Chapter 5
Seaweed Acclimation to Salinity and Desiccation Stress

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Dedicated to my mentor Professor Dr. Gunter O. Kirst on the occasion of his 70th birthday.

5.1 Variability of Salinity in Seaweed Habitats

Salinity is a technical term in marine sciences and represents the sum of all dissolved salts of a defined body of water. Therefore, marine biologists for a long time used the expression *promille* (‰) or *parts per thousands* (ppt) to describe salinity concentration. In contrast, since 1978 oceanographers defined salinity in the Practical Salinity Scale as dimensionless Practical Salinity Units (PSU) which is the conductivity ratio of a seawater sample to a standard KCl solution. In October 2010, the Intergovernmental Oceanographic Commission (IOC), International Association for the Physical Sciences of the Oceans (IAPSO), and the Scientific Committee on Oceanic Research (SCOR) jointly adopted a new standard for the calculation of the thermodynamic properties of seawater. This new standard, called TEOS-10, uses Absolute Salinity S_A (mass fraction of salt in seawater) to describe the salt content of seawater. Ocean salinities now have units of g dissolved salts kg^{-1} water (http://www.teos-10.org; Wright et al. 2010). Therefore, all salinities throughout the text are expressed as Absolute Salinity S_A.

The chemical composition of the dissolved salts is relatively constant throughout the open oceans due to intensive mixing, and it varies only between 33 and 37 S_A, gradually decreasing from the subtropics toward the tropics and polar seas. In contrast, salinity strongly varies in nearshore waters and estuaries where river freshwater mixes with marine water bodies. Here horizontal and vertical gradients between 0 and 33 S_A can be measured. The degree of salt dilution in estuaries

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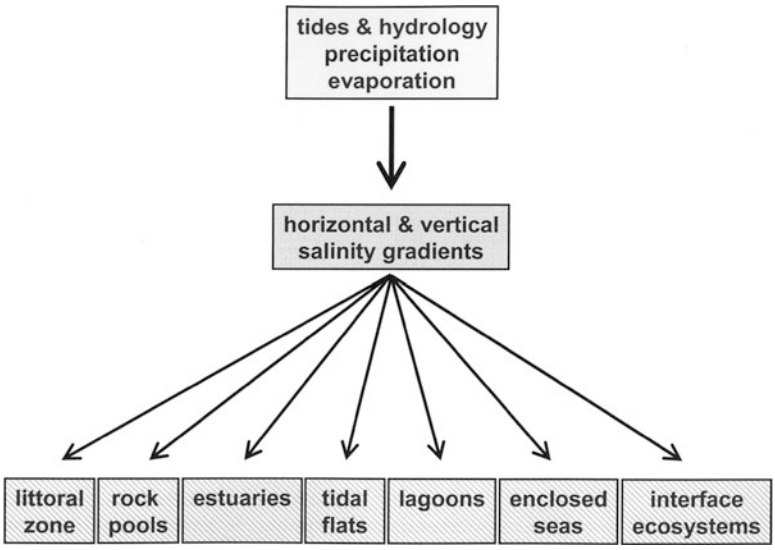

Fig. 5.1 Illustration of the abiotic factors influencing salt concentration resulting in horizontal and vertical salinity gradients in various aquatic systems

largely depends on the rate and volume of freshwater runoff, and can be dramatic. The water of the Amazon, for example, can be recognized at the surface of the open Atlantic Ocean for approximately 300 km (Ryther et al. 1967). Since the geochemistry of the river's catchment area is typically highly variable as well, it strongly affects besides the salt concentration also the salt composition in estuaries. In addition, tidal flows, hydrological conditions, wind, precipitation, and evaporation strongly influence salt concentration of the respective water bodies (Fig. 5.1). Consequently, salinity is typically a local rather than a global parameter and can be highly variable in coastal regions.

Since most seaweeds are sessile organisms that preferentially grow attached to hard substrata such as rocks, gravel, or as epiphytes on salt marsh plants, on mangroves, and on mussel colonies, they are mainly confronted with salinity fluctuations and desiccation when exposed at low tides. At lowest water levels, hyposaline conditions may be present due to the mixing of seawater with rain, snow, or melt water, while hypersaline stress may occur due to evaporation during high insolation in summer or freezing-out of freshwater in winter. In addition, in estuaries and fjords which often exhibit extensive seaweed communities (Schramm and Nienhuis 1996), rivers or freshwater runoff mix with seawater resulting in diurnally and seasonally fluctuating salinity gradients. In Arctic waters, seaweed species can be strongly affected by melt water influx and calving glaciers (Hanelt et al. 2001; Karsten et al. 2003a).

Most seaweeds such as kelps inhabit the sublittoral which is characterized by quite stable environmental factors, and hence these more deepwater plants only

rarely experience salinity and desiccation stress. One of these occasions, however, is spring low tides where sublittoral seaweed species may be exposed for some time to salinity changes and desiccation (Lüning 1990). Besides the intertidal zone and estuaries, there are other habitats occupied by seaweeds which are affected by salinity changes or gradients (Fig. 5.1). Rock pools at low tide often exhibit strong salinity fluctuations due to precipitation or evaporation. Seaweeds living on tidal flats (e.g., on mussel beds) or in interface ecosystems (salt marshes, mangroves) are regularly affected by the tidal flows and hence to a combination of salinity and desiccation stress. In mangroves epiphytic red algae exposed to the prop roots can be observed completely covered with salt crystals. Semi-enclosed seas with only small connections to the open oceans mostly exhibit strong horizontal salinity gradients or hypersaline conditions depending on the hydrology of the catchment area and precipitation. The best studied semi-enclosed system is the Baltic Sea with its strong, but stable horizontal salinity gradient from almost freshwater conditions in the Northeast to brackish/marine conditions in the western part. Since the largest part of the Baltic Sea shows a salinity between 4 and 7 S_A only, biodiversity of the aquatic flora (incl. seaweeds) and fauna is generally strongly reduced, because this salinity range (ß-mesohalinum 5–10 S_A) is too low for marine species and too high for freshwater organisms (Remane 1940). In warm-temperate to subtropical coastal regions such as South Australia, lagoons are typical shallow water systems that are separated from the open ocean by some form of barrier (e.g., sand spit) resulting in often hypersaline conditions because of the high prevailing evaporation. A typical example is the Coorong lagoon in South Australia with salinities >100 S_A, where high abundances of charophyte algae can occur (Bisson and Kirst 1983). Marine and brackish waters are consequently almost infinitely variable in the amplitude and frequency of their saline changes, which of course has various consequences for the physiological performance of seaweeds.

5.2 Effects of Salinity Stress and Desiccation on the Physiology of Seaweeds

Since decades, the effects of salinity and desiccation on the ecophysiological performance of seaweeds have been described to outline the species-specific width of tolerance, i.e., upper and lower limits for survival, as well as mechanisms for acclimation (Kirst 1990 and references therein). Nevertheless, both salinity stress and desiccation reflect two different forms of water deprivation. While under salt stress conditions, seaweed cells are still in full contact to liquid water of diminished water potential, desiccation leads to strong cellular dehydration. Typical physiological parameters which have been studied under such stress conditions include rate of survival, growth, photosynthesis, respiration, and reproduction.

5.2.1 Rate of Survival

The rate of survival can be easily estimated by following seaweed growth or photosynthesis activity under defined stress conditions, or using classical vital staining techniques for individual cells such as Evans Blue (Russell 1985; Thomas and Kirst 1991). Only dead cells with damaged membranes will take up this stain and hence appear blue under the microscope. These days a range of commercially available fluorescence dyes such as the so-called Live–Dead kits are available which in combination with an epifluorescence microscope allow the evaluation and visualization of even smallest damages on membrane integrity.

The available data on cell viability in relation to salinity indicate that members of eulittoral green algal genera such as *Ulva* (incl. *Enteromorpha*), *Acrosiphonia*, *UIothrix, Cladophora*, and *Prasiola* are generally much less affected than sublittoral red and brown seaweeds (Russell 1985, 1987, Kain and Norton 1990; Thomas and Kirst 1991; Karsten et al. 1991a). While even under triple seawater concentration >75% of green algal cells remain intact, mortality in sublittoral brown algae such as *Phaeurus antarcticus* increased to almost 100% (Karsten et al. 1991a). This rather euryhaline feature of eulittoral green seaweeds may be considered as important environmental factor for controlling vertical species zonation on the shore (Russell 1987).

5.2.2 Growth

From an ecological perspective, growth represents the most relevant physiological process to describe the performance of seaweeds in their respective habitat because it optimally integrates all positive and negative environmental effects on the organism and hence reflects the acclimation potential (Gustavs et al. 2009). A careful analysis of growth patterns allows the evaluation of salinity tolerance limits, growth optima, and acclimation abilities of individual seaweed species, and, thus facilitates the interpretation of natural distributions. Knowledge of physiological limits is necessary to estimate and understand distribution limits within an unknown community, i.e., in a competitive situation. Additionally, the shape of the growth rate curve in dependence of any abiotic factor characterizes a seaweed species as steno- or euryoecious, or in case of salinity as steno- or euryhaline, which definitively affects its competitive strength.

Until now, numerous ecophysiological studies have been undertaken on the salinity effects on growth in seaweeds (Jacob et al. 1991; Karsten et al. 1991a, 1993b, 1994, 1996b; Karsten and West 1993; Kirst 1990 and references therein, Thomas and Kirst 1991). All these papers clearly indicate that eulittoral seaweeds from polar to tropical regions grow between low salinities (5–10 S_A) and double seawater concentration (66–68 S_A), in most cases with conspicuous optima under normal seawater conditions. The supralittoral *Prasiola crispa* ssp. *antarctica* even

grows between 0.3 and 105 S_A (Jacob et al. 1991). In contrast to the generally broad salinity tolerance of upper-shore seaweeds, those from the sublittoral usually exhibit the narrowest tolerance limits (Russell 1987). Salinities commonly encountered in areas of abundance are most favorable for growth (Bird et al. 1979; Bird and McLachlan 1986). This is well reflected in the growth pattern of the green alga *Ulva pertusa* from an eelgrass bed in a semi-protected bay at the southwest coast of Korea (Choi et al. 2010). *Ulva pertusa* exhibited optimum growth at 20 S_A, a situation encountered in the field during the rainy season when this species often forms blooms in eelgrass beds. Growth under hyposaline conditions, i.e., in aquatic systems such as estuaries or the eastern part of the Baltic Sea, may be governed by the availability of certain inorganic ions which increase the lower tolerance limits of seaweeds. In this context, the presence of Ca^{2+} plays an essential role in cell signaling, as structural component of seaweed cell walls and membranes, and as cation to balance organic anions in the plant vacuole (Kauss 1987; Tazawa et al. 1987; Verret et al. 2010). Whole seaweed thalli have been reported to exhibit differential salinity tolerances. Particularly young apical growing parts of species of the genera *Cladophora*, *Ceramium*, *Phycodrys*, and *Plumaria* are more sensitive to hyposaline conditions than older, basal parts (Russell 1987). Kirst (1990) speculated that this observation is a secondary effect of Ca^{2+} availability, as particularly fast-growing cells depend on this cation, for example, for cell wall formation.

If not only growth but also survival is considered, most seaweeds show a remarkable physiological potential. While the red alga *Porphyra umbilicalis* exhibits optimum growth between 7 and 52 S_A, it survived without cell division even in sixfold fully marine salinities for 2 weeks (Wiencke and Läuchli 1980). Similar observations have been reported in the studies of elongation growth in the siphonous green alga *Valonia macrophysa* (Gutknecht et al. 1978). Near the limits of salinity tolerance, growth of most studied seaweeds is typically strongly reduced or even completely inhibited in order to funnel all available metabolic energy into the process of osmotic adjustment, which guarantees survival under fluctuating salinities. Besides these energetic considerations, high inorganic ion concentrations under salt stress conditions exert negative, i.e., inhibiting effects on seaweed growth. This is reflected in conspicuous changes in size and morphology of seaweeds under long-term salt stress (Russell 1987).

If additional environmental factors that greatly affect the growth rate of seaweeds as well are included in the investigation of the growth–salinity relationship (e.g., radiation (UV), temperature, etc.), the emerging picture becomes very complex. Therefore, only a few macroalgal species have been investigated in this respect, such as the green algae *Cladophora glomerata* and *C. rupestris* (Thomas et al. 1988), the red alga *Polysiphonia lanosa* (Reed 1983), or the kelp species *Laminaria groenlandica* and S*accharina latissima* (Druehl 1967). Low salinity may be compromised by temperature as shown for North Pacific *L. groenlandica* which cannot tolerate the combination of low salinity and high temperature conditions encountered in areas subjected to snow-melt runoff, whereas *S. latissima* can. Both species, however, do well in areas subjected to winter rain runoff where

cold conditions prevail (Druehl 1967). All available data indicate that growth of seaweeds primarily depends on radiation and temperature conditions, and the more these environmental factors approach species-specific optimum requirements, the broader is the salinity range tolerated. In addition, if seaweeds are exposed to desiccation during low tides, growth is completely inhibited. The physiological strategy is to cope with and survive this stress condition by maximum reduction of all metabolic activities.

5.2.3 *Photosynthesis and Respiration*

Besides growth, photosynthesis and respiration are two central physiological processes in seaweeds which are strongly affected by salinity changes (Kirst 1990). Under extreme hypo- or hypersaline conditions, photosynthesis and respiration are typically completely inhibited and in many cases confirm the growth–salinity relationships described above. Whether salinity stress is extreme depends on the habitat and vertical zonation of the respective seaweed species, because sublittoral taxa are generally much more sensitive and hence stenohaline than their eulittoral, mainly euryhaline, counterparts (Russell 1987). A relatively salt-insensitive photosynthesis and respiration seem to be a prerequisite for the successful occupation of the eulittoral habitat and may ensure long-term survival and reproduction under large amplitudes of salinity in combination with other environmental factors (Gessner and Schramm 1971). A similar relation regarding photosynthesis as a function of desiccation was reported in various seaweeds from different tidal heights (Wiltens et al. 1978). In addition, photosynthesis and respiration exhibit different responses under a range of salinities as documented for a set of eulittoral green algae from Antarctica (Karsten et al. 1991a; see also Chap. 13 by Wiencke and Amsler). While most investigated species showed optimum photosynthesis at 34 S_A and decreasing rates between 7 and 17 S_A as well as between 51 and 68 S_A, respiration was much less affected. Similarly, under desiccation photosynthesis of seaweeds is much more affected than respiration (Wiltens et al. 1978).

The photosynthetic and respiratory responses following exposure to moderate and high changes in salinity are inconsistent among seaweeds. Frequently, a transient stimulation of respiration and a stimulatory or inhibitory effect on photosynthesis have been observed (Kirst 1990). The time required for a more or less complete recovery is species specific and lasted for several hours to days for seaweeds (Kirst 1990).

Using chlorophyll fluorescence kinetics, the underlying processes leading to salt-induced inhibition of photosynthesis were studied in various eulittoral green and red alga (*Prasiola*, *Ulva*, *Porphyra*) (Wiltens et al. 1978; Fork and Öquist 1981; Satoh et al. 1983; Smith et al. 1986). These investigations were aimed primarily at measuring the effects of desiccation on photosynthesis. The two stresses (increasing salinity and desiccation) are comparable since they result in a reduction of the cellular water potential. During desiccation, however, cellular ionic concentrations

increase and the ion ratios remain constant. In contrast, during salinity stress algal cells may not only increase ionic concentrations but also undergo changes in ion ratios owing to selective uptake. This has to be taken into account when comparing the results obtained with species under salt or desiccation stress.

The primary photosynthetic mechanism is affected at the electron transport stage between PS I and PS II. The sensitive site in *Porphyra* and *Ulva* species is most likely between plastoquinone and P 700 (Wiltens et al. 1978). In *Porphyra perforata* there seem to be at least three sites in the photosynthetic apparatus that are inhibited by high salinity, namely the photoactivation of electron flow on the reducing side of PS I, the electron flow on the water side of PS II and the transfer of light energy between the pigment complexes (Satoh et al. 1983). These authors concluded that a free electron flow at all three sites is essential to avoid photodamage through chronic photoinhibition, which will occur if only one site is blocked because of, e.g., the accumulation of highly reactive oxygen species(ROS; see also Chap. 6 by Bischof and Rautenberger).

More recent data on cyanobacteria indicate a salt-induced inactivation of both oxygen evolution in PSII and electron transport in PSI (Allakhverdiev and Murata 2008). The site of inactivation is the electron-donating side of PSII, i.e., the oxygen-evolving machinery, due to the influx of uncontrolled Na^+ and Cl^- ions with resultant dissociation of extrinsic proteins from photosystems (Allakhverdiev and Murata 2008).

5.3 Processes of Osmotic Acclimation

Hypersaline conditions in the external medium affect seaweeds in two ways: first, the water potential is strongly reduced leading to dehydration of cells, and secondly, high concentrations of some inorganic ions exert toxic effects on cellular metabolism. Similarly, desiccation stress will also result in strong dehydration of cells. Since both hypersalinity and desiccation affect the internal osmotic potential, the acclimation responses of seaweeds are comparable.

Water is taken up in all living cells by osmosis, which is driven by the water potential gradient, i.e., only if the intracellular water potential is lower than in the external medium there is a water influx. Consequently, marine seaweeds have to create an internal osmotic potential higher than that of seawater to gain and retain constant water content of the cells, which is necessary to maintain turgor as the driving force for growth. Seaweeds typically respond to external salinity changes with osmotic acclimation processes which involve the control of cytoplasmic and vacuolar concentrations of osmotically active compounds (Kirst 1990). Therefore, osmotic acclimation is a fundamental mechanism of salinity tolerance of these plants that conserves the stability of the intracellular milieu (homeostasis), and it is essential for maintaining an efficient functional state in the cells (Kirst 1990). Most seaweeds use inorganic ions and small organic osmolytes to create the internal osmotic potential.

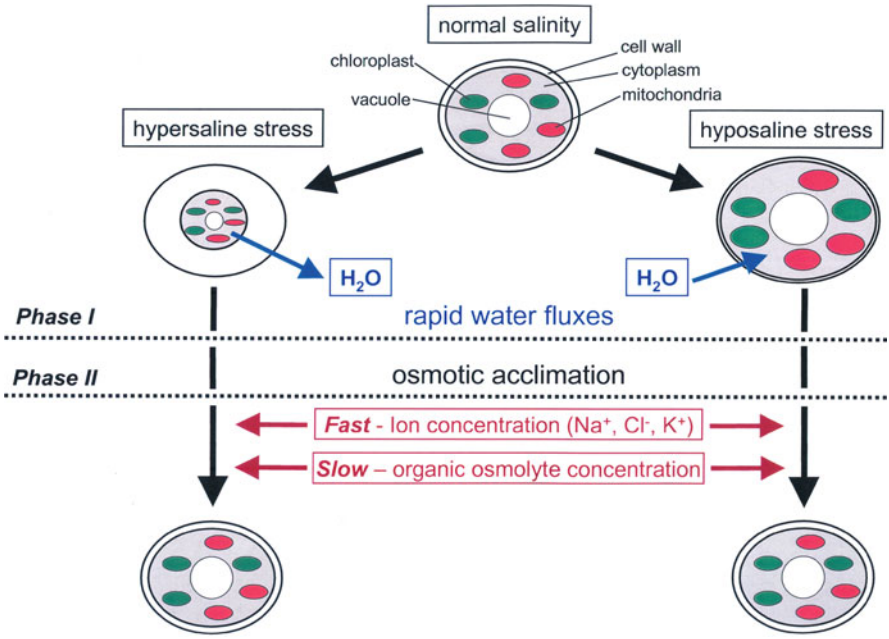

Fig. 5.2 Schematic summary of the processes of osmotic acclimation in seaweeds after hypersaline and hyposaline stress leading to a new steady state

The response of seaweeds to salinity changes is a well-regulated biphasic process. Phase I is characterized by rapid changes in turgor pressure caused by massive water fluxes in or out of the organism following the osmotic gradient. Phase II represents the process of osmotic acclimation, i.e., adjustment of the intracellular concentrations of the osmotically active inorganic ions and organic osmolytes until a new steady state is achieved (Fig. 5.2). Both phases are part of a feedback loop that comprises the osmotic acclimation.

The water fluxes during phase I are rapid processes with half times in seaweeds lasting from minutes to hours (Zimmermann and Steudle 1978), resulting in water influx under hyposaline conditions or water efflux during hypersaline stress, both of which mitigate at least transiently the osmotic stress (Fig. 5.2). Most importantly, these processes follow passive "osmometer" behavior, and depend on physicochemical properties of the cell-wall membrane complex such as the hydraulic conductivity (water permeability, water channels). So far it has been assumed that all phase I processes are not under metabolic control (Kirst 1990), but since the discovery of the so-called aquaporins as membrane-located water channels it seems that cells can control their water content (Törnroth-Horsefield et al. 2006). These channels are widely distributed in all kingdoms of life. They form tetramers in the plasmalemma, and facilitate the transport of water through the lipophilic membrane. Probably, many water channels at the cytoplasmic membrane open for short time during salt stress, and not only water but also small water-soluble solutes are taken up or lost during phase I.

In contrast to the phase I, phase II is a rather slow process in seaweeds lasting from several hours up to 2–3 days (Kirst 1990, Karsten et al. 1996b). As a result, the internal osmotic potential becomes adjusted by changing the concentrations of ions and organic osmolytes to restore the original turgor pressure. These processes are under direct metabolic control.

5.3.1 Inorganic Ions

To adjust inorganic ion concentrations to homeostatic conditions, ions are actively extruded under hypersaline conditions but must be imported under hyposaline stress. The main ions involved in osmotic acclimation are K^+, Na^+, Cl^- and to a lesser extent sulfate, nitrate, or phosphate. The ionic composition of algal cells and in particular of their vacuoles varies widely depending on cellular number and volume of vacuoles (Kirst 1990). In most seaweeds, the Cl^- concentration usually follows the fluctuation in salinity. With respect to the Na^+ content, particularly siphonous green algae such as members of the genera *Caulerpa*, *Halimeda* or *Bryopsis* with their huge vacuolar system accumulate this cation. In contrast, other seaweeds with small vacuoles and high cytoplasmic fraction prefer to accumulate K^+ under salinity stress (Kirst 1990). In contrast to the toxic properties of Na^+ (and Cl^-), K^+ is fully compatible to metabolic activities, although an explanation for this fact is still missing (Maathuis and Amtmann 1999). Both cations exhibit similar physico-chemical properties, as the smaller Na^+ together with its rather large hydration shell mimics the size of K^+. Consequently, uptake systems for K^+ have difficulties discriminating between both ions, and high external Na^+ amounts may result in K^+ deficiency. Inside the cell, Na^+ can compete for K^+-binding sites of proteins, which contribute to their stabilization, resulting in the inhibition of K^+-dependent metabolic processes (Hagemann 2011). Therefore, all organisms tend to ensure a defined usually high K^+/Na^+ ratio in the cytoplasm (Maathuis and Amtmann 1999).

During osmotic adjustment, the changes in and control of ion composition are achieved by regulating the activity of specific transport systems, which are particularly well studied in cyanobacteria (Hagemann 2011) and probably act also in seaweeds. Ion concentrations in algae are mainly regulated by ion-selective carriers driven by the membrane potential (Gimmler 2000). In addition, facilitated diffusion via ion-selective channels may play a role during rapid changes and recovery of ionic composition (Kirst 1990).

The generally low cytoplasmic Na^+ concentrations observed in most seaweeds clearly indicate that active sodium ion export mechanisms exist in these plants. The complete genome sequence of the cyanobacterial strain *Synechocystis* 6803 showed six different genes, annotated as Na^+/H^+ antiporters (Kaneko et al. 1996), of which at least three were functioning (Inaba et al. 2001). The occurrence of rather large gene families for Na^+/H^+ antiporters clearly point to a group of proteins that fulfill many important functions in osmotic acclimation of cyanobacteria and probably of

seaweeds as well. Various studies showed that proton-pumping activity can be directly switched on in salt-treated cyanobacteria due to increased respiratory activity or because of stimulated activities of specific ATPases (Wiangnon et al. 2007, Hagemann 2011). Other Na^+-export systems such as primary active sodium pumps or transporters might exist in seaweeds, but experimental proof is still lacking.

The much higher concentration of intracellular K^+ than in the external medium clearly points to an active uptake of this important cation from the seaweed exterior. In the genome sequences of cyanobacteria, various K^+ transporters and putative K^+ channels have been assigned as potential candidates for performing the uptake of this cation (Hagemann 2011), and more recently it was reported that all cyanobacteria possess the structural genes for a functional ATP-dependent K^+ transport system consisting of a K^+ permease, an ATPase that provides the energy, and a structural stabilisator (Ballal et al. 2007). Most interestingly, this complex K^+ transport system requires but does not transport Na^+ (Matsuda et al. 2004). Such a direct activation by the presence of Na^+ would nicely explain how the K^+ transport activity is rapidly enhanced under salt stress.

In contrast to Na^+ and K^+ transport mechanisms much less is known about Cl^- extrusion/uptake systems. Although Cl^- was involved in the osmotic acclimation of various eulittoral green algae from Antarctica, the concentration remained relatively low even under hypersaline treatments (Karsten et al. 1991b). This is in accordance with the related temperate green algae such as *Ulva prolifera* (as *Enteromorpha prolifera*) (Young et al. 1987). Except *Acrosiphonia arcta*, all other green algal species studied from Antarctica have typically small cells and a large cytoplasmic:vacuolar ratio (Karsten et al. 1991b). They resemble cytoplasm-rich microalgae, which also tend to maintain low Cl^- values in their cytoplasm (Dickson and Kirst 1986). Cl^- like Na^+ has adverse effects on many enzymes (Gimmler et al. 1984). In addition, ribosomes of plants are not functional in the presence of high Cl^- contents (Ritchie 1988).

In the green alga *Acetabularia* spp. a negative membrane potential was measured and interpreted as primarily caused by an electrogenic Cl^- pump (Wendler et al. 1983). Nevertheless, in eukaryotic algae and cyanobacteria Cl^- transport is still badly understood. In contrast, in some bacteria and other eukaryotes such as invertebrates and humans, various genes encoding Cl^- channels or Cl^-/H^+ exchangers have been described (Jentsch 2008). The underlying proteins assemble to dimers, with each monomer containing an ion translocation pathway (Jentsch 2008). From the available data on these organisms it is reasonable to assume similar Cl^- transport systems for seaweeds.

Although K^+, Na^+ and Cl^- represent the major inorganic ions involved in osmotic acclimation, some seaweeds such as *Laminaria digitata* use NO_3^- to satisfy osmotic and nitrogen requirements (Davison and Reed 1985a). However, there are strong seasonal changes in the cytoplasmic composition of major inorganic and organic osmolytes in this kelp. *Laminaria digitata* accumulates high nitrate concentrations in spring. During summer, this anion is completely metabolized, and the gap in the osmotic potential is filled through the biosynthesis

and accumulation of the polyol mannitol. This seasonal increase in mannitol concentration compensates for the intracellular decrease in nitrate rather than for changes in external salinity (Davison and Reed 1985a).

In some green seaweeds such as *Codium decorticatum* (Bisson and Gutknecht 1975), *Acrosiphonia arcta* and *Ulva rigida* (Karsten et al. 1991b) SO_4^{2-} plays an important role as an osmolyte. Some brown seaweeds of the genus *Desmarestia* even contain this anion in the form of free sulfuric acid inside the vacuole resulting in a pH of 1–2 (Eppley and Bovell 1958; Anderson and Velimirov 1982).

The energy requirements for ion transport in seaweed species such as *Ulva lactuca* are equivalent to 10–30% of the energy provided by respiration (Ritchie 1988). It seems that all inorganic ion transporters in seaweeds are generally quite fast systems with much lower requirements for metabolic energy compared with the cost for biosynthesis or degradation of organic osmolytes (Kirst 1990). The formation of an intracellular osmotic potential of 1 osmol kg^{-1} requires 1.2 mol ATP when created by KCl uptake only, but 66 mol ATP when based on sorbitol or mannitol biosynthesis.

5.3.2 Organic Osmolytes

The main physiological strategy of all seaweeds studied so far under saline conditions is to keep particularly the Na^+ and Cl^- concentrations in the cytoplasm as low as possible. Since protein and organelle function (e.g., ribosomes, mitochondria), enzyme activity, membrane integrity and structural macromolecules in seaweeds are adversely affected by these ion concentrations (Kirst 1990), it is now generally accepted that the biosynthesis and accumulation of organic osmolytes in the cytoplasm, although energetically costly, permits the generation of low water potentials without incurring metabolic damage (Karsten et al. 1996a; Yancey 2005; Eggert and Karsten 2010).

In many, but not all, cases the organic osmolytes are identical with the main photosynthetic product, and thus there are preferences in different seaweed groups. Polyols such as mannitol are characteristic for most Phaeophyceae, while the Chlorophyta contain typically sucrose, some taxa in addition to proline, glycine-betaine, or dimethylsulphoniumpropionate (DMSP) (Kirst 1990). Although mannitol is present as the sole osmotically significant low-molecular weight organic solute in most brown seaweeds, some species are capable of synthesizing a second polyol (Davison and Reed 1985b). The top-shore Phaeophyceae *Pelvetia canaliculata* contains the heptitol volemitol as an additional intracellular organic solute (Kremer 1976), while the lower shore brown alga *Himanthalia elongata* from the Northern hemisphere as well as various Southern hemisphere Fucales (e.g., *Bifurcariopsis capensis*, *Hormosira banksii*, *Xiphophora chondrophylla*) accumulate the hexitol altritol as isomeric solute in addition to mannitol (Chudek et al. 1984; Wright et al. 1987).

In the Rhodophyta a multitude of low-molecular-weight carbohydrates have been identified in recent years (Eggert and Karsten 2010). The main photosynthetic

product in members of most orders of red seaweeds is clearly the heteroside floridoside (α-D-galactopyranosyl-(1-2)-glycerol), while most members of the Ceramiales (Florideophyceae) generally synthesize and accumulate instead of floridoside, the chemically related digeneaside (α-D-mannopyranosyl-(1-2)-glycerate) (Kremer 1978).

Already Lindberg (1955) provided evidence that, in addition to floridoside, members of the Bangiales (Bangiophyceae) contain an isomeric form of floridoside, isofloridoside (α-D-galactopyranosyl-(1-1)-glycerol). Wickberg (1958) later reported isofloridoside in *Porphyra umbilicalis* as a uniform mixture of D- and L-forms. More recently, the chemical structures and configurations of all three heterosides from *Porphyra perforata* were investigated, and the occurrence of floridoside along with both D- and L-isofloridoside was verified (Meng et al. 1987), while the strong involvement of all these compounds in the process of osmotic acclimation was experimentally proven in *P. columbina* (Karsten et al. 1993a). More interesting is the observation of different heteroside patterns in *Porphyra* species from different biogeographic regions in Europe, Africa, North America, Asia, and Australia (Karsten 1999). The composition of the three compounds varied among the species studied. In *P. columbina* from Australia, L-isofloridoside was always quantitatively dominant, while floridoside was the major component in *P. dioica* from the North Sea. D-Isofloridoside was usually present in small concentrations, except in *P. perforata* from the Pacific coast of the USA where it occurred in equal concentrations along with floridoside and L-isofloridoside (Karsten 1999). These results point to species-specific different enzymatic activities of the underlying anabolic pathways, which are, however, not completely understood.

The consistent presence of digeneaside together with a new compound was noted in some members of the genus *Hypoglossum* (Delesseriaceae, Ceramiales), and a chemical survey in members of this taxon resulted in the identification of digalactosylglycerol (2,3-dihydroxypropyl (α-D-galactopyranosyl)-(1→6)-ß-D-galactopyranoside), which was strongly involved in osmotic acclimation (Karsten et al. 2005). Surprisingly, digalactosylglycerol has never been reported for any other seaweed species before, even though it most probably represents the main photosynthetic product in *Hypoglossum barbatum* and *H. heterocystideum* (Karsten et al. 2005).

The disaccharide trehalose was detected in several members of the Ceramiales. While some taxa of this order such as *Aglaothamnion* spp. exhibited only trehalose, others such as *Delesseria sanguinea* showed trehalose together with digeneaside. Although more recent data indicate that only a few Ceramialean taxa are capable of synthesizing trehalose (Karsten et al. 2007), the summarized results in the review of Craigie (1974) point to other Florideophycean species also forming this disaccharide. Therefore, this compound may be more widely distributed among the Rhodophyta than currently thought.

Most interesting is the fact that in some Ceramiales such as in the mangrove-associated genera *Bostrychia* and *Caloglossa* as well as in some early diverging red algal lineages the polyols mannitol, sorbitol, and dulcitol can be found (Karsten and

West 1993; Karsten et al. 1994, 1999, 2003b; Eggert and Karsten 2010), which are otherwise uncommon for red algae.

With the exception of digeneaside that plays no more than a minor role in osmotic acclimation of Rhodophyta (Karsten et al. 2005; Eggert and Karsten 2010), all other low-molecular-weight carbohydrates act as organic osmolytes. Since these organic compounds can be accumulated and tolerated at high intracellular concentrations, and permit the generation of low water potentials without incurring metabolic damage (Yancey 2005), the term "compatible solute" was introduced by Brown and Simpson (1972). In general, the intracellular concentrations of these organic osmolytes are actively adjusted by photosynthesis driven de novo biosynthesis or by remobilization of storage products and are directly proportional to external salinity (Kirst 1990).

Although differing in their chemical structure, compatible solutes in seaweeds have some features in common: they are typically highly soluble, in most cases have no net charges at physiological pH, and are non-inhibitory at high concentrations (Kirst 1990; Karsten et al. 1996a). The interactions of these organic compounds with intracellular macromolecules are not completely understood and several mechanisms have been suggested. Bisson and Kirst (1995) discussed different models to explain the protection of enzyme systems: (1) binding of the solute to the protein, (2) colligative action of the solute, (3) buffering of potentially damaging changes in solution properties, (4) inhibition of conformational changes resulting in formation of inter- or intramolecular disulfide bridges, and (5) preferential exclusion of the solute from the protein surface. These models can be basically summarized into two types: (1) those that hypothesize direct solute–protein interactions and (2) those that postulate that protein stability is mediated by solute-induced changes in water structure (Roberts 2005; Yancey 2005). However, there is little experimental evidence in seaweeds for any of these models.

Desiccation tolerant seaweeds such as supralittoral green algal *Prasiola* or brown algal *Pelvetia* species typically exhibit morphological adaptations such as thick cell walls that protect against rapid water loss and the capability to synthesize and accumulate polyols as "water-keeping" substances (Jacob et al. 1991, 1992).

5.3.3 *Biosynthesis of Organic Osmolytes*

Kremer and Kirst (1981) showed that exogenously applied inorganic ^{14}C is rapidly taken up by various red algae and assimilated into floridoside, which is thus acting as a major photosynthetic product. The biosynthesis of floridoside is initiated by a condensation reaction of l-glycerol-3-P and UDP-galactose resulting in floridoside-P. This reaction is mediated by a respective heteroside-P synthase (Kremer and Kirst 1981). Floridoside-P is subsequently de-phosphorylated by a specific phosphatase. In this anabolic pathway l-glycerol-3-P serves as precursor, and the condensation reaction takes place at the C-2 position of glycerol. In contrast, the biosynthesis of D- and L-isofloridoside in the Bangiales is unknown.

The biosynthesis of digeneaside in red algae is initiated by a condensation reaction of L-glycerate-P and UDP-mannose resulting in digeneaside-P. This reaction is mediated by digeneaside-P synthase, and followed by a specific digeneaside phosphatase which de-phosphorylates digeneaside-P (Kirst 1990).

All members of the mangrove red algal genus *Caloglossa* synthesize the polyol mannitol under salinity stress (Karsten and West 1993). The biosynthesis of mannitol was experimentally verified for the first time in *Caloglossa leprieurii* (Karsten et al. 1997). This species exhibits four enzymes that control the size of the mannitol pool. Mannitol-1-P dehydrogenase (Mt1PDH; EC 1.1.1.17) reduces fructose-6-P to mannitol-1-P. Mannitol-1-phosphatase (Mt1Pase; EC 3.1.3.22) subsequently dephosphorylates mannitol-1-P and releases mannitol in the anabolic pathway. The catabolic pathway includes the conversion of mannitol to fructose by mannitol dehydrogenase (MtDH; EC 1.1.1.67) and further to fructose-6-P by hexokinase (HK; EC 2.7.1.1.). Both pathways involved in mannitol metabolism are known as the so-called mannitol cycle (Karsten et al. 1997).

A current study on the key enzyme mannitol-1-P dehydrogenase in the brown alga *Ectocarpus siliculosus* took advantage of the recently published genome (Cock et al. 2010; Rousvoal et al. 2011). Applying a biochemical and for the first time a genomic approach, the latter authors documented a salt-induced gene expression and upregulation for this enzyme, i.e., hypersaline conditions stimulated the formation of mannitol. Similarly, Iwamoto et al. (2003) documented the biochemical and kinetic properties of purified mannitol-1-P dehydrogenase from *Caloglossa continua*, and also reported strong enzyme regulation by salinity.

In contrast to the mannitol metabolism, the biosynthesis of sorbitol and dulcitol as well as of trehalose in red seaweeds is unstudied. Nevertheless, a trehalose-6-phosphate synthase gene was recently screened out from a large DNA fragment library constructed from *Porphyra yezoensis* (Dai et al. 2004), indicating the genotypic presence of a trehalose biosynthesis key enzyme. This example strongly supports the usefulness of various recent genomic projects on different seaweed taxa, which will give a deeper look into the molecular mechanisms of biosynthesis and regulation of organic osmolytes.

5.3.4 Antioxidants

Since seaweeds perform oxygenic photosynthesis using water as an electron donor they steadily release molecular oxygen, which can be accumulated and easily chemically converted to potentially damaging reactive oxygen species (ROS). The sources and production sites of ROS are mainly related to photosynthetic activities, such as pseudocyclic photophosphorylation and the Mehler reaction, which stimulate the accumulation of hydrogen peroxide (Asada 1994; see Chap. 6 by Bischof and Rautenberger). Besides these internal processes, formation of ROS might also be induced under hypersaline conditions as reported for *Ulva fasciata* (Sung et al. 2009). These authors undertook a gene expression study and

documented a salt-induced upregulation of various antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, and catalase, which efficiently detoxified ROS.

5.3.5 Ultrastructural Changes

In many seaweeds, the separation of the plasmalemma from the cell wall rather than cell volume changes under hypersaline conditions is the most damaging aspect of plasmolysis (Reed 1990). However, in some species such as the red alga *Caloglossa leprieurii*, the elastic cell walls accommodated marked shrinkage of the protoplasm when treated with hypersaline media resulting in a concomitant swelling and hence prevention of plasmolysis (Fig. 5.2, Mostaert and King 1993). These authors suggested that the enormous flexibility of the cell wall thickness may be a protective mechanism in response to fluctuating salinities.

Besides the adverse ionic influences on particular sites of the photosynthetic machinery, the cellular ultrastructure may also be affected by salt stress resulting in reduced photosynthesis and respiration. In *Porphyra umbilicalis* changes in the thylakoid structure of the chloroplasts after salt treatment have been described (Wiencke 1982). Hypersaline conditions typically result in cell shrinkage due to water loss with thylakoids and membranes appressed, and under hyposaline conditions cellular organelles are typically swollen. This disturbance of fine structure may cause a disruption of energy transfer in photosynthetic electron flow (Satoh et al. 1983). In contrast to chloroplasts, mitochondrial cristae ultrastructure is less affected by salinity changes (Kirst 1990), and this well explains the better functioning of respiration compared to photosynthesis under the respective stress.

Another ultrastructural observation is the formation of vacuoles after hypo- or hypersaline stress in seaweeds such as *Porphyra umbilicalis*, which under normal conditions contain only very few of these organelles (Wiencke and Läuchli 1980). The vacuoles formed under hypersaline conditions may serve as compartments to sequester metabolically toxic ions, mainly Na^+ and CI^- (Wiencke et al. 1983). Concomitantly, the fine structure of the tonoplast changes is probably related to the intensity of ion transport across it (Wiencke and Läuchli 1983).

5.3.6 Self Protection

In addition to active processes, which compensate for osmotic stress, *Laminaria* blades form multiple-layered, mat-like canopies at neap tides, protecting the individual thalli against desiccation and salinity changes (Lüning 1990). Macroalgal canopies of *Ulva* sp. in the upper littoral zone in southern Spain form under emersed conditions sheet-like, multiple-layered structures in which the top layer usually bleaches due to strong insolation, desiccation, and other abiotic stresses, thereby providing photoprotection and moisture for subcanopy thalli (Bischof et al. 2002).

5.4 Salinity Ecotypes

Habitats with strong horizontal salinity gradients such as estuaries or the Baltic Sea promote genetic adaptation and eventually speciation. Indeed, the geographic semi-isolation from the Atlantic and the steep horizontal salinity gradient of the Baltic Sea have caused local genetic adaptation in several seaweeds and contributed to the formation of so-called ecotypes. Ecotypes (*sensu* Turesson, 1922) are genetically distinct populations of a species that are locally adapted to particular environmental conditions. The ability of populations to adapt to a novel habitat is a key component of the speciation process. Ecotypes are recognized as physiologically distinct variants of a species. The concept of ecotypes is widely accepted in relation to ecophysiological diversity in seaweeds when considering salinity stress responses. Several Baltic seaweeds were physiologically and morphologically compared with their marine counterparts and a number of salinity ecotypes have been described such as *Ceramium strictum* (Rueness and Kornfeldt 1992), *Delesseria sanguinea* and *Membranoptera alata* (Rietema 1993), and *Fucus vesiculosus* (Nygard and Dring 2008). But data from other regions also clearly point to strong ecotypic differentiation between populations of the same seaweed species such as estuarine and marine isolates of *Pylaiella littoralis* (Bolton 1979), *Polysiphonia lanosa* (Reed 1984), *Bostrychia radicans*, and *B. moritziana* (Karsten et al. 1993b, 1994). Although the kelp genus *Laminaria* is stenohaline (Bartsch et al. 2008), ecotypic differentiation in terms of growth under different salinities has been reported in North Atlantic populations of *Saccharina latissima* originating from Long Island Sound, New York, and Cape Neddick, Maine (Gerard et al. 1987), and might be considered as a mechanism to adapt to locally environmentally unfavorable conditions. However, in most seaweeds the underlying molecular mechanisms of ecotypic differentiation are unstudied.

One exception is *Bostrychia tenuissima*, which is restricted to Southern Australia and New Zealand. Previous studies have revealed two distinct patterns in the presence of osmotically active polyols. Southern Australian populations only have sorbitol whereas northern Australian populations have in addition to sorbitol also dulcitol. These polyol patterns led to speculation on ecotypic differentiation in both population types (Karsten et al. 1995). Using molecular approaches, a 100% congruence was found between polyol patterns and three plastid haplotypes observed among all isolates studied, which experimentally proved for the first time a genetic basis for different ecotypes of the same seaweed species (Zuccarello et al. 1999).

5.5 Outlook

Since the review of Kirst (1990) on salinity tolerance in algae not much scientific progress has been made in seaweeds. In strong contrast, in cyanobacteria many molecular mechanisms involved in osmotic acclimation have been successfully

addressed (Hagemann 2011). The main reason for this huge gap in knowledge between both organism groups is related to available genome information. Without genomic data or other modern approaches such as metabolomics or proteomics, it is still difficult to evaluate molecular mechanisms, such as ion transport across membranes, biosynthesis of organic osmolytes, gene expression, and regulation. Therefore, the establishment of more genomes of model seaweeds is urgently needed to get a fundamental understanding of salinity and desiccation stress responses. A first step has been recently taken with the brown alga *Ectocarpus siliculosus* (Cock et al. 2010), so that deeper insight into the molecular biology of osmotic acclimation can be expected in the near future.

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