Chapter 11

Trade-offs and Synergies in the Structural and Functional Characteristics of Leaves Photosynthesizing in Aquatic Environments

Stephen Christopher Maberly*
Lake Ecosystems Group, Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, UK

and

Brigitte Gontero
Enzymology of Supramolecular Systems, Aix Marseille Univ, CNRS, BIP, Marseille, France

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*Author for correspondence, e-mail: scm@ceh.ac.uk

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Summary

Aquatic plants, comprising different divisions of embryophytes, derive from terrestrial ancestors. They have evolved to live in water, both fresh and salty, an environment that presents unique challenges and opportunities for photosynthesis and growth. These include, compared to air, a low water stress, a greater density, and attenuation of light, and a more variable supply of inorganic carbon, both in concentration and chemical species, but overall a lower carbon availability, and the opportunity to take up nutrients from the water. The leaves of many aquatic plants are linear, dissected, whorled, or cylindrical with a large volume of air spaces. They tend to have a high specific leaf area, thin cuticles, and usually lack functional stomata. Exploiting the availability of chemicals in their environment, freshwater macrophytes may incorporate silica in their cell wall, while seagrasses contain sulphated polysaccharides, similar to those of marine macroalgae; both groups have low lignin content. This altered cell wall composition produces plants that are more flexible and therefore more resistant to hydraulic forces (mechanical stress arising from water movement). Aquatic plants may have enhanced light harvesting complexes conferring shade adaptation, but also have mechanisms to cope with high light. Aquatic plants have evolved numerous strategies to overcome potential carbon-limitation in water. These include growing in micro-environments where CO_2 is high, producing leaves and roots that exploit CO_2 from the air or sediment and operating concentrating mechanisms that increase CO_2 (CCM) around the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase. These comprise C_4 metabolism, crassulacean acid metabolism, and the ability to exploit the often high concentrations of HCO_3^-, and ~50% of freshwater macrophytes and ~85% of seagrasses have one or more CCM. Many of these adaptations involve trade-offs between conflicting constraints and opportunities while others represent ‘synergies’ that help to maximize the productivity of this important group of plants.

I. Introduction

Aquatic embryophytes (bryophytes, lycophytes, monilophytes, and angiosperms) are a heterogeneous group of plants with many common features. They have arisen as a result of convergent evolution from terrestrial ancestors to adapt to life in marine and fresh waters. In the ocean, seagrasses cover an area of about 3.10^{11} m^2 (Duarte et al. 2005) and have an average net productivity rate that ranges from 500 to 1200 g C m^{-2} $year^{-1}$ (van der Heijden and Kamenos 2015). This corresponds to a net global productivity of about 0.4 Pg C $year^{-1}$, which is about 1% of marine primary productivity. Nevertheless, seagrass beds help to support coastal fisheries and are important habitats for fish, birds, invertebrates, and marine mammals, play an important role in nutrient cycling (Waycott et al. 2009) and keep waterbone pathogens in check (Lamb et al. 2017). They are also globally important for organic carbon sequestration, contributing about 30–40 Tg C $year^{-1}$, or nearly 20% of global oceanic carbon burial (Duarte et al. 2005).

In fresh waters, macrophytes are important primary producers, particularly in shallow lakes and rivers. They can also be regarded as ‘ecological engineers’ producing a structurally complex environment that is very different from the open water and an important habitat for numerous organisms including invertebrates and fish (Chambers et al. 2008; Kovalenko et al. 2012). They also help to prevent turbid, phytoplankton-dominated systems as part of the alternative stable states that can exist in shallow lakes (Scheffer et al. 1993). Their presence in shallow lakes increases food-chain length (Ziegler et al. 2015) probably through their contribution to productivity, environmental variability, and their provision of a refuge for higher trophic levels. In some shallow environments, such as the Amazonian floodplains, macrophytes can contribute a large portion of the net primary productivity of the system (Silva et al. 2013).

Macrophytes comprise a range of life forms in a gradient ranging from emergent plants with leaves that photosynthesize in air (not considered in this chapter), through species with leaves floating on the water surface but rooted in the sediment, free-floating plants with leaves on the water surface, to completely submerged plants with leaves photosynthesizing in water, either free-floating in the water column or, more commonly, rooted in the sediment. The latter group can be broadly divided into two types: (i) rosette plants (often called isoetids) with leaves that are short, stiff, and often cylindrical and (ii) tall caulescent shoots (often called elodeids) with leaves with a range of morphologies (Sect. 3). Seagrasses typically belong to the caulescent, elodeid group of submerged plants. The life form system of Wiegleb (1991) gives a more detailed breakdown of hydrophyte growth forms. While most aquatic plants are rhizophytes with roots penetrating the sediment, some, such as the bryophytes, are haptophytes, attached to, but not penetrating hard surfaces.

In this chapter, we compare air and water as environments for photosynthesis and growth and describe how the structure and function of aquatic leaves allows them to thrive in aquatic environments. We deal primarily with submerged embryophytes, freshwater macrophytes, and seagrasses, but make occasional reference to macrophyte and microphyte algae.

II. Adaptation of Aquatic Plants to the Environmental Challenges and Opportunities in Water

A. *Evolution of Aquatic Embryophytes*

All aquatic embryophytes evolved from terrestrial ancestors that in turn evolved from aquatic green algae, probably within the Zygnematophyceae (Wickett et al. 2014), and therefore contain evolutionary traces of adaptations to life in both air and water. For example, terrestrial bryophytes inherited features from their algal ancestors, such as desiccation tolerance and lignin-like cell wall polymers, that allowed them to colonize land (Graham et al. 2014) while present day macrophytes possess visible features of their terrestrial past such as cuticles and stomata (functional and non-functional), xylem (Sculthorpe 1967) and leaves themselves.

Aquatic angiosperms are found in 17% of angiosperm families, comprising over 6000 species (Cook 1990; den Hartog and Kuo 2006), but represent less than 2% of all angiosperms (Les et al. 1997). Freshwater angiosperms have arisen at least 100 times (Les and Tippery 2013) and are represented predominantly by monocotyledons (Liliopsida) rather than dicotyledons (Magnoliopsida) (Les and Schneider 1995). In fresh waters, the cline between terrestrial, wetland, and submerged environments, blurred by episodic and seasonally variable water levels, produces a gradient that probably facilitated movement of plants between land and water. Indeed, there is evidence for progressive evolution from emergent to floating-leaved then to submerged leaves in aquatic lineages (Du and Wang 2014). Fossil and phylogenetic data show that invasion of the aquatic habitat occured very early in the evolution of the angiosperms and that they existed in the Cretaceous 120 million years ago (Friis et al. 2001; Gomez et al. 2015; Les 2015).

In coastal and marine waters, there are 72 species of seagrasses within 13 genera and five families if euryhaline genera such as *Ruppia* are included. All belong to the monocotyledon, largely freshwater order Alismatales (Les et al. 1997; den Hartog and Kuo 2006); Short et al. 2007; Papenbrock 2012; Les and Tippery 2013) and so probably evolved from freshwater ancestors. Seagrasses are believed to have evolved relatively recently, around 64–72 million years ago (Olsen et al. 2016) or possibly only 16–41 million years ago (Chen et al. 2012). The apparent evolutionary barrier to colonization of marine systems by embryophytes, indicated by low species diversity, is not clear. It does not appear to be linked to salinity *per se* since seagrasses have a range of mechanisms to deal with this factor (Touchette 2007). It has been suggested to be caused by difficulties of efficient pollination and potentially to the lack of co-evolution with insects, a group that is also limited in its success in marine environments (Van der Hage 1996). It could also result from competition with large macroalgae that were already present in marine systems in contrast to fresh waters where macroalgae, essentially members of the family Characeae, are relatively small and occupy a different niche, or simply result from a physical barrier caused by the hydraulic forces.

B. *Comparison of Air and Water as Environments for Photosynthesis and Growth*

The fluids of air and water provide contrasting environments for the photosynthesis, growth, and survival of photoautotrophs. These properties (Maberly and Spence 1989) set contrasting ecological challenges and opportunities (Table 11.1) that have resulted in different evolutionary solutions to maximize fitness in aquatic and terrestrial plants (Maberly 2014).

The most obvious distinction between air and water is water availability itself. In fresh water, the water potential is, by definition, 0 MPa and in seawater about −2.5 MPa. In air, it can range from 0 at 100% humidity to as low as −200 MPa in very dry air, setting extreme challenges for leaf water balance. Despite the many structural, morphological, and physiological attributes of terrestrial plants, water availability is still a major factor controlling plant productivity on land (Hsu et al. 2012). A second environmental difference is the greater density of water, compared to air, that reduces the need for investment in structural material, but increases the drag on a plant in flowing water. For a given velocity, the drag and lift forces on an object in water are about 29-times greater than in air (Denny 1993). Thus, plants in rivers with a relatively low water velocity of 1 m s^{-1} experience the same forces as plants in air with a wind speed of 100 km h^{-1}. Plants, in littoral regions where waves are breaking, or in fast flowing water such as in rapids, often inhabited in sub-tropical and tropical areas by freshwater macrophytes of the family Podostemaceae (Koi et al. 2015), will experience much greater forces.

A third difference is the generally low levels of underwater light. This results from reflection at the air-water interface, but mainly by the much greater, and spectrally-selective, attenuation of light in water compared to air (Table 11.1). This is caused by light absorption by water molecules themselves, plus the ability of water to dissolve substances, such as coloured dissolved organic matter ((Kirk 2011). Furthermore, because of the density of water, particles including phytoplankton, sediment, and non-living organic material can be kept in suspension, scattering light and increasing attenuation. Finally, growth of epiphytes on aquatic leaves because of the absence of desiccation and presence of dissolved nutrients, can reduce light availability even further (Sand-Jensen 1977). As a consequence, light controls macrophyte depth limits (Krause-Jensen and Sand-Jensen 1998), macrophyte productivity at the whole population level

Table 11.1 Challenges, opportunities, and responses for plant leaves in the environments (Env) of air (A) and water (W)

Feature	Env	Attribute	Environmental opportunity	Response to opportunity	Environmental challenge	Response to challenge
Water availability	A	Low	–	–	Risk of desiccation and restriction of productivity	Transpiration. Cuticle, stomata, sub-stomatal chambers, C_4, CAM
	W	High	No risk of desiccation	High productivity; epidermal chloroplasts, thin leaves	Competition with algae	Allelochemicals
Density	A	Low	Low aerodynamic drag	Tall plants possible	Structural support required	Lignin and secondary thickening
	W	High	Support of plant by water	Low investment in structural components	High hydrodynamic drag	Flexible leaves and shoots
Light availability	A	High	High incident light in unshaded habitats	High productivity in absence of water stress	Possibility of photoinhibition and UVB damage	Hairs, cuticle, epidermal pigments, xanthophyll cycle, antioxidants
	W	Low	Less likely to be photoinhibited or photodamaged	Low investment in photoprotection mechanisms	Restriction of photosynthesis and colonization depth	Epidermal chloroplasts
Temperature variability	A	High	Possible promotion of diurnal photosynthesis and restriction of nocturnal respiration	–	Temperature stress, elevated photorespiration at high temperature	Stomatal control of cooling, C_4, CAM
	W	Low	Low risk of temperature stress	–	–	–
Gas diffusion rate	A	High	Rapid gas exchange between leaf and air	High productivity in absence of water stress	–	–
	W	Low	Generation of micro-habitats with high CO_2	Exploitation of particular habitats	Restriction of photosynthesis by CO_2 and O_2 storage	CCMs: HCO_3^- use, C_4, CAM

(continued)

Table 11.1 (continued)

Feature	Env	Attribute	Environmental opportunity	Response to opportunity	Environmental challenge	Response to challenge
O_2 availability	A	High	No restriction of aerobic respiration	–	Photorespiration risk	C_4, CAM
	W	Low	Potential reduced photorespiration	–	Reduced O_2 supply to below-ground organs	Lacunal connection shoot to root, pressurised ventilation
Inorganic carbon availability	A	High	Relatively constant, not strongly limiting	–	–	–
	W	Low	High CO_2 in some habitats/sites & HCO_3^- present	Exploitation of particular habitats; use of HCO_3^-	Highly variable and can be depleted to near zero	CCMs: HCO_3^- use, C_4, CAM
Nutrient solubility	A	Low	–	–	Supply largely from soil water	Vessels to supply nutrients to leaf taken up by roots
	W	High	Nutrients available to leaves and roots	Shoot and root uptake	Competition with algae	Allelochemicals

(Sand-Jensen et al. 2007), and can also control the overall productivity of some freshwater systems (Karlsson et al. 2009). Although aquatic environments are essentially shade environments, wave focussing at the water surface can produce bursts of high light with a duration of milliseconds (Schubert et al. 2014). These differ from sunflecks, well-known in terrestrial vegetation (Pearcy 1990), in producing levels of surface light that can be five-times greater than surface light and consequently have the potential to cause photoinhibition and photodamage.

A fourth difference is the heat capacity of water that is around 3500-times greater than that of air on a volume basis (Maberly and Spence 1989). This tends to reduce the magnitude of diel temperature variation in water compared to air. Also, because in fresh water, ice is slightly less dense than liquid water at 4 °C, it forms at the surface in winter, thereby protecting freshwater plants from freezing in all but the shallowest or extremely cold environments.

A fifth difference relates to the availability of inorganic carbon, a key requirement for photosynthesis (Table 11.1). The solubility of CO_2 in water decreases with temperature, but at 15 °C, the molar concentrations in air and water are similar. In contrast, oxygen is about 30-times less soluble in water than in air and this might suggest that aquatic photosynthesis would be favored over that in air because of reduced photorespiration. Furthermore, CO_2 is the only form of inorganic carbon in air, while in water it is supplemented by bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). Bicarbonate and carbonate are produced by dissolution of limestone and weathering of silicates (Pagani et al. 2009) and hence their concentration is catchment-dependent and highly variable among fresh waters (Talling 1985), but relatively constant in the oceans. The different forms of inorganic carbon are interconnected by equilibria controlled largely by pH. In fresh water, HCO_3^- is the dominant form of inorganic carbon at pH values between the two carbonate dissociation constants at about 6.4 and 10.4. In seawater, the equivalent dissociation constants are about pH 6.0 and 9.1 and the concentration of HCO_3^- is around 2 mmol L^{-1}, which is about 140-times greater than the concentration of CO_2 at air-equilibrium.

However, these apparent photosynthetic advantages of inorganic carbon in water are not always realized because of other characteristics that reduce inorganic carbon availability. First, although atmospheric concentrations of CO_2 have varied substantially over geological time (Pearson and Palmer 2000), they are relatively stable over decades with current seasonal ranges of the order of 2% of the annual mean and annual growth of less than 1% (Thoning et al. 1989). In contrast, concentrations of CO_2 are highly variable in productive aquatic systems where rates of biological transformation between organic and inorganic carbon can greatly exceed rates of re-supply from the atmosphere, input from rivers, and entrainment from depth. Consequently, inland waters can be both substantially under- or over-saturated with CO_2 relative to the atmosphere at different times of year (Cole et al. 1994; Maberly 1996).

Another major constraint on the supply of CO_2 to photosynthesizing leaves in water is the approximately 10,000 lower rate of diffusion of CO_2 compared to that in air, caused by the greater density of water (Raven 1970). Consequently, a transport limitation is imposed on leaf photosynthesis (Black et al. 1981) causing the $K_{½}$ for CO_2 uptake in water to be between 100 to 200 µmol L^{-1}, roughly six- to eleven-times air-equilibrium concentrations (Maberly and Madsen 1998).

The high solubility of many ions and compounds in water provides an opportunity for nutrients, such as nitrogen and phosphorus, to be taken up by shoots as well as roots. This also produces a challenge because nutrients in the water column are a resource for planktonic and attached algae that may compete with the plants for these and other resources (Table 11.1). High solubility also allows allelochemicals and chemical signalling to produce an aquatic "smellscape" that affects interactions between different types of organism, potentially altering the competitive balance between plants and algae directly or indirectly via food-web interactions (van Donk and van de Bund 2002; Gross 2003).

III. Response of Leaf Morphology, Structure, and Composition to Aquatic Environments

Section 2 above describes some of the environmental opportunities and challenges faced by aquatic plants (outlined in Table 11.1) and the consequent biological responses are outlined below in Sects. 3 and 4.

A. Leaf Morphology

Aquatic leaves vary hugely in area (Pierce et al. 2012) from the very small leaves of free-floating lemnids with a leaf area of <1 mm^2 as in *Wolfia arrhiza*, through leaves of typical area of around 300 mm^2, to the very large floating leaves of water lilies of over 44,000 mm^2 for *Nuphar alba* and exceptionally nearly 5 10^6 mm^2 in the giant water lily *Victoria amazonica*. The morphology of aquatic leaves is less variable than those of terrestrial plants. A number of different leaf forms are widespread as discussed in classic texts such as those of Arber (1920) and Sculthorpe (1967). For example, in freshwater macrophytes, the 'isoetid' leaf shape is common, comprising short, stubby, cylindrical leaves with a large lacunal volume found in the eponymous lycophyte *Isoetes* (Fig. 11.1a) and also in dicotyledons such as *Littorella*, *Lobelia*, and *Subularia* and monocotyledons such as *Eleocharis*. Dissected whorled leaves are found in many genera of dicotyledon macrophytes, including *Myriophyllum* (Fig. 11.1b), *Limnophila*, and *Ceratophyllum* while dissected leaves are also found in *Cabomb*a and *Ranunculus* (*Batrachium*). Whorled, but mainly entire, leaves are present in monocotyledon genera such as *Egeria* (Fig. 11.1c), *Elodea, Hydrilla*, and *Lagarosiphon* and also in dicotyledon genera such as *Hippuris*. Rarely, large entire leaves are produced as in the monocotyledon *Ottelia* (Fig. 11.1d) and the submerged leaves of the dicotyledon *Nuphar lutea*. Within the large monocotyledon genus *Potamogeton*, there is a diversity of form including larger entire leaves such as in *P. lucens* (Fig. 11.1e), small linear leaves as in *P. maackianus* (Fig. 11.1f), and filiform leaves as in *P. pectinatus* (now *Stukenia pectinata*). Linear strap-like leaves are common, including those of the monocotyledons *Vallisneria* (Fig. 11.1g) and *Sparganium*. For the eudicots at least, small variations in a common development program can produce large changes in morphology (Runions et al. 2017). In seagrasses, all of which are monocotyledons, there is an even lower diversity of leaf-form with linear strap-like leaves being widespread, e.g., in *Posidonia*, *Thalassia* (Fig. 11.1h), and *Zostera*, although some genera contain species with linear leaves, e.g., *Amphibolis* or ovate leaves as in species of *Halophila* (Fig. 11.1i; (Kuo and den Hartog 2006).

Thus, there is a high degree of convergent evolution in the leaf form of aquatic embryophytes, implying common responses to environmental pressures. This is supported by the different leaf morphologies commonly seen in amphibious freshwater plants with terrestrial-like floating or emergent leaves produced in air and typical aquatic leaves produced underwater (Arber 1920; Sculthorpe 1967; Maberly and Spence 1989).

B. Leaf Structure

One of the necessary developments in the invasion of land by aquatic plants was the evolution of stomata, which occurred about

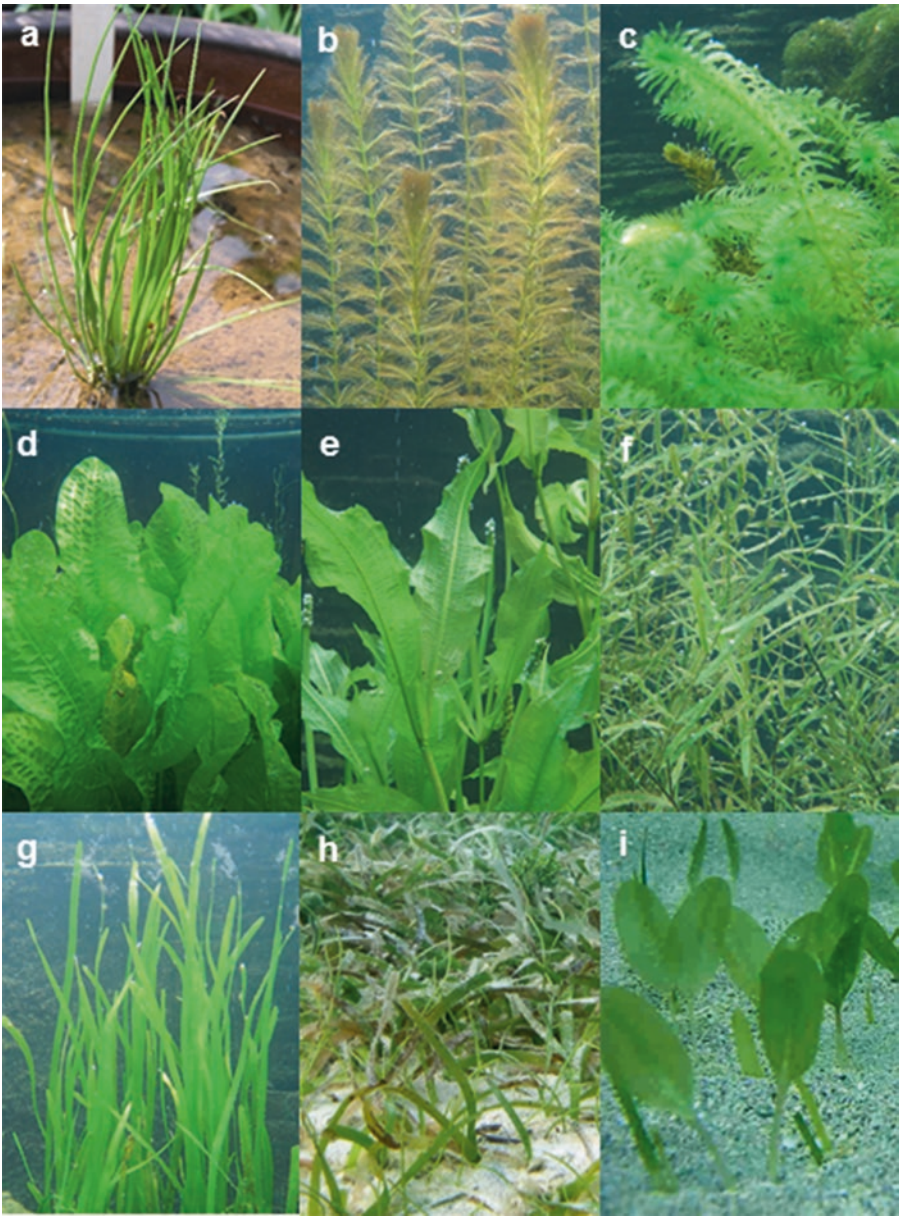

Fig. 11.1. Leaf form in freshwater macrophytes. (**a**) *Isoetes sinensis* (leaves in air), (**b**) *Myriophyllum verticillatum*, (**c**) *Egeria densa*, (**d**) *Ottelia acuminata*, (**e**) *Potamogeton lucens*, (**f**) *Potamogeton maackianus*, (**g**) *Vallisneria spiralis*, (**h**) *Syringodium filiforme* and *Thalassia testudinum*, and (**i**) *Halophila ovalis.* Photograph (**a**) was taken by Qing-Feng Wang, (**h**) by Ole Pedersen, and (**i**) by Marion Cambridge; the remainder were taken by the authors at the Botanical Garden of the Chinese Academy of Sciences in Wuhan

400 million years ago (Edwards et al. 1998; Ruszala et al. 2011). Since stomata control gas exchange including water loss, they are obsolete in aquatic environments. Indeed, most submerged leaves do not possess stomata, or, where they do exist, they are non-functional (Sculthorpe 1967). However, leaves of aquatic plants that float at the surface of the water, such as water lilies (e.g., *Nymphaea violacea*), have stomata at the

upper surface (Rudall and Knowles 2013). Stomata are also only present on the upper surface of the floating leaves of the aquatic pteridophyte, *Marsilea*, while stomata are present on both adaxial and abaxial surfaces of its aerial leaves (Lin and Yang 1999). In the seagrass *Zostera marina,* the genome of which has been sequenced (Olsen et al. 2016), the stomatal genes appear to have been lost. However, in another seagrass, *Thalassia testudinum,* paracytic stomata were observed in plants grown under 'stressed' conditions (Benzecry 2013). These contrasting data indicate that the loss of stomata is not universal in the seagrasses and there could be phylogenetic differences since *Zostera* belongs to the Zosteraceae while *Thalassia* is a member of the Hydrocharitaceae.

The lack of, or very low, water stress allows the cuticle to be very thin in many freshwater plants and seagrasses, typically about 0.1 μm (Frost-Christensen et al. 2003; Kuo and den Hartog 2006). It also allows chloroplasts to occur in epidermal cells (Fig. 11.2). This structure is typical of most aquatic plants, some of which have thin leaves with laminae of only two or three cell layers (Fig. 11.2g, h). Both laminar (Fig. 11.2c, d) and cylindrical leaves (Fig. 11.2e, f) can have large lacunal volumes although, unlike the sub-stomatal cavities of terrestrial leaves, these are not directly connected to the external environment. Floating leaves, in contrast, are thick exceeding 0.5 mm with many cells and a dorsi-ventral mesophyll structure similar to terrestrial leaves (Fig. 11.2a) or a homogenous mesophyll structure (Ronzhina and P'Yankov 2001). Within the seagrasses, small species such as *Halophila* (Figs. 11.1 and 11.2) and *Halodule* tend to have thin leaves while larger plants such as *Thalassia* and *Posidonia* tend to have thicker leaves (Papenbrock 2012) Figs. 11.1 and 11.2) presumably because they have to withstand greater hydraulic forces.

Leaf thickness in freshwater macrophytes and seagrasses has been compared to terrestrial plants by Enriquez et al. (1996). The median thickness of these aquatic leaves was about 130 μm while the median thickness of terrestrial herbs was 240 μm. Linked to this, the thickness of leaves of submerged freshwater macrophytes and seagrasses is much less than that of leaves that photosynthesize in air (Fig. 11.3). Since there is a negative relationship between leaf thickness and specific leaf area (m^2 of projected leaf area per kg dry mass; (Vile et al. 2005), specific leaf area tends to be greater in submerged freshwater macrophytes than in terrestrial leaves. An extensive meta-analysis of leaf mass per area, the converse of specific leaf area, was undertaken by Poorter et al. (Poorter et al. 2009). They showed that aquatic leaves, particularly those of freshwater macrophytes, had a much lower leaf mass per unit area than terrestrial leaves (Fig. 11.4). Similar differences can be seen in comparisons of aquatic, floating, and terrestrial leaves (Klančnik et al. 2014). Some of the ecological and physiological consequences of this are discussed in Sect. 4. Leaf construction costs, the amount of energy required to produce a unit weight or area of leaf (Williams et al. 1987), varies with the structure of aquatic leaves and their ecological habitat (Ronzhina and Ivanov 2014). Construction cost was lower in submerged compared to floating leaved rooted hydrophytes and lower still in free-floating leaves. Leaves with large cells had lower construction costs than leaves with more, but smaller, cells (Ronzhina and Ivanov 2014).

Riparian plants close to the air-water interface essentially photosynthesize in air but are frequently inundated and so have to survive, and preferably photosynthesize, during periods of flooding. Some species have hydrophobic leaves that trap an air film around them when submerged (Colmer and Pedersen 2008). This has been dubbed a 'plant plastron' by Raven (2008) by analogy with some aquatic insects. The plastron can

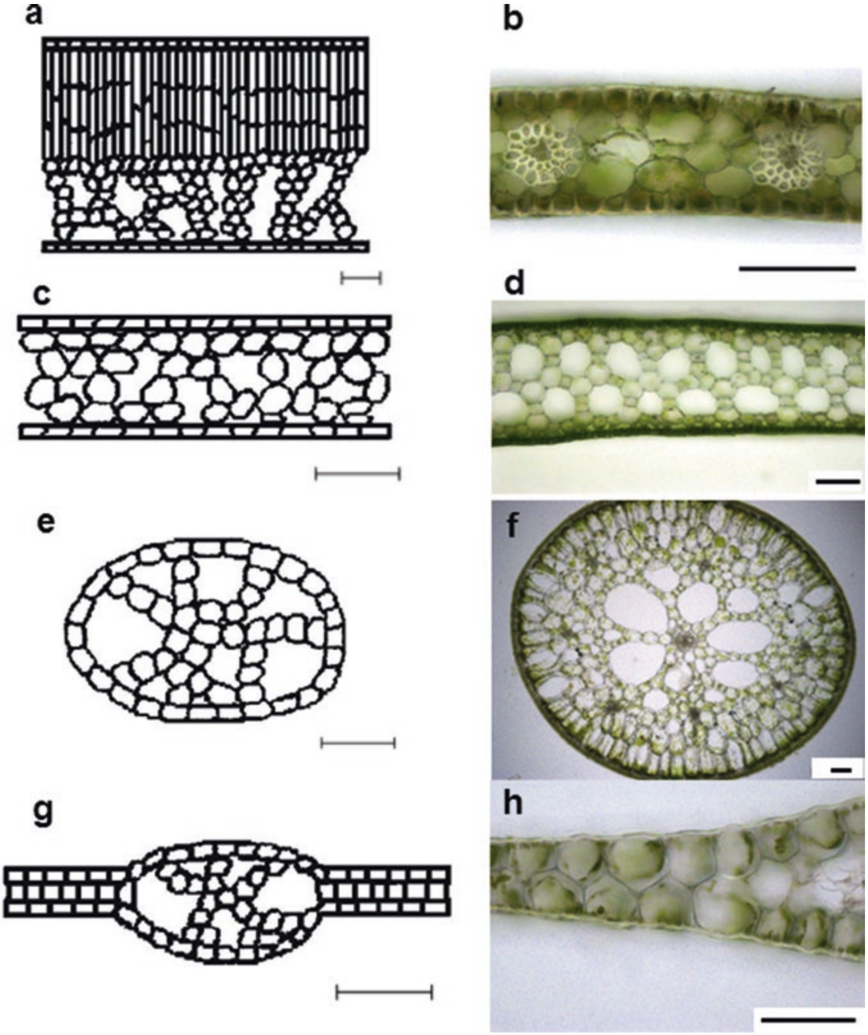

Fig. 11.2. Cross-sections of leaves of freshwater macrophytes (left-hand column) and seagrasses (right-hand column). (**a**) *Nuphar lutea* floating leaf, (**b**) *Amphibolis antarctica*, (**c**) *N. lutea* submerged leaf, (**d**) *Posidonia australis*, (**e**) *Ranunculus trichophyllous subsp. eradicatus*, (**f**) *Syringodium isoetifolium*, (**g**) *Potamogeton compressus*, and (**h**) *Halophila ovalis*. (Panels **a**, **c**, **e** and **g** reproduced with permission from Ronzhina and P'Yankov (2001). Panels **b**, **d**, **f** and **h** courtesy of Lukasz Kotula. Scale bar 100 μm)

increase the rate of CO_2 uptake by leaves in water by up to sixfold and the rate of oxygen influx to the leaf, helping to support respiration in the dark (Verboven et al. 2014; Voesenek and Bailey-Serres 2015).

The low solubility of oxygen in water, coupled with a 10,000-fold lower rate of diffusion, leads to an oxygen supply rate that is 300,000 times lower in water than air (Verberk et al. 2011). This, along with high rates of organic carbon breakdown in some environments such as the sediment, can lead to oxygen concentrations that are at or close to zero. This can prevent aerobic respiration of roots and rhizomes causing toxic products of glycolysis and fermentation, such as ethanol, to form. It can also produce redox shifts in heavy metal ions causing them to become more toxic (Crawford 1992).

The extensive lacunae of aquatic leaves, which are often continuous between shoots and roots, facilitate the transport of oxygen

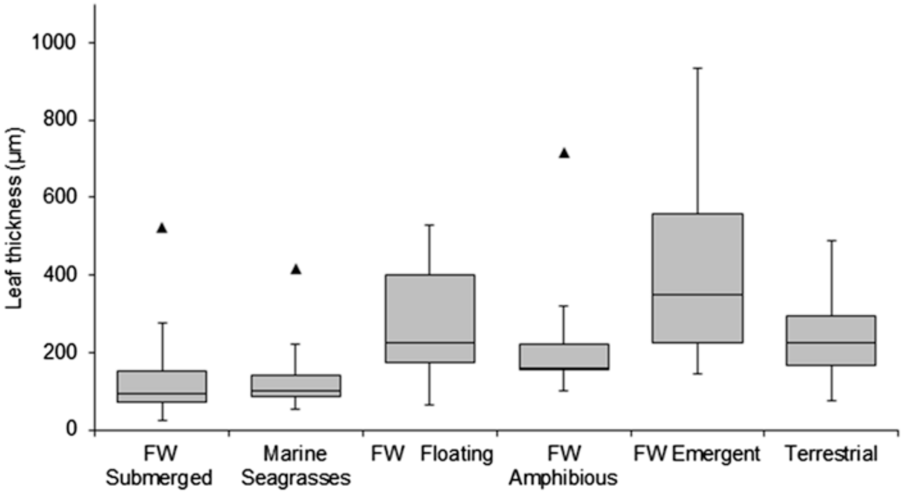

Fig. 11.3. Leaf thickness for leaves from different habitats calculated following Vile et al. (2005) for data from Pierce et al. (2012). Box and whisker plots show the lower and upper quartiles shaded in grey and the median as the horizontal line. The length of the whiskers is 1.5 times the interquartile range. The triangles represent the extreme outliers. Data for freshwater submerged (n = 33), freshwater floating (n = 19), freshwater secondary (n = 9), and terrestrial leaves (n = 506) were derived from (Pierce et al. 2012), data for marine seagrasses (n = 9) were from (Borum et al. 2015), and data from freshwater emergent plants (n = 6) were from (Colmer and Pedersen 2008). *FW* freshwater

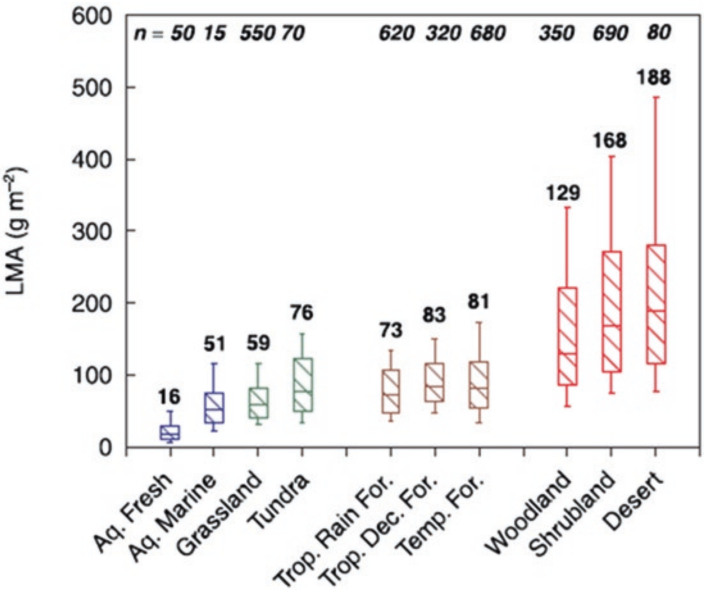

Fig. 11.4. Leaf mass per unit area (LMA, the inverse of specific leaf area) for different types of aquatic and terrestrial leaves. The bottom and top part of the box indicate the 25th and 75th percentile, respectively, the two whiskers the 10th and the 90th percentile, and the horizontal line within the box the median value. The median value is also printed above the box plots. The total number of species present in each functional group or habitat is indicated at the top of the figure. *Aq.* Aquatic, *Trop.* Tropical, *Dec* Deciduous, *For.* Forest, *Temp.* Temperate. (Reproduced with permission from Poorter et al. 2009)

into the sediment (Sand-Jensen et al. 1982; Soana and Bartoli 2013). In aquatic plants with leaves in the air, pressurized ventilation can occur (Dacey 1980, 1981; Grosse et al. 1991). This arises from thermo-osmosis driven by a temperature difference between the ambient environment and a leaf, which forces gas molecules to diffuse into the warmer compartment. The process requires pores that are small enough to generate Knudsen diffusion, around 1 μm, which in the case of *Nuphar lutea* occurs in young, newly emerging floating leaves within a monolayer of cells that separates the palisade and spongy parenchyma (Schroder et al. 1986). The flow, into the young leaves and out via the old leaves, at rates of up to five litres per hour, can supply substantial amounts of oxygen to the roots and rhizomes, helping them to reduce damage in flooded sediments. However, the oxic conditions in the sediment around the roots produced by diffusion or ventilation may decrease the availability of phosphorus through changes in redox state (Wigand et al. 1997) see Sect. 3.3.1).

C. *Leaf Composition*

Leaf composition is influenced by the growth environment of the plant. Unlike most terrestrial leaves where essential mineral resources derive largely from uptake from the soil, aquatic leaves can access nutrients from the sediment and directly from the water.

1. *Nitrogen and Phosphorus*

Many early studies focused on the extent to which water or the sediment supplied the nitrogen and phosphorus requirements of aquatic plants (Denny 1980; Barko et al. 1991). Differences in nutrient availability in the sediment porewater and overlying water have a major effect on the contribution of roots and leaves to nutrient uptake. Thus, in conditions typical of nutrient-rich Danish streams, Madsen and Cedergreen (2002) showed that the water could completely satisfy the nutrient requirement of macrophytes since experimental removal of the roots, or enriching the water with additional nutrients, had no effect on the relative growth rate of the four species of plant tested.

Early work established critical average yield-limiting elemental composition for freshwater macrophytes of 0.13 and 1.3 as a percentage of dry mass for phosphorus and nitrogen, respectively (Gerloff and Krombholz 1966). These values represent the lower limits of content (yield-limiting), while rate-limiting contents for phosphorus are about 1.6- and 3-times greater for photosynthesis and growth respectively (Colman et al. 1987) and presumably similar for nitrogen. A survey of nutrient contents of 344 plant samples from 65 rivers and lakes in NE Scotland was undertaken by Demars and Edwards (2007). They found that only a few samples were close to being yield-limited, but many were potentially rate-limited, especially by phosphorus availability. There was a large taxon-specific variation in nutrient content: aquatic bryophytes (lacking roots) had lower tissue contents of nitrogen and particularly phosphorus than vascular plants. This suggests that, in addition to anchoring plants in the sediment, roots may play an important role in supplying nutrients to the plant when concentrations in the water are low. Furthermore, macrophytes can store excess phosphorus and levels exceeding 1% of dry mass have been recorded (Thiébaut 2008). These reserves can then prevent or reduce phosphorus limitation when demand exceeds supply. Overall, the flexibility afforded by alternative nutrient supply, from sediment or water, may be one reason why nutrient limitation appears not to be a major factor controlling the growth of freshwater plants.

2. *Cell Walls*

Cellulose, hemi-cellulose, and pectin are major components of angiosperm cell walls, as is lignin in terrestrial plants. Lignin is a complex phenolic polymer that increases mechanical strength and reduces the permeability of the vessel walls that transport water from the roots to the leaves. The content of lignin varies greatly in angiosperms: grasses contain 5–10% on a dry mass basis and some tropical hardwoods contain more than 40%; an average lignin content of 20% has been estimated for modern land plants (Novaes et al. 2010). Some aquatic species have a similar lignin content to modern land plants, however in many it is lower, although highly variable among species, ranging between 0.3% and 20% for aquatic species and between 2% and 14% for wetland species (Schoelynck et al. 2010). For example, the lignin content in submerged *Berula erecta* is only 0.8–1.4% while in *Nuphar lutea* it is about 1.2% in submerged leaves and 2% in floating leaves (derived from Fig. 11.1 in (Grasset et al. 2015)).

The cell walls of seagrass leaves and shoots also have low lignin contents (Espineira et al. 2011) and contain sulphated polysaccharides (anionic polymers), like marine macroalgae, but unlike freshwater and terrestrial plants (Aquino et al. 2005; Silva et al. 2012). In seagrasses, sulphated polysaccharides are made up of galactose units (Papenbrock 2012). The carbohydrate sulfotransferases encoding genes appear to be an ancient eukaryotic feature (Collen et al. 2013) that was lost in land plants, possibly because sulphate concentrations in soils are generally much lower than in the ocean, but regained in seagrasses (Olsen et al. 2016). In marine macroalgae, sulphated polysaccharides confer rheological properties such as flexibility (Kloareg and Quatrano 1988) and presumably perform a similar function in seagrasses.

In fresh waters, leaves have direct access to minerals in water and have a higher mineral content than terrestrial leaves (Ronzhina et al. 2009). The silica concentration in fresh water can be high (on average 200 µM in rivers; (Meybeck 1979)) and some freshwater macrophytes use silica in their cell walls since this produces some rigidity at a 10- to 20-fold lower energy cost than the production of lignin or cellulose (Raven 1983). The biogenic silica content varies between 7 and 28 mg g^{-1} dry matter for true aquatic species and between 2 and 14 mg g^{-1} dry matter for wetland species (Schoelynck et al. 2010). Across aquatic and wetland species, there is an antagonist relationship between biogenic silica, lignin, and cellulose content. Interestingly, the freshwater macrophyte *Egeria densa* acclimated for 3 weeks to higher hydrodynamic stress (exposure to 0.5 m s^{-1} water flow compared to low water movement) showed ‘thigmomorphogenetic’ responses. The composition of silica and lignin in leaves and stems increased at the higher flow, causing a greater resistance to breaking and a greater tensile strength, although flexibility was unaltered (Schoelynck et al. 2015).

There is a varied response of aquatic plants to the mechanical stress experienced in their environment (Schutten et al. 2004; Bornette and Puijalon 2011; Miler et al. 2012). Two broad strategies have been identified to minimize the risk of shoot breakage, ‘avoidance’ that minimizes the forces experienced by a plant and ‘tolerance’ that maximizes resistance to breakage (Puijalon et al. 2011). In a survey of 30 species, Puijalon et al. (2011) found an inverse correlation between the two strategies, implying that they are alternatives such that each involves a cost as well as a benefit. For example, ‘avoidance’ strategies may incur a cost resulting from self-shading (see Sect. 5), while ‘tolerance’ strategies require a greater resource allocation to structural compounds.

3. *Storage Compounds*

Starch is a polymer of glucose and a primary product of photosynthesis that serves as a storage compound supporting metabolism and growth when carbon and energy demands outstrip supply (Fondy and Geiger 1982; Weise et al. 2011). Its metabolism is highly influenced by key environmental factors such as day length, temperature, and nutrient availability (Zeeman et al. 2004). In land plants, starch content is extremely variable and ranges between 1 (e.g., in the fern *Polypodium punctatum*) and 40% (e.g., in the lycophyte *Selaginella rupestris*; (Sharkey et al. 2004). The starch content of freshwater plants is generally between 0.3% and 2.5% (Steinbachova-Vojtıskova et al. 2006; Cao et al. 2011; Grasset et al. 2015; Yang and Liu 2015). In seagrasses, starch contents as high as 14% have been reported (Touchette and Burkholder 2000) representing around 65% of the total non-structural carbohydrate content. For a given species, starch content varies with nutrient content. For example, in *B. erecta* and *N. lutea,* starch content decreased as the habitat nutrient content increased, presumably as starch can accumulate when biomass is limited by availability of nutrients such as nitrogen and phosphorus (Grasset et al. 2015). Ronzhina et al. (2009) found a slightly, but significantly, greater average non-structural polysaccharide (starch and fructosans) content in aquatic compared to terrestrial leaves, possibly caused by the greater lignin and structural polysaccharide content of terrestrial leaves.

4. *Regulation of Leaf Form and Structure*

Amphibious aquatic plants that live in habitats with fluctuating water levels have to survive and preferably thrive in air and water. Some species produce leaves of similar overall morphology (homophyllous) while others produce leaves of very different appearance in the two environments (heterophyllous; (Maberly and Spence 1989). Leaves of low stature plants, such as the isoetid *Littorella uniflora*, only differ slightly in morphology when growing in air or water. In a study of this species growing on the shores of a reservoir with seasonal fluctuating water levels, Robe and Griffiths (1998) showed that leaves produced in air were nearly twofold longer and 1.6-fold thinner than leaves produced underwater. Submerged leaves had a few stomata that appeared to be non-functional and terrestrial leaves had a tenfold greater stomatal density and 3.7-fold lower lacunal volume.

In heterophyllous amphibious plants, leaves produced in air are often similar to terrestrial leaves with thick cuticles, functional stomata, and sub-stomatal cavities, while leaves produced in water are typically aquatic in morphology and structure. A range of different environmental factors are known to trigger the switch between aquatic and terrestrial leaves (Maberly and Spence 1989; Wells and Pigliucci 2000). Low versus high temperature (Johnson 1967), short versus long daylength (Kane and Albert 1982), high versus low concentration of CO_2 (Bristow 1969), and high versus low water potential (Deschamp and Cooke 1983) favor the production of submerged versus aerial leaves and vice versa. Phytochrome, affected by the ratio of red to far-red light, is involved in the production of aerial leaves in *Hippuris vulgaris.* Aerial leaves are produced by a low red:far-red ratio in the field and the laboratory even when underwater (Bodkin et al. 1980). This mechanism of sensing the environment is not present in the seagrass *Z. marina*, which has lost the genes coding for phytochrome production (Olsen et al. 2016), but appears to be present in the brackish water seagrass *Ruppia maritima* that responds to different red:far-red ratios (Rose and Durako 1994).

Some or all of these responses to environmental cues are probably mediated by hormones. Gibberellic acid often promotes the formation of submerged leaves, while abscisic acid, frequently produced in response to water stress, often promotes the formation of aerial leaves (Maberly and Spence 1989; Wells and Pigliucci 2000). Another plant hormone, ethylene, is involved in shoot elongation, a flooding response in some species including *Callitriche platycarpa*, *Ranunculus sceleratus*, and *Rumex palustris* (Jackson 1985). *Potamogeton pectinatus* (*Stukenia pectinata*) lacks the ability to produce ethylene, but responds to exogenously supplied ethylene (Summers and Jackson 1998). The seagrass *Z. marina*, in contrast, appears to lack ethylene responsive genes (Golicz et al. 2015; Olsen et al. 2016) but, apart from tidal fluctuations, seagrasses do not experience the same seasonal and episodic changes in water level as freshwater and riparian macrophytes.

IV. Resource Acquisition and Responses to Aquatic Environments

A. Light Acquisition

The aquatic environment is basically a shade environment because of a reflection loss at the air-water interface and the absorption of light by water and dissolved and suspended material, which can be exacerbated further by growth of epiphytes on leaf surfaces (Sand-Jensen 1977; Sect. 2.2). The rooting depth limit of freshwater macrophytes is controlled by light and varies between 2.2% of surface light in bryophytes, 5% in charophytes and caulescent (elodeid) angiosperms, 12.9% in *Isoetes*, and 16.3% in rosette (isoetid) angiosperms (Middelboe and Markager 1997). The differences among groups are partly caused by the proportional extent of roots with a large respiratory burden in isoetids and the ability of some species, such as caulescent angiosperms, to elongate their shoots at low light and therefore grow into shallower water with higher light levels. Seagrasses only have a depth limit of 16 to 18% of surface light (Lee et al. 2007), although in the generally more transparent marine waters, it has been suggested that this could be caused by the effects of pressure, rather than low light, at depth (Beer and Waisel 1982).

Enriquez (2005) studied the light absorption efficiency in *Thalassia testudinum* and other seagrasses. As expected, leaf light absorption efficiency decreased with pigment content per unit area as a consequence of the package effect. The lower pigment light absorption efficiency of *T. testudinum* compared to the more typical terrestrial leaf of *Mentha aquatica* might be explained by the absence of palisade cells and spongy mesophyll cells in seagrasses. However, the thin, flat leaves of tropical seagrasses were more efficient than *M. aquatica,* perhaps because of greater scattering within the leaf that helps to offset the package effect.

Individual submerged freshwater and marine macrophytes are generally shade-adapted with median compensation points for net photosynthesis (I_c) at a photon irradiance of about 16 μmol m^{-2} s^{-1} and the onset of light-saturation (I_k) at about 130 μmol m^{-2} s^{-1} (Binzer et al. 2006). In a compilation of data from temperate and tropical seagrasses, the median values of I_c and I_k were 30 and 116 μmol m^{-2} s^{-1} respectively (Lee et al. 2007). Freshwater macrophytes from deeper, low-light environments are more shade-adapted than those from shallower water (Spence and Chrystal 1970a, b). The genome of the seagrass *Z. marina* contains ten genes for LHCB1, one of three proteins that form the trimers of the photosystem II light-harvesting complex. The number of genes for LHCB1 in *Z. marina* is greater than in *Spirodela polyrhiza* and *Arabidopsis thaliana* (Olsen et al. 2016), consistent with enhancing performance at low light. Although individual leaves may be saturated by low levels of light, an individual leaf within a dense stand of plants maybe shaded by other leaves and therefore receive very low light so that the stand may not be fully light-saturated even at maximum light levels (Binzer et al. 2006). Individual leaves or shoots, however, may experience high light and photoinhibition may therefore occur that, although photoprotective, (Adams et al. 2013), will reduce net photosynthesis. When

H. verticillata, grown at moderate light, was exposed to full sunlight for 15 minutes, photosynthesis was photoinhibited by about 50% at an inorganic carbon concentration of 0.6 mmol L^{-1} but was not inhibited at 2 mmol L^{-1} because carbon fixation provided a greater sink for the light energy absorbed (White et al. 1996). When three species of freshwater macrophyte, grown at low light, were exposed to light from 1.4-fold to 14-fold higher, a photoprotective response was triggered that down-regulated their light harvesting machinery (decreased chlorophyll content) and the photosynthetic electron transport chain (Hussner et al. 2010).

Enzymes and molecules that scavenge reactive oxygen species (ROS) are also involved in photoprotection. The amphibious plant *Lobelia cardinalis* relies on the xanthophyll cycle for the pre-emptive dissipation of excitation energy as heat preventing ROS formation (Nielsen and Nielsen 2006). In contrast, another amphibious species, *Nesaea crassicaulis,* when grown in water appeared to lack the xanthophyll cycle but had very high levels of anthocyanin that is a powerful antioxidant and acts as sunscreens and quencher of free radicals a (Nielsen and Nielsen 2006).

Ultraviolet radiation at the water surface can be substantial but it is rapidly attenuated with depth, especially in water with high concentrations of coloured dissolved organic carbon (Morris et al. 1995). However, no significant differences were found in the content of UV-B screening pigments (flavonoids) in floating versus submerged leaves of the amphibious macrophytes *Ranunculus trichophyllum* and *Potamogeton alpinus* (Germ et al. 2002). The authors concluded that, in these species, levels of screening pigments were saturating and sufficient to prevent damage at current or elevated (17%) levels of UV-B. In contrast, differences in UV-absorbing compounds were found on a unit area basis between submerged and floating or emerged leaves of *Sagittaria sagittifolia* and *Ranunculus lingua* (Klančnik et al. 2014). In the seagrass *Z. marina*, in contrast to the LHCB1 genes, a gene involved in UV-B-sensing and triggering photo-protective responses, UVR8 (Rizzini et al. 2011), appears to have been lost (Olsen et al. 2016), although associated genes such as COP1 are present. Olsen et al. (2016) hypothesized that the loss of UVR8 is linked to the attenuation of UV radiation in aquatic environments since UVR8 is present in another Alismatales that floats on the water surface, *Spirodela*. More sequenced genomes are required from a range of different aquatic plants to test this and other hypotheses.

B. Carbon Acquisition

The potential problems of acquiring inorganic carbon in aquatic environments are outlined in Sect. 2.2. Aquatic embryophytes exhibit a large range of anatomical, morphological, biochemical, physiological, and ecological carbon acquisition strategies to minimize this constraint (Klavsen et al. 2011; Maberly and Gontero 2017). This diversity of strategies reflects the various costs and benefits involved in acquiring inorganic carbon in different aquatic environments and contrasts with terrestrial plants where there are only three carbon dioxide acquisition strategies, all based on carboxylation enzymes as described below.

1. C_3 Metabolism

Terrestrial and aquatic leaves assimilate CO_2 by a common pathway known as the reductive pentose phosphate pathway, the Calvin-Benson-Bassham cycle or the C_3 cycle. This pathway uses the products of the light reactions of photosynthesis, ATP and NADPH, to produce carbon skeletons that lead to the production of sucrose and starch and involves 13 reactions catalyzed by 11 enzymes. In the first step, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) carboxylates ribulose-1,5-bisphosphate (RuBP) with CO_2 to produce two molecules of phosphoglyceric acid (PGA), each with three carbon atoms. Rubisco can also oxygenate RuBP producing one molecule of PGA and one molecule of phosphoglycolate leading to photorespiration when the concentration of O_2 at its active site is high relative to CO_2 (Bowes et al. 1971; Bowes and Ogren 1972). The terrestrial evolutionary history of aquatic embryophytes means that the properties of their Rubisco enzyme are likely to be more similar to that of their direct land plant ancestors than to the microalgal and macroalgal photoautotrophs (Tabita et al. 2008) with which they grow and compete. Similarly, carboxylation in plants is unlikely to involve other enzymes (e.g., use of carbon monoxide dehydrogenase/Acetyl-CoA synthase; (Tabita et al. 2008; Berg 2011; Raven et al. 2012; Hadj-Saïd et al. 2015; Kroth 2015), in contrast to archaea and eubacteria. In order to be fully active, Rubisco must be activated by a non-substrate CO_2, or carbamylated at the ϵ-amino group of a specific lysine residue at position 201 (numbered from the plant spinach enzyme) with the divalent cation Mg^{2+} (Lorimer and Miziorko 1980). Although the regulatory properties of Rubisco activase have not been examined in aquatic organisms, the regulation of this activase is probably similar to that employed by terrestrial shade plants (Gontero and Salvucci 2014).

While the properties of Rubisco in aquatic plants are likely to be similar to their terrestrial forebears, the levels of activity tend to be lower reflecting the lower resource availability in water than in air. A survey of 21 freshwater plants found a median Rubisco activity of about 150 µmol h^{-1} mg^{-1} Chla (range of 12 to 464 µmol h^{-1} mg^{-1} Chla; (Beer et al. 1991). Rubisco activity was lower in submersed than in emergent freshwater leaves (Beer et al. 1991; Fig. 11.5), in agreement with previous observations (Farmer et al. 1986). Moreover, Rubisco activity from seagrasses was similar to that of submersed freshwater species (Beer et al. 1991). From compiled data for C_3 land plants, the average activity of Rubisco from 11 species was higher with a median of about 220 µmol h^{-1} mg^{-1} Chla (ranging from 80 to 622 µmol h^{-1} mg^{-1} Chla; Fig. 11.5).

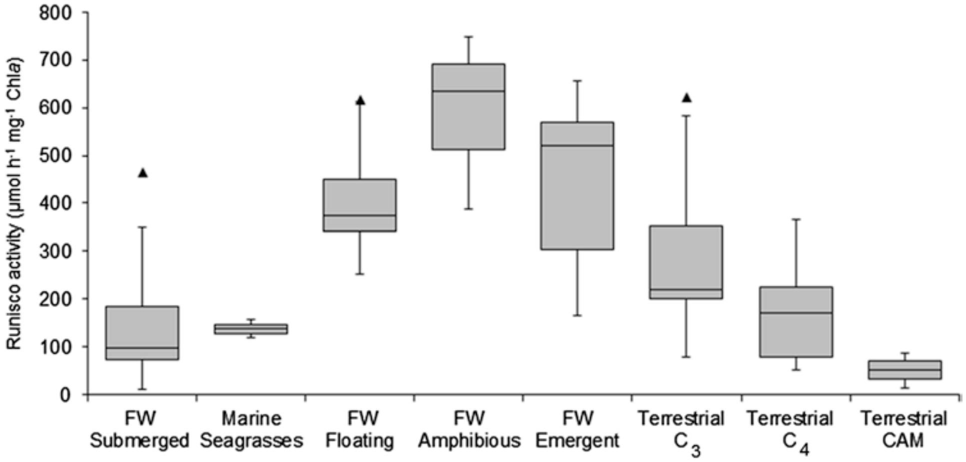

Fig. 11.5. Activity of Rubisco per unit chlorophyll *a* in different plant types. Box and whisker plots show the lower and upper quartiles shaded in grey and the median as the horizontal line. The length of the whiskers is 1.5 times the interquartile range. The maximum outlier is shown by a closed triangle. (Data for aquatic plants from Beer et al. (1991) and terrestrial plants predominantly from Vu et al. (1984). FW = freshwater)

2. Avoidance Strategies

Avoidance strategies (*sensu* Klavsen et al. (2011) are employed by plants that live and grow in microhabitats with locally high CO_2 concentrations, such as above the sediment surface close to sites where decomposition of organic matter produces CO_2, and hence avoid problems caused by low CO_2 availability. For example, the low-growing aquatic moss *Fontinalis antipyretica* is restricted to CO_2 as a carbon source (Bain and Proctor 1980) yet grows in a lake, Esthwaite Water UK, where surface concentrations of CO_2 are close to zero in the summer. It survives by growing just above the sediment surface where concentrations of CO_2 are 2- to 10-times air-equilibrium (Maberly 1985a, b). About 20% of freshwater macrophytes lack a CO_2-concentrating mechanism (CCM) but, based on plant stature, grow close enough to the sediment to have the possibility of benefitting from a high-CO_2 microenvironment (Fig. 11.6).

3. Exploitation Strategies

Exploitation strategies (*sensu* Klavsen et al. (2011)) involve morphological or anatomical features that give access to higher concentrations or higher availability of CO_2. Examples include floating leaves that exploit the more constant and available concentrations of CO_2 in the atmosphere. These can make a major contribution to carbon uptake of amphibious plants (Janauer and Englmaier 1986; Prins and Deguia 1986; Madsen and Breinholt 1995), but they may not be able to prevent overall carbon limitation, so restricting some species to sites with high concentrations of CO_2 (Nielsen and Borum 2008). The high concentrations of CO_2 in the sediment can be exploited by short isoetids, because extensive lacunae in roots and leaves form a continuous pathway for CO_2 to diffuse from the sediment to the leaves. This was first shown by Wium-Andersen (1971) for *Lobelia dortmanna* and can account for a large, but variable proportion of carbon uptake in many isoetids (Raven et al. 1988; Madsen et al. 2002). The rate of diffusion restricts the length of leaf over which this process can occur. Accordingly, Bagger and Madsen (2004) found that leaves were longer in populations of *L. uniflora* growing in sediment with high concentrations of CO_2. The thick cuticle of some isoetids, especially *Lobelia dortmanna*, reduces exchange of CO_2 with the water, but acts to trap CO_2 within the leaf. In *Isoetes australis*, achlorophyllous leaf bases in the sediment, comprising 34% of shoot surface area, are additional important points of CO_2 entry to the photosynthesizing leaves (Pedersen et al. 2011b). Overall, about 30% of the tested species have access to the atmosphere via floating or emergent leaves but only about 3% have access via lacunae to CO_2 within the sediment (Fig. 11.6).

4. Amelioration Strategies

C_4 Metabolism

Amelioration strategies involve additional, energy-requiring processes. Some land plants, mainly those living in high light, hot, dry and/or saline environments, have evolved adaptations in which CO_2 is first fixed by a supplementary pathway, namely crassulacean acid metabolism (CAM) or C_4 photosynthesis. These CCMs elevate the CO_2 concentration around Rubisco, thereby suppressing photorespiration (Giordano et al. 2005; Raven et al. 2011, 2012; Meyer and Griffiths 2013).

In both pathways, the first carboxylation step is achieved by the oxygen-insensitive enzyme phosphoenol pyruvate carboxylase (PEPC) that catalyzes carboxylation of phosphoenol pyruvate with HCO_3^- to yield a four-carbon molecule, oxaloacetate, that is subsequently converted to malate (or aspartate). Malate (or aspartate) is then decarboxylated to produce CO_2 near Rubisco by one of three enzymes: NADP malic enzyme

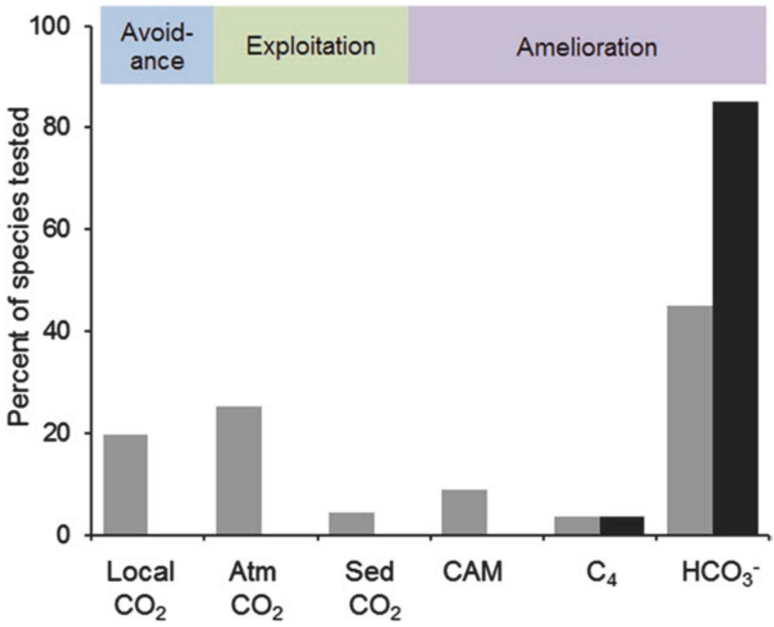

Fig. 11.6. Carbon acquisition strategies in freshwater macrophytes (grey histograms) and seagrasses (black histograms); data from (Maberly and Madsen 2002; Koch et al. 2013; Borum et al. 2015). Local = leaves that have access to elevated levels of CO_2 in their immediate vicinity; Atm = species with access to CO_2 in the atmosphere; Sed = species that receive the bulk of CO_2 from sediments via anatomical conduits and from their own respiratory CO_2; CAM = species that utilize crassulacean acid metabolism to acquire CO_2 during the night for subsequent use in light-driven chloroplast-localized photosynthesis during the day; C_4 = plants that utilize C_4 photosynthesis to acquire CO_2 more efficiently during the day; and HCO_3^- = plants that utilize bicarbonate as a source of carbon for photosynthesis

(ME), NAD-ME, or PEP carboxykinase (PEPCK). In terrestrial plants, C_4 metabolism has long been associated with a radial leaf anatomy (Kranz anatomy), involving two types of cells, to separate physically synthesis of the four carbon acids from decarboxylation in order to prevent futile cycling. Fixation of CO_2 by PEPC occurs in mesophyll cells that surround bundle sheath cells where decarboxylation occurs and where Rubisco is present (Gutierre et al. 1974; Hatch 1987; Edwards et al. 2001). Detailed phylogenetic and carbon isotope studies have resolved C_3, C_4 and C_3–C_4 intermediate relationships within many taxonomic groups and showed that the C_4 pathway of photosynthesis has evolved many times, suggesting that compartmentation is an intrinsic feature maximizing the efficiency of the process (Sage 2004; Sage et al. 2012). Interestingly, about a decade ago, variants of the C_4 pathway within a single cell were discovered in terrestrial plants growing in salty, semi-desert regions, including *Bienertia cycloptera* and *Borszczowia aralocaspica* (family Chenopodiaceae) (Voznesenskaya et al. 2001; Voznesenskaya et al. 2002; Edwards et al. 2004) and more recently *Bienertia sinuspersici* (Offermann et al. 2011). These results show that Kranz anatomy is not essential for C_4 metabolism.

In aquatic systems, a number of 'amelioration strategies' involving CCMs exist to counteract the limitations in inorganic carbon availability (Raven 1970; Maberly and Madsen 1998, 2002; Giordano et al. 2005; Klavsen et al. 2011). It is likely that C_4 photosynthesis arose in aquatic systems, in response to limitations in dissolved CO_2, before its advent in terrestrial ones, based on

PEPCK in the green macroalga *Udotea flabellum* (Reiskind and Bowes 1991), and more controversially, and geologically later, in some marine diatoms putatively based on PEPC (Roberts et al. 2007; Reinfelder 2011; Clement et al. 2016).

Like the single-cell C_4 metabolism in some terrestrial plants, aquatic C_4 metabolism takes place in a single cell. The best characterized freshwater C_4 plant is the submerged aquatic monocotyledon *Hydrilla verticillata* (Hydrocharitaceae); (Holaday and Bowes 1980; Salvucci and Bowes 1981; Bowes et al. 2002). C_4 metabolism in *H. verticillata* is facultative, being induced by various environmental conditions that reduce the availability of CO_2 and promote photorespiration (Reiskind et al. 1997). In *H. verticillata*, physical separation of PEP carboxylation and decarboxylation is achieved within a single cell since PEPC is confined to the cytosol and Rubisco to the chloroplast.

The regulatory patterns and properties of the enzymes involved in C_4 metabolism have been studied in detail in *H. verticillata*. When C_4 metabolism is induced in *H. verticillata*, the activities of PEPC, pyruvate phosphate dikinase (PPDK), NADP–ME, and asparagine and alanine aminotransferases are elevated, while Rubisco activity remains constant (Magnin et al. 1997). One out of the three PEPC isoforms was expressed uniquely in C_4 leaves and transcript levels for this isoform increased (Rao et al. 2002). Genes for two isoforms of PPDK, as well as genes that encode a transporter, an aminotransferase, and two chaperonins, were also up-regulated (Rao et al. 2006). The activity of NADP-ME increased tenfold in C_4 plants (Magnin et al. 1997) and a specific chloroplast isoform (HVME1) was induced. This isoform had specific and unusual regulatory properties that are likely to facilitate C_4 metabolism in this species (Estavillo et al. 2007). Furthermore, the catalytic efficiency of recombinant HVME1 was twofold higher than that of rice but lower than the plastid forms of maize, and the K_m for malate was higher than that for the maize enzyme but fourfold lower than that found in rice. Bowes and co-workers concluded that NADP-ME is the decarboxylating enzyme in *H. verticillata* (Holaday and Bowes 1980). Although both NAD- and NADP-ME increased under limiting CO_2 conditions (Table 4 in Salvucci and Bowes 1981), 95% of the total NAD-ME activity was found in the mitochondrial fraction. Therefore, if NAD-ME was the decarboxylating enzyme, CO_2 would be released in the cytosol causing a futile cycle since PEPC is also present within this compartment. In contrast, the NADP-ME isoform, HVME1, is specifically induced in the chloroplast, the site where CO_2 is concentrated (Reiskind et al. 1997) and it was therefore concluded that it was the main decarboxylating enzyme in this species.

Egeria densa, another species from the Hydrocharitaceae, also exhibits a NADP-ME C_4 syndrome, under high light and high temperature, that can also be triggered by exogenous application of the plant stress hormone abscisic acid (Casati et al. 2000). The kinetic properties of the purified NADP-ME that was induced were similar to those of terrestrial C_3 plants. The partially purified PEPC synthesized during the induction of C_4 photosynthesis had a K_m value (*ca* 8 μM) for HCO_3^- that is lower than those reported in the literature for other PEPC enzymes (Casati et al. 2000). PEPC isoforms are not only induced in leaves (Casati et al. 2000), but also in stems in *E. densa* under low CO_2 conditions (Gu et al. 2015). Similar results were obtained for PPDK and NADP-ME, possibly indicating that the stem could act as a photosynthetic organ as has been described in some terrestrial plants such as tobacco and celery (Hibberd and Quick 2002) and several species of trees (Berveiller and Damesin 2008). As is often the case for an enzyme catalyzing the first step of a pathway, PEPC is finely regulated: phosphorylation and feedback inhibition by malate are well established in terrestrial plants (Chollet et al. 1996) and possibly also occur in aquatic systems (Casati et al. 2000; Lara et al. 2002; Gu et al. 2015). Recently, two further Hydrocharitaceae, *Ottelia alismoides* and *O. acuminata,* were also shown to exhibit the C_4 syndrome in the absence of Kranz anatomy (Zhang et al. 2014; Shao et al. 2017). When grown at low CO_2, the activity of PEPC and NAD-ME was higher than when grown at high CO_2. If NAD-ME is the decarboxylating enzyme in these species it will be the first reported case for an aquatic plant. Four carbon acids accumulate in other freshwater macrophytes, such as *Elodea canadensis* (Degroote and Kennedy 1977), but there is currently no evidence for turnover and transfer of CO_2 from these metabolites into the C_3 pathway.

In seagrasses, the capacity to perform C_4 photosynthesis seems limited to a few species. Evidence is strongest for *Cymodocea nodosa* (Beer et al. 1980), but *Halophila stipulacea* and *Thalassia testudinum* may have a facultative C_4 metabolism induced by low CO_2 concentrations (Koch et al. 2013; Larkum et al. 2017); further investigation is required to test this possibility. If confirmed, this would be another example of C_4 photosynthesis in aquatic plants lacking Kranz anatomy since there is no indication of Kranz anatomy in any seagrasses (e.g., Fig. 11.2). Presently, about 4% of tested freshwater macrophytes and seagrasses show the capacity for C_4 metabolism (Fig. 11.6).

CAM

The other supplementary pathway in terrestrial plants, CAM, is very similar to C_4 photosynthesis, but PEPC and Rubisco are temporally regulated, the former being active at night and the latter during the day. At night, the four-carbon compound malic acid is stored in vacuoles and decarboxylated during the following day. The features of CAM are therefore: net uptake of CO_2 and accumulation of malic acid during the night, diel change in acidity and starch content of the cells, opposite to one another, a high PEPC activity during the night, and a high PEPC:Rubisco activity ratio (Osmond 1984; Winter et al. 2015).

For decades, CAM was regarded as a water conserving measure in plants from environments with intermittent water availability. The intriguing discovery by J. Keeley that this metabolism was also present in an aquatic plant (*Isoetes howellii*; (Keeley 1981) was first rejected but later confirmed (Keeley 2014). In these plants, there is net CO_2 uptake accompanied by malic acid accumulation during the night (Keeley et al. 1983; Keeley and Busch 1984). CAM has subsequently been found in all other species of *Isoetis* and other submerged macrophytes including *L. uniflora* (Madsen 1987a) and *Crassula helmsii* (Newman and Raven 1995; Klavsen and Maberly 2009). Other freshwater species show some evidence for diurnal acid fluctuations that may be related to CAM (Webb et al. 1988) including *Vallisneria spiralis* (Keeley 1998) and *V. spinulosa*, *Nechamandra alternifolia*, and *E. densa* (Yin et al. 2017). Recently, it was shown that *O. alismoides* (Zhang et al. 2014) and *Deinostema violaceum* (a member of the Plantaginaceae, like *L. uniflora*; (Yin et al. 2017) are able to operate CAM facultatively. CAM has been found in about 9% of tested aquatic species, which is comparable to the percentages estimated for terrestrial plants (Silvera et al. 2010).

Crassulacean acid metabolism is under environmental control in aquatic plants. Typically CAM is up-regulated when leaves are in water and down-regulated when leaves are in air in *Isoetes howellii* and *L. uniflora* (Keeley and Busch 1984; Aulio 1986; Robe and Griffiths 2000); the opposite to expectations for a water-conserving measure and to what occurs in some terrestrial CAM plants (Cushman and Borland 2002). After about 3 days emersion, CAM activity in *L. uniflora* aquatic leaves decreased by 70% and new terrestrial leaves had no CAM activity and exhibited high Rubisco activity (Robe and Griffiths 2000). Recently, using a range of approaches, including titratable acidity measurements, mRNA levels, and activity measurements of C_3 and C_4 enzymes, it was shown that *Isoetes sinensis* possesses a stronger CAM activity under submerged than under terrestrial conditions (Yang and Liu 2015), although, unlike other species of *Isoetes*, it appeared not to be completely down-regulated in leaves grown in air (Yin et al. 2017). CAM is also down-regulated when *L. uniflora* is grown at low light (43 µmol photon m^{-2} s^{-1} of photosynthetically active radiation) versus high light (450 µmol photon m^{-2} s^{-1}) and CAM activity is reduced when this species is grown at high concentrations of CO_2 (Madsen 1987b). Activity can also change seasonally (Klavsen et al. 2011; Klavsen and Madsen 2012).

CAM in aquatic ecosystems is correctly considered to be a carbon-conserving strategy that increases net inorganic carbon acquisition. For instance, many species with CAM live in soft-water lakes or in shallow seasonal pools of moderate alkalinity where the availability of CO_2 is low (Sondergaard and Sand-Jensen 1979; Keeley et al. 1983; Madsen 1985). Aquatic CAM is beneficial in this habitat because, unlike the situation for many terrestrial CAM plants, uptake of external CO_2 is not suppressed in the light allowing CO_2 to take place over 24 hours and to exploit nocturnal concentrations of CO_2 that are often elevated relative to those during day. A major carbon-conserving advantage of aquatic CAM is its ability to reduce respiratory loss of carbon by converting CO_2 to malate. For example, in *C. helmsii*, CAM activity was equivalent to 74% of night-time respiration in spring and over 200% in summer (Klavsen and Maberly 2009). CAM also has a beneficial effect by increasing rates of photosynthesis and suppressing photorespiration in *Isoetes australis* (Pedersen et al. 2011a).

About 98% of the malic acid that is decarboxylated during the day to produce endogenous CO_2 is re-assimilated in *Littorella* and *Isoetes*. This high efficiency is likely to be related to internal storage in the extensive lacunal system of the leaves, their relatively thick cuticles, and the resistance of the surrounding liquid medium. In these species, the maximum CO_2 concentration in the lacunal air ranged from 0.8 to 1.5%, which is comparable to the values for terrestrial plants (Madsen 1987a). Last, it has been suggested that CAM could function as a nitrogen conserving mechanism because an increased concentration of CO_2 at the active site of Rubisco makes the enzyme more efficient (Sage and Kubien 2003). However, for *L. uniflora* at least, this hypothesis could not be confirmed (Baattrup-Pedersen and Madsen 1999).

There is no suggestion so far that any seagrasses operate a CAM system (Koch et al. 2013). Some accumulation of ^{14}C label in four carbon acids has been detected in *Thalassia hemprichii*, *Thalassodendron ciliatum*, and *Halophila stipulacea* that did not, however, appear to be linked to CAM metabolism (Beer et al. 1980). Nonetheless, more research is warranted to test the possibility that a form of CAM is in operation in some species.

Bicarbonate Use

Bicarbonate is the most abundant form of inorganic carbon in seawater and many fresh waters (Sect. 2.2) (Maberly and Gontero 2017). However, some form of active transport is required to acquire HCO_3^- from the surrounding aqueous medium because the plasmalemma is impermeable to HCO_3^- and the aquatic leaf has a negative internal membrane potential (Denny and Weeks 1970) creating a large electrochemical gradient opposing passive HCO_3^- entry. A widespread mechanism of HCO_3^- acquisition in freshwater macrophytes is the generation of low and high pH at the abaxial and adaxial leaf surfaces respectively (the so-called polar leaf). This mechanism has similarities to that associated with the well-studied banding on the giant cells of some freshwater green

Fig. 11.7. Marl on the upper surface of a *Potamogeton lucens* leaf, indicated by the arrow, growing among *Hydrilla verticillata* and *Myriophyllum spicatum*. Photograph taken by the authors

macroalgae (charophytes) *Chara* and *Nitella* (Lucas and Smith 1973). The formation of marl (calcite) on the upper surface of aquatic leaves of species within certain genera, e.g., *Elodea*, *Egeria*, *Hydrilla* and *Potamogeton* (Fig. 11.7), has been known for decades and studied as a mechanism of carbon uptake by scientists such as Arens, Ruttner, and Steemann-Nielsen in the middle of the twentieth century (see review by Prins (1989). Extensive studies by Prins and co-workers (1980, 1982; Prins and Elzenga 1989) have elucidated the various mechanisms involved. The cells of the lower epidermis often, but not always, have extensively folded plasma membranes with the appearance of transfer cells. The proposed mechanism involves active extrusion of protons at the lower epidermis, aided by the larger plasma membrane area, generating a local reduction of pH (down to pH 4). This converts HCO_3^- into CO_2 within the boundary layer that can then diffuse into the leaf. In order to maintain electrochemical balance, cations are transported through the apoplast from the lower to the upper side of the leaf and a net export of OH^- occurs at the upper epidermis generating high pH values (pH 10 to 11) causing HCO_3^- to be converted to carbonate and calcite to be precipitated the marl seen in (Fig. 11.7). The precipitation of carbonate also leads to the generation of CO_2 close to the cell surface in *Chara corallina* that may be taken up (McConnaughey 1991) and a similar process may occur at the upper leaf surface. This is not the only mechanism involved in HCO_3^- utilization since some species within genera such as *Ranunculus* or *Myriophyllum* are effective users of HCO_3^- (Maberly and Spence 1983) but do not possess the physical leaf features described above, as is also true for *Vallisneria spiralis* (Prins et al. 1980). However, there might still be spatial horizontal patterns of high and low pH that act in a similar way, analogous to the banding in some species of *Chara*. Alternatively, or in addition, HCO_3^--H^+ co-transport might be involved in HCO_3^- uptake, but more work is needed to elucidate the mechanisms involved that are likely to vary among species.

Of the species of freshwater macrophyte tested, about 45% have the ability to use HCO_3^- (Maberly and Madsen 2002; Fig. 11.6). Less extensive work has been performed on HCO_3^- use in seagrasses compared to freshwater macrophytes. Compiled data (Carr and Axelsson 2008; Koch et al. 2013; Borum et al. 2015) suggest that about 85% of the 27 species that have been studied are able to use HCO_3^- (Fig. 11.6). This is greater than the 57% found in the freshwater macrophytes tested, but the marine habitat has a higher HCO_3^- concentration than many fresh waters, so the ecological advantage may be greater in the oceans. Detailed studies of the mechanism of HCO_3^- use in seagrasses have been performed using inhibitors by a number of workers. For example, in *Z. marina* and *Ruppia cirrhosa*, HCO_3^- use mainly involves HCO_3^- dehydration in external acid zones, catalyzed by periplasmic carbonic anhydrase, followed by diffusion into the leaf (Hellblom et al. 2001; Hellblom and Axelsson 2003). In addition, non-catalyzed

HCO_3^- dehydration or direct uptake of HCO_3^- has also been suggested to be involved. In a study of nine seagrass species (Borum et al. 2015), seven were definitely able to use HCO_3^- and of these, five were suggested to operate a mechanism involving external acidification. In the two additional species, internal conversion of HCO_3^- to CO_2 was inferred. External carbonic anhydrase played a role in four of the species with the ability to use HCO_3^- (see Table 3 in Borum et al. 2015). Fewer studies of this nature have been undertaken on freshwater macrophytes.

The more constant concentration of CO_2 in the oceans and the closer coupling of the aqueous CO_2 concentration with the atmospheric CO_2 content makes it possible to assess whether seagrass photosynthesis is currently saturated by inorganic carbon and likely to be increased by changing atmospheric CO_2 content. Borum et al. (2015) found that the rate of net photosynthesis in eight of the nine species they tested, the exception being *Zostera polychlamys*, was limited at pre-industrial concentrations of CO_2 (280 ppm). This suggests that relief of carbon-limitation in future elevated CO_2 environments might alter the competitive interactions among the species in non-light-limited situations. Experiments to determine carbon limitation in stream macrophytes showed that *in situ* rates of photosynthesis were 35% of CO_2-saturated rates for a species unable to use HCO_3^- and 60% for a species able to use HCO_3^- despite the stream having a high CO_2 concentration of about 220 μmol L^{-1} (Madsen and Maberly 1991). A similar type of assessment, but based on growth carried out in two contrasting Danish lakes with lower CO_2 concentrations than the stream, showed that a species restricted to using CO_2 was unable to grow while a species able to use HCO_3^- grew slowly but the rate was stimulated by about 50% when supplied with higher CO_2 concentrations (Vadstrup and Madsen 1995). These experiments, and others like them, show that freshwater macrophytes without the ability to use HCO_3^- can be severely limited by carbon availability at typical environmental concentrations of CO_2 and that although HCO_3^- helps to reduce this it may not remove carbon-limitation completely.

V. Trade-Offs, Synergies, and Future Prospects

The contrasting environmental challenges and opportunities of air and water as environments for photosynthesis and growth have been described in the text and summarised in Table 11.1. These represent responses to a single environmental factor while in reality a leaf has to respond simultaneously to multiple environmental conditions. These may require a trade-off, where one response is optimised for one environmental factor but is less beneficial for another or a synergy where a particular feature has more than one benefit. Consequently, different characteristics are beneficial in different environments, although this is complicated by environmental variability that may alter the cost-benefit balance of a trade-off, and by acclimation at a range of time-scales that will help to optimize fitness by altering morphology and physiology. It is this interaction among environmental conditions, characteristics, trade-offs, and synergies that control fitness and the ecological distribution of species. In this penultimate section, three examples of different possible types of trade-offs, and three examples of synergies, are discussed.

A. Trade-Offs

Structural or physiological characteristics typically have energy or opportunity costs as well as fitness benefits. As a result, they confer an advantage under some, but not all, environments. As a first example, the flexible nature of many aquatic plants reduces the mechanical stress they experience in flowing water and thus reduces the risk of shoots breaking or being uprooted. However, the reconfiguration of shoots can have negative consequences by increasing self-shading and therefore reducing light availability to the plant (Sand-Jensen 1998).

A second example concerns the morphology of many aquatic leaves. Madsen (1991) showed that entire, as opposed to dissected, leaves were more common in oligotrophic compared to eutrophic lakes and more common in streams and marine systems than in lakes. This was attributed to trade-offs between minimizing boundary-layer thickness, and hence maximising rate of material exchange, and preventing mechanical damage at high flow. In seagrasses, the general pattern of thin leaves in some small species and thicker leaves in larger species is probably linked, at least in part, to a similar trade-off.

A third example relates to the use of HCO_3^- in aquatic plants. This has costs and benefits that find different balances in different environments. The most obvious cost relates to the extra energy required to produce and operate the machinery required for HCO_3^- use (Raven and Lucas 1985; Raven et al. 2008). The relevance of this cost in the 'real world' is supported by the lack of HCO_3^- use in red macroalgae growing in low light environments in the ocean (Maberly 1990) and in bryophytes (Bain and Proctor 1980) that are frequently found at the depth-limit in lakes (Middelboe and Markager 1997). If energy was the only cost, one would expect HCO_3^- to be ubiquitous in optically shallow water, but this is not the case as was observed (Maberly et al. 2015) in a transect down a fairly shallow river fed by a spring with very high HCO_3^- concentrations (>4 mmol L^{-1}). Concentrations of CO_2 were very high close to the source, and the species found there were restricted to those that use CO_2. A few kilometers downstream, where concentrations of CO_2 were much lower but the concentration of HCO_3^- was virtually the same, all the species tested were able to use HCO_3^- (Maberly et al. 2015). A similar response also occurs within a species, *Elodea canadensis*, grown at high light and very high concentrations of CO_2 (2200 μM), down-regulated its ability to use HCO_3^- (Sand-Jensen and Gordon 1986). An additional cost of using HCO_3^- relates to the affinity of using CO_2 (Maberly and Madsen 1998). At a given limiting concentration of CO_2, the CO_2-dependent rate of photosynthesis is around twofold higher in species restricted to CO_2 than in ones also able to use HCO_3^-. This difference appears to be related to a lower internal permeability in species able to use HCO_3^- (Madsen and Maberly 2003). This is teleologically consistent with, although not yet proved to be linked to, the prevention of futile cycling in species able to use HCO_3^-: a low permeability reduces the efflux of inorganic carbon actively taken up by the leaf. It is also possible that species limited to the use of CO_2 have a lower leaf construction cost than those species that also use HCO_3^-. There are virtually no data on this, but in Table 11.1 in Ronzhina and Ivanov (2014), the single submerged species tested that lacks the ability to use HCO_3^-, *Utricularia vulgaris*, had the lowest area-based construction cost. Clearly, this is worthy of further study.

B. *Synergies*

Some structural and physiological features have a synergistic benefit for more than one environmental challenge. For example, the lack of water stress allows chloroplasts to be present in the epidermis of the leaves of many submerged plants (e.g., Fig. 11.2). This is likely to be beneficial both for the supply of light and carbon although it has been noted (Black et al. 1981) that the large external transport resistance around the leaves of freshwater macrophytes means that internal diffusion resistances are relatively small in comparison.

In a second example, flexible shoots reduce the risk of breakage but also reduce water flow rate within the plant stand and so promote sedimentation. Sediment within a patch has been found to be 4.1 cm above the river bed on average (Sand-Jensen and Pedersen 2008) and since this comprises fine organic sediment, it has a high carbon, nitrogen, and phosphorus content. This may make an important contribution to nutrient availability to plants in streams and rivers that are not already eutrophic (Sand-Jensen 1998). However, in rivers with sufficient nutrients in the water, high organic carbon content in the sediment can cause a negative trade-off by limiting oxygen supply to the roots (Moller and Sand-Jensen 2011) and, in some species at least, reducing the strength of anchorage provided by the roots, increasing the risk that the whole plant might be washed out of the system (Sand-Jensen and Moller 2014).

In a third example, floating or emergent leaves give access to the much more reliable supply of CO_2 in the atmosphere. This is a common feature in many emergent amphibious plants and makes them among the most productive plants in the world (Westlake 1975). Moreover, access to the atmosphere also helps to minimize the common problem of restricted oxygen supply to roots and rhizomes by providing a pathway for atmospheric oxygen to reach roots and rhizomes, especially in species with ‘forced ventilation’(Dacey 1980). The transpiration stream may also promote the transport of nutrients from root to shoot at a rate greater than that possible by acropetal transport (Pedersen 1993).

C. *Future Prospects*

As is hopefully apparent from this chapter, there is a large and growing body of information about how aquatic leaves respond to the challenges and opportunities presented by their environment and hence maximize their fitness. There are also some clear knowledge gaps, some of which are highlighted below. Unlike most terrestrial plants (but the C_4/CAM *Portulaca oleracea* is an exception; (Koch and Kennedy 1980)), some aquatic plants can operate more than one CCM or carbon acquisition strategy. There are some apparent, but presently unexplained, associations between the different CCMs in freshwater macrophytes and seagrasses. All species known to have the ability to perform C_4 photosynthesis are also able to use HCO_3^-. While it is true that HCO_3^- is the substrate for PEPC, the link between these two facts is not necessarily straightforward and direct. One can speculate that the association could be linked to the low concentrations of CO_2 and high concentrations of O_2 that can occur in productive beds where C_4 plants may be found. While both processes act as a CCM, the C_4 pre-fixation by PEPC may help to reduce oxygen sensitivity but HCO_3^- provides the continued supply of carbon. In contrast, species known to have the ability to perform CAM are largely restricted to CO_2. It is possible to speculate that species with the ability to undergo CAM tend to rely on a relatively thick cuticle to trap CO_2 produced by decarboxylation of malate within the plant. This might be an effective strategy in species such as the isoetids that can obtain carbon from the sediment where it will also serve to increase internal concentrations of CO_2, but will be detrimental to plants that rely on HCO_3^- in the water. One interesting exception is *O. alismoides* that appears to operate three CCMs, HCO_3^- use, C_4 photosynthesis, and, facultatively, CAM (Zhang et al. 2014; Shao et al. 2017). If these mechanisms are all confirmed to be present, it will raise interesting questions about how these different processes are regulated and interact.

Unlike terrestrial plants, including *A. thaliana*, few aquatic plants have been sequenced, the recently published sequence of *Z. marina* (Olsen et al. 2016) being an exception as are the genomes of two species of two floating freshwater plants, *Lemna minor* (Van Hoeck et al. 2015) and *Spirodela polyrhiza* (Wang et al. 2014). Genomic sequencing and analysis of more aquatic species and their comparison with terrestrial species will lead to novel phylogenetic and functional insights. Along with analyses of proteomes, transcriptomes and metabolomes, it will dramatically increase our understanding of how aquatic plants respond to their environment at the level of their morphology, physiology, and biochemistry.

VI. Conclusions

The challenges and opportunities faced by aquatic plants photosynthesising in water are very different to those of their terrestrial ancestors photosynthesising in air. This has led to distinctive differences in the morphology, structure and resource acquisition processes of aquatic compared to terrestrial leaves. The contrasting properties of air and water, and the biological characteristics that they trigger, can be useful to highlight fundamental processes. For example, the interrelationships between CCMs discussed above are also relevant to discussions about the evolution of terrestrial C_4 photosynthesis and CAM (Sage 2002). In another example, the discovery of CAM in aquatic plants (Keeley 1981) and the subsequent realization that its function was linked to carbon-conservation, rather than water-conservation, has also illuminated the carbon-conserving function of CAM in terrestrial plants. Within the field of food security, the possibility of introducing genes for C_4 photosynthesis into C_3 crops such as rice (Leegood 2013) has relied in part on the extensive work on the best-characterized plant with single-cell photosynthesis, *Hydrilla verticillata* (Bowes 2011). Aquatic leaves are also part of the global spectrum of leaf and shoot types. In a recent analysis, Díaz et al. (2016) showed that aquatic leaves lie on one of the major axes that they identified. They fell within the group of ephemeral, small plants with low investment in vegetative structures along with small terrestrial ruderals such as *Arabidopsis thaliana*. Recent work that related the size of terrestrial leaves (Wright et al. 2017) to leaf-energy budgets and water-loss could be confronted with data from aquatic leaves where other challenges are likely to be dominant as a result of the physical properties of water.

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