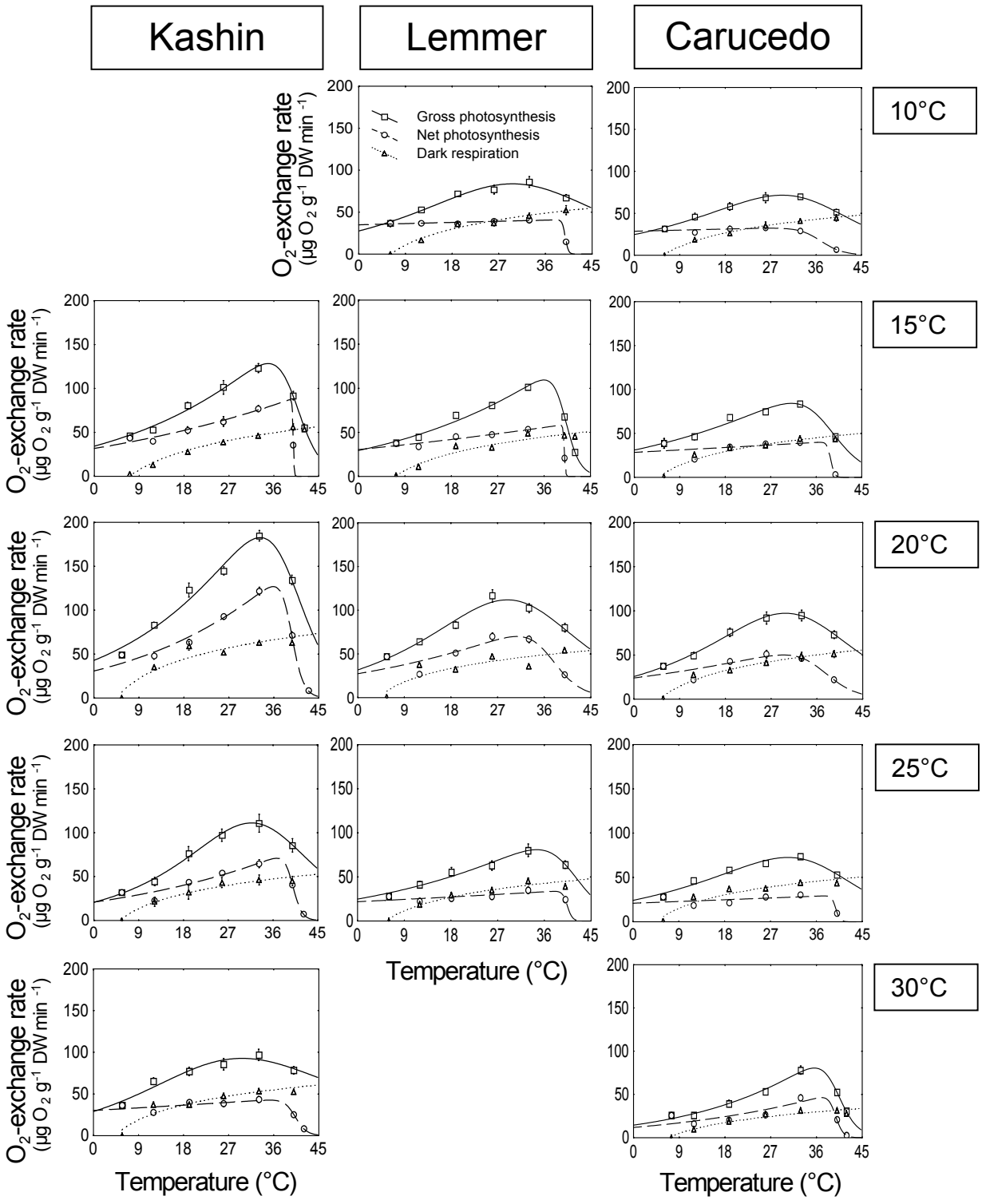


Figure 6. Effect of incubation temperature on the irradiance-saturated (gross and net) photosynthesis and dark respiration for three clones of *P. pectinatus*, grown at a range of different water temperatures (i.e. 10 to 30°C). Photosynthesis data were fitted to the equations proposed in Johnson et al. (1974; equation [1] and [2] in the text). Respiration values were fitted to a hyperbolic function. The curves shown here are based on mean oxygen-exchange rates (n=3) ± standard errors, while parameter estimates used for statistical analysis (see Table 3 and 4) were derived from each replicate curve separately (not shown).

The ambient rate of gross photosynthesis (Pm amb) tended to decrease at low temperatures (especially for Kashin and Carucedo; Table 3), but did not vary significantly between 20 and 30°C. For net photosynthesis, higher rates of ambient oxygen production were recorded at 20°C (especially for Kashin and Lemmer), but values at all other growth temperatures were similar (Table 3). Kashin had the highest Pm amb values for both gross and net photosynthesis (Table 3).

Table 3. Photosynthesis parameters (gross and net) for three clones of *P. pectinatus*, grown at different water temperatures. Values are means ± SE (n=3). The effect of clone and temperature (for T: 15-25°C) was tested by two-way ANOVA. Significant differences between the clones are indicated with different capital letters (Tukey a posteriori test, $P < 0.05$). Significant treatment differences within clones are indicated with lower case letters (one-way ANOVA, Tukey a posteriori test, $P < 0.05$) or lower case letters in italics (transformation of the data did not improve homogeneity of variances and multiple pairwise comparisons were made using separate t-tests with case-specific variance estimates, EER < 0.05).

Clone	T_{gr}	Gross photosynthesis			Net photosynthesis		
		T_{opt}	Pm at T_{opt} (µg O_2 g^{-1}DW min^{-1})	Pm amb (µg O_2 g^{-1}DW min^{-1})	T_{opt}	Pm at T_{opt} (µg O_2 g^{-1}DW min^{-1})	Pm amb (µg O_2 g^{-1}DW min^{-1})
	(°C)	(°C)			(°C)		
Kashin	10	A –	A –	A –	A –	A –	A –
	15	34.8 ± 0.3^{a}	127.9 ± 5.8^{b}	65.1 ± 3.7^{b}	38.2 ± 0.6^{a}	84.9 ± 6.2^{b}	48.3 ± 3.1^{b}
	20	33.3 ± 0.4^{ab}	183.9 ± 5.9^{a}	117.7 ± 5.3^{a}	35.9 ± 0.4^{a}	127.6 ± 2.3^{a}	72.9 ± 3.8^{a}
	25	31.7 ± 0.3^{b}	111.2 ± 9.4^{bc}	96.5 ± 7.9^{a}	36.7 ± 0.4^{a}	71.4 ± 5.4^{b}	50.7 ± 1.9^{b}
	30	30.2 ± 0.1^{b}	95.2 ± 6.4^{c}	95.2 ± 6.4^{a}	35.2 ± 0.3^{a}	42.7 ± 3.7^{c}	41.1 ± 3.6^{b}
Lemmer	10	A 29.4 ± 2.2^{ab}	B 84.6 ± 6.8^{bc}	B 47.5 ± 2.4^{c}	A 34.5 ± 3.3^{abc}	B 40.5 ± 2.2^{b}	B 36.4 ± 2.8^{b}
	15	36.0 ± 0.1^{a}	109.5 ± 1.7^{ab}	54.1 ± 1.2^{bc}	39.6 ± 0.2^{a}	58.7 ± 2.9^{a}	40.0 ± 0.9^{b}
	20	28.9 ± 0.1^{b}	112.3 ± 6.1^{a}	93.0 ± 4.8^{a}	30.8 ± 0.2^{c}	70.3 ± 4.3^{a}	54.5 ± 3.3^{a}
	25	35.1 ± 0.6^{ab}	82.4 ± 6.0^{c}	64.2 ± 6.1^{b}	37.6 ± 0.0^{b}	33.7 ± 3.5^{b}	29.7 ± 3.1^{b}
	30	–	–	–	–	–	–
Carucedo	10	B 29.6 ± 1.2^{b}	C 72.1 ± 4.0^{a}	B 40.2 ± 3.4^{b}	B 27.0 ± 1.6^{c}	C 32.6 ± 2.0^{b}	C 30.2 ± 2.6^{bc}
	15	31.4 ± 1.3^{ab}	84.8 ± 2.3^{a}	54.4 ± 2.8^{ab}	35.2 ± 2.4^{ab}	39.2 ± 1.1^{ab}	32.7 ± 2.6^{abc}
	20	29.9 ± 0.3^{b}	97.2 ± 6.8^{a}	77.1 ± 5.6^{a}	29.7 ± 0.8^{bc}	51.3 ± 4.5^{a}	43.2 ± 4.1^{a}
	25	30.3 ± 0.7^{b}	81.8 ± 8.4^{a}	76.6 ± 8.8^{a}	38.3 ± 0.1^{a}	29.5 ± 1.9^{b}	26.5 ± 1.3^{c}
	30	35.6 ± 0.2^{a}	80.8 ± 6.0^{a}	67.8 ± 4.2^{a}	37.6 ± 1.2^{a}	48.0 ± 5.6^{a}	37.5 ± 2.2^{ab}

T_{gr}: growth temperature, **T_{opt}**: optimal temperature, **Pm at T_{opt}**: maximum light-saturated photosynthetic rate, **Pm amb**: ambient rate of light-saturated photosynthesis (i.e. for corresponding combinations of growth and incubation temperature), –: no data available.

Dark respiration

Dark respiration had not begun to fall, even by the highest incubation temperatures (40 or 42°C) and therefore we regarded the fitted rate of dark respiration at 40°C (R_d max) as a maximum. Growth temperature had a relatively small effect on R_d max and Kashin had the highest values of the three clones (Table 4).

Ambient rates of dark respiration (R_d amb) showed an increase between 10 and 20°C, before levelling off (Table 4). The highest R_d amb value was found for the clone from Kashin (Table 4).

Table 4. Dark respiration parameters for three clones of *P. pectinatus*, grown at different water temperatures. Values are means ± SE (n=3). The effect of clone and temperature (for T: 15-25°C) was tested by two-way ANOVA. Significant differences between the clones are indicated with different capital letters in the same typeface (Tukey a posteriori test, $P < 0.05$). Significant treatment differences within clones are indicated with lower case letters (one-way ANOVA, Tukey a posteriori test, $P < 0.05$)

T_{growth} (°C)	Dark respiration					
	R_d max ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)	**R_d amb** ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)	**R_d max** ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)	**R_d amb** ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)	**R_d max** ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)	**R_d amb** ($\mu g\ O_2\ g^{-1}DW\ min^{-1}$)
	Kashin		**Lemmer**		**Carucedo**	
10	***A*** –	A –	***B*** 51.7 ± 4.2^{a}	AB 13.5 ± 0.2^{c}	***B*** 45.2 ± 3.6^{a}	B 12.7 ± 1.1^{d}
15	52.8 ± 2.9^{ab}	22.8 ± 0.9^{b}	47.8 ± 0.7^{a}	20.9 ± 0.2^{b}	47.3 ± 2.2^{a}	25.3 ± 1.0^{c}
20	69.7 ± 2.0^{a}	47.5 ± 1.3^{a}	51.3 ± 2.2^{a}	35.8 ± 1.5^{a}	52.9 ± 4.1^{a}	35.0 ± 2.7^{ab}
25	49.8 ± 6.8^{b}	38.3 ± 5.6^{a}	45.2 ± 3.3^{a}	34.5 ± 2.4^{a}	48.0 ± 0.5^{a}	37.6 ± 0.3^{a}
30	57.7 ± 3.6^{ab}	50.2 ± 3.2^{a}	–	–	31.8 ± 1.9^{b}	26.6 ± 1.6^{bc}

R_d max: fitted rate of dark respiration at 40°C. **R_d amb**: ambient rate of dark respiration (i.e. for corresponding combinations of growth and incubation temperature), –: no data available.

Chlorophyll

No systematic trends could be detected in the effect of temperature on either total chlorophyll concentration or the chlorophyll a/b ratio (Table 5). Clonal differences existed for the total chlorophyll concentration (lowest values for Carucedo), while the chlorophyll a/b ratios did not vary significantly among clones (Table 5).

Table 5. Total chlorophyll concentration (a+b) and chlorophyll a/b-ratio (a/b), for three clones of *P. pectinatus* grown at different water temperatures. The effect of clone and temperature (for T: 15 to 25°C) was tested by two-way ANOVA. Significant differences between the clones are indicated with different capital letters in the same typeface (Tukey a posteriori test, $P < 0.05$). Significant treatment differences within clones are indicated with lower case letters (one-way ANOVA, Tukey a posteriori test, $P < 0.05$) or lower case letters in italics (transformation of the data did not improve homogeneity of variances and multiple pairwise comparisons were made using separate t-tests with case-specific variance estimates, EER < 0.05). –: no data available.

T_{growth} (°C)	Chlorophyll					
	a+b ($\mu g\ g^{-1}$ FW)	**a/b** ($\mu g\ \mu g^{-1}$)	**a+b** ($\mu g\ g^{-1}$ FW)	**a/b** ($\mu g\ \mu g^{-1}$)	**a+b** ($\mu g\ g^{-1}$ FW)	**a/b** ($\mu g\ \mu g^{-1}$)
	Kashin		**Lemmer**		**Carucedo**	
10	***AB*** –	A –	***A*** 538 ± 24^{b}	A 2.61 ± 0.09^{ab}	***B*** 403 ± 35^{c}	A 2.77 ± 0.06^{b}
15	726 ± 94^{a}	2.67 ± 0.15^{a}	800 ± 42^{a}	2.97 ± 0.12^{a}	595 ± 47^{ab}	3.48 ± 0.18^{a}
20	638 ± 16^{a}	3.15 ± 0.16^{a}	807 ± 12^{a}	2.91 ± 0.13^{ab}	739 ± 19^{a}	2.93 ± 0.14^{ab}
25	706 ± 43^{a}	2.63 ± 0.32^{a}	653 ± 18^{b}	2.25 ± 0.07^{b}	526 ± 29^{bc}	2.49 ± 0.11^{b}
30	563 ± 80^{a}	2.47 ± 0.09^{a}	–	–	711 ± 39^{a}	2.46 ± 0.22^{b}

Discussion

Photosynthesis and respiration

Photosynthetic acclimation to higher or lower water temperatures is frequently accomplished by a shift in optimal temperature, which results in comparable rates of photosynthesis at incubation temperatures that equal the particular growth temperatures (e.g. Berry & Björkman, 1980). However, optimal temperatures for neither net nor gross photosynthesis varied in correspondence with the growth temperature for any of the investigated clones of *P. pectinatus* (i.e. there was no acclimative shift in T_{opt}; Table 3). In addition, the clones showed the highest maximum photosynthetic capacity (Pm at T_{opt}) when grown at 20°C, and an overall depression of the photosynthetic capacity at all other growth temperatures (Table 3). Although this might indicate that *P. pectinatus* lacks the ability to photosynthetically acclimate to a wide range of temperature (Berry & Björkman, 1980), differences in photosynthetic capacity (both gross and net) at corresponding growth and incubation temperatures (i.e. Pm amb), were less marked (Table 3). Especially for the clones from Kashin and Carucedo, this resulted in some capacity for acclimation to higher water temperatures, since the recorded Pm values for ambient gross photosynthesis were not significantly different at 20 and 30°C (Table 3). We did not find the expected lower thermal optima for the higher-latitude clones (Table 3), although the high photosynthetic capacities (both Pm at T_{opt} and Pm amb) for Kashin (Table 3) might be of adaptive value, sustaining more rapid growth where the growing season is short (Eagles, 1967).

For terrestrial plant species it has been suggested that respiratory acclimation to higher growth temperatures can be accomplished by a downward shift of the thermal response curve (e.g. Laurigauderie & Körner, 1995). When growth and incubation temperatures correspond, full or partial acclimation should then lead to comparable or slightly increasing rates of dark respiration (e.g. reduced variation in R_d amb). However, in all three clones of *P. pectinatus*, R_d amb strongly increased between 10 and 20°C, but levelled off or even decreased at higher water temperatures (Table 4), suggesting that there is some degree of acclimation of respiratory processes to higher temperatures (as in e.g. Tranquillini et al., 1986; Larigauderie & Körner, 1995). Highest maximum respiration rates (R_d max) were found for the high-latitude clone from Kashin (Table 4), similar to the response of terrestrial plants, where ecotypes native to cooler environments often respire faster (Billings et al., 1971; Lechowicz et al, 1980). These differences may be due to the presence of higher concentrations of mitochondria that can sustain the higher costs for maintenance or promote a more rapid growth at lower temperatures (Miroslavov & Kravkina, 1991).

Morphology, biomass allocation and productivity

For all clones except Kashin, stem length decreased at temperatures above 15°C (Fig. 3a). This finding contrasts with the results obtained for several other aquatic macrophytes, which showed an increase in length of the main shoot with temperature (Barko & Smart, 1981; Barko et al., 1982). We suggest, however, that the low thermal optimum could be of adaptive value to *P. pectinatus* in early spring, when elongation of emerging shoots towards the water surface is stimulated by low light conditions, and may benefit from cooler temperatures. Both stem and internode length decreased at temperatures above 15°C (Fig. 3a and c), while the number of nodes increased up to a plateau at 20 to 25°C (Fig. 3b). We therefore conclude that differences in the stem length were mediated by

differences in the internode length, rather than by number of nodes produced (similar to Barko et al., 1982; Bulder et al., 1987). The reduction in stem length at higher water temperatures was also correlated with an increased number of shoots per plant (Fig. 3a and d), suggesting that all clones produced a higher number of shorter shoots.

Variation in the proportional allocation of dry matter is normally associated with the maintenance of an appropriate balance between carbon assimilation and nutrient uptake (Berry & Raison, 1981). Leaf area ratio (LAR) increased with increasing water temperature in all clones, but the relative contribution of specific leaf area (SLA) and leaf mass ratio (LMR) to these changes differed (Fig. 4b and c, Fig. 5a). The increase of LAR probably minimised the reduction in photosynthetic capacity (per unit plant mass) at temperatures above 20°C. Clones also differed in the variation of proportional allocation of biomass with temperature. All clones invested less biomass in stem tissue at higher water temperatures (<SMR; Fig. 5b), but in Kashin and Lemmer, this was balanced by a higher proportion of dry mass in leaves (>LMR; Fig. 5a), rather than in roots and rhizomes, as seen in other clones (>BMR; Fig. 5c). The latter is surprising, since diffusion is generally enhanced at higher temperatures and consequently plants may need less root biomass to fulfil nutrient requirements (Berry & Raison, 1981). Irrespective of water temperature, the clone from Kashin showed a clearly distinct pattern of biomass allocation: high LMR (and high SLA) resulted in a high LAR (Fig. 4b and c, Fig. 5a), while the low SMR probably resulted from the production of only a limited number of very short shoots (Fig. 3a and d, Fig. 5b).

We consider that the total accumulated plant biomass at the end of the experimental period can be used as a fitness surrogate that shows the extent to which *P. pectinatus* can cope with variation in water temperature. Plant biomass was not significantly different for a relatively wide range of growth temperatures (20-30°C for Kashin and Lemmer; 15-30°C for St. Petersburg, Stigsholm and Carucedo; Fig. 5d), probably reflecting some capacity in all clones for physiological and morphological acclimation to higher water temperatures. We therefore conclude that *P. pectinatus* is thermally tolerant.

Conclusions

The overall thermal response with respect to morphology, growth and gas-exchange varied little between clones of *P. pectinatus*. We found some degree of photosynthetic and respiratory acclimation to higher water temperatures, while morphological acclimation (increase of LAR and SLA) contributed to comparable rates of ambient gross photosynthesis at temperatures above 20°C. The interplay of the plastic responses in both physiological and morphological plant characteristics resulted for all clones in a comparable biomass yield over a relatively wide range of water temperatures. We therefore confirmed that *P. pectinatus* is thermally tolerant rather than locally adapted. Nevertheless, a clone obtained from the northern distributional limit of *P. pectinatus* (Kashin) was similar to other range-margin populations in differing in many ways from the centre of the species distribution, perhaps due to genetic drift, inbreeding or strong selection (Woodward, 1988; Jonas & Geber, 1999).

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CHAPTER 5

Clonal variation in morphological and physiological responses to irradiance and photoperiod for the aquatic angiosperm *Potamogeton pectinatus* L.

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Summary

Cosmopolitan plants are exposed to contrasting gradients in irradiance and photoperiod across latitude and may cope with these differences by local specialisation and/or phenotypic plasticity. We investigated the relative contribution of both mechanisms to variation in plant growth for three clones of the aquatic angiosperm *Potamogeton pectinatus* L., originating from 42.5 to 68°N. Plants were grown at a factorial combination of two irradiances (50 and 350 µmol m^{-2} s^{-1}) and three photoperiods (13, 16 and 22 h) and morphology, gas-exchange rate and biomass accumulation were recorded.

The overall response to variation in irradiance and photoperiod was similar for all three clones. Differences in irradiance resulted in strong acclimative changes in morphological and physiological plant characteristics. At low irradiance, pronounced vertical shoot extension compensated for the limited plasticity in leaf area production. In addition, photosynthetic capacity, apparent quantum yield and total chlorophyll concentration increased at low growth irradiance. As a result, biomass yield at the end of the experimental period was largely comparable for both irradiance treatments.

Similar to the effect of low irradiance, a decrease in photoperiod resulted in plastic changes in morphology (increase of leaf biomass per unit plant biomass) and physiology (increase of photosynthetic capacity) that can be interpreted as an acclimative response to a reduction in the daily irradiance. However, the acclimative responses did not fully compensate for differences in photoperiod, since at 13 and 16 h photoperiod biomass was significantly lower than at 22 h. In short, we showed that *P. pectinatus* is phenotypically plastic and not locally specialised to differences in irradiance and photoperiod.

Introduction

Irradiance and photoperiod are different aspects of the light climate that have a large impact on many aspects of plant life (Björkman, 1981; Salisbury, 1981). The genetically fixed and phenotypically plastic

responses to variation in instantaneous irradiance are described by the well-established concept of 'sun' and 'shade' strategies. While 'sun' plants show physiological and developmental characteristics that promote growth at high irradiance, 'shade' plants minimise the detrimental effect of low light by exhibiting features that increase light capture and photosynthetic efficiency (Boardman, 1977; Björkman, 1981). Besides these responses that relate to the instantaneous quantum flux, terrestrial angiosperms are known to be sensitive to changes in the daily-integrated level of irradiance. This aspect of the light climate, which depends on the interplay of both irradiance and photoperiod, may also affect various morphological and physiological plant processes (Lambers et al., 1998). For example, in several grass species a lower daily irradiance due to shortening of the photoperiod resulted in an increase in photosynthetic capacity (Burian & Winter, 1976; Ryle, 1966). Independent of its influence on daily irradiance, photoperiod also controls vegetative growth processes (Hay, 1990) and serves as an environmental cue for life-cycle events that require a precise seasonal timing (e.g. induction of flowering; Salisbury, 1981; Thomas & Vince-Prue, 1981).

Aquatic macrophytes show light-response patterns that are largely comparable to those of terrestrial species (Spence, 1974; Jeffrey, 1981). Nevertheless, the aquatic environment can modify some aspects of the light climate, which has an impact on plant performance. While an increase in water depth hardly affects the length of the photoperiod, the instantaneous irradiance level can be strongly reduced, especially under turbid conditions (Van Vierssen & Hootsmans, 1994). In comparison with terrestrial species, submerged aquatic macrophytes have been categorised as obligate 'shade' plants. In accordance with this, they normally show low light-compensation points and low photosynthetic rates (Bowes, 1987; Bowes & Salvucci, 1989). Surprisingly, these characteristics also apply to aquatic macrophytes that inhabit shallow surface waters and potentially receive full sunlight (Bowes & Salvucci, 1989). Moreover, freshwater angiosperms have a varying capacity to acclimate to the lower irradiances of their natural environment. Since many aquatic plants show a high degree of plasticity in morphological traits, strong photomorphogenic effects may reduce the need for acclimative changes in physiological processes like photosynthesis. Besides acclimative responses to the instantaneous level of irradiance, aquatic macrophytes can react to variation in daily irradiance, e.g. by increasing the photosynthetic rate in response to a decrease in this abiotic factor (Santamaría & Van Vierssen, 1995). However, closely related species may show contrasting responses to variation in daily irradiance (Koch & Dawes, 1991), which illustrates that for aquatic plants the available information on this aspect of the light climate is far from unequivocal.

Existing studies on the responses of aquatic macrophytes to variation in the light climate most often dealt with irradiance and photoperiod as separate factors. This is surprising because most aquatic macrophytes are widely dispersed (Sculthorpe, 1967) that experience contrasting latitudinal gradients in both aspects of the light climate. Although specific habitat characteristics may modify the light climate to some extent, plants growing in higher latitudes are generally exposed to lower irradiances and longer photoperiods than their southern counterparts (Kirk, 1994). Therefore one might expect broadly distributed aquatic angiosperms to show ecotypic differentiation across latitude (i.e. contrasting physiological and/or morphological responses to changes in irradiance and photoperiod). Alternatively, strong phenotypic plasticity may result in high tolerance to both spatial and temporal variation in irradiance and photoperiod.

Here we focus on the submerged aquatic angiosperm fennel pondweed (*Potamogeton pectinatus* L.), which shows a pseudo-annual life cycle and predominantly reproduces by means of subterranean tubers (Van Wijk, 1988; Van Wijk, 1989). *P. pectinatus* has a cosmopolitan distribution and in the northern hemisphere the species occurs from tropical to sub-arctic regions (Casper & Krausch, 1980;

Wiegleb & Kaplan, 1998). Three clones of *P. pectinatus* from largely different latitudes were selected to characterise clonal variation in morphology, growth and photosynthesis in response to differences in irradiance and photoperiod. More specifically we considered the following research questions: (1A) Does *P. pectinatus* show ecotypic differentiation based on contrasting responses to photoperiod and irradiance, that allows individual clones to perform best at the prevailing light conditions of their natural habitat? (1B) Or does *P. pectinatus* show a high acclimative potential that allows clones from all sites to perform well at different combinations of irradiance and photoperiod? (2) What is the relative contribution of morphological and physiological plant traits to acclimative responses to irradiance and photoperiod?

Material and methods

Origin, propagation and storage of plant material

Three clonal lines of the aquatic angiosperm *P. pectinatus* were selected to represent different climatic regions. Each line originated from a single tuber, collected at the following localities that greatly differed in latitude: Kashin Island (KAS, Korovinskaya Bay [68.24°N, 53.88°E], Russia), Babbelaar (BAB, Lake Lauwersmeer [53.34°N, 6.22°E], The Netherlands) and Lake Carucedo (CAR [42.48°N, 6.77°E], Spain). Hereafter, countries of origin and the acronyms will refer to the clones.

To produce enough tubers for the experiment, plants were propagated at the Centre for Terrestrial Ecology (Heteren [51.92°N, 5.85°E], The Netherlands), while during the European winter season they were grown at the Plant Protection Research Institute (Pretoria [25.75°S, 28.20°E], South Africa). Individual clones were raised outdoors in polyethylene tanks (1m^3), supplemented with tap water over a mixture of sand and potting clay (3:1 by dry mass [DM]). Following collection from the tanks, all tubers were surface-sterilised with 5% sodium hypochlorite to remove harmful micro-organisms (Madsen, 1985), before storage in darkness at 4°C (i.e. conditions that prevent unplanned sprouting).

Growth conditions

For all clones, tuber sprouting and subsequent plant growth was performed at a factorial combination of two irradiances (50 and 350 µmol m^{-2} s^{-1}) and three photoperiods (13, 16 and 22 h). Plants were grown in glass aquaria (30x40x40 cm) filled with tap water (96 l), whose temperature was maintained at 20°C by separate heating and cooling units. The aquaria (two per treatment) were distributed among three phytotrons, in which the photosynthetically active radiation (PAR) was provided by 24 fluorescent light tubes (TLD 50W/85o HF, Philips Nederland B.V., Eindhoven, The Netherlands). PAR was measured at 2 cm below the water surface with an under water quantum sensor (LI-192SA, LICOR, Lincoln, NE, USA). The low irradiance level (50 µmol m^{-2} s^{-1}) was achieved by covering the aquaria with neutral density shading nets. Per clone, 180 tubers of a standardised size (50-150 mg fresh mass [FM]) were randomly assigned to the treatments (e.g. 30 tubers per treatment). Each sub-set of tubers was pre-sprouted in a plastic tray filled with washed aquarium sand and all trays were randomly distributed over the two aquaria per treatment. After one week, sprouted tubers (24 per clone per treatment) were selected and transferred individually to plastic beakers (volume: 150 cm^3), filled with a mixture of potting clay and aquarium sand (1:3 by DM) and covered with a 2 cm layer of aquarium sand. The beakers were randomly distributed over the two aquaria per treatment. Whenever

necessary, the aquaria were replenished with tap water and the pH was adjusted weekly to 7.3 with small volumes of 3 M sulphuric acid.

Experimental design

On day 14, 42 and 56 after the tubers were set to sprout, 5 randomly selected plants (per clone and per treatment) were washed free of sediment and divided into separate fractions (leaves, stems, roots/rhizomes). For plants harvested at day 56, the stem length (of the longest shoot) and the number of nodes (of the longest shoot) were recorded prior to biomass determination (after drying for 48 h at 70°C).

On day 28, an additional 9 plants (per clone and per treatment) were harvested for oxygen-exchange measurements and leaf area determination (prior to DM estimation of the separated plant fractions, as before). To calculate the total leaf area per plant, a video image processing system (COMEF 2.1, OEG GmbH. Frankfurt a/d Oder, Germany) was used to measure leaf area on a leaf sub-sample of 100-180 mg FM. The collected sub-samples were stored at –20°C and used to determine chlorophyll a and b concentrations (according to Porra et al., 1989) within four weeks after sampling. To estimate the DM of the collected leaf sub-sample, a FM to DM linear regression based on the remaining leaf material was used.

Oxygen-exchange measurements

Measurements were performed simultaneously in three closed replicate systems (each consisting of a Perspex cuvette and an electrode chamber connected with PVC and Tygon® tubing to give a total volume of 422 ml) that were completely submerged in a set of two glass aquaria (30x40x40 cm) filled with tap water (96 l each). Each system included a peristaltic pump (Masterflex pump drive 7520-45, pump heads 7519-05, Cole Parmer Instrument Co., Vernon Hills, IL, USA) working at a flow rate of 750 ml min^{-1} and readings from a Cellox 325 oxygen probe (WTW, Weilheim, Germany) were recorded (every 10 s) with a micrologger (Campbell Scientific Ltd, Leicestershire, UK) connected to a personal computer. Photosynthetically active radiation (PAR) was provided by a SON-T AGRO lamp (Philips Nederland B.V., Eindhoven, The Netherlands) that was mounted above one of the aquaria. Different irradiance levels were created by varying the distance between the lamp and the water surface and by using neutral density shading nets with various mesh-sizes. PAR was measured with an underwater quantum sensor (type and brand, as before) at three points along the inside of each cuvette. We thereby aimed at the following mean photon flux densities: 25, 50, 75, 100, 150, 200, 300, 400 and 500 µmol m^{-2} s^{-1}.

In each of the three cuvettes, we inserted three intact plants that were in apparent good condition. Prior to the oxygen-exchange measurements, a single addition of 20 g bicarbonate was made to the water of each aquarium. This produced a dissolved inorganic carbon concentration (ΣDIC: 4.6 mM) that was high enough to saturate photosynthesis of *P. pectinatus* (Sand-Jensen, 1983). After the addition of bicarbonate, the pH of the water was 7.7, corresponding with a relative distribution of 95% HCO_3^- and 5% CO_2 (Prins & Elzenga, 1989). Measurements of dark respiration were started at an initial oxygen concentration of 9-10 mg l^{-1}, after covering the aquaria with black plastic foil. Subsequently, the dissolved oxygen concentration in the water was reduced to 3-4 mg l^{-1} (by bubbling with nitrogen gas) and photosynthesis was measured at increasing light intensities. All oxygen-exchange measurements were performed during 30 to 45 minutes, until the dissolved oxygen

concentration had increased or decreased with at least 0.5 mg l^{-1}. Between measurements, the cuvettes were opened and the medium inside the oxygen-exchange systems was completely replenished with surrounding water from the aquaria.

Curve fitting and parameter estimation

Oxygen-exchange rates per unit time and biomass were calculated by linear regression (initial lag-phases were excluded). For the description of light-response curves in aquatic systems, several models have been proposed (e.g. Jassby & Platt, 1976; Iwakuma & Yasuno, 1983; Cossby et al., 1984). On the basis of reviewed literature, Hootsmans & Vermaat (1994) concluded that most models are equally good and no objective criterion can be found to reject the relatively simple rectangular hyperbola. We therefore fitted our biomass-related oxygen-exchange rates to the following equation describing a rectangular hyperbola, in which an extra term accounting for dark respiration was included (e.g. Santamaría et al., 1994):

$$P = \frac{Pm \times I}{K_{0.5} + I} - R_d$$

where P is the rate of net photosynthesis (in µg O_2 g^{-1} DM min^{-1}), Pm is the irradiance-saturated rate of gross photosynthesis (in µg O_2 g^{-1} DM min^{-1}), I is the independent variable irradiance (in µmol m^{-2} s^{-1}), $K_{0.5}$ is the half-saturation constant (in µmol m^{-2} s^{-1}) and R_d is the rate of dark respiration (in µg O_2 g^{-1} DM min^{-1}). The apparent quantum yield α (in µg O_2 m^2 s µmol^{-1} g^{-1} DM min^{-1}) was calculated as Pm divided by $K_{0.5}$ (e.g. Hootsmans & Vermaat, 1994).

Statistical analysis

All data were statistically analysed using STATISTICA (Statsoft Inc. 1999). Prior to the analyses, data were log-transformed whenever necessary to ensure residuals normality and homogeneity of variances (Sokal & Rohlf, 1995). For all variables (including parameters related to oxygen-exchange measurements) differences between the clones, treatments (irradiance and photoperiod) and their interactions were tested by means of three-way ANOVAs. Analysis of variance was followed by a chosen subset of post-hoc comparisons (using Tukey a posteriori tests), which included contrasts between the investigated clones, and contrasts between all combinations of irradiance and photoperiod within clones.

Results

Morphology and biomass allocation

Both irradiance and photoperiod had a significant effect on stem length, internode length and the number of nodes (Table 1). Growth at low irradiance or increasing photoperiod resulted in longer stems for all clones (Fig. 1A). This was primarily due to an increase of the mean internode length (data not shown), but also to a small increase of the number of nodes per shoot (data not shown). The

Dutch clone (BAB) produced longest shoots and the Russian one (KAS) the shortest, while the Spanish clone (CAR) showed intermediate shoot lengths (significant clone effect; Table 1, Fig. 1A).

Table 1. Three-way ANOVAs on plant parameters related to shoot and leaf morphology, with clone (C), irradiance (I) and photoperiod (P) as main effects. For all main effects and their interactions, the degrees of freedom, variance ratios and significance levels (NS: not significant, *P < 0.05, **P < 0.01, ***P < 0.001) are shown. SL: length of the longest stem, NO: number of nodes on the longest stem, MIL: mean internode length, LAR: leaf area ratio, SLA: specific leaf area.

Effect	df(effect)	SL	NO	MIL	LAR	SLA
C	2	94.2 ***	82.9 ***	32.5 ***	117.6 ***	87.3 ***
I	1	248.9 ***	53.4 ***	157.9 ***	71.1 ***	34.4 ***
P	2	128.6 ***	77.1 ***	62.7 ***	45.3 ***	25.6 ***
CxI	2	31.2 ***	1.6 NS	20.8 ***	1.0 NS	2.2 NS
CxP	4	3.6 **	1.2 NS	2.1 NS	4.9 ***	4.2 *
IxP	2	0.6 NS	11.3 ***	3.7 *	14.2 ***	7.9 ***
CxIxP	4	0.8 NS	3.6 **	1.0 NS	0.4 NS	0.8 NS
	df(error)	72	72	72	144	144

Irradiance and photoperiod had a limited effect on both leaf area ratio (LAR: leaf area per unit plant mass; Fig. 1B) and specific leaf area (SLA: leaf area per unit leaf mass; Fig. 1C). However, at short photoperiod a decrease in irradiance resulted in a pronounced increase of both LAR and SLA, as indicated by the significant irradiance x photoperiod interaction (Table 1; Fig. 1B and C). The Spanish clone (CAR) showed significantly lower LAR (Fig. 1B) and SLA values (Fig. 1C) than both other clones (significant clone effect; Table 1).

Table 2. Three-way ANOVAs on plant parameters related to biomass allocation and biomass yield (at day 56), with clone (C), irradiance (I) and photoperiod (P) as main effects. For all main effects and their interactions, the degrees of freedom, variance ratios and significance levels (NS: not significant, *P < 0.05, **P < 0.01, ***P < 0.001) are shown. LMR: leaf mass ratio, SMR: stem mass ratio, BMR: belowground mass ratio.

		Biomass allocation			**Biomass**
Effect	df(effect)	LMR	SMR	BMR	Yield
C	2	90.3 ***	381.6 ***	4.6 *	27.5 *
I	1	67.3 ***	706.9 ***	347.6 ***	27.2 ***
P	2	34.5 ***	203.0 ***	5.1 **	34.9 ***
CxI	2	8.5 ***	73.5 ***	6.2 **	8.5 NS
CxP	4	5.4 ***	5.0 ***	2.5 *	0.8 ***
IxP	2	16.4 ***	5.6 **	8.7 ***	8.8 ***
CxIxP	4	1.4 NS	1.2 NS	1.7 NS	1.9 NS
	df(error)	324	324	324	72

Clone, irradiance and photoperiod affected biomass allocation in a non-additive way (significant main effects and two-level interactions; Table 2). At low irradiance, biomass allocation to leaves and stems increased (i.e. higher LMR [leaf mass per unit plant mass] and SMR [stem mass per unit plant mass]; Fig. 2A and B) at the expense of the proportional investment of biomass in roots and rhizomes (i.e. lower BMR [belowground mass per unit plant mass]; Fig. 2C). At short photoperiods (13 h), SMR

increased at the expense of LMR, while BMR was only slightly affected (Fig. 2). Responsiveness to irradiance and photoperiod varied among clones, although the trends were maintained (e.g. LMR was less responsive to irradiance for CAR, and SMR was less responsive to irradiance for KAS; Fig. 2A and B). While plants from KAS invested most of their resources into leaves and less in stems, the opposite was found for CAR (significant clone effects; Table 2, Fig. 2A and B). The highest proportion of biomass allocated to belowground plant parts was found for the Dutch clone (BAB), while both other clones (KAS and CAR) showed lower and comparable BMR values (significant clone effect; Table 2, Fig. 2C).

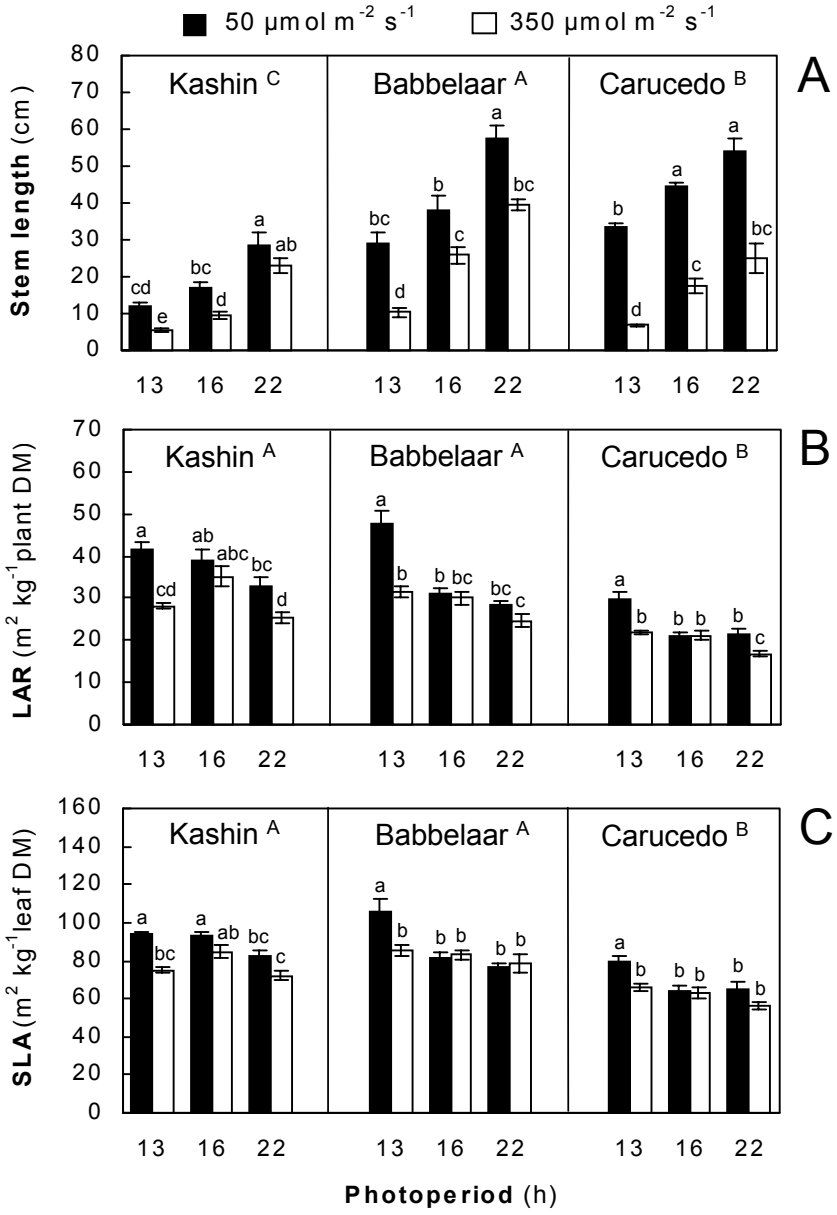

Figure 1. The effect of irradiance and photoperiod on shoot morphology and leaf area production, for three clones of *P. pectinatus* obtained from different latitudes. **(A)** stem length, **(B)** leaf area ratio (LAR: leaf area per unit plant mass), **(C)** specific leaf area (SLA: leaf area per unit leaf mass). Shown values are means ± standard errors, recorded after 28 (LAR and SLA) or 56 (stem length) days of growth. The effect of clone, irradiance and photoperiod was tested by a three-way ANOVA. Different capital letters indicate significant differences between clones and different lower case letters significant treatment effects within clones (Tukey a posteriori test, $P < 0.05$).

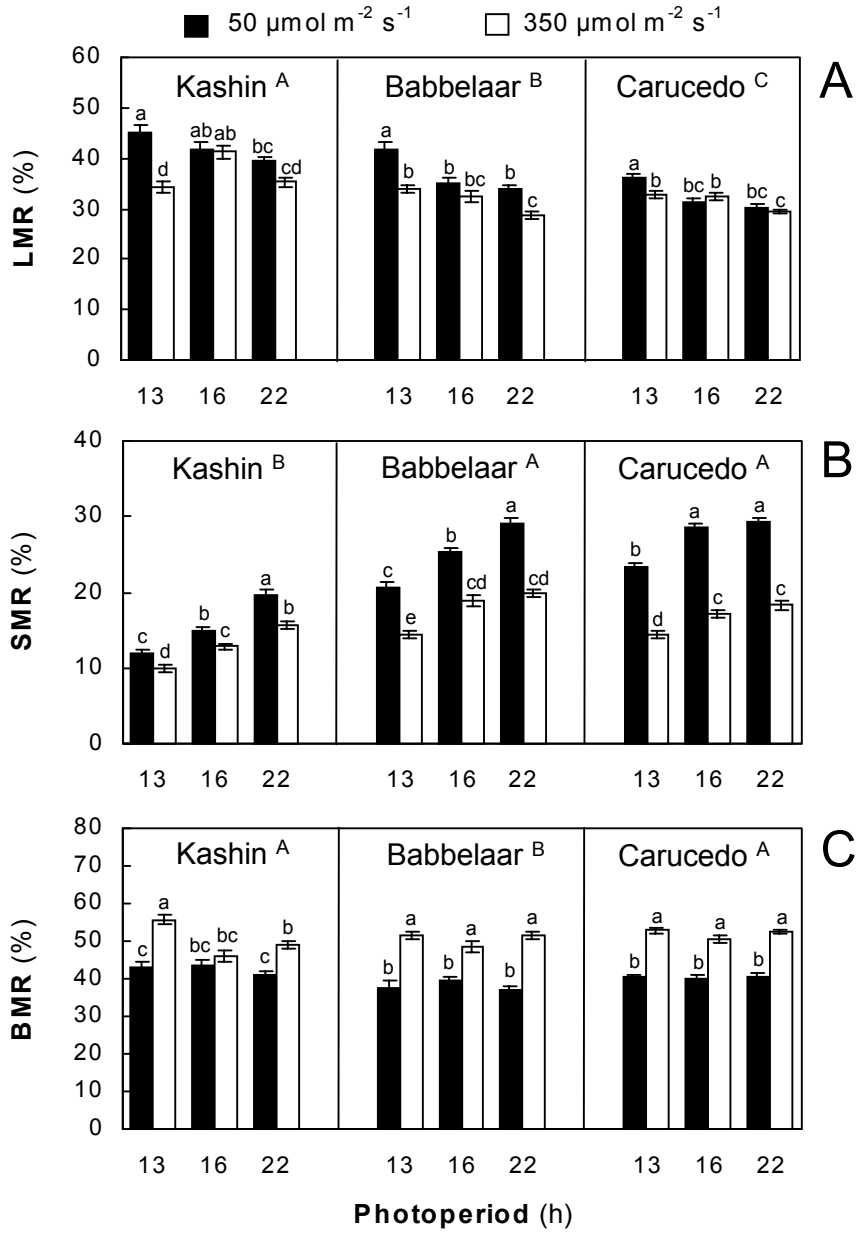

Figure 2. The effect of irradiance and photoperiod on biomass allocation, for three clones of *P. pectinatus* obtained from different latitudes. Shown values are means ± standard errors (n=19). **(A)** leaf mass ratio (LMR, leaf mass per unit plant mass), **(B)** stem mass ratio (SMR, stem mass per unit plant mass), **(C)** belowground mass ratio (BMR, belowground mass per unit plant mass). The effect of clone, irradiance and photoperiod was tested by a three-way ANOVA. Different capital letters indicate significant differences between clones and different lower case letters significant treatment effects within clones (Tukey a posteriori test, $P < 0.05$).

Photosynthetic light-response and chlorophyll determination

All light-response curves were fitted in a satisfactory manner to the rectangular hyperbola ($R^2 > 0.95$ in all cases). To emphasise the differences between the various experimental treatments, we only show light-response curves fitted on mean oxygen-exchange rates (Fig. 3), while parameter estimates were derived from individual replicate curves (Fig. 4).

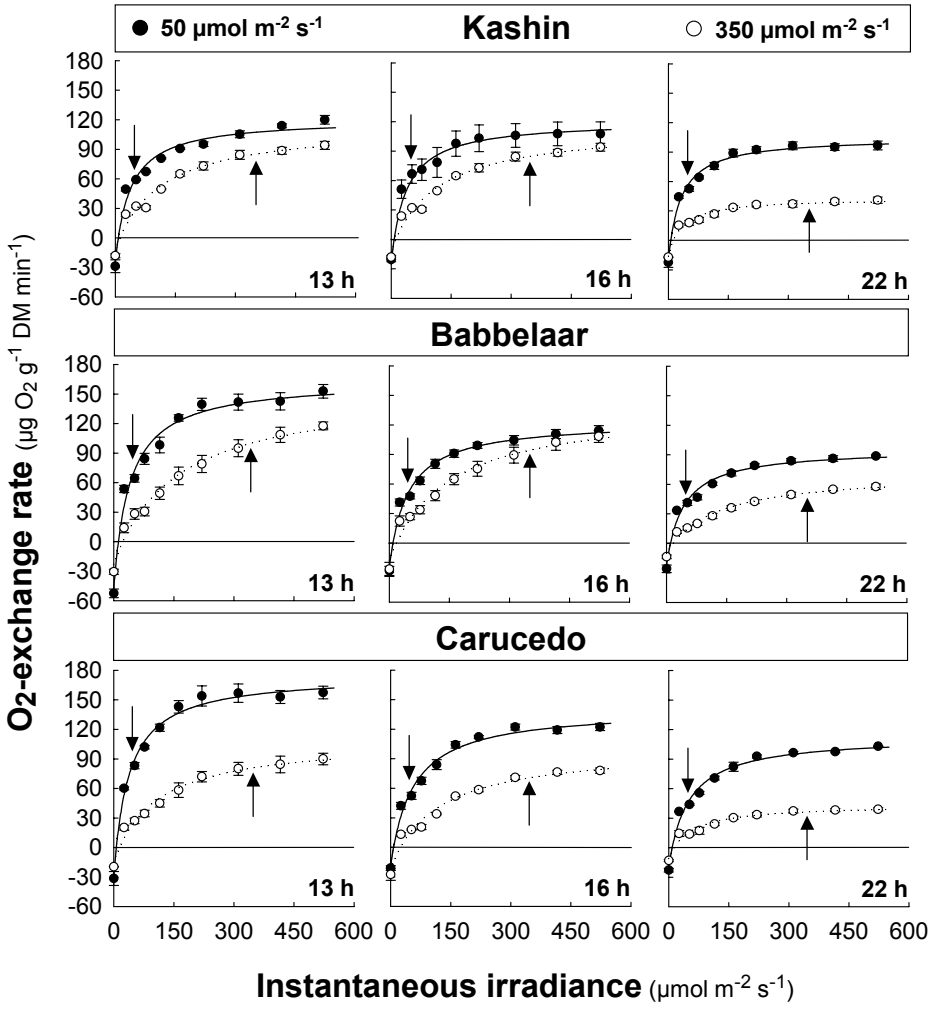

Figure 3. Fitted light-response curves for three clones of *P. pectinatus* obtained from different latitudes and grown at a factorial combination of two irradiances and three photoperiods. The curves shown are based on mean oxygen-exchange rates (n=3) ± standard errors, while parameter estimates used for statistical analysis (see Fig. 4) were derived from each replicate curve separately (not shown). Arrows indicate the photosynthetic rate at the irradiance the plants were grown. Non-visible error bars are obscured by data point symbols.

Table 3. Three-way ANOVAs on parameters related to oxygen-exchange and chlorophyll concentration, with clone (C), irradiance (I) and photoperiod (P) as main effects. For the main effects and all its interactions, the degrees of freedom, variance ratios and significance levels (NS: not significant, *P < 0.05, **P < 0.01, ***P < 0.001) are shown. Pm: irradiance-saturated photosynthetic rate, α: quantum yield, R_d: rate of dark respiration. a+b: total chlorophyll concentration, a/b: chlorophyll a/b-ratio.

		Oxygen-exchange			Chlorophyll	
Effect	df(effect)	Pm	α	R_d	a+b	a/b
C	2	31.9 ***	8.6 ***	2.1 NS	16.8 ***	1.4 NS
I	1	203.1 ***	396.8 ***	28.1 ***	241 ***	30.3 ***
P	2	202.1 ***	7.3 **	5.8 **	7.5 **	1.9 NS
CxI	2	20.5 ***	1.6 NS	1.6 NS	27.6 ***	3.6 *
CxP	4	2.7 *	6.4 ***	2.9 *	3.4 *	0.9 NS
IxP	2	43.6 ***	7.9 **	4.1 *	3.2 NS	18.8 ***
CxIxP	4	2.4 NS	0.6 NS	0.4 NS	5.4 **	3.6 *
	df(error)	36	36	36	36	36

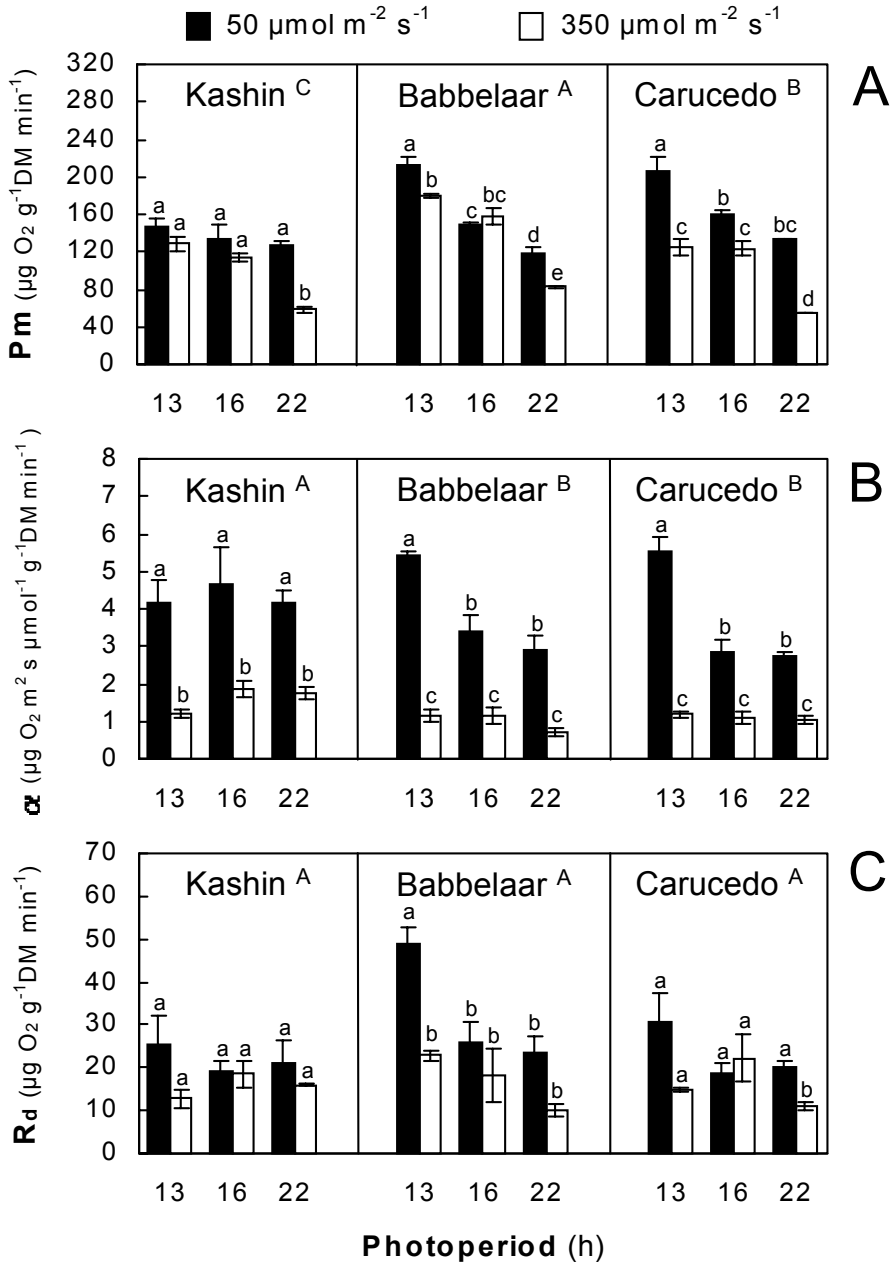

Figure 4. The effect of irradiance and photoperiod on parameter estimates derived from fitted light-response curves, modeling the photosynthetic performance of three *P. pectinatus* clones from contrasting latitudes. **(A)** irradiance-saturated gross photosynthesis (Pm), **(B)** quantum yield (α), **(C)** rate of dark respiration (R_d). The effect of clone, irradiance and photoperiod was tested by a three-way ANOVA. Different capital letters indicate significant differences between clones and different lower case letters significant treatment effects within clones (Tukey a posteriori test, $P < 0.05$).

The investigated clones showed a higher rate of irradiance-saturated gross photosynthesis (Pm) when they were grown at low irradiance, although this effect varied among photoperiods (significant irradiance effect and irradiance x photoperiod interaction; Table 3, Fig. 4A). All three clones showed an increase in Pm when plants were grown at longer photoperiod (significant photoperiod effect; Table 3, Fig. 4A), although for the Russian clone (KAS) this was not significant at low light intensity (based on multiple comparisons, Fig. 4A). Pm was highest for the Dutch clone (BAB), intermediate for the Spanish (CAR) and lowest for the Russian clone (KAS)(significant clone effect; Table 3, Fig. 4A).

A strong increase in apparent quantum yield (α) was found when the clones were grown at low irradiance (significant irradiance effect; Table 3, Fig 4B). For the Russian clone (KAS), the increase in α at low irradiance was independent of photoperiod, while for both other clones the increase was larger at 13 h photoperiod (based on multiple comparisons; Fig 4B). The clone from Kashin showed the highest apparent quantum yields, which were lower but comparable for both other clones (significant clone effect; Table 3, Fig. 4B).

A decrease in irradiance resulted for all clones in higher rates of dark respiration (R_d)(significant irradiance effect; Table 3), although multiple comparisons among photoperiods were frequently not significant due to high variation in measurements (Fig. 4C). Dark respiration was highest at low irradiance combined with short photoperiod (13 h) and lowest at high irradiance combined with long photoperiod (22h)(significant irradiance x photoperiod interaction; Table 3, Fig. 4C). We did not find significant clonal differences in R_d (non-significant clone effect; Table 3, Fig. 4C).

Table 4. The effect of irradiance and photoperiod (P) on total chlorophyll concentration (a+b) and chlorophyll a/b-ratio (a/b) for three clones of *P. pectinatus* obtained from different latitudes. Significant differences between the clones are indicated by different capital letters (three-way ANOVA, Tukey a posteriori test, $P < 0.05$). Different lower case letters stand for significant treatment differences within clones (one-way ANOVA, Tukey a posteriori test, $P < 0.05$).

Clone	P	Chlorophyll			
		a+b (µg g^{-1} FM)		**a/b** (µg $µg^{-1}$)	
	(h)	50 µmol/m^2s^{-1}	350 µmol/m^2s^{-1}	50 µmol/m^2s^{-1}	350 µmol/m^2s^{-1}
Kashin	**13**	B 702 ± 19^{a}	495 ± 4^{bc}	A 3.12 ± 0.08^{ab}	3.06 ± 0.07^{ab}
	16	728 ± 42^{a}	402 ± 12^{cd}	2.59 ± 0.06^{ab}	3.16 ± 0.02^{ab}
	22	615 ± 29^{ab}	329 ± 29^{d}	3.60 ± 0.08^{a}	2.22 ± 0.21^{b}
Babbelaar	**13**	A 654 ± 113^{ab}	672 ± 19^{ab}	A 3.14 ± 0.10^{ab}	3.07 ± 0.03^{ab}
	16	922 ± 30^{a}	552 ± 35^{b}	3.38 ± 0.18^{a}	3.16 ± 0.16^{ab}
	22	741 ± 36^{ab}	538 ± 87^{b}	3.36 ± 0.17^{a}	2.54 ± 0.16^{b}
Carucedo	**13**	A 1091 ± 31^{a}	487 ± 26^{c}	A 3.27 ± 0.12^{b}	3.38 ± 0.06^{ab}
	16	835 ± 47^{ab}	421 ± 30^{c}	3.53 ± 0.12^{ab}	2.53 ± 0.14^{c}
	22	971 ± 81^{b}	285 ± 11^{c}	3.84 ± 0.11^{a}	2.21 ± 0.05^{c}

Growth at low irradiance resulted for all clones in higher chlorophyll concentrations in the leaves (significant irradiance effect; Table 3 and 4). In contrast, differences in photoperiod had a limited, though significant effect on the total chlorophyll concentration (significant photoperiod effect; Table 3 and 4). The Russian clone (KAS) showed the highest chlorophyll concentration, while those from The Netherlands (BAB) and Spain (CAR) showed lower but comparable values (significant clone effect; Table 3 and 4).

The chlorophyll a/b-ratio was significantly affected by irradiance, but not by photoperiod (Table 3). The influence of irradiance did however vary among photoperiods (significant irradiance x photoperiod

interaction; Table 3). For all clones, this resulted in a decrease of the chlorophyll a/b-ratio at a combination of long photoperiod (22 h) and high irradiance (Table 4). The chlorophyll a/b-ratio did not differ among clones (non-significant clone effect; Table 3 and 4).

Biomass yield

In general, low irradiance resulted for all clones in a decrease of the biomass yield at the end of the experiment (significant irradiance effect; Table 2, Fig. 5). However, the effects of irradiance were frequently small and non-significant (Fig. 5), and varied among photoperiods (irradiance x photoperiod interaction; Table 2). A decrease of the photoperiod from 22 to 16 h, resulted in a significant decrease of the biomass yield for all clones (Significant photoperiod effect; Table 2, Fig. 5). However, a further reduction of the photoperiod (to 13 h) did not result in an additional decrease in plant biomass (Fig. 5). The clone from The Netherlands (BAB) showed the highest biomass yield, while intermediate and lower values were recorded for the Russian (KAS) and Spanish (CAR) clones (Table 2, Fig. 5).

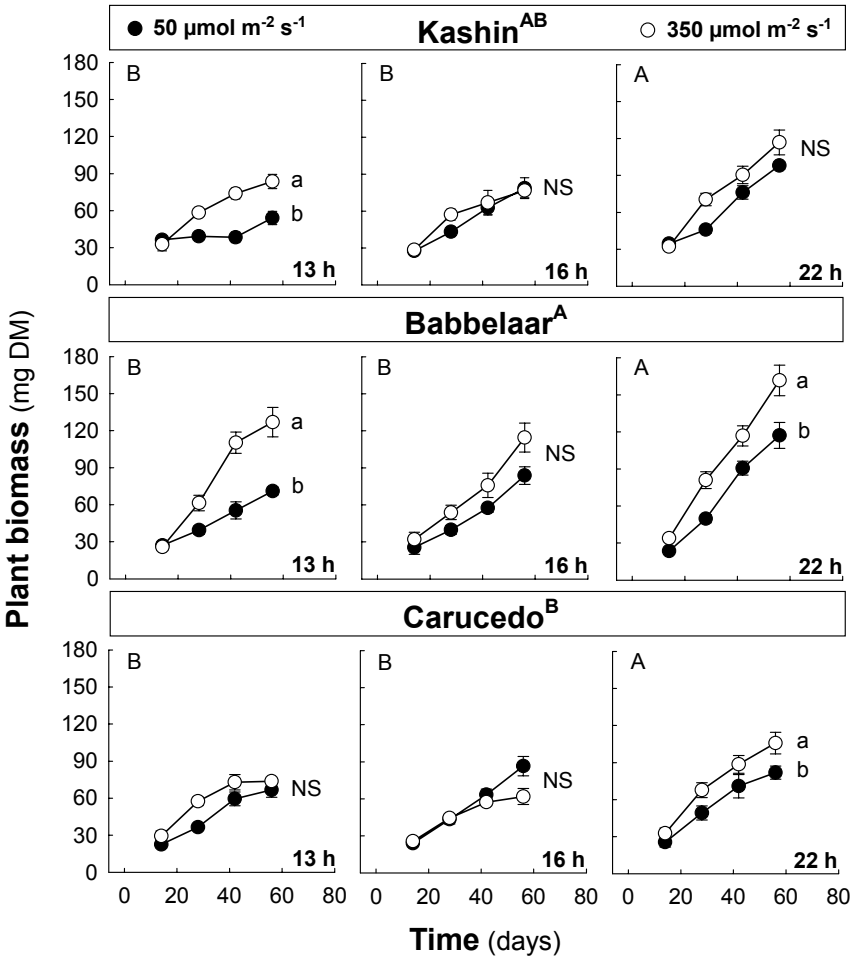

Figure 5. Total biomass accumulation as a function of time, recorded for three clones of *P. pectinatus* grown at a factorial combination of two irradiances and three photoperiods. The effect of clone, irradiance and photoperiod was tested by a three-way ANOVA. Different capital letters next to the clone names indicate significant differences between the clones (Tukey a posteriori test, $P < 0.05$). Different capital letters in the figures stand for significant differences among photoperiods and different lower case letters indicate significant irradiance effects (at day 56) within clones and photoperiods (Tukey a posteriori test, $P < 0.05$).

Discussion

Morphological responses

Under conditions of low irradiance (Björkman, 1981; Lambers et al., 1998) or short photoperiod (e.g. Ryle, 1966; Jablonski & Geiger, 1987; Carlen et al., 1999), terrestrial plants frequently enhance the capture of light by an acclimative increase of the leaf area ratio (LAR). At low irradiance this increase predominantly results from an increase of the specific leaf area (SLA)(Björkman, 1981, but see Dijkstra 1989), while at short photoperiod an increase of both SLA and LMR (leaf mass ratio) may contribute to a higher LAR (e.g. Jablonski & Geiger, 1987; Carlen et al., 1999). Although the investigated clones of *P. pectinatu*s showed a limited capability for compensatory changes in LAR, the synergistic effect of reduced irradiance and short photoperiod (13 h) did result in an acclimative increase (Fig. 1B). Under these conditions, both SLA and LMR contributed to the higher LAR (Fig. 1C and 2A).

In line with previous research on the light-response of *P. pectinatus* (Vermaat & Hootsmans, 1994), plants that were grown at low irradiance allocated a higher proportion of dry matter to shoots, at the expense of roots and rhizomes (Fig. 2). Although low irradiance tended to increase the LMR (Fig. 2A), the higher allocation of dry matter to shoots was primarily explained by an increase in the stem mass ratio (SMR; Fig. 2B). This change in the pattern of biomass allocation, together with a more pronounced elongation of internodes (data not shown), resulted in strong vertical shoot extension (Fig. 1A). Several other aquatic macrophytes showed a similar type of morphological response (Barko & Smart, 1980; Barko et al., 1982) which involves the photosynthetically active tissue to be concentrated at the water surface, thereby enhancing light capture under conditions of high turbidity. In addition, our data support the hypothesis that photomorphogenic responses that result in canopy formation may be ecologically analogous to acclimative increases of LAR (Barko & Smart, 1980; Barko et al., 1982). Although the increase of shoot length at low irradiance was apparent in all clones, it was more pronounced in clones originating from lower latitudes (Fig. 1A). As competition for light is likely to be more intense in more productive southern habitats where plant density is frequently higher; canopy formation may result in a larger competitive advantage (Schmitt et al., 1995).

The length of the photoperiod did not affect the allocation of biomass to above- and belowground plant parts (Fig. 2). However, plants that were grown at short photoperiods allocated a higher proportion of their resources to leaves (higher LMR; Fig 2A) at the expense of stems (lower SMR; Fig. 2B). For the vegetative part of the life cycle, Spencer et al. (1993) who grew *P. pectinatus* at 10 and 16 hours photoperiod, observed similar changes in biomass allocation. Although the increase in LMR at short photoperiod was rather limited (i.e. 8.5% from 22 to 13 h photoperiod; grand average of two irradiances and three clones), it probably represents a mechanism by which the plants increase the photosynthetic productivity under reduced daily irradiance. However, there seems to be a trade-off between LMR and shoot length, since increases in LMR were accompanied by decreases in SMR and shoot length (Fig. 1A, 2A and 2B). Comparably, decreases in shoot length at shorter photoperiods have also been reported for terrestrial plants (Björkman, 1981).

Photosynthetic responses

For shade-acclimated terrestrial plants, higher photosynthetic rates at low irradiance have been frequently reported to result from a decrease in respiratory costs associated with lower investments in photosynthetic capacity (Boardman, 1977; Björkman, 1981; Lambers et al., 1998). However, in the present experiment we found an increase in Pm when plants were grown at low irradiance (Fig. 4A; similar to Hootsmans et al. [1996], but in contrast with Hootsmans & Vermaat [1995]). One explanation could be that the investigated clones had a low potential to make efficient use of high light, which resulted in photodamage of the antenna pigments. However, the photosynthetic capacities of high-irradiance grown plants (i.e. 114 μg O_2 g^{-1} DM min^{-1}, grand average across clones and photoperiods; Fig. 4A) were not critically depressed, but at the upper end of the range reported for a number of submerged freshwater angiosperms (i.e. between 35-120 μg O_2 g^{-1} DM min^{-1}; Van et al., 1976; Barko & Smart, 1981; Pokorný et al., 1984; Best & Dassen, 1987). We therefore suggest that the investigated clones possess the inherent ability to increase their photosynthetic capacity at low growth-irradiance. In agreement with this, we found that the rates of dark respiration were higher for plants grown at low irradiance (Fig. 4C). This probably reflects the higher activity of the photosynthetic apparatus (Pm and α), which requires more respiratory energy for biosynthesis and maintenance.

Many plants minimise the detrimental effects of low irradiance through an increased investment of energy and resources in light harvesting. In *P. pectinatus*, a higher light absorption through increases in total chlorophyll concentration (Table 4) and LAR (Fig. 1B), resulted in a higher apparent quantum yields (α) at low irradiance (Fig. 4B). In other studies on light acclimation of *P. pectinatus*, the apparent quantum yield did not increase with decreasing irradiance, despite the fact that the total chlorophyll concentration increased (Hootsmans et al., 1996; Hootsmans & Vermaat, 1994). This discrepancy may be explained by high variation in measurements, which hampered the accuracy by which the initial slope of the light-response curve could be estimated. More accurate photosynthetic measurements, performed for other aquatic angiosperms, have shown an increase in α with decreasing irradiance (Goldsborough & Kemp, 1988; Küster et al., 2000; Santamaría & Van Vierssen, 1995).

Acclimation to low irradiance generally involves a decrease in chlorophyll a/b-ratio, which represents a higher investment in the light-harvesting complex (LHC) that contains more chlorophyll b (e.g. Evans, 1988). However, we found little variation in the chlorophyll a/b-ratio among plants grown at different irradiances (Table 4; similar to Hootsmans et al., 1996). In addition, the pattern of variation interacted with photoperiod in a non-additive way. For example, at long photoperiod (22 h) the chlorophyll a/b ratio increased for plants grown at low irradiance, opposite to the expected response (Table 4). All in all, our results and evidence on other species suggests that changes in chlorophyll a/b-ratio are of minor importance for light acclimation among aquatic angiosperms (Bowes & Salvucci, 1989 and references therein).

The photosynthetic performance of the Russian clone (KAS) was largely unresponsive to differences in photoperiod (Fig. 4). However, both other clones showed a progressive increase of the photosynthetic capacity (Pm) at decreasing photoperiods (Fig. 4a). The latter was also observed for the aquatic macrophyte *Ruppia drepanensis* (Santamaría & Van Vierssen, 1995) and for plants native to the terrestrial environment (Denne & Smith, 1971; Burian & Winter, 1976). The increase in Pm probably reflects a higher activity of the Calvin Cycle, by which the plants partly compensated for the decrease in daily irradiance (Robinson, 1984). In addition, an increase of the apparent quantum yield

(α) may also result in a higher rate of carbon fixation at decreasing photoperiods. Indeed, such a response was found for the Dutch (BAB) and Spanish (CAR) clones, but only when they were grown at low irradiance (Fig. 4B). Finally, since short photoperiods inevitably result in longer dark periods, plants would benefit from reduced rates of dark respiration (e.g. Santamaría & Van Vierssen, 1995). However, for the clones from The Netherlands (BAB) and Spain (CAR), plants grown at short photoperiod showed a slight increase in dark respiration (Fig. 4C), which is probably related to an increase in both the capacity (Pm; Fig. 4A) and the activity of the photosynthetic apparatus (Fig. 3).

Biomass yield

We considered biomass yield at the end of the experimental period as a fitness surrogate to evaluate to what extent the plastic response of *P. pectinatus* to variation in irradiance and photoperiod can be considered adaptive. For all investigated clones, growth at low irradiance resulted in decreased biomass yield; however, differences between both treatments were relatively small and frequently not significant (Fig. 5). This probably resulted from the strong acclimative changes in the photosynthetic performance of plants grown at low irradiance (i.e. increase of Pm and α; Fig. 4A and B). These changes gave rise to comparable photosynthetic rates at the incubation irradiances that coincide with the irradiance level at which the plants were grown (Fig. 3). Under low irradiances induced by high water turbidity the clones' ability to form a canopy through an increase in shoot length will probably result in an additional increase in carbon fixation. However, since we modulated irradiance by means of shading nets, light conditions in the aquaria were vertically uniform. We therefore underestimated the effectiveness of shoot elongation as an acclimative response (i.e. its effect on the net carbon intake). In short, our data showed that *P. pectinatus* clones from different geographic regions are shade tolerant and can cope with considerably low irradiances.

For all investigated clones, we found that a reduction in photoperiod from 22 to 16 h resulted in a decrease in biomass yield (Fig. 5). This indicates that the increase in Pm was not high enough to fully compensate for the decrease in daily irradiance at shorter photoperiods. However, a further reduction in photoperiod from 16 to 13 h did not result in an additional decrease in plant biomass (Fig. 5). For the Dutch (BAB) and Spanish (CAR) clones that were grown at low irradiance, the observed increase in Pm, α and LAR probably explains the compensation of the biomass yield at short photoperiod (13 h)(Fig. 1B, Fig 4A and B). However, such interpretation is questionable because the Russian clone (KAS) and the plants from all clones grown at high irradiance did not show such assimilative changes in Pm α and LAR, while their biomass yield did not vary between photoperiods (16 versus 13 h).

Conclusions

Our data show that all three clones originating from different latitudes have the capacity to largely compensate for the effect of low irradiance, through the interplay of acclimative responses in both morphological and physiological characteristics. These include, canopy formation mediated by pronounced vertical shoot extension and higher Pm and α values that resulted in an increase of the photosynthetic rate at low light. As a consequence, biomass yield at the end of the experiment was largely comparable for plants grown at different irradiances. Although a decrease in photoperiod also resulted in acclimative responses in morphology (i.e. increase of LMR) and photosynthesis (i.e. increase of Pm), the biomass yield at the end of the experiment was lower at a short (13 and 16 h)

photoperiod. Since all investigated clones showed comparable responses to variation in irradiance and photoperiod, we reject the hypothesis that *P. pectinatus* is locally specialised. More so, this investigation indicates that the clones possess a high capacity for acclimation to differences in irradiance and a partial capacity to compensate for differences in photoperiod.

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CHAPTER 6

Latitudinal variation in life-cycle characteristics of *Potamogeton pectinatus* L.: tuber sprouting and the effect of temperature and photoperiod on growth

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Summary

During the period of active growth, high-latitude populations of broadly distributed plants are exposed to lower temperatures and longer photoperiods than their southern counterparts. To cope with this, species may consist of different ecotypes that are locally adapted to the prevailing climatic conditions. The present study focussed on the response of various life-cycle characteristics to differences in temperature and photoperiod for six clones of the aquatic angiosperm *Potamogeton pectinatus* L., obtained from 49 to 68°N.

In the first part of the experiment, latitudinal variation in tuber sprouting was investigated at a range of different water temperatures (10, 15, 20 and 25°C). For all clones, sprouting characteristics were comparable at higher water temperatures (20 and 25°C), while at lower temperatures the maximum frequency of tuber sprouting (at 10°) and the rate of tuber sprouting over time (at 10 and 15°C) decreased with increasing latitude. This pattern of ecotypic differentiation may represent a mechanism to avoid juvenile plant damage at high-latitude locations, where thermal conditions are unpredictable early in the growth season.

In the second part of the experiment we investigated biomass allocation and growth at factorial combinations of five water temperatures (as above) and two photoperiods (16 and 22 h). For all clones, the allocation of biomass to various plant organs was largely insensitive to differences in temperature and photoperiod. While the biomass yield increased with increasing water temperature (saturating above 20°C), an extension of the photoperiod did not have a significant influence. As a consequence, we conclude that higher-latitude clones of *P. pectinatus* do not have the capacity to compensate for the growth-limiting effect of low water temperature, by making use of the increased light availability associated with a prolonged photoperiod.

Introduction

Temperature and photoperiod have a large impact on many stages of the plants' life cycle and during the period of seasonal growth they exhibit opposite patterns of variation across latitude (Berry & Raison, 1981; Salisbury, 1981). As a consequence, northern populations of broadly distributed plants are exposed to lower temperatures and longer photoperiods than their southern counterparts. To cope with this geographic variation in abiotic conditions, species may consist of ecological races that differ in their response to temperature and photoperiod (Berry & Raison, 1981; Salisbury, 1981). On the other hand, high phenotypic plasticity that results in tolerance to latitudinal variation in climatic factors may also contribute to a species' wide distribution (Bradshaw, 1965).

Only a limited number of studies focus on thermal differentiation among populations of broadly distributed plants. For example, in several herbaceous species the thermal requirements for seed germination were positively correlated with the latitude of provenance, which has been interpreted as a mechanism to avoid seedling mortality in northern regions, where thermal conditions are more unpredictable early in the growth season (Sawada et al., 1994; Minggang et al., 2000). At latitudes that differ in prevailing air-temperatures, productivity is largely determined by the thermal sensitivity of physiological processes like photosynthesis and respiration (Berry & Björkman, 1980). In some populations native to colder regions, a higher growth rate was achieved by an overall increase of the photosynthetic rate (Joshi & Palni, 1998), while in others the thermal optimum for gas-exchange was lowered (Billings et al., 1971). Finally, there is some evidence that geographically distant populations may differ in the thermal response of reproductive processes like vernalisation and flowering (Heide, 1994; King et al., 1995).

Published work on ecotypic differentiation in photoperiodic plant responses has primarily focused on reproduction. For example, in European varieties of *Lolium perenne* the critical daylength for floral induction was positively correlated with the latitude of the seed source (Aamlid et al., 2000). Plant morphology and growth may also show latitudinal variation in photoperiodic responses. For example, in several broadly distributed woody and herbaceous species, the optimal daylength for leaf elongation, tillering and biomass yield increased with increasing latitude (Håbjørg, 1976; Håbjørg, 1978). In addition, plant productivity of high-latitude provenances may be stimulated by longer photoperiods, thus compensating for the detrimental effects of low temperature (Hay & Heide, 1983).

Studies on latitudinal variation in plant responses to temperature and photoperiod focussed nearly exclusively on terrestrial plant life. This is surprising, since many species native to the aquatic environment are widespread and therefore represent an excellent tool for investigations on ecotypic differentiation and phenotypic plasticity across latitude. Since waterbodies buffer daily and seasonal oscillations in air-temperature, several authors have argued that temperature does not fundamentally control the distribution of aquatics (Sculthorpe, 1967; Pip, 1989). Although this might be true for marine plants, freshwater macrophytes frequently inhabit shallow habitats where seasonal extremes may vary between 0 and 40°C (Bowes & Salvucci, 1989) and are thus comparable to the thermal ranges experienced by terrestrial plants (Berry & Raison, 1981). Similarly, changes in the light absorption characteristics of the water column may result in considerable variation in the instantaneous quantum flux among lakes and/or seasons (Spence, 1975; Jeffrey, 1981), but the length of the photoperiod is hardly affected and remains comparable to the terrestrial environment (Van Vierssen & Hootsmans, 1994).

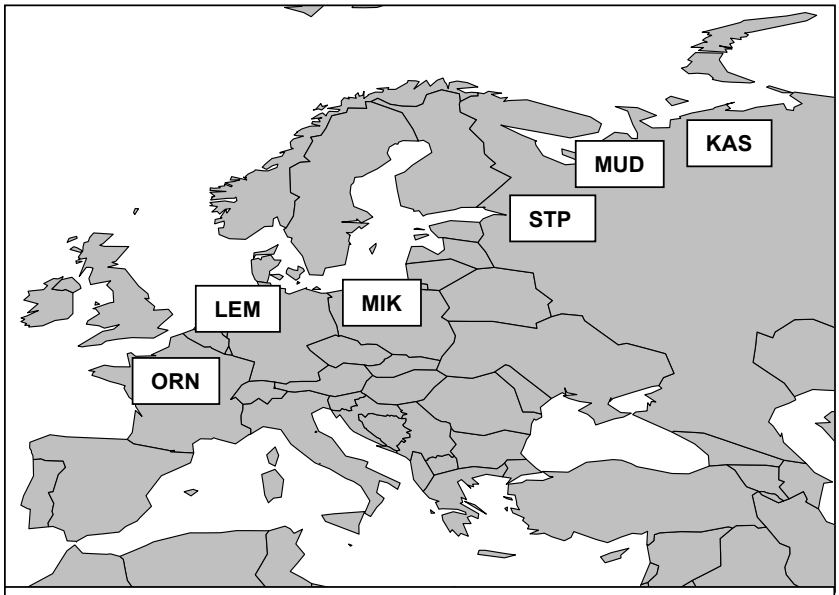
Here we focus on clonal variation in plant responses to temperature and photoperiod, for the submerged angiosperm fennel pondweed (*Potamogeton pectinatus* L.). *P. pectinatus* is a pseudo-annual that shows a cosmopolitan distribution and can be found in nearly all climatic zones (Casper & Krausch, 1980; Wiegleb & Kaplan, 1998). Most often, the species occurs in permanently flooded aquatic ecosystems that may differ in water depth, trophic status and alkalinity (Kantrud, 1990). In temperate areas, seasonal growth largely depends of the sprouting of subterranean tubers (Van Wijk, 1989); a process controlled by water temperature and maximised between 15 and 25°C (Spencer & Ksander, 1992; Van Wijk, 1989; Madsen & Adams, 1988). However, since previous experiments were predominantly performed with *P. pectinatus* tubers collected from mild-temperate climates, it is unclear whether thermal requirements for sprouting differ across latitude. We therefore performed an experiment in which tuber sprouting was measured at a range of different water temperatures (i.e. 10 to 25°C) for six clones obtained from 49 to 68°N. By analogy with the findings for seed germination in broadly distributed terrestrial plants (cf. Sawada et al., 1994; Minggang et al., 2000), we hypothesised that at low temperature, tuber sprouting would be negatively correlated with the latitude of origin.

In a previous experiment we showed that *P. pectinatus* can be considered thermally tolerant, since clones obtained from a latitudinal gradient showed a comparable biomass yield over a relatively broad range of water temperatures (15/20 to 30°C; Pilon & Santamaría, 2002). However, in sub-arctic regions temperature frequently drops below this tolerance range, which potentially limits growth of *P. pectinatus*. This detrimental effect may be compensated by an increase in growth at longer photoperiods (cf. Hay & Heide, 1983), although the combined effect of temperature and photoperiod on biomass yield was never studied in *P. pectinatus*. We therefore performed an experiment in which clones from contrasting latitudes (i.e. the same that were used to characterise tuber sprouting) were grown at a combination of different water temperatures (10 to 25°C) and photoperiods (16 and 22 h.). We hypothesised that clones from northern regions would have a higher capacity to make efficient use of the increased light availability associated with a longer photoperiod, thus compensating for the growth limiting effect of low temperature.

Material and Methods

Origin and propagation of plant material

Six clonal lines of *P. pectinatus* were selected, each originating from a single tuber collected at localities that greatly differed in latitude (i.e. 49 to 68°N, Fig. 1). To produce enough tubers for the experiment, all clones were propagated under common garden conditions at the Centre for Terrestrial Ecology (Heteren, The Netherlands [51.92°N, 5.01°E]; growing season from May to September 1998). Plants were grown outdoors in polyethylene tanks ($1m^3$) filled with tap water over a mixture of sand and potting clay (3:1 by dry mass [DM]). Following collection from the tanks, all tubers were surface-sterilised with 5% sodium hypochlorite (Madsen, 1985) and stored in darkness at 4°C, until the start of the experiment (March 1999). The stratification period was long enough to ensure full break of dormancy and prevent possible interactions with the thermal dependence of tuber sprouting (Van Wijk, 1989). At the time of the experiments, all clonal lines had been multiplied under the described conditions for 6 growth seasons, thus minimising the probability that clonal variation is due to environmental carry-over effects.



Clone	Country	Location	Latitude	Longitude
KAS	Russia	Korovinskaya Bay, Kashin Island	68.24°N	53.88°E
MUD	Russia	Sukhoye Bay, Mudyug Island	64.92°N	40.30°E
STP	Russia	St. Petersburg, pond in park	59.95°N	30.36°E
MIK	Poland	Lake Mikolajski	53.80°N	21.57°E
LEM	Netherlands	Lake IJsselmeer, near Lemmer	52.84°N	5.71°E
ORN	France	River Orne, near La Foret-Auvray	48.82°N	0.33°E

Figure 1. Localities from where the investigated clones of *P. pectinatus* were obtained.

Tuber sprouting at different water temperatures

Per clone, 192 tubers of 30 to 70 mg fresh mass (FM) were pre-selected and randomly assigned to the different temperature treatments (i.e. 10, 15, 20 or 25°C). Within treatments, all tubers (48 per clone) were randomly distributed among the wells of 14 micro-titer plates (Greiner Labortechnic GmbH). Each well (24 per plate) contained a single tuber that was covered with a layer of washed aquarium sand. All plates were randomly distributed among four glass aquaria (30x40x40 cm) filled with tap water (96 l), whose temperature (i.e. 10, 15, 20 or 25°C) was maintained by separate heating and cooling units. The aquaria were placed in a phytotron with an average irradiance of 157 ± 0.8 µmol m^{-2} s^{-1} (Philips TLD 50W/85o HF, combined with neutral density shading nets) and a photoperiod of 16 h. For 38 days, tuber sprouting was checked every two to three days. Tubers were considered sprouted when the apical meristem tips showed significant elongation (two times the initial size). Per clone and per temperature, the rate of tuber sprouting (over time) and the maximum frequency of tuber sprouting (%) was calculated.

Growth at different water temperatures and photoperiods

When at least 12 tubers per clone and per treatment had successfully sprouted, they were transferred individually to plastic beakers (volume: 150 cm^3) filled with a mixture of aquarium sand and potting clay (3:1 by DM). Before returning the beakers to the aquaria, the sediment was covered with a layer of aquarium sand to prevent nutrient leakage into the water. All plants continued to grow at the water

temperature (i.e. 10, 15, 20 or 25°C) and irradiance level (i.e. 157 ± 0.8 µmol m^{-2} s^{-1}) that was used for tuber sprouting. However, half of the plants (6 per clone per treatment) were randomly selected and placed in a phytotron that sustained a photoperiod of 16 h, while the other half was exposed to a 22-h photoperiod. Both photoperiods were chosen to represent the maximum range of summer daylengths encountered by clones from Central to Northern Europe (Kirk, 1994). The pH of the water was adjusted weekly to 7.3 with H_2SO_4, and water levels were maintained by adding tap water as necessary. After 7 weeks of growth (counted from the day the tubers were set to sprout) all plants were washed free of sediment and the length of the main stem was measured for a randomly selected sub-sample of 3 plants per clone per treatment. Subsequently, all harvested plants were divided into separate fractions (leaves, stems, roots and rhizomes, tubers) for which the dry mass was determined (after 48h at 70°C).

Statistical analysis

The effect of clone, temperature and their interaction on the maximum frequency of tuber sprouting (expressed as binomial data) was analysed by general linear modelling (GLM). A binomial error distribution with a logit link function was used, with clone as a categorical factor and temperature as a continuous factor. Deviances from the model were scaled with the square root of the ratio between deviance and degrees of freedom, to correct for the effects of data overdispersion in the statistical test. Subsequently, we assessed whether the maximum frequency of tuber sprouting was correlated with the latitude from where the clones were obtained, using logistic regressions on binomial tuberisation events (separately within each temperature). Both analyses were performed in the GENMOD procedure of SAS (SAS Institute Inc., 1989).

The effect of water temperature and clone on the rate of tuber sprouting was analysed by using Cox proportional hazard regressions, with days to sprouting as dependent variable (e.g. Allison, 1995). First, we carried out a stratified regression, based on the likelihood of pooled versus separate Cox regressions, with clone as a grouping variable. Then we performed separate regressions for the effect of temperature (within each clone) and for the effect of latitude (within each temperature) on the rate of tuber sprouting. All regressions were performed in STATISTICA (Statsoft Inc., 1999).

Data related to plant growth were analysed by three-way ANOVAs, with clone, temperature and photoperiod as main factors. Tukey a posteriori tests were used to explore differences among clones. Prior to the analysis, data were log-transformed or arcsine-transformed whenever necessary to ensure normality and homogeneity of variances (Sokal & Rohlf, 1995). Analysis of variance was performed in STATISTICA (Statsoft Inc. 1999).

Results

Tuber sprouting at different water temperatures

The model used to analyse maximum sprouting frequencies for different clones and water temperatures fitted the data well (deviance = 12, $P > 0.99$). Maximum frequency of tuber sprouting differed among clones ($\chi^2 = 35.20$, $P < 0.0001$), but not among temperatures ($\chi^2 < 0.01$, $P > 0.99$). The latter probably resulted from high and largely comparable sprouting frequencies at temperatures between 15 and 25°C (i.e. 85 to 100%, Fig. 2). However, at the lowest water temperature (10°C),

clonal differences in maximum tuber sprouting were more pronounced (i.e. 33 to 100%, Fig. 2), which was also reflected by a significant clone x temperature interaction (χ^2 = 3.28, P < 0.01). As a consequence, only at 10°C a significant (negative) relationship between latitude and the maximum frequency of tuber sprouting was found (χ^2 = 26.64, P < 0.0001; Fig. 3).

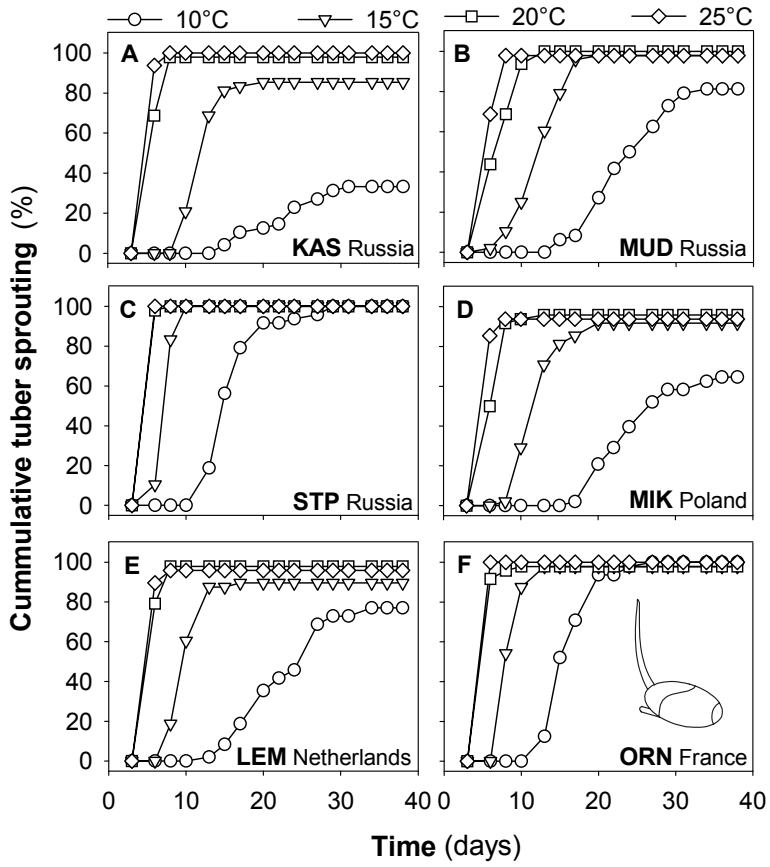


Figure 2. Effect of water temperature on the cumulative frequency of tuber sprouting over time, for six clones of *P. pectinatus* (for abbreviations of clone names, see Fig. 1).

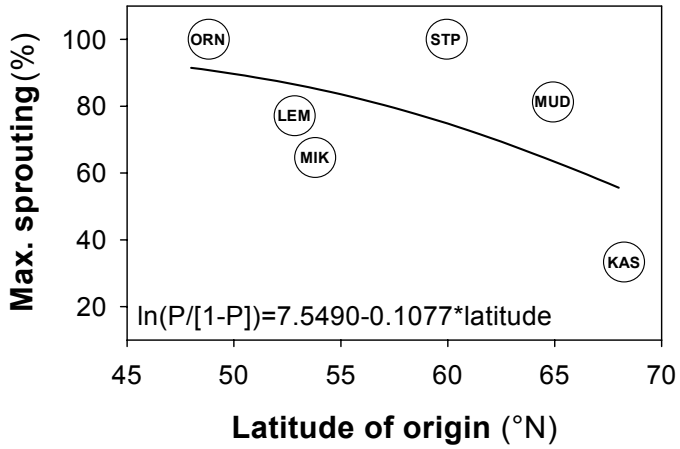


Figure 3. Relationship between the maximum sprouting frequency (at 10°C) and the latitude of origin of six *P. pectinatus* clones (for abbreviations of clone names, see Fig. 1). A logistic regression of binomial sprouting events on latitude is also shown (χ^2 = 26.64, P < 0.0001).

A significant clone x temperature interaction showed that the thermal response of the rate of tuber sprouting varied among clones (χ^2 = 3743.9, P < 0.01). Cox proportional hazard regressions, modelling the effect of water temperature on the rate of tuber sprouting were significant for all clones, with increasing sprouting rates at increasing water temperatures (Table 1A). Cox proportional hazard regressions of latitude versus the rate of tuber sprouting were only significant at lower water temperatures (i.e. 10 and 15°C), with sprouting rates decreasing at higher latitude (Table 1B).

Table 1. Effect of water temperature (10, 15, 20 and 25°C; **A**) and latitude of origin (49 to 68°N; **B**) on the rate of tuber sprouting (parameter estimates) for six clones of *P. pectinatus*. Days to sprouting of individual tubers were fitted to Cox proportional hazard regressions. Model significance for individual regressions are indicated in the table ($^{\$}$P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

A	**Model significance**		**Temperature**
	df	χ^2	parameter estimates ± SE
KAS	1	104.90 ****	1.30 ± 0.15
MUD	1	168.54 ****	1.43 ± 0.13
STP	1	117.63 ****	1.13 ± 0.13
MIK	1	153.05 ****	1.48 ± 0.14
LEM	1	130.00 ****	1.25 ± 0.13
ORN	1	137.10 ****	1.27 ± 0.13
B	**Model significance**		**Latitude of origin**
	df	χ^2	parameter estimates ± SE
10°C	1	5.10 *	-0.025 ± 0.011
15°C	1	13.65 ***	-0.034 ± 0.009
20°C	1	3.25 $^{\$}$	-0.016 ± 0.009
25°C	1	0.66 ^{NS}	-0.007 ± 0.009

Table 2. F-values and significance levels (*P < 0.05, **P < 0.01, ***P < 0.001) for factorial ANOVAs on the effect of the independent variables clone (C), temperature (T), photoperiod (P) and their interactions on plant morphology and growth.

		Morphology	**Biomass allocation**				**Biomass**
Effect	df(effect)	SL	LMR	SMR	RRMR	TMR	plant
C	5	66.0 ***	88.6 ***	250.7 ***	17.0 ***	14.4 ***	23.3 ***
T	3	144.2 ***	92.3 ***	87.9 ***	24.2 ***	7.2 ***	1496.0 ***
P	1	33.2 ***	36.9 ***	41.2 ***	8.7 **	6.6 *	1.5 ^{NS}
CxT	15	6.2 ***	20.2 ***	11.6 ***	14.3 ***	12.7 ***	4.0 ***
CxP	5	3.7 **	4.7 ***	24.6 ***	2.0 ^{NS}	5.5 ***	3.3 **
TxP	3	10.2 ***	26.1 ***	11.9 ***	2.5 ^{NS}	2.5 ^{NS}	11.1 ***
CxTxP	15	3.1 ***	3.4 ***	11.2 ***	2.8 ***	2.1 *	1.4 ^{NS}
	df(error)	96	239	239	239	239	239

SL: length of the main stem, **LMR**: leaf mass ratio, **SMR**: stem mass ratio, **RRMR**: root and rhizome mass ratio, **TMR**: tuber mass ratio

Growth at different water temperatures and photoperiods

The independent factors clone, temperature and photoperiod significantly affected the length of the main stem (Table 2). Maximum stem length was achieved at 15 to 20°C and then saturated or decreased at higher water temperatures (Fig. 4A). The increase in stem length at intermediate water temperatures (i.e. 15 and 20°C) tended to be lower at short photoperiod, particularly for the three Russian clones (KAS, MUD, STP) (Fig. 4A; significant second and three order interactions, Table 2). Longest stems were produced by the clone from France (ORN), while shortest shoots were found for a clone from Russia (KAS) and Poland (MIK) (Fig. 4A; significant clone effect, Table 2).

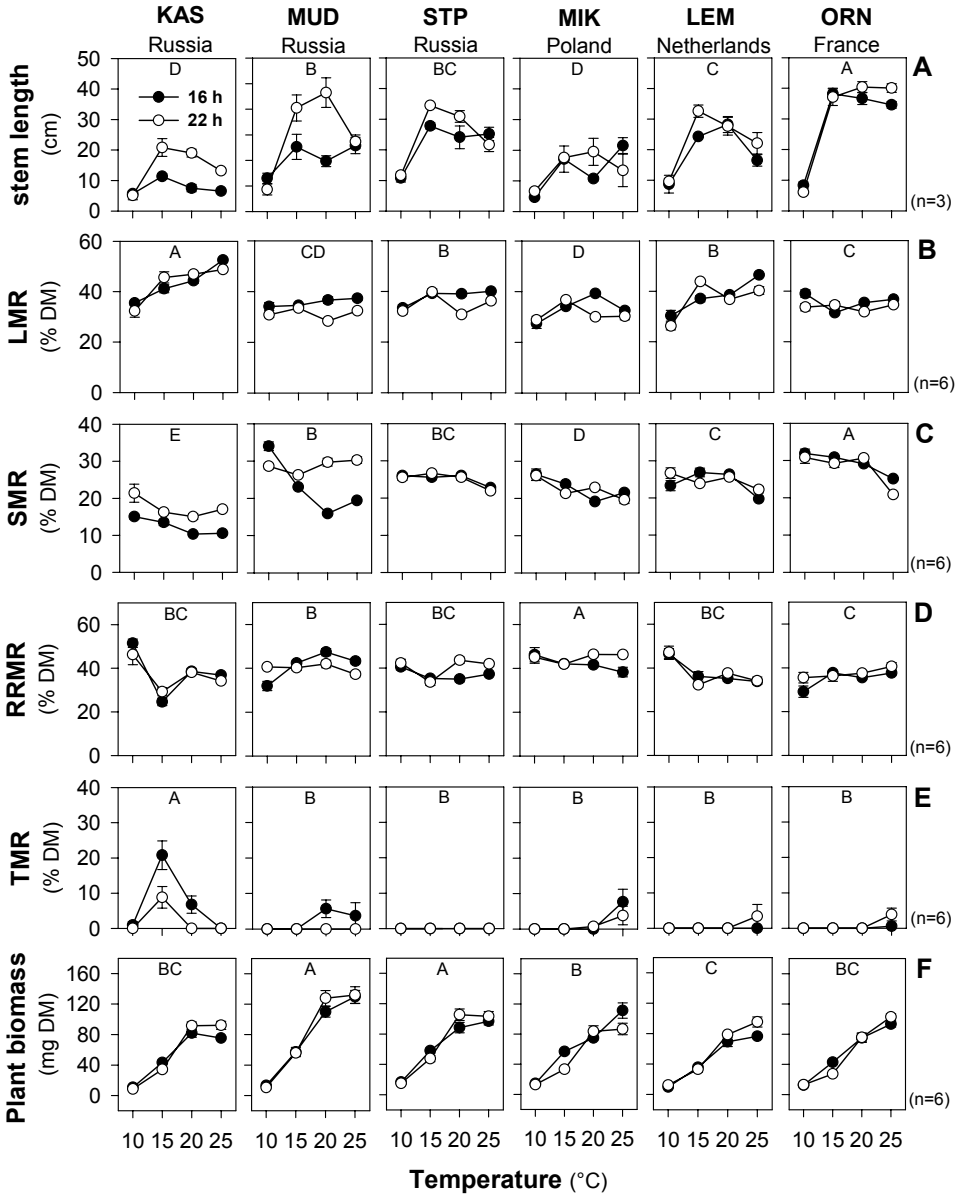


Figure 4. Reaction norms for plants grown at different combinations of water temperature and photoperiod for six clones of *P. pectinatus* (for abbreviations of clone names, see Fig. 1). (**A**): length of the main stem, (**B**): leaf mass ratio (LMR), (**C**): stem mass ratio (SMR), (**D**): root and rhizome mass ratio (RRMR), (**E**): tuber mass ratio (TMR), (**F**): total plant biomass. Shown values are means ± standard error. Non-visible standard errors are smaller than the size of the datapoints. Different capital letters indicate significant differences between the clones (Tukey a posteriori tests, $P < 0.05$).

Clone, temperature and photoperiod significantly affected all variables related to biomass allocation (Table 2). For one of the Russian clones (KAS) and the Dutch clone (LEM), leaf mass ratio (LMR) increased and root and rhizome mass ratio (RRMR) decreased with increasing water temperature, while in all other clones LMR and RRMR were largely independent of temperature (Fig. 4B and D; significant clone x temperature interactions, Table 2). The stem mass ratio (SMR) decreased with increasing water temperature, particularly for the Russian clone from Mudyug (MUD) exposed to short photoperiod (Fig. 4C; significant two and three order interactions, Table 2). For the Russian clone from Kashin (KAS), highest tuber mass ratio (TMR) was observed at 15°C, while in all other clones the production of tubers was infrequent and only occurred at 20 and 25°C (Fig. 4D; significant clone x temperature interaction, Table 2). The northernmost clone (KAS) showed the highest allocation of biomass to leaves (LMR, Fig. 4B) and tubers (TMR, Fig 4D), at the expense of stems (SMR, Fig. 4C), while the southernmost clone (ORN) allocated the highest percentage of total biomass to stems (SMR, Fig. 4C) at the expense of roots and rhizomes (RRMR, Fig. 4D). Finally, the clone from Poland (MIK) had the highest biomass allocation to roots and rhizomes (RRMR, Fig. 4D), at the expense of leaf tissue (LMR, Fig. 4B).

Total plant biomass increased with increasing water temperature (significant temperature effect, Table 2), but levelled off at 20°C (Fig. 4F). The effect of temperature varied among photoperiods (significant temperature x photoperiod interaction, Table 2), although the overall effect of photoperiod on plant biomass was not significant (Table 2). In addition, the response of biomass accumulation to differences in temperature and photoperiod varied among clones (significant clone x temperature and clone x photoperiod interactions, Table 2). In both cases, and with the exception of an increase in biomass with temperature, variation among combinations of clone x temperature x photoperiod did not show any discernible trend. Two Russian clones (MUD and STP) showed the highest plant biomass, which was lowest for the Dutch clone (LEM, Fig. 4F).

Discussion

Clonal variation in tuber sprouting

The present study shows that tuber sprouting in *P. pectinatus* is largely dependent of water temperature, which was also found for other aquatic macrophytes that rely on asexual propagules for regeneration (Kadono, 1982; Van Wijk & Trompenaars, 1985; Spencer et al., 2000). In accordance with the thermal response of many plant physiological processes (Berry & Raison, 1981), we showed that the rate of tuber sprouting increased with increasing water temperature (Table 1A). Especially at 20 and 25°C, this resulted in a high sprouting success (i.e. 93 to 100%) within a few days from the start of the experiment (Fig. 2). Although the overall effect of temperature on maximum tuber sprouting was not significant, still highest frequencies were recorded between 15 and 25°C (Fig. 2). Other studies that investigated tuber sprouting in *P. pectinatus* reported a similar range of thermal tolerance for populations from Central Europe and North America (Madsen & Adams, 1988; Van Wijk, 1989; Spencer & Ksander, 1992).

Intuitively one might expect tubers obtained from higher-latitude locations to sprout more readily at lower water temperatures. Although this expectation was supported by preliminary findings of Van Wijk (1989), who showed that at 10°C tubers of *P. pectinatus* from Finland sprouted better than those from the Netherlands; the more comprehensive data set presented here, indicates the opposite. At

lower water temperatures, both the maximum frequency of tuber sprouting (at 10°C) and the rate of tuber sprouting (at 10 and 15°C) decreased with increasing latitude (Table 1, Fig. 3). A similar relationship was found for broadly distributed plants from the terrestrial environment, as seeds collected from higher latitudes germinated at a more narrow range of higher temperatures (Sawada et al., 1994; Minggang et al., 2000). Such thermal response might be of critical importance for the survival of sub-arctic range-margin populations (here represented by the Russian *P. pectinatus* clone from Kashin [KAS]), since juvenile plant damage resulting from low water temperatures is more likely to occur at higher latitudes.

Clonal variation in growth characteristics

The investigated clones produced longest stems when they were raised at relatively low water temperatures (i.e. 15 or 20°C, Fig. 4A). Although this is in line with previous observations on *P. pectinatus* (Pilon & Santamaría, 2002), other aquatic macrophyte species produced longest stems at considerably higher water temperatures (i.e. 24 to 32°C; Barko & Smart, 1981; Barko et al., 1982). We suggest that the low thermal optima for shoot growth could be of adaptive value in areas with low spring temperatures, where shoot elongation stimulated by low irradiance would then be accelerated. Furthermore, our data showed that for *P. pectinatus* clones from higher latitudes (KAS and MUD), the increase of shoot length at intermediate water temperatures was most pronounced at long photoperiod (Fig. 4A). Similar latitude-related variation in height increment in response to differences in photoperiod was found for ecotypes of *Poa pratensis* (Aamlid, 1992) and might indicate that in broadly distributed species shoot elongation is optimised to the local photoperiod (Håbjørg, 1978; Salisbury, 1981).

Changes in biomass allocation in response to differences in abiotic conditions are related to the maintenance of an appropriate balance between shoot and root functions. At lower temperatures, nutrient availability decreases and both terrestrial (Berry & Raison, 1981) and aquatic plants (Barko & Smart, 1981; Barko et al., 1982) may respond by increasing root-to-shoot ratios. In contrast, at short photoperiods the radiant energy available for photosynthesis is reduced and plants are expected to invest a higher proportion of their resources in shoots as compared to roots. Except for the Russian clone from Kashin (KAS) and the Dutch clone (LEM), biomass allocation was largely unresponsive to differences in water temperature and/or photoperiod (Fig. 4B to E). The previous indicates that in *P. pectinatus*, plasticity in biomass allocation has limited importance for survival across climatic gradients. Alternatively, changes in morphology, independent of differences in biomass allocation, might be effective to cope with geographic variation in abiotic conditions. For example, at low temperature the production of more fine roots could result in a higher efficiency for nutrient uptake (Chapin III, 1974), while at short photoperiod an increase of the specific leaf area (SLA) could lead to a higher capacity for light harvesting (Carlen et al., 1999).

Biomass allocation among different plant parts is not only determined environmentally, but also genetically. In broadly distributed plants, genetic variation among distant populations can be related to the climatic conditions at the original growing site (Reinaertz, 1984; Sawada et al., 1994). Although in the present experiment significant clonal variation in biomass allocation was found (data of all treatments pooled); no consistent pattern of variation with latitude was apparent. The Russian clone from the northern distributional range-margin of *P. pectinatus* (KAS) clearly differed from all other clones (Fig. 4). Its low stem mass ratio probably resulted from the production of very short stems (Fig. 4A and C; similar to Pilon & Santamaría, 2002), while the high tuber mass ratio (at 15°C, Fig. 4E)

indicates an early onset of reproduction (similar to Pilon et al., 2002). After 7 weeks of growth, only the sub-arctic clone from Kashin (KAS), which is normally exposed to particularly short growth seasons, had already allocated a substantial proportion of its resources to tubers (similar to Pilon et al., 2002).

For all investigated clones of *P. pectinatus*, final biomass yield increased with increasing water temperature, but levelled off above 20°C (Fig. 4F). We therefore expect maximum biomass productivity to occur within the thermal range reported for most temperate aquatic angiosperms, among which *P. pectinatus* (24 to 32°C; Pilon & Santamaría, 2002; Vermaat & Hootsmans, 1994; Barko & Smart, 1981; Barko et al., 1982). In contrast with the findings for terrestrial angiosperms (e.g. Sale & Orr, 1987), we did not detect a significant increase in biomass yield when the clones were raised at long photoperiod (similar to Spencer & Anderson, 1987). As a consequence, the hypothesis that higher-latitude clones would profit from a 22-h photoperiod, which could compensate for the decrease in growth at temperatures below 15°C (cf. Hay & Heide 1983), was not confirmed. As an alternative, we suggest that *P. pectinatus* clones from sub-arctic regions adjust their phenology, i.e. they show a compressed life cycle (with earlier reproduction) and grow later in the season to avoid unfavourable water temperatures (similar to Criddle et al., 1994 for terrestrial plants).

Conclusions

Our investigation showed that across a wide range of latitude, *P. pectinatus* tubers show comparable sprouting characteristics at largely different water temperatures (i.e. 15-25°C). However, at lower water temperatures both the maximum frequency of tuber sprouting (10°C) and the rate of tuber sprouting (10 and 15°C) were negatively correlated with the latitude of origin of the clones. This may prevent tuber sprouting early in the season, when water temperature may periodically drop below the tolerance range for plant survival. We thus confirmed the hypothesis that ecotypic differentiation in sprouting characteristics exists among *P. pectinatus* clones from different latitudes. Furthermore, lower water temperatures resulted in an overall decrease in biomass yield, but none of the investigated clones showed an increased growth at longer photoperiod. Consequently, we rejected the hypothesis that higher-latitude clones make more efficient use of increased light availability associated with a prolonged photoperiod, thus compensating for the effect of low temperature.

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CHAPTER 7

Plant performance across latitude: the role of plasticity and local adaptation

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Summary

Geographic variation can lead to the evolution of different local varieties within a given species, therefore influencing its distribution and genetic structure. We investigated the contribution of plasticity and local adaptation to the performance of a common aquatic plant (Fennel pondweed *Potamogeton pectinatus*) at contrasting climates, using reciprocal transplants at three experimental sites across a latitudinal cline in Europe. Plants from 54 clones, originally collected from 14 populations situated within four climatic regions (sub-arctic, cold-temperate, mild-temperate and Mediterranean) were grown in three different localities within three of these regions (cold-temperate: Norway; mild-temperate: The Netherlands; Mediterranean: Spain). Tuber production was highest for the Central European clones, independently of the experimental site where the clones were grown. Selection coefficients indicated that populations at the European centre of the species distribution perform better at all sites. These results are more in agreement with the observation that range-marginal populations show decreased fitness due to genetic processes (such as genetic drift and inbreeding) than with processes of differentiation due to local adaptation. However, marginal populations showed changes in life-history traits (compressed life cycles in the north, perenniality in the south) that may allow them to perform better locally (at the distribution range limits). Our results thus suggest that local adaptation may overlap spatially with centre-periphery gradients in performance caused by genetic factors.

Introduction

Common plant species with wide distributions may perform well across a wide range of environmental conditions (Joshi et al., 2001). However, the capacity of individual genotypes to perform well over the full range of conditions is frequently limited (De Witt et al., 1998; Joshi et al., 2001). Common terrestrial species are often characterised by both phenotypic plasticity and large genetic variation (Bradshaw, 1984) and by local specialisation to particular environmental conditions (Joshi et al., 2001; Van Tienderen, 1990). In contrast, aquatic plant species tend to have broad distributions (Sculthorpe,

1967) and limited genetic variation (Barret et al., 1993), suggesting the widespread occurrence of general-purpose genotypes among them (Santamaría, 2002; Pilon et al., 2002). General-purpose genotypes are able to maintain a high fitness over broad ranges of environmental conditions through compensatory plastic responses in morphology, physiology and/or phenology (adaptive plasticity; Schlichting, 1986; Dudley & Schmitt, 1995; Pilon & Santamaría, 2002).

One of the largest sources of environmental variation, particularly at large (continental) scales is climate. This is reflected in world-wide vegetation zones, which follow the latitudinal patterns of variation in the prevailing environmental conditions (e.g. Walter, 1973); and in global patterns of plant distribution, which are largely explained by species-specific responses to temperature and precipitation (e.g. Woodward & Williams, 1987; Woodward, 1988; 1990). Solar irradiance and temperature decrease with increasing latitude, while summer photoperiod increases (Kirk, 1983). Since these variables are known to influence many aspects of plant life (Berry & Raison, 1981; Björkman, 1981; Salisbury, 1981), widely distributed plant species may be expected to show phenotypic variability across latitude. Genetically based latitudinal variation in phenology (Potvin, 1986), growth (Chapin III, 1974; Sawada et al., 1994; Li et al., 1998; Clevering et al., 2001) and sexual reproduction (Aizen & Woodcock, 1992; Winn & Gross, 1993; Clapham, 1998) has been reported among terrestrial species. In general, ecotypes originating from higher latitudes showed relatively small statures, early flowering and decreased fecundity due to lower growth rates or short developmental cycles (Chapin III & Chapin, 1981; Potvin, 1986; Winn & Gross, 1993; Li et al., 1998). While some information is available for seagrasses (Phillips et al., 1983) and marine macro-algae (Strömgren, 1986; Orfanidis & Breeman, 1999), latitudinal responses in freshwater angiosperms has received little attention. Because the latter are among the most widely distributed plants (Santamaría, 2002; Sculthorpe, 1967) and represent a key component of shallow and littoral aquatic ecosystems (Carpenter & Lodge, 1986), we were interested in determining the extent to which their broad distributions are related to local specialisation of populations from different climatic regions.

The contribution of environmental and genetic variation to phenotypic variability and performance can be assessed using reciprocal-transplant experiments, where significant home versus away advantages are assumed to reflect local specialisation and, more liberally, local adaptation to conditions prevailing at the home site (e.g. Joshi et al., 2001). Reciprocal transplants have typically been performed over small scales and in terrestrial species (Bradshaw, 1984; Schmid, 1985; Linhart & Grant, 1996), often revealing the existence of local adaptation (e.g. McGraw & Antonovics, 1983; Nagy & Rice, 1997). Population differentiation in morphological and physiological traits has also been reported for a few aquatic species, typically involving laboratory experiments rather than outdoor transplants (e.g. Koch & Dawes, 1991; Koch & Seeliger, 1988; Barret et al., 1993). Recently, Joshi et al. (2001) extended this replant-transplant approach to a European scale, aiming at detecting local specialisation by adaptation to conditions at home sites and correlated differences in selection against away strains and climatic distances between sites. They found overall performance of three grassland species to decline with increasing transplanting distance, being highest for home replants (Joshi et al., 2001). However, climatic distance was not correlated with selection indices and only accounted for 18% of the variation related to geographic distance, suggesting that the observed pattern of variation could be caused by factors other than climate (such as biotic influences). Since Joshi et al. (2001) performed their experiment directly in local field sites, they were not able to evaluate the contribution of local (e.g. edaphic or biotic conditions) *versus* climatic environmental variation to the observed patterns of local specialisation. In contrast, we attempted to minimise local variation by standardising most environmental variables (e.g. soil characteristics and water chemistry) not related to climatic

conditions (i.e. temperature, irradiance and photoperiod). Furthermore, we attempted to evaluate the relative contribution of plasticity to the observed patterns of phenotypic variation and to remove the influence of maternal carry-over effects (Lacey, 1998; Rossiter, 1998) by multiplying all sampled genotypes under standardised conditions during a complete growth season prior to transplanting their clonal propagules to all our experimental sites.

Material and methods

Study species

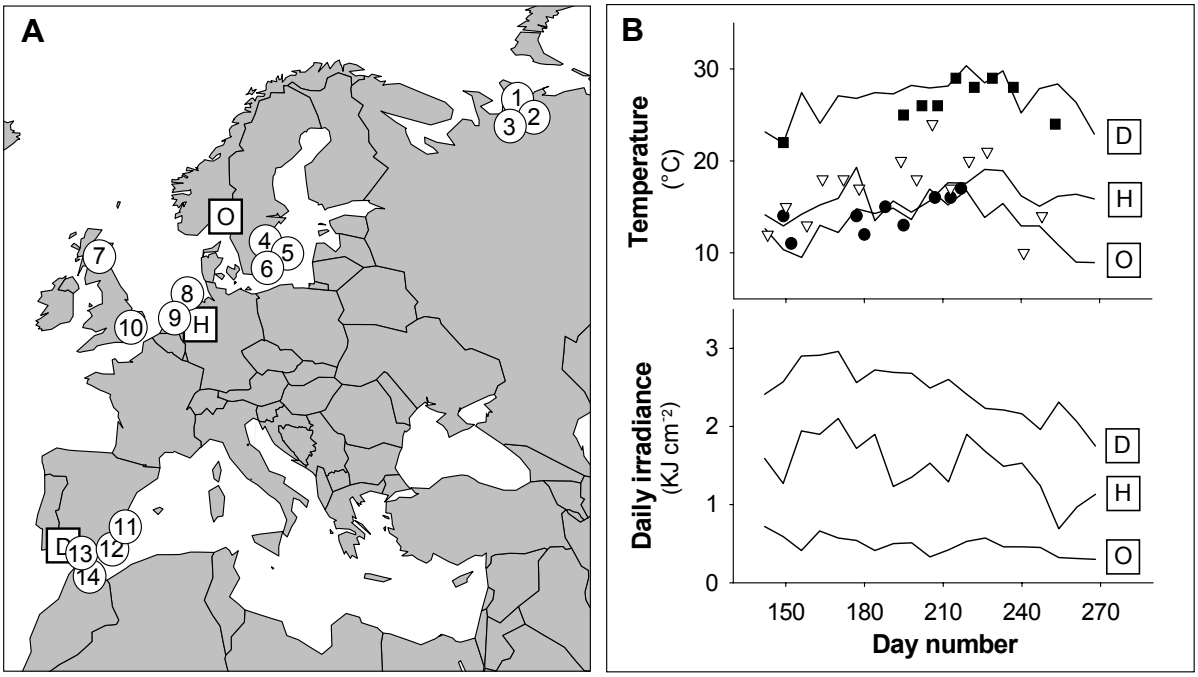
Fennel pondweed (*Potamogeton pectinatus* L.) is a submerged angiosperm with a pseudo-annual life cycle and a cosmopolitan distribution (Casper & Krausch, 1980; Wiegleb & Kaplan, 1998). Plants grow during spring-summer and survive the winter by means of subterranean tubers (specialised asexual propagules). Under mild climatic conditions, *P. pectinatus* may grow as a perennial (Yeo, 1965; Van Wijk, 1988). Seed production shows large inter-annual variability and its contribution to yearly population recruitment is generally considered to be minimal in temperate regions (e.g. Van Wijk, 1989a). Instead, seeds might be important for dispersal and establishment after disturbances (Van Wijk, 1989a). Given the limited importance and reliability of short-term sexual fecundity estimates, we considered that asexual population growth rate (*r*; a surrogate of clone survivorship) represents a better fitness-correlate than fecundity (see also Crone, 2001).

Reciprocal transplant experiment

In summer-autumn 1998 and spring 1999, tubers and/or rhizome fragments were collected from at least four spaced (>10 m) individuals at each of 14 localities in Europe and North Africa, grouped within five discrete latitudinal ranges taken to represent different climatic regions (Fig.1A and C). In summer 1999, we grew a single tuber or rhizome fragment from each sampled individual (i.e. one putative clone) under outdoor, common-garden conditions in Heteren (The Netherlands), in order to obtain clonal propagules (tubers). The Mediterranean populations failed to produce tubers under such circumstances (since they are probably perennial); hence we kept the plants growing during the following winter in indoor aquaria and utilised rhizome fragments as propagules. Growing the plants indoors during the winter was also necessary for a few Swedish clones, due to their low tuber production. For such clones, both tubers (from the previous autumn) and rhizome fragments (from living plants) were used as propagules in the transplant experiment. Out of five clonal fragments collected from Loch Gelly (UK), only three clonal lines became established during the propagation phase; hence, we were only able to use three clones for this population.

In northern regions (UK, Sweden and Russia), *P. pectinatus* hybridises with two closely related species of the subgenus *Coleogeton*. The resulting hybrids, *P.* x *suecicus* (*P. pectinatus* x *P. filiformis*) and *P.* x *bottnicus* (*P. pectinatus* x *P. vaginatus*), are very difficult to discriminate from *P. pectinatus* on the basis of morphological traits (Preston et al., 1998; Preston et al., 1999). For this reason the identity of the collected clones was investigated by means of molecular tools (RFLP on nuclear and chloroplast DNA, extracted from freshly grown material; King et al., 2001). In addition, sequencing data were used to discriminate between different *P. pectinatus* clones, i.e. to make sure that the

putative clones selected for the experiment (i.e. from all geographic regions) indeed represent different genotypes.



C

Region	No.	Population	Latitude	Longitude	Climatic region	No. clones
Northern Russia	1	Zelonii Island	68.28°N	54.15°E	Sub-arctic	4
	2	Gusinec Shar	68.25°N	53.72°E		4
	3	Srednii Shar	68.10°N	53.63°E		3
Sweden	4	Sandviken	58.80°N	17.68°E	Temperate-cold	4
	5	Vassviken	58.82°N	17.63°E		4
	6	Sillensvik	58.82°N	17.65°E		4
Scotland	7	Loch Gelly	56.15°N	3.30°W	Temperate	3
Netherlands & UK	8	Lauwersmeer	53.33°N	6.23°E	Temperate-mild	4
	9	IJsselmeer	52.85°N	5.68°E		4
	10	Ouse Washes	52.79°N	0.37°E		4
Spain & Marocco	11	Santa Fé	38.22°N	0.58°W	Mediterranean	4
	12	El Hondo	38.12°N	0.42°W		4
	13	Veta la Palma	36.93°N	6.22°W		4
	14	Sidi Moussa	35.24°N	2.94°W		4

Experimental site	Country	Latitude	Longitude	Climatic region
Oslo	Norway	59.92°N	10.77°E	Temperate-cold
Heteren	The Netherlands	51.92°N	5.85°E	Temperate-mild
Donaña	Spain	37.00°N	6.45°W	Mediterranean

Figure 1. (**A**) Geographic origin of *P. pectinatus* populations used in the transplant experiment (circles with population numbers) and the location of the three experimental sites where all selected clones were grown (squares). In the squares 'O' refers to Oslo (Norway), 'H' to Heteren (The Netherlands) and 'D' to Doñana (Spain). (**B**) Weekly averages of mean air temperature (lines), mean water temperature (symbols) and daily irradiance at the three experimental sites for the period between May 15th and September 24th 2000. (**C**) Geographic characteristics of the *P. pectinatus* populations and the experimental sites.

For the experiment (summer 2000), we grew plants from the 54 clonal lines at three distant localities, situated at different latitudes and having contrasting climates (Doñana, SW Spain; Heteren, The Netherlands and Oslo, SE Norway; Fig. 1). The region of origin of the clones corresponded to the three experimental localities, except for the clones originating from Scotland (situated between two of

the experimental sites; Fig. 1A and C) and Northern Russia (situated in the northern range-limit of the species). The design was not fully balanced, due to shortage of tubers from a few clones, although we aimed to use five replicates per clone and growing site. In total 613 plants were finally grown (204 in Norway, 207 in The Netherlands and 202 in Spain), corresponding to a factorial combination of 3 sites x 54 clones using an average of 3.8 replicates.

Each replicate consisted of an individual plant, grown from a tuber or a rhizome fragment, planted in 4 l pots (upper diameter: 21 cm) filled with a mixture of sand and potting clay (in a dry weight ratio of 3:1) covered with 2 cm of washed aquarium sand. At each experimental site, the pots were randomly interspersed among 9 containers (of 110 cm length x 95 cm width x 65 cm height) filled with water. To minimise variability in water chemistry (given the higher nutrient load and the higher mineral content that characterise the available surface water in Spain and The Netherlands respectively), water was obtained from a nearby oligotrophic lake in Norway and pumped from nearby sandy aquifers in The Netherlands and Spain. Total P and total N concentrations in the sediment mixture were 290 mg P kg^{-1} and 650 mg N kg^{-1}, representing a supply of 1396 mg P and 5109 mg N per pot, enough to prevent nutrient-limitation of plant growth (Van Wijk, 1989b). The water supplied a minimal amount of nutrients in comparison with the sediment, hence the variability caused by differences in nutrient supply related to the different water sources used were irrelevant. Although we aimed at placing the containers in open sunlight, in Norway and Spain they received some shading in the afternoon hours, caused by nearby trees and buildings respectively. Each container was covered with neutral-density shading net (Agronet®-N35; reducing light availability by 35%) to reproduce shading conditions typical of the shallow lakes inhabited by *P. pectinatus* and limit the amount of debris and insects falling into the water.

Plant propagules were planted in mid-May 2000, by inserting them carefully through the first 2 cm of washed aquarium sand into the clay-sand sediment mixture. Plants were cultivated until the end of September, when they were harvested by washing off the sediment using pressurised water and a sieve with a mesh-size of 2 mm. In the laboratory, we measured the length of the longest shoot and the number of ramets per plant, and separated the aboveground (shoots) and belowground (roots + rhizomes) fractions to measure their dry weight (after 24h at 70°C). Tubers were weighed individually (fresh weight) and a randomly chosen subsample of tubers was used for dry weight determinations (as above; N=240). Based on this subsample, the dry weight of the remaining tubers was estimated from linear regressions relating dry weight to fresh weight (separately for each region from where the clones originated; R^2 always above 0.95).

In pseudo-annual plants, asexual rates of population growth (r) are best estimated as the product of asexual fecundity (*af*, calculated here as the average number of tubers per clone, estimated separately for each experimental site) and survival probability (p, calculated here as the proportion of plants that produced at least one ramet or one viable tuber, for each clone and experimental site), i.e. as $r = p \times af$. We then used r as a measure of plant performance or fitness, and calculated selection coefficients for each clone i relative to the best-performing clone at each particular experimental site as $s_i = 1 - (r_i / r_{max})$. s_i thus ranges from 0 (for the most successful clone at each site) to 1 (indicating complete selection against a given clone). This procedure may underestimate the performance of perennial plants growing in mild climates, since clones that produced no tubers have a $s_i = 1$. For this reason, fitness estimates of plants that produced no tubers but did not senesce by the end of the experiments (i.e. the Mediterranean clones growing in Spain) were also calculated using asexual rates of population growth based on the average number of ramets per plant (i.e. the number of potentially

independent clonal individuals) instead of the number of tubers. Fitness estimates using tubers or tubers+ramets were almost identical; hence, we will only report on the latter.

Statistical analysis

All variables measured (total biomass, vegetative biomass and tuber biomass; number of tubers and tuber size; shoot length, shoot-root ratio and number of ramets; and clonal survival) and fitness estimates were analysed by means of hierarchical, mixed-models ANOVAs, performed in the General Linear Models module of STATISTICA 5.5 (Statsoft, 1999). Dependent variables were $\log_{10}$ or square root transformed as necessary to assure normality and remove heteroscedasticity. In the ANOVAs, the fixed factor 'experimental site' was factorially crossed with the fixed factor 'region of origin', with the random factor 'population' (which was nested within 'region of origin') and with the random factor 'clone' (which was nested within 'population'). We regarded 'experimental site' and 'region of origin' as fixed factors because we were interested in their interaction with each other. In addition, we added a factor coding the type of propagule from which the individual plants grew (tubers vs. rhizome fragments) and two continuous variables to account for the size of the propagule ('initial tuber fresh weight' and 'length of the initial rhizome fragment'). For this analysis, we only used data from those plants that became established (i.e. that produced at least one ramet or one viable tuber). Survival and fitness estimates were obtained as a single value per clone and experimental site; hence, mixed-model ANOVAs left out the factor 'clone' and its interactions, and the covariables 'type of propagule', 'average initial tuber fresh weight' and 'average length of the initial rhizome fragment' were entered as the average values per clone per site.

Environmental ('site') and genetic (decomposed in 'region', 'population' and 'clone') main effects were interpreted to represent the general superiority of plants growing at, or originating from a particular site; while interaction effects indicate that different strains respond differently at different sites (similar to Joshi et al., 2001). In the site x region matrix (Table 2), a home effect is represented by values found on the diagonal in bold and an increasing distance effect is reflected by values farther away from the diagonal in bold.

Results

During the experimental period (May-September 2000), pronounced climatic differences existed between the experimental sites in Norway, The Netherlands and Spain. *P. pectinatus* clones that were grown at higher latitudes experienced lower air and water temperatures and lower levels of daily irradiance than those grown in the south (Fig. 1B).

Environmental ('site') and genetic ('region', 'population' and 'clone') effects, as well as their interactions on life-history characters were statistically significant in most cases (Table 1). Environmentally induced variation occurred in all variables measured, reflecting an increase in performance with decreasing latitude (Fig. 2). Genetic effects occurred mostly at regional level, with almost no significant differences among populations and few among clones (Table 1). Most genetic x environmental variation was explained by region x site interactions, with few significant interactions at population and clone levels (Table 1). Propagule size and type had limited effects on plant growth, affecting significantly only a few variables (Table 1).

Table 1. Results of mixed-model General Linear Modelling, analysing the influence of environmental and genetic effects on life-history traits, survival and selection coefficients of 54 clones of *P. pectinatus* collected over a broad latitudinal range. NS: not significant, $P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001. S: site, R: region, P: population, C: clone.

	Main effects				Interactions			Propagule type	Propagule size	
	S	R	P	C	S x R	S x P	S x C		tuber	rhizome
df(factor)	2	4	8	36	8	16	64	1	1	1
df(error)	19-21	9-10	19-23	73-76	17	77-80	284-296	284-296	284-296	284-296
Total biomass	163.4 ***	16.7 ***	0.3 NS	1.7 *	2.3 $	1.7 $	1.0 NS	2.2 NS	0.2 NS	2.9 $
Vegetative biomass	103.0 ***	21.1 ***	0.2 NS	1.6 *	3.1 *	1.6 $	1.0 NS	2.6 NS	0.2 NS	3.3 $
Tuber biomass	121.5 ***	11.7 ***	0.6 NS	1.9 *	7.7 ***	1.8 *	1.2 NS	0.6 NS	0.8 NS	0.5 NS
Number of tubers	272.6 ***	11.5 ***	0.8 NS	1.7 *	13.1 $	1.6 $	1.2 NS	2.1 NS	0.1 NS	2.0 $
Tuber size	59.0 ***	13.3 ***	2.2 $	2.3 **	21.0 ***	0.3 NS	1.1 NS	0.3 NS	13.2 ***	0.3 NS
S/R-ratio	16.3 ***	3.3 $	1.8 NS	1.6 *	6.9 ***	0.9 NS	1.2 NS	3.9 *	1.0 NS	1.8 NS
Shoot length	25.1 ***	8.2 **	1.2 NS	0.9 NS	2.5 $	1.6 $	2.1 ***	1.3 NS	5.7 *	2.0 NS
Number of ramets	184.7 ***	14.4 ***	1.7 NS	1.3 NS	4.8 **	1.4 NS	1.2 NS	0.7 NS	0.1 NS	2.1 NS
df(factor)	2	4	9		8	18		1	1	1
df(error)	20-21	11-13	21-24		19-21	115-118		115-118	115-118	115-118
Survival	8.5 **	8.4 **	2.7 *		0.7 NS	0.9 NS		1.0 NS	7.5 **	24.6 ***
Selection coefficient	10.7 ***	1.4 NS	3.5 **		5.3 **	1.4 NS		0.1 NS	20.1 ***	8.3 **

Biomass yield (total, vegetative and tubers) increased significantly when the plants were grown at sites of decreasing latitude (Table 1, Fig. 2A and D). Total biomass yield and tuber production were higher for Central-European clones (Netherlands/UK and Scotland) and lower for northern (Swedish/Russian) and southern (Spanish/Moroccan) clones. Vegetative biomass followed the same trend, except for the Mediterranean clones grown in Spain, which had the highest biomass yield observed in the experiment (Fig. 2A). Even in their 'native' experimental site, however, most Mediterranean plants (i.e. most clonal replicates of the Mediterranean clones) failed to produce tubers (Fig. 2D).

The Swedish and Mediterranean clones produced fewer but larger tubers (Fig. 2E and F) and showed also the largest plasticity in tuber size, with was significantly reduced when the plants grew in 'less favourable' experimental sites (further north than Spain for the Mediterranean clones and further north than The Netherlands for the Swedish clones; Fig. 2F).

Shoot length increased with decreasing latitude of origin, being maximal for the Mediterranean clones. It also tended to decrease from southern to northern experimental sites (Fig. 2C). The relative differences among experimental sites varied for the different regions of origin. For the Mediterranean clones, differences in shoot length were large between the experimental site in Norway and those in The Netherlands and Spain, which did not differ significantly among themselves (Scheffé post-hoc tests: $P<10^{-5}$, $P<10^{-8}$ and $P=0.99$, respectively). For the Russian clones, the pattern was reversed: differences were large between the experimental site in Spain and those in The Netherlands and

Norway, which were not significantly different (Scheffé post-hoc tests: P=0.037, P=0.0001 and P=0.99, respectively). For the Dutch/English and Swedish clones, differences between sites were more homogeneous; no significant variation was detected for the Scottish clones.

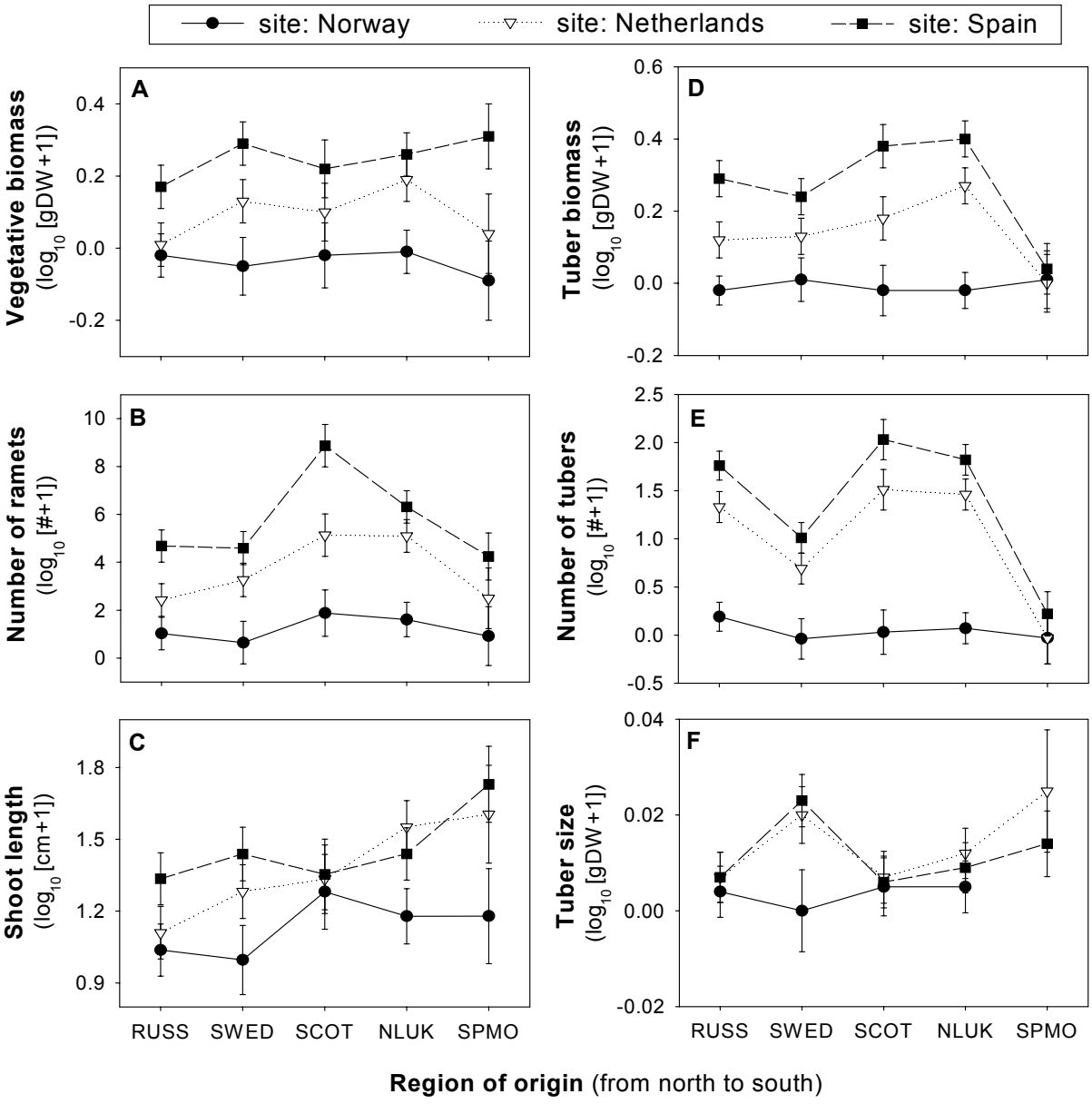


Figure 2. Vegetative biomass (**A**), number of ramets (**B**), shoot length (**C**), tuber biomass (**D**), number of tubers per plant (**E**) and tuber size (**F**) for *P. pectinatus* clones originally collected at five different regions across Europe (RUSS: Northern Russia, SWED: Sweden, SCOT: Scotland, NLUK: The Netherlands / United Kingdom, SPMO: Spain / Morocco), when grown under common-garden conditions at three experimental sites (Norway, The Netherlands and Spain). Shown are log-transformed means ± standard error. Means were computed at the covariate's mean (log_{10} tuber mass: 0.017; log_{10} rhizome length: 0.28).

Ramet production increased from experimental sites in the north to those in the south (Fig. 2B), while shoot-to-root ratio tended to be lower at the Norwegian site (not shown). Variation among regions of origin was correlated for both variables. Scottish and Dutch/English clones showed the highest ramet production (Fig. 2B) and lowest plasticity in shoot-to-root ratio (not shown). Russian,

Swedish and Mediterranean clones showed larger plasticity in shoot-to-root ratio (not shown) and a narrower plastic range in ramet production (Fig. 2B).

Seed production was very low. Only a few plants produced seeds, and they were in all cases Mediterranean clones (2 out of 16 clones) growing in Spain. On average, 14% of all Mediterranean plants growing in Spain produced seeds (not shown).

Plant survival varied significantly among sites, and among the regions and populations where the clones originated (Table 1). However, none of the interactions with experimental site were significant (i.e. all clones responded similarly when grown at all experimental sites). Survival was significantly lower in Norway than in The Netherlands and Spain ($P<0.003$, Scheffé post-hoc tests), which did not differ significantly ($P=0.44$). The Mediterranean clones showed a significantly lower survival than all other clones ($P<10^{-7}$ for all post-hoc comparisons involving the Mediterranean clones, Scheffé post-hoc tests), that did not differ significantly among themselves ($P>0.18$; data not shown). The type of propagule from which the plants grew had no significant effects on survival; propagule size was, on the other hand, positively correlated with survival (for both types of propagules; Table 1).

Selection coefficients did not vary significantly among sites, but they varied among the regions and populations where the clones originated and were significantly affected by the site x region interaction (Tables 1 and 2). At all experimental sites, Central-European clones (from Scotland and The Netherlands/UK) had the highest fitness, while those from the Mediterranean and Swedish regions had the lowest. Russian clones had intermediate fitness in Spain and The Netherlands and higher fitness (though not significantly different to the Central-European clones) in the Norwegian site (Scheffé post-hoc tests; Table 2).

Table 2. Selection coefficients for *P. pectinatus* genets obtained from different geographic regions and grown at three different sites. Different letters indicate significant differences between regions for a given experimental site, with lettering order indicating increasing selection coefficients (Scheffé a posteriori test, $P < 0.05$). A 'home' effect is shown on the diagonal (in bold and italics) and a 'distance' effect is reflected by changing values away from the diagonal. Confidence limits (95%) are indicated between brackets. N = number of genets.

Region of origin	Experimental site						
	Norway		Netherlands		Spain		N
Russia	***0.523*** ^{a}	(0.31-0.73)	0.666 ^{ab}	(0.55-0.78)	0.612 ^{ab}	(0.49-0.74)	12
Sweden	***0.987*** ^{b}	(0.96-1.02)	0.889 ^{bc}	(0.81-0.97)	0.908 ^{b}	(0.86-0.95)	12
Scotland	0.848 ^{ab}	(0.69-1.01)	***0.360*** ^{ab}	(0.15-0.57)	0.161 ^{ab}	(-0.35-0.68)	3
Netherlands/UK	0.732 ^{ab}	(0.57-0.90)	***0.464*** ^{ab}	(0.32-0.61)	0.455 ^{ab}	(0.32-0.59)	12
Spain/Morocco	1.000 ^{b}		0.997 ^{c}	(0.99-1.00)	***0.902*** ^{c}	(0.85-0.95)	16
Mean	0.83	(0.75-0.90)	0.75	(0.67-0.82)	0.70	(0.62-0.77)	55

Discussion

Our results show that most *P. pectinatus* clones can grow and reproduce asexually at distant latitudes. Sub-arctic and temperate clones grew and produced tubers at the three experimental sites (Norway, the Netherlands and Spain). For all clones, independent of the region and population of origin, biomass yield and tuber production increased when grown at decreasing latitude. This might suggest

that, in Europe, optimal conditions for *P. pectinatus* growth are encountered in the Mediterranean region. It should be noted, however, that habitat availability in that region is limited by the scarcity of permanent waterbodies (since most shallow lakes dry up during summer) or by major fluctuations in water depth (Gafny & Gasith, 1999). Hence, *P. pectinatus* is most abundant in Central Europe (Casper & Krausch, 1980), although the potential for clonal growth and multiplication is higher in the south (see also Yeo, 1965).

Tuber production was highest for the Central-European clones (i.e. those originally collected at temperate-mild regions: The Netherlands and the UK, 52-56°N), independently of the experimental site where they were grown. Selection coefficients indicated that populations at the European centre of the species distribution perform better at all sites (the second component of survivorship, plant survival during the growth season, did not vary significantly among non-Mediterranean clones). Local adaptation thus seems to play a minor role. Our results are more in agreement with the observation that range-marginal populations show decreased fitness due to genetic processes, such as genetic drift and inbreeding (Jonas & Geber, 1999). It is noteworthy that a different component of asexual fitness, ramet production, shows a comparable pattern of variation among clones.

However, in our high-latitude experimental site (Norway), Russian clones showed increased fitness (though not significantly higher than the Dutch and Scottish clones). For these clones, marginal changes in survival seem to compensate for the decreased tuber production when growing under the limiting (northern-range) conditions typical of cold-temperate and sub-arctic sites. Our results are consistent with common-garden experiments demonstrating that high-latitude populations of *P. pectinatus* complete their life cycle and produce tubers earlier (Pilon et al., 2002). Compressed life cycles are generally predicted to increase survival in the northern range (due to the short summers and the unpredictable conditions in late spring and early autumn) at the cost of reduced plant growth and propagule size when growing in more favourable environments.

Mediterranean clones failed to produce tubers by mid-September at the three experimental sites. Though they had a biomass yield comparable to all other clones (except the Russian), they had a higher proportion of non-senescent biomass and much thicker rhizomes at harvest. In the temperate and sub-arctic region, surviving without tubers (i.e. as a perennial) is rarely possible due to the severity of the winter season (with strongly reduced photoperiods, low water temperatures and frequent frost damage in shallow areas); hence, Mediterranean clones would not be able to establish. In Mediterranean climates (with extended spring-autumn growth seasons and mild winters), on the other hand, perenniality and/or delayed tuberisation are likely to result in increased competitive ability.

Hence, Mediterranean clones seem to be locally adapted: owing to their perennial life cycle they would be unable to become established in temperate to sub-arctic populations, but they may have a competitive advantage in climates with mild winters. Correlated changes in shoot length, shoot-to-root ratio and tuber size are consistent with this hypothesis. Shoot length increased with decreasing latitude of origin of the clones. Early elongation of shoot internodes is a well-known response to shading, and high plasticity in this trait confers a competitive advantage in environments where competition for light is a significant selection factor (e.g. Dudley & Schmitt, 1995; Schmitt et al., 1995). In southern sites, characterised by high population densities and high biomass standing crop (due to the mild winters and better growth conditions during spring-summer; Santamaría, personal observation), this trait will probably confer a competitive advantage to the local clones. Mediterranean clones showed larger plasticity in shoot-to-root ratio (a trait also involved in light-competition responses) than the Central-European clones, and they produced larger tubers, which are known to confer a competitive advantage in dense populations (Spencer & Ksander, 1995; Rodriguez-Gironés

et al., unpublished manuscript). It is however remarkable that the Swedish clones show a similar pattern of variation; until more information becomes available on the relative contribution of tuber size and shoot-to-root allocation to competition and plant survival under different climatic regimes, it is difficult to interpret these traits more accurately.

Our data seem thus open to conflicting interpretations. On the one hand, variation in life-history traits and fitness estimates suggests that clones originating from temperate regions have general-purpose genotypes able to grow optimally at very distant latitudes. On the other hand, range-limit populations show changes in their life history (compressed life cycles in the north, perenniality and more investment in seeds in the south) that may allow them to perform better locally. This discrepancy may be caused by limitations in the duration of our experiment, which does not allow for long-term estimates of fitness (i.e. over several clonal generations) that would take into account variable life histories among different clones. Alternatively, we may be looking at the spatial overlap of two different processes. Local adaptation may be constrained, but still arise among populations showing centre-periphery gradients in plant performance due to genetic effects (as described above). Under such circumstances, the likelihood of finding observable effects reflecting local adaptation (i.e. home versus away advantages) will be highest in experimental manipulations over large geographic areas and strong environmental gradients, particularly those covering the full gradient between both distributional range limits in broadly distributed species (as we did here).

Finally, it is worth noting that tuber size is likely to interact with the results reported in this paper. We standardised tuber and rhizome size to minimise their influence on the outcome of the experiment. It may be, however, that tuber size is one of the traits influencing survival in a given region. Indeed, we have observed significant variation in the size of newly produced tubers, among clones (genetic effects), among experimental sites (plastic effects) and among their combinations (genetic variation in the reaction norm). Unfortunately, it is very difficult to predict and interpret the effect of propagule size for two reasons. Firstly, there is a trade-off between the increasing construction costs of larger propagules and the increased plant fitness they confer (through increased sprouting success, competitive ability and resistance to partial damage; Spencer & Ksander, 1995; Santamaría & Rodriguez-Gironés, 2002; Westoby et al., 1992; 1996). Hence, information on both sides of the trade-off is needed for an interpretation of tuber size variation. For example, Swedish clones that produce larger tubers might benefit from the higher initial growth rates they confer, which is of particular importance in cold regions with short summers. However, the high construction costs of large tubers might constrain their production in the colder and shorter summers of sub-arctic Russia. Secondly, tuber size shows broad plastic variation (e.g. larger plants tend to produce larger tubers). Plants might thus optimise the fitness of their asexual descendants through plastic changes in tuber size (adaptive phenotypic plasticity). In this respect, progress in the understanding of the regulation of propagule size and its effects on plant fitness will contribute significantly to the understanding of plant local adaptation as a response to spatial heterogeneity.

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CHAPTER 8

Summarising discussion

The performance of broadly distributed plants is potentially constrained by geographic variation in climatic factors. Several patterns of response have been proposed that can be considered of adaptive value if variation in abiotic conditions is pronounced. Firstly, species may possess a high capacity for phenotypic plasticity that results in increased tolerance to variation in abiotic factors (Via et al., 1995). Secondly, locally adapted genotypes may evolve that show phenotypic characteristics that are only suited for a restricted set of environmental conditions (Van Tienderen, 1991). Thirdly, populations may avoid unfavourable conditions by showing changes in phenology or life-cycle strategy (e.g. Phillips et al., 1983; Criddle et al., 1994; Clevering et al., 2001).

As compared to terrestrial species, many aquatic plants are widespread and have the ability to thrive in different climatic regions (Sculthorpe, 1967; Santamaría, 2002). To evaluate to what extent phenotypic plasticity, local adaptation and differences in phenology might contribute to the globally wide distribution of the aquatic macrophyte fennel pondweed (*Potamogeton pectinatus* L.), a series of experiments were performed that focused on geographic variation in life-history traits. In the following sections the results of these experiments will be summarised and discussed in relation to the widespread occurrence of *P. pectinatus*. In addition, some recommendations for further research will be given at the end of this chapter.

Geographic variation in life-history traits of *P. pectinatus*

Vegetative growth and asexual reproduction under standardised conditions

Field studies have shown that among Western European populations of *P. pectinatus* differences exist in phenology, biomass productivity and reproductive allocation (Ozimek et al., 1986; Kautsky, 1987; Van Wijk, 1988). However, these investigations did not allow for the recognition clear latitudinal trends, as specific habitat conditions largely influenced phenotypic variation in life-history traits. To investigate the existence of genetically fixed and latitude-correlated differences in growth and reproduction, two laboratory experiments were performed with *P. pectinatus* clones obtained from 24-68°N *(Chapter 2)*. In the first experiment characteristics related to asexual propagation were investigated (for fifteen clones). The results showed that the investment of dry matter in tubers increased with increasing latitude of origin, while both the aboveground biomass and the length of the longest shoot decreased (Fig. 1). In addition, clones obtained from higher latitudes produced more but smaller tubers (Fig. 1). The observed clines in asexual reproduction may point to a latitude-correlated shift in the timing of

tuberisation. At higher latitudes a decrease in seasonal length probably selected for clones with a compressed life cycle and an early and high investment of biomass in tubers. This change in phenology constrained the size of the tubers, as a lower shoot biomass had to supply photosynthates for the formation of a higher number of propagules. In contrast, at lower latitudes the length of the growth season is prolonged, which allows asexual reproduction to be postponed until a larger vegetative plant biomass is reached. The higher availability of photosynthates then results in the development of bigger tubers that probably have a higher change of survival and competitive ability. Similar positive relationships between the length of the growth season and propagule size have been described for various species native to the terrestrial environment (e.g. Galen & Stanton, 1991; Aizen & Woodcock, 1992). Moreover, it is noteworthy that our experiment did not fully account for differences in the length of the reproductive period. While for higher-latitude clones the production of tubers was probably completed after 90 days of growth (an experimental period corresponding to a relatively short growing season), lower-latitude clones might have produced a higher total tuber biomass if the experimental period had been longer (as depicted in Fig. 1).

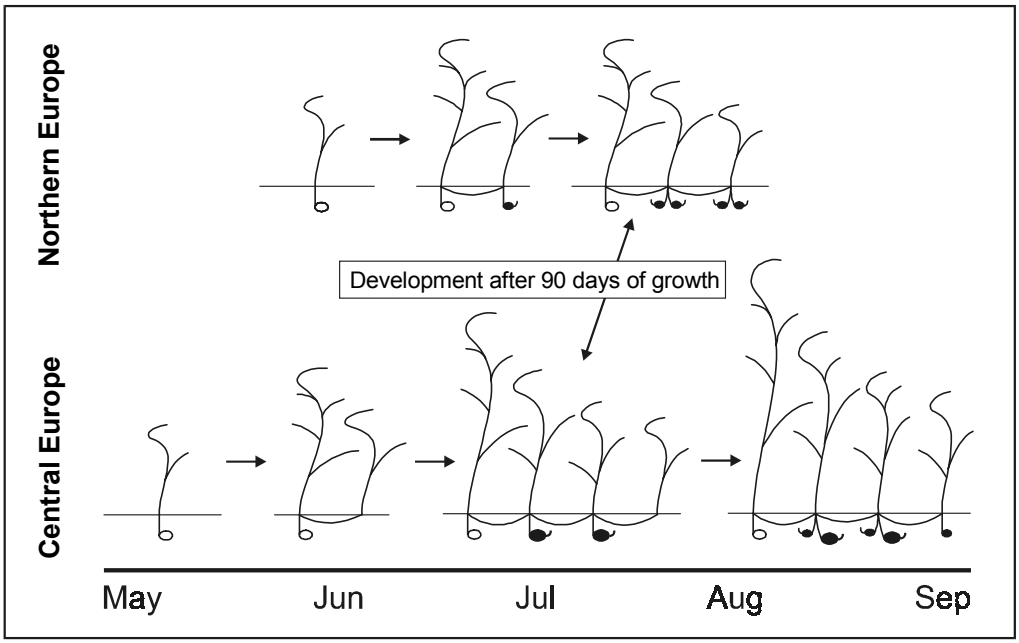

Figure 1. Hypothesised seasonal growth pattern of *P. pectinatus* in Central and Northern Europe (from May to September). This figure is based on data presented in *Chapter 2* and *7*.

The second experiment concentrated on characteristics related to vegetative growth, morphology and photosynthesis for eleven clones of *P. pectinatus*. Despite the detection of significant clonal differences (which may point to small-scale adaptation to conditions other than climate), the number of latitude-correlated traits was limited (i.e. only the number of leaves per plant increased with latitude). This may indicate that variation in vegetative growth is not correlated with latitudinal differences in reproductive phenology. However, it is important to note that part of the clones were raised under conditions that largely differed from those they would experience in their natural habitat. Consequently, latitudinal variation in vegetative growth may relate to differences in the reaction norm of morphological and physiological characteristics, rather than to the phenotypic value of these traits

under standardised conditions. For this reason a considerable part of this thesis focused on laboratory experiments in which clones of *P. pectinatus* from different geographic regions were exposed to a range of conditions, representing latitudinal variation in climatic factors (i.e. temperature, irradiance and photoperiod). This approach enabled us to test whether the investigated clones showed local adaptation, or whether they had the ability to tolerate contrasting environmental conditions trough adaptive phenotypic plasticity.

Plant responses to variation in temperature

Temperature is a climatic factor that has a pronounced effect on the rate of many plant developmental processes and shows variation on different temporal and spatial scales. Small thermal oscillations occur on a diurnal basis, whilst there is more pronounced variation over the seasons. However, most conspicuous differences in the temperature exist across latitude, between the extremes of arctic and desert climates (Berry & Raison, 1981). Although the water column can buffer submerged plants against rapid fluctuations in air-temperature, seasonal extremes may vary between 0 and 40°C (Bowes & Salvucci, 1989). In addition, predominant lake water temperatures differ by 10 to 15°C between Northern and Southern Europe (Santamaría, 2002). Consequently, the aquatic environment can be considered thermally heterogeneous, which may constrain water plants in their performance.

In the centre of its distributional range (i.e. Central to Northern Europe), *P. pectinatus* shows a pseudo-annual life cycle, which means that in spring population re-growth largely depends on the sprouting of subterranean tubers (Van Wijk, 1989). While previous investigations showed that tuber sprouting is controlled by water temperature (Spencer & Ksander, 1992; Van Wijk, 1989; Madsen & Adams, 1988a), it was unclear whether the thermal requirements for sprouting differed across latitude. *Chapter 6* reports on an experiment in which tuber sprouting at different water temperatures (varying between 10 and 25°C) was investigated for six clones of *P. pectinatus* obtained from a wide range of latitude (49-68°N). The results showed that at higher water temperatures (20 and 25°C) the sprouting characteristics were comparable for all investigated clones. However, at lower water temperatures the maximum frequency of tuber sprouting (at 10°C) and the rate of tuber sprouting over time (at 10 and 15°C) decreased with increasing latitude of origin. This pattern of thermal response might be of adaptive value to prevent juvenile plants from damage at northern localities, where the temperature conditions are largely unpredictable early in the growth season. Similar findings were obtained for terrestrial species, in which seeds collected from higher latitudes germinated at a more narrow range of higher temperatures (Sawada et al., 1994; Minggang et al., 2000).

Seasonal differences in temperature vary with latitude and plants may respond to this by acclimative changes in physiological processes (Berry & Björkman, 1981). While for terrestrial and marine plants seasonal acclimation in photosynthesis and respiration received some attention (e.g. Drew, 1978; Singh et al., 1996), no such studies were available for species native to freshwater ecosystems. *Chapter 3* reports on an experiment in which seasonal acclimation in gas-exchange was examined for three freshwater macrophyte species, among which *P. pectinatus*. To measure the thermal response of photosynthesis and respiration, shoots of all three species were collected from the field at monthly intervals. Among others, the photosynthetic optimal temperature (T_{opt}) was used as an indicator for the degree of thermal acclimation (e.g. Santamaría & Van Vierssen, 1997). Although all species showed significant temporal variation in T_{opt}, the observed differences were small ($\leq$ 5.4°C) and not directed towards higher values at the end of the growth season (i.e. when actual water temperatures are higher) (Fig. 2A). Moreover, a simple model showed that also the shape of the

temperature-response curves (at sub- and supra-optimal temperatures) was not optimised to seasonal differences in water temperature. The only consistent seasonal trend in gas-exchange was a gradual decrease in the maximum photosynthetic (at T_{opt}) and respiratory rate over time. In the absence of acclimative responses to seasonal variation in water temperature we may conclude that for the investigated species costs involved in the attainment of physiological changes overweight the potential benefits. Secondly, seasonal differences in water temperature might be of minor importance as compared to the loss of photosynthetic capacity due to ageing.

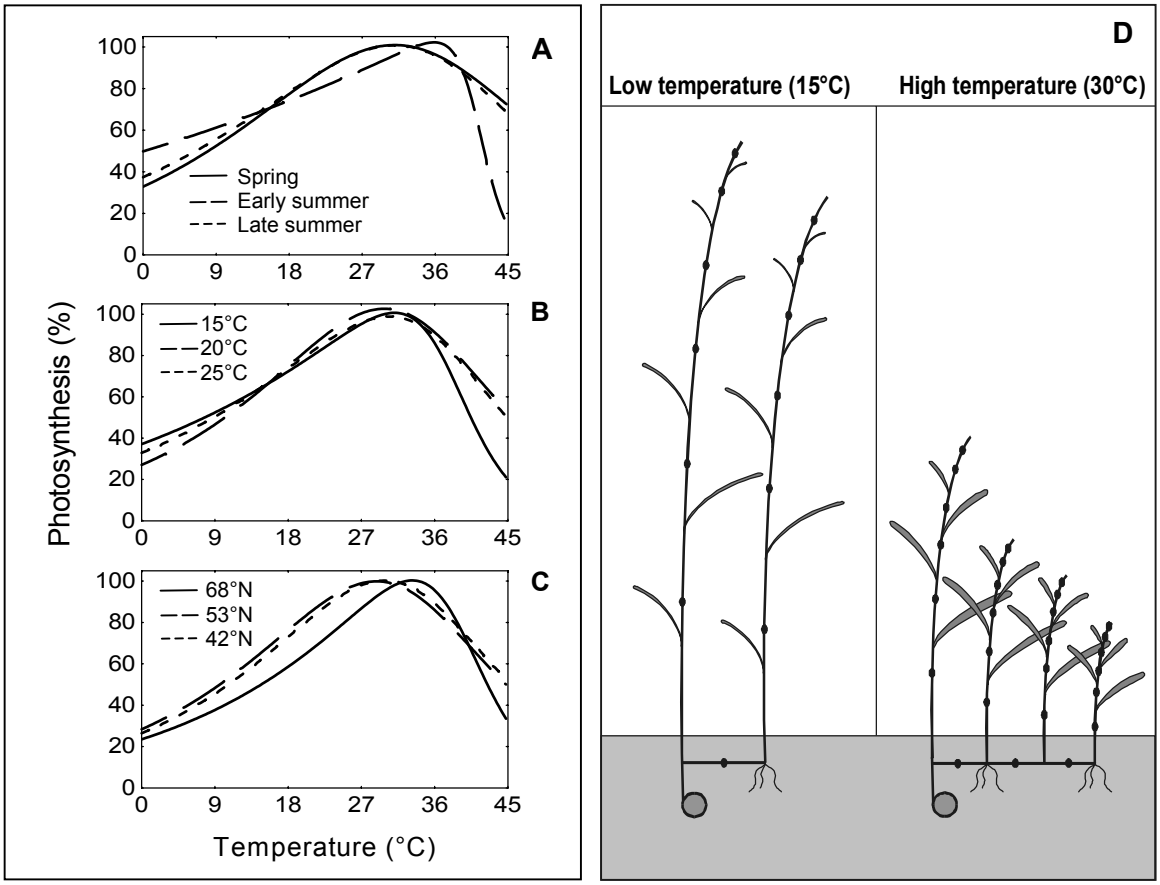

Figure 2. Variation in the relative rate of gross photosynthesis for: (**A**) *P. pectinatus* plants collected at different periods during the growth season, (**B**) a *P. pectinatus* clone that was grown at different temperatures. (**C**) *P. pectinatus* clones that were originally collected at different latitudes. (**D**) Schematic representation of the morphology of *P. pectinatus* plants grown at different water temperatures. This figure is based on data presented in *Chapter 3*, *4* and *6*.

Although gas-exchange of *P. pectinatus* was not optimised to seasonal variation in water temperature, plants native to different climatic regions might differ in their thermal optima and/or in their plastic responses to temperature. For three *P. pectinatus* clones obtained from distant latitudes (42 to 68°N) the thermal responses of photosynthesis and respiration were measured for plants grown at temperatures varying between 10 and 30°C *(Chapter 4)*. The results showed that irrespective of the latitudinal origin of the clones, optimal temperatures for net and gross photosynthesis were high (i.e. varying between 27 and nearly 40°C) and did not vary in correspondence with the growth temperature (Fig. 2B). In addition, among clonal differences in T_{opt} were small and the expected geographic pattern of variation (i.e. lower thermal optima for higher-latitude clones) was not found (Fig. 2C). Nevertheless,

at corresponding growth and incubation temperatures above 20°C differences in photosynthetic capacity (Pm) and respiration were less marked than at lower temperatures. Although this suggests some degree of thermal acclimation, it is clear that *P. pectinatus* can not adjust its physiological response to a wide range of temperature. A plausible explanation for this limited acclimation capacity could be that biochemical modifications necessary to enhance carbon uptake constrain the photosynthetic and respiratory reactions from being thermally optimised (as proposed by Bowes & Salvucci, 1989).

Morphological adjustments or changes in the pattern of biomass allocation can also improve plant performance under contrasting thermal regimes. The extent to which modifications in these characteristics (rather than changes in photosynthesis and respiration) contribute to biomass productivity at various temperatures was also investigated for *P. pectinatus* clones obtained from a wide range of latitude *(Chapter 4* and *6)*. The results showed that the overall pattern of thermal response in morphology and biomass allocation (between 10 and 30°C) was comparable for all investigated clones. Both the stem and internode length increased between 10 and 15°C and decreased again at higher growth temperatures, whereas the number of shoots per plant continued to increase up to at least 25°C (Fig. 2D). In general, the partitioning of biomass was significantly affected by temperature, but the observed differences were relatively small and the pattern of variation was not consistent among clones. A progressive increase of LAR (leaf area ratio) and SLA (specific leaf area) with increasing temperature partly compensated for the loss of photosynthetic capacity at higher growth temperatures (enabling comparable rates of ambient gross photosynthesis). As a consequence, biomass yield at the end of the experimental period was not significantly different for a relatively wide range of temperature (between 15/20 to 30°C). From this it is concluded that growth of *P. pectinatus* is thermally tolerant, while a lower threshold for normal development exists at about 15°C (similar to the findings of Madsen & Adams, 1988b; Vermaat & Hootsmans, 1994).

In summary, while seasonal variation in temperature did not result in physiological acclimation *(Chapter 3)*, *P. pectinatus* clones grown at contrasting temperatures showed some capacity for photosynthetic and respiratory acclimation to higher growth temperatures (*Chapter 4)*. However, plastic changes in morphology (increase of LAR and SLA), were probably more important to achieve a comparable plant biomass over a wide range of temperature *(Chapter 4)*. In addition, variation in temperature experienced over latitude is reduced by phenological changes, since higher-latitude clones sprout later in the season when water temperatures are higher *(Chapter 6)*.

Plant responses to variation in light climate

The instantaneous level of irradiance and the length of the photoperiod are different aspects of the light climate that have a large impact on plant performance (Björkman, 1981; Salisbury, 1981). Moreover, the combination of both these abiotic factors determines the daily-integrated level of irradiance, which may also affect various plant physiological and morphological characteristics (Lambers, 1998). During the growth period (spring and summer), irradiance and photoperiod show opposite patterns of variation across latitude. Although the optical properties of the water column may modify the light climate to some extent, aquatic macrophytes experience lower irradiance levels and longer photoperiods in northern as compared to southern locations.

Previous studies suggested that distant populations of *P. pectinatus* might differ in their response to the level of irradiance, although this was never tested experimentally (Hootsmans & Vermaat, 1994; Vermaat & Hootsmans, 1994; Hootsmans et al., 1996). Furthermore, no information existed on

geographic variation in the growth responses of *P. pectinatus* to differences in photoperiod. Therefore an experiment was performed in which three *P. pectinatus* clones originating from contrasting latitudes (42.5 to 68°N) were grown at a factorial combination of two irradiance levels (50 and 350 µmol m^{-2} s^{-1}) and three photoperiods (13, 16 and 22 h) *(Chapter 5)*. For all investigated clones, differences in irradiance resulted in strong acclimative changes in plant morphological and physiological characteristics. At low quantum flux, the capacity for light capture increased by pronounced vertical shoot extension (mediated by internode elongation), which added to a limited increase in the production of leaf area (Fig. 3). In addition, low-light grown plants increased their productivity by increases in irradiance-saturated photosynthesis (Pm), apparent quantum yield (α) and total chlorophyll concentration (Fig. 3). Due to these plastic responses, the observed differences in biomass yield between both irradiance treatments were relatively small for all investigated clones.

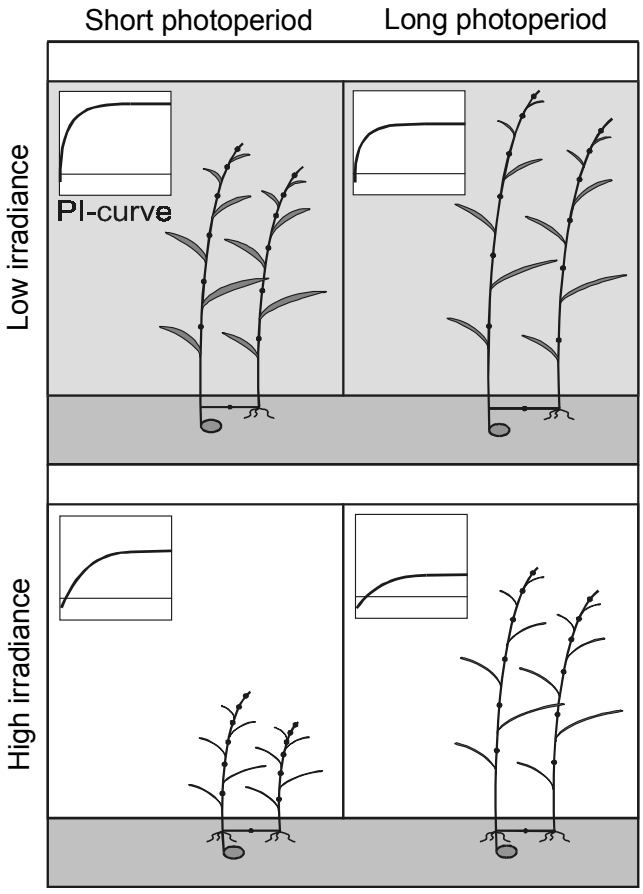

Figure 3. Schematic representation of morphology and the relationship between gross photosynthesis and irradiance (PI-curve) for *P. pectinatus* plants grown at different combinations of irradiance and photoperiod. This figure is based on data presented in *Chapter 5*.

Similar to the effect of low irradiance, also a decrease in photoperiod resulted for *P. pectinatus* in plastic changes in morphology and photosynthesis *(Chapter 5)*. At shorter photoperiods a higher proportion of resources was allocated to the leaves (i.e. increased leaf mass ratio, LMR), which probably represented a mechanism by which the photosynthetic productivity was increased. These higher LMR-values were counterbalanced by decreases in stem mass ratio (SMR) and shoot length

(Fig. 3). Moreover, the synergistic effect of a short photoperiod (13 h) and low irradiance level resulted for two of the three clones in acclimative increases in LAR and α (Fig. 3). However, the most consistent acclimative change in photosynthesis was a progressive increase of Pm at decreasing photoperiods (Fig. 3). Nonetheless, plastic responses in morphology and photosynthesis did not fully compensate for the differences in photoperiod, because at 13 and 16 h photoperiod plant biomass was significantly lower than at 22 h.

The results described in *Chapter 5* revealed that *P. pectinatus* clones obtained from distant latitudes responded in a largely comparable manner to differences in photoperiod. However, this might change when plants would become exposed to variation in the thermal regime. In this context, it is conceivable that higher-latitude clones of *P. pectinatus* show local adaptation to cool and long-day conditions characteristic of the north (as was found for a high-latitude cultivar of *Poa pratensis*; Hay & Heide, 1983). At lower temperatures this might result in a higher capacity to profit of an increased light availability associated with a prolonged photoperiod. To test this hypothesis, an experiment was performed in which clones from contrasting latitudes (49-68°N) were grown at a factorial combination of two photoperiods (16 and 22 h) and five water temperatures (10, 15, 20, 25 and 30°C) *(Chapter 6)*. The results showed that irrespective of the origin of the clones and the thermal regime they were exposed to, biomass allocation and growth at contrasting photoperiods were much smaller than those produced by differences in the thermal regime. Consequently, we concluded that in the northern distributional range of *P. pectinatus*, the growth limiting effect of low temperature ($<$15°C) can not be compensated by an increased light availability resulting from longer photoperiods.

In summary, the experiment presented in *Chapter 5* did not confirm that distant populations of *P. pectinatus* respond differently to variation in irradiance (cf. Vermaat & Hootsmans, 1994; Hootsmans & Vermaat, 1994; Hootsmans et al., 1996). Also the reactions to differences in photoperiod were comparable among all clones, even when they were grown at a range of different water temperatures. Consequently, we reject the hypothesis that *P. pectinatus* is locally adapted to the light climate that is considered to be predominant at the latitude of origin. Instead, our results showed that adaptive phenotypic plasticity allows the species to tolerate considerable variation in the light regime.

Plant performance across latitude; a transplantation experiment

The laboratory studies described above showed, that latitude-correlated changes in phenology and high phenotypic plasticity might enable *P. pectinatus* to grow successfully at contrasting environmental conditions *(Chapter 3* to *6)*. However, the question remained whether this ability can be maintained *in situ*, i.e. when plants are grown at distant localities with contrasting climate and in consideration of the reproductive phase. For this reason an experiment was performed in which 54 clonal lines of *P. pectinatus* (originating from 14 populations situated within four climatic regions) were grown in Norway (Oslo), The Netherlands (Heteren) and Spain (Doñana) *(Chapter 7)*. To minimise small-scale differences in variables not related to climate (e.g. soil and water chemistry) all plants were raised under standardised conditions. In correspondence with the findings of *Chapter 4* to *6*, all clones had the ability to grow at localities that largely differed in climate (i.e. water temperature, daily irradiance), although the biomass yield at the end of the experimental period decreased with increasing latitude. Independent of the location where the plants were grown, the highest reproductive output (i.e. total tuber biomass and the number of tubers per plant) was observed for clones originating from Central Europe (Fig. 4). The fact that Northern European clones produced fewer tubers is probably related the finding that range-margin populations may show decreased fitness due to genetic drift and inbreeding

(Woodward, 1988; Jonas & Geber, 1999). The Mediterranean clones were largely unable to reproduce by means of tubers, though they did produce (a few) seeds when they were grown in Spain (Fig. 4). Hence, even if seeds were transported to temperate and sub-arctic regions, establishment would be rarely possible as in pseudo-annual populations regeneration largely depends on the sprouting of subterranean tubers. Instead, Mediterranean clones showed an increase in perenniality (higher proportion of non-senescent biomass at harvest), which may indicate local adaptation to mild climatic conditions. The production of taller shoots (Fig. 4) also pointed to local adaptation, since at southern localities competition for light becomes more important due to higher population densities (e.g. Dudley & Schmitt, 1995; Schmitt et al., 1995). The results obtained for *P. pectinatus* agree well with the findings of an experiment in which clones of the cosmopolitan wetland species *Phragmites australis* were reciprocally transplanted across Europe. Similar to our study, Spanish clones showed life-cycle characteristics (i.e. increased perenniality, longer shoots, thicker rhizomes and reduced flowering) that can be considered of adaptive value in climates with reduced seasonality (Clevering et al., 2001).

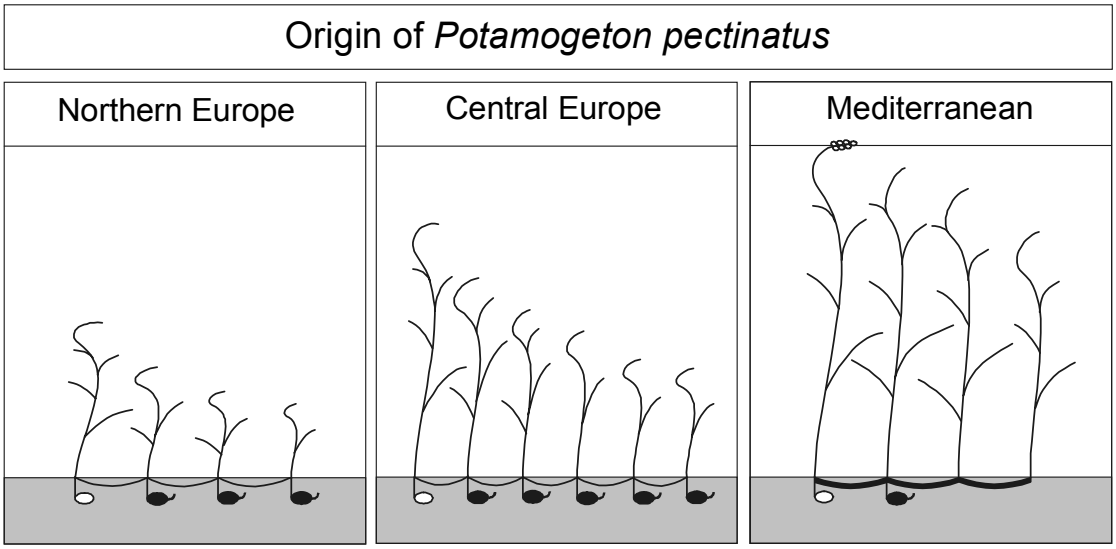

Figure 4. Morphology of *P. pectinatus* plants originally obtained from different geographic regions, grown under common-garden conditions in Norway, The Netherlands and Spain. This figure is based on data presented in *Chapter 7*.

Conclusions and ecological implications

Local adaptation and phenotypic plasticity have frequently been assumed to be mutually exclusive evolutionary mechanisms that contribute to the capacity of plants to cope with contrasting environmental conditions (Joshi et al., 2001; Nagy & Rice, 1997). This thesis has shown that this predetermined distinction is rather artificial, as the ability of *P. pectinatus* to thrive in different climatic regions depends on both mechanisms and changes in phenology. In addition, our results revealed that investigations that seek to evaluate to what extent local adaptation, phenotypic plasticity and changes in phenology contribute to the distribution of (cosmopolitan) plants, should focus on various life-cycle stages. This, because the involvement of the described evolutionary mechanisms may vary depending of the plant process under study (as outlined below).

At northern localities, where the length of the growth season is restricted, genetically fixed changes in phenology result for *P. pectinatus* in a compression of the life cycle. To prevent young plants to be damaged from low spring temperatures, tubers of higher-latitude clones possess a higher thermal threshold for sprouting *(Chapter 6)*. In addition, northern clones show early reproduction, which constrains the size of the tubers *(Chapter 2)*. Furthermore, adaptive phenotypic plasticity allows *P. pectinatus* to grow at contrasting environmental conditions *(Chapter 3* to *6)*. Although thermal acclimation in gas-exchange is constrained, plastic changes in morphology are of special importance to attain a comparable biomass between 15/20 and 30°C *(Chapter 3, 4* and *6)*. *P. pectinatus* can also cope with considerable differences in the light climate. Increased light capture through canopy formation and acclimative changes in photosynthesis result in a relatively high biomass yield at low irradiance (i.e. 2.5% of full sunlight in temperate regions) *(Chapter 5)*. Similarly, photoperiods varying between 13 and 22 h did result in plastic changes in morphology and physiology that limited the loss of biomass productivity at shorter days *(Chapter 5)*. Despite the fact that changes in phenology and high phenotypic plasticity allowed *P. pectinatus* to grow and reproduce in different climatic regions, the Mediterranean clones showed local adaptation resulting from increased perenniality and the absence of asexual reproduction *(Chapter 7)*.

In *P. pectinatus*, the strong reliance on phenotypic plasticity may be related to the clonal growth habit of the species. Since the aquatic environment does not facilitate sexual reproduction, because pollination success is frequently low (Duarte et al., 1994) and incompatible mating types have a higher change of occurrence in typically small populations (Barret et al., 1993), the evolution of locally adapted genotypes is likely to be constrained. As a consequence, survival of *P. pectinatus* in Central to Northern Europe probably depends on long-lived general-purpose genotypes that can cope with climatic variation through high plasticity. This may be a general trend for (widely distributed) submerged aquatic macrophytes, since many species show prolific asexual multiplication, reduced sexual reproduction and high plasticity (Barret et al., 1993). In contrast, for terrestrial plants local adaptation in response to variation in abiotic conditions was frequently found on small (e.g. Nagy & Rice, 1997; Gauthier et al., 1998), but also large spatial scales (e.g. Sawada et al., 1994; Joshi et al., 2001). This may have been facilitated by the higher frequency of sexual reproduction that is observed in terrestrial as compared to aquatic plants (Santamaría, 2002; Barret et al., 1993), although more research is needed to confirm this idea.

Recommendations for further research

Although this thesis focused on life-cycle stages that are of key-importance for the survival of *P. pectinatus* in regions that differ in climate (i.e. tuber sprouting, vegetative growth and asexual reproduction), the question on how variation in abiotic factors may affect sexual reproduction was not addressed. In part this was a consequence of practical limitations, as in both laboratory and common-garden experiments, flowering and subsequent seed set was rarely observed (*Chapter 2* to *7)*. Although in Central to Northern Europe, regeneration of *P. pectinatus* heavily relies on the sprouting of tubers; seed production is of importance to increase genetic diversity in largely clonal populations (Hangelbroek et al., unpublished manuscript). In addition, long-distance dispersal by water birds and thus the colonisation of new areas may critically depend on it. Consequently, future researchers of *P. pectinatus* should make an effort to elucidate which (climatic) factors are of importance to trigger and

seasonally synchronise sexual reproduction. In addition, the existence of reproductive ecotypes should be investigated across broad geographic regions.

The results presented in this thesis showed that adaptive phenotypic plasticity could allow *P. pectinatus* to grow and reproduce at localities that largely differ in climate. Furthermore, recent and ongoing investigations revealed that waterfowl-mediated transport of seeds might result in long-distance dispersal over ranges of at least 600 km (Charalambidou, unpublished data). However, successful establishment in new areas not only depends on the previous, but also on the competitive ability of the dispersed genotype. As all our experiments were performed with individually grown plants, nothing is known about inherent differences in competitive strength. To enrich our knowledge on this topic, it would be of interest to study intraspecific competition between *P. pectinatus* clones originating from different geographic regions, and interspecific competition between 'native' and 'non-native' *P. pectinatus* clones and other aquatic macrophyte species.

Finally, more studies are needed that evaluate the generality of environmental tolerance and latitude-correlated changes in phenology in explaining the broad distributional range of aquatic (and terrestrial) macrophytes. Comparative studies that also make use of species with more restricted geographic ranges are recommended.

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Samenvatting

Deze samenvatting is bedoeld voor niet-vakgenoten die zich in korte tijd op de hoogte willen stellen van de globale inhoud van dit proefschrift.

De groei van hogere planten wordt in sterke mate beïnvloed door klimatologische factoren zoals temperatuur, lichtintensiteit en daglengte. Doordat deze factoren variatie vertonen in afhankelijkheid van breedtegraad, en veel planten slechts onder bepaalde condities kunnen groeien, worden de meeste soorten in hun geografische verspreiding beperkt. Als gevolg hiervan kennen we vele vegetatietypen, die qua plantensamenstelling sterk van elkaar verschillen (variërend van toendra-vegetaties in subarctische streken tot tropische vegetaties nabij de evenaar). Er zijn echter ook soorten waarvan de verspreiding over het aardoppervlak niet gehinderd lijkt te worden door uiteenlopende klimatologische omstandigheden. Een drietal evolutionaire mechanismen biedt een mogelijke verklaring voor de totstandkoming van deze kosmopolitische plantengroei. Ten eerste zouden wijdverspreide planten kunnen bestaan uit afzonderlijke populaties, waarvan het functioneren is afgestemd op de omstandigheden zoals die heersen in een beperkt deel van het verspreidingsgebied (lokale adaptatie). Ten tweede zouden de betreffende soorten door veranderlijke kenmerken (adaptieve fenotypische plasticiteit) een grote mate van tolerantie kunnen bezitten met betrekking tot variatie in klimatologische omstandigheden. Ten derde is het mogelijk dat planten de timing van bepaalde levensprocessen hebben afgestemd op de lengte van het groeiseizoen en zodoende ongunstige condities kunnen vermijden (verandering in fenologie).

Naast landplanten met een wereldwijde verspreiding, bestaan er opvallend veel waterplanten die zich prima thuis voelen in uiteenlopende klimaatgebieden. Juist om deze reden zijn ze zeer geschikt om te bepalen welke van de eerder genoemde evolutionaire mechanismen een rol spelen bij de handhaving van een wereldwijde verspreiding. Ter beantwoording van deze vraag, richtte het in dit proefschrift beschreven onderzoek zich op het functioneren van een waterplant die ook in Nederland algemeen voorkomt: het schedefonteinkruid (*Potamogeton pectinatus* L.). Deze geheel onder het wateroppervlak groeiende kosmopolitische soort, die te vinden is in ondiepe zoete en brakke wateren, plant zich veelal ongeslachtelijk voort door middel van ondergrondse wortelknolletjes (tubers). Voorafgaand aan het eigenlijke onderzoek werden in uiteenlopende klimaatgebieden (op breedtegraden variërend van 24 tot 68° N.Br.) tubers van het schedefonteinkruid verzameld. De hieruit opgekweekte planten werden in de gelegenheid gesteld om aan het einde van het groeiseizoen wortelknolletjes te vormen en na de voltooiing van enkele groeicycli werden per afzonderlijke kloon vele genetisch identieke tubers verkregen Door deze ongeslachtelijk vermeerdering werden de eigenschappen die bepalend zijn voor de overleving in het gebied van herkomst behouden. De tubers werden vervolgens gebruikt om laboratorium- en veldexperimenten uit te voeren waarin diverse klonen werden blootgesteld aan variatie in (klimatologische) factoren. Op deze wijze kon bepaald worden of planten die normaliter groeien in heel verschillende klimaatgebieden, lokaal geadapteerd zijn, fenotypisch plastisch (en tolerant) zijn, of variatie vertonen in fenologie.

De resultaten gaven aan dat niet een enkel mechanisme verantwoordelijk kan worden geacht voor het wereldwijde ‘succes’ van het schedefonteinkruid, maar dat alle drie de genoemde evolutionaire ‘oplossingen’ een bijdrage leveren aan het voorkomen van de plant in uiteenlopende klimaatgebieden.

In subarctische streken beperkt de relatief lage watertemperatuur de lengte van het groeiseizoen tot ongeveer 3 maanden. Als aanpassing aan deze omstandigheid, hebben noordelijke klonen fenologische veranderingen ondergaan waardoor ze hun levenscyclus in een korter tijdsbestek kunnen voltooien. Zo kiemen tubers later in het seizoen om te voorkomen dat jonge planten beschadigd raken door temperaturen die in het (subarctische) voorjaar geregeld beneden de tolerantiegrens kunnen zakken *(Hoofdstuk 6)*. Ook zorgen klonen in het noordelijke verspreidingsgebied al vroeg in het groeiseizoen voor nageslacht (door middel van tubers) en stellen op deze wijze het voorbestaan van de populatie veilig. De bladbiomassa is dan echter nog te gering om veel koolhydraten te produceren en de tubers blijven dan ook relatief klein. Zuidelijke klonen daarentegen hebben te maken met een langer groeiseizoen, waardoor ze hun ongeslachtelijke voortplanting kunnen uitstellen totdat een grotere vegetatieve biomassa is bereikt, waarmee uiteindelijk grotere tubers geproduceerd kunnen worden *(Hoofdstuk 2)*.

Laboratoriumexperimenten toonden aan dat het groeiproces van planten afkomstig uit diverse klimaatgebieden op een vergelijkbare wijze werd beïnvloed door variatie in omgevingsfactoren (temperatuur, lichtintensiteit, daglengte). De afzonderlijke klonen leverden dus niet de beste 'prestaties' wanneer ze werden blootgesteld aan omstandigheden die representatief waren voor het klimaat in het gebied van herkomst. Veelmeer was er sprake van morfologische en fysiologische veranderingen waardoor verschillen in biomassaproductie, die anders onder uiteenlopende omstandigheden waren ontstaan, werden gereduceerd. Hieruit kunnen we concluderen dat de groei van het schedefonteinkruid in verschillende klimaatgebieden waarschijnlijk niet verklaard kan worden door lokale adaptatie, maar dat fenotypische plasticiteit zorgt voor een grote mate van tolerantie. Echter, de mate waarin plasticiteit werd waargenomen verschilde in afhankelijkheid van de bestudeerde eigenschap. Hoewel eerder onderzoek had laten zien dat planten hun productiviteit kunnen optimaliseren door de gaswisseling (fotosynthese en ademhaling) af te stemmen op de temperatuur die ze tijdens de groei ondervinden, is deze eigenschap bij het schedefonteinkruid slechts in beperkte mate ontwikkeld (*Hoofdstuk 3* en *4)*. Dit gebrek aan fysiologische flexibiliteit werd gedeeltelijk gecompenseerd door morfologische veranderingen. Zo werd bijvoorbeeld bij een hogere opkweektemperatuur een groter bladoppervlak aangelegd (per eenheid plantgewicht), waardoor de afname van de fotosynthese-snelheid boven de 20°C kon worden afgeremd *(Hoofdstuk 4)*. Hoewel het plastisch vermogen van het schedefonteinkruid zodanig was dat alle klonen konden opgroeien bij zeer uiteenlopende temperaturen (15-30°C), ligt de ondergrens voor een normale vegetatieve ontwikkeling waarschijnlijk tussen de 10 en 15°C *(Hoofdstuk 4* en *6)*.

Ongeacht de geografische herkomst, bleken de onderzochte klonen tolerant te zijn voor verschillen in lichtintensiteit. Een zeer lage instraling (2,5% van vol zonlicht) bracht morfologische veranderingen teweeg die tot doel hadden om meer licht te onderscheppen. Zo zorgde een geprononceerde stengelstrekking ervoor dat de bladbiomassa in de bovenste waterlaag geplaatst werd, waar de lichtintensiteit normaliter het hoogst is. Bovendien werd de lichtabsorberend vermogen verder vergroot door een toegenomen concentratie chlorofyl in de bladeren. Ook het fotosynthese-apparaat onderging diverse veranderingen. Zo nam niet alleen de capaciteit van de fotosynthese toe (bij lichtverzadiging), maar ook de efficiëntie bij een lage lichtintensiteit. Als gevolg hiervan kon ondanks een verminderde instraling de productie van koolhydraten grotendeels op peil gehouden worden *(Hoofdstuk 5)*. Ook een reductie van de daglengte had voor de onderzochte klonen allerlei fenotypische veranderingen tot gevolg. Planten die bij een korte dag werden opgekweekt trachtten het effect van een geringe dagelijkse instraling te minimaliseren door relatief meer bladweefsel te maken. Wanneer bovendien ook nog de intensiteit van het licht gereduceerd werd (bij een daglengte van 13 uur) nam ook het

bladoppervlak (per eenheid plantgewicht) en de efficiëntie van de fotosynthese bij lage lichtintensiteit toe *(Hoofdstuk 5)*.

De beschreven laboratoriumexperimenten lieten zien dat veranderlijke kenmerken ervoor zorgen dat het schedefonteinkruid kan opgroeien onder uiteenlopende abiotische omstandigheden. Het was echter onduidelijk of de planten dit vermogen behouden wanneer ze blootstaan aan daadwerkelijke verschillen in klimaat. Om deze reden werd een experiment uitgevoerd waarin 54 klonen (afkomstig uit verschillende klimaatgebieden, variërend van subarctisch tot mediterraan) werden opgekweekt in Oslo (Noorwegen), Heteren (Nederland) en Donaña (Sevilla, Spanje) *(Hoofdstuk 7)*. Hoewel de veronderstelde klimatologische tolerantie werd bevestigd doordat de getransplanteerde klonen tot volle wasdom konden komen op alle drie de locaties, verschilden de mediterrane planten toch van alle andere klonen. Terwijl planten afkomstig uit noordwest Europa aan het einde van het groeiseizoen (september) tubers hadden gevormd, waarna de bovengrondse biomassa begon af te sterven, was dit niet het geval voor de klonen uit Spanje en Noord-Afrika (Marokko). Deze produceerden nauwelijks tubers en vertoonden bovendien geen tekenen van verval (desintegratie van de bovengrondse delen). Dit duidde er op dat deze zuidelijke klonen lokaal geadapteerd zijn, waardoor ze gebruik kunnen maken van het milde mediterrane klimaat om gedurende het gehele jaar te groeien. Bovendien zorgen de gunstige klimatologische condities er waarschijnlijk voor dat de noodzaak om tubers te vormen (die zorgen voor ondergrondse overleving gedurende het noordelijke winterseizoen) is gereduceerd.

Samenvattend, kunnen we stellen dat in subarctische streken fenologische aanpassingen resulteren in een korte levenscyclus, waardoor watertemperaturen onder de 15°C vermeden worden. Bovendien bezitten planten in een belangrijk deel van het verspreidingsgebied een grote mate van fenotypische plasticiteit, die zorg draagt voor tolerantie ten aanzien van klimatologische verschillen. Tenslotte vertoont het schedefonteinkruid in het gebied rond de Middellandse Zee lokale aanpassing aan de milde klimatologische omstandigheden. Verder onderzoek moet uitwijzen of de hier beschreven resultaten algemene geldigheid hebben voor andere wijdverspreide waterplanten. Mocht dit zo zijn, dan hebben aquatische kosmopolieten een andere strategie ontwikkeld dan terrestrische soorten, die in diverse klimaatgebieden vaak minder plastisch en meer lokaal geadapteerd lijken te zijn.

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Jörn

Curriculum vitae

Jörn Pilon werd op 25 januari 1968 in Rotterdam geboren en bracht het grootste deel van zijn jeugd door in Oostvoorne. Na het behalen van het VWO-diploma, startte hij in 1987 met een studie biologie aan de Universiteit Utrecht (UU). In het kader van zijn afstuderen bewerkte hij een tweetal doctoraalonderwerpen bij de Vakgroep Botanische Oecologie en Evolutiebiologie (BOEV, UU). Onder leiding van dr. Peter Bakker (projectgoep Fytopathologie) bestudeerde hij de door Pseudomonaden geïnduceerde resistentie tegen *Fusarium*-infectie bij de anjer. Bij dr. Pieter Poot (projectgroep Oecofysiologie van Planten) werd vervolgens onderzoek gedaan naar de fotosynthese-karakteristieken van verschillende sextypen van de smalle weegbree. Na het behalen van zijn doctoraaldiploma (1993), werkte hij gedurende een jaar als gastmedewerker bij de projectgroep Oecofysiologie van Planten (BOEV, UU) aan de bladwas-samenstelling van aan UV-b blootgestelde *Poa*-grassen. Van januari 1995 tot december 1998 volgde een aanstelling als Onderzoeker-in-Opleiding (OiO) bij het Centrum voor Limnologie van het Nederlands Instituut voor Oecologisch Onderzoek (NIOO-KNAW) in Nieuwersluis. Binnen de nieuw opgerichte werkgroep Plant-Dier Interacties werd een start gemaakt met het in dit proefschrift beschreven onderzoek. Aangezien de voortgang van de werkzaamheden aanvankelijk ernstig belemmerd werd door perikelen omtrent de bouw van klimaatkamers, werd de aanstelling in 1999 met anderhalf jaar verlengd. Na het aflopen van dit dienstverband kwam hij tot afronding van dit proefschrift tijdens een aanstelling als gastmedewerker bij het Centrum voor Limnologie. Naast zijn werkzaamheden als onderzoeker is Jörn Pilon sinds 1990 tevens actief als freelance natuur- en reisfotograaf. Zijn foto's worden veelvuldig gepubliceerd in zeer uiteenlopende media (boeken, tijdschriften, folders, kaarten, verpakkingen etc.) en een gedeelte van zijn beeldarchief is voor commerciële doeleinden in beheer bij een aantal binnen- en buitenlandse beeldbanken.

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