The osmotic adjustment of marine microalgae

and the complex role osmolytes play in this process

Thesis of Liz Kendall
Supervisor: Dr. Gieskes
Department of Marine Biology,
The University of Groningen
April 1996
Summary:

Algae inhabit a wide variety of both marine and freshwater habitats. These habitats differ in regard to various factors such as chemical composition, the organisms that live there, the light which may radiate into that particular area, the temperature of the sites depending on where the environment is located, just to name a few. One factor that varies from environment to environment is the salinity. This paper will look at the mechanisms utilized by marine algae to cope with the changes in salinity content in their habitats and most importantly how they use different osmolytes to carry out this process.

Marine algae “osmotically adjust” themselves to external salinity changes, in a biphasic manner. Firstly, this includes changes in turgor pressure or large internal water fluxes in response to osmotic gradients. Secondly, an internally regulated osmotic adjustment occurs with the use of both inorganic and organic osmolytes.

Compatible solutes are ions and molecules used by many organisms to osmotically adjust and they play a double role in the process of osmotic adjustment. They act as osmolytes and also protect the cellular enzymatic activities under salinity stress. They are called “compatible solutes” because they protect the cellular enzymatic activity. The main compatible solutes are polyols (including amino acids, carbohydrates and sugars), quaternary ammonium derivatives or tertiary sulphonium compounds. Certain species and taxonomic classes use specific compatible solutes and some even use combinations of them.

Glycerol is an important compatible solute because it plays a major role with glycolysis and interactions with enzymes of the Krebs cycle. β-dimethylsulphoniopropionate (DMSP) is a compatible solute that is produced in large quantities by marine microalgae as an osmoregulator. This compatible solute has also been suggested to be a cryoprotectant in marine microalgae that inhabit the cold waters of the polar region. Therefore, microalgae utilize DMSP for both osmoregulation and as a cryoprotectant.

Some algae, e.g. the genus Dunaliella and some Chlamydomonas species, have an enhanced tolerance to salt. These salt-tolerant algae are referred to as halophils and their salt optima in relation to their growth rate differs from the normal salt tolerant species. They are salt-tolerant because they have an extreme tolerance of low water availability. Special types of marine microalgae with a similar salinity tolerance inhabit the Great Salt Lake.

There are two main metabolic pathways that marine algae utilize in order to osmotically adjust under salinity stress. The first metabolic pathway is termed hyperosmotic stress by which an abundance of enzymes is synthesized and then osmolyes are produced. Where the cellular metabolism is reduced, reserve products are remobilized and redistributed within the compartments of the cell. The second metabolic pathway is termed hypoosmotic stress in which polymeric reserve products are utilized, synthesis of enzymes is inhibited, degradative pathways are inhibited and organic osmolytes are released into the medium to protect the cell from rupturing by increased osmotic stress. These pathways function together in synchrony to enable marine micro-algae to survive in their environment.
<table>
<thead>
<tr>
<th>Table of Contents:</th>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Different terms used</td>
<td>3</td>
</tr>
<tr>
<td>Inorganic and organic osmolytes</td>
<td>4</td>
</tr>
<tr>
<td>Ionic relations</td>
<td>5</td>
</tr>
<tr>
<td>Organic relations</td>
<td>6</td>
</tr>
<tr>
<td>Osmolyte concentration change coordination</td>
<td>7</td>
</tr>
<tr>
<td>Compatible solutes</td>
<td>8</td>
</tr>
<tr>
<td>Osmolyte evolution</td>
<td>9</td>
</tr>
<tr>
<td>Osmotic acclimation</td>
<td>11</td>
</tr>
<tr>
<td>Turgor sensing</td>
<td>12</td>
</tr>
<tr>
<td>Water fluxes</td>
<td>15</td>
</tr>
<tr>
<td>Compartmentation/Vacuoles</td>
<td>15</td>
</tr>
<tr>
<td>Specific osmolytes in specific algae</td>
<td>16</td>
</tr>
<tr>
<td>Glycerol</td>
<td>17</td>
</tr>
<tr>
<td>Glycerol's enzymatic protection</td>
<td>19</td>
</tr>
<tr>
<td>Proline and glycine betaine</td>
<td>21</td>
</tr>
<tr>
<td>$\beta$-dimethylsulphoniopropionate (DMSP)</td>
<td>22</td>
</tr>
<tr>
<td>Salt tolerant algae</td>
<td>23</td>
</tr>
<tr>
<td>Some exceptional cases</td>
<td>25</td>
</tr>
<tr>
<td>Metabolic pathways</td>
<td>27</td>
</tr>
<tr>
<td>Ecological implications of osmoregulation</td>
<td>28</td>
</tr>
<tr>
<td>Conclusions</td>
<td>29</td>
</tr>
<tr>
<td>Reference List</td>
<td>31</td>
</tr>
</tbody>
</table>
Introduction:

Regulation of cellular internal ionic composition is a physico-chemical process necessary for the survival of a variety of organisms. This process, which is commonly referred to as osmotic adjustment (Reed & Barron, 1983), has evolved in many organisms in highly specialized ways. The internal and external ionic composition of cells is related to the cells' osmotic pressure and turgor pressure and can be measured in a variety of ways including calculations of the extracellular space and tissue composition (reviewed in Dainty, 1963, 1976; found in Zimmermann, 1978), electrical (electromechanical) potential measurements, as well as measurements of the cellular dimensions and morphology (reviewed in Zimmermann & Steudle, 1977; found in Zimmermann, 1978).

In order for marine microalgae to survive in their natural environment they must constantly adjust themselves physically to their external world. This thesis focuses on how osmotically adjustment occurs specifically in marine microalgae. A brief description of the theoretical mechanisms microalgae utilize in order to osmotically adjust to external salinity changes as well as the inorganic and organic osmoregulators or "osmolytes" will also be covered. The goal of this study is to gain a basic understanding of the complex mechanisms and theories governing osmotic adjustment in marine microalgae.

The main factors that contribute to marine algal growth and distribution are temperature, light, water movements, nutrients, but also salinity (Soeder & Stengel, 1974). Temperature is important in the determination of general geographical distributions, growth rates and the metabolic physiology of algae. Marine algae are dependent on light for photosynthesis and photoautotrophic growth. These processes, however, are sensitive to temperature and light intensities which can stimulate or inhibit metabolism and growth.

Biochemical reactions of cells are carried out with nutrient components in an internal water-solvent medium (Walsby, 1982). Cell water compositions are therefore important in allowing the cell to carry out the biochemical reactions continuously occurring. Furthermore, the cell's water volume is important in determining pH values, osmotic pressure, ionic strength, the accumulation of other solutes and the cell's membrane potential.

Cells lacking continuous cell walls (wall-less cells) utilize contractile vacuole complexes
in order to get rid of excess fluid arising from an osmotic influx of water (Hausmann and Patterson, 1984). Such an influx arises when an osmotic gradient exists between a cell, high in internal solutes, and an external environment which is lower. This is the normal situation in freshwater environments, but it is also found in marine waters. This causes the cell to swell and ultimately rupture (Kirst, 1980). Vacuole contents are discharged at a specific spot on the cell surface and the contraction cycle of the vacuole is the sum of the filling (diastole) and expulsion (systole) of the contents of the vacuole (Kirst, 1980).

Many organisms have evolved sophisticated mechanisms that balance their osmotic strength with that of their environment. Although a large number of organic molecules could fulfill this function, an organism may fill this need by using only a few of such compounds (Rudulier, 1984). Compatible solutes accumulate intracellularly when in the presence of high levels of extracellular solutes and are known to protect cells from dehydration (Chambers et al., 1987).

A cell will do one of the following things if subjected to water stress when exposed to a concentrated solution with a low water availability: either thermodynamically equilibrate with the solution, for example by losing water, or it will suffer a temporary water loss, but invest energy into accumulating a solute to create a concentration thermodynamically equal in measure with the external water activity (Brown, 1978), where the water activity roughly correlates negatively with the salinity concentration. Such a solute can be a retained metabolite or a substance accumulated extracellularly where the solute accumulation lowers the intracellular water activity (increases the concentration) so that entering water obtains thermodynamic equivalence with the external medium (Brown, 1978).

In the process of osmoregulation the main criterion is that the osmoregulator is retained or “pooled” within the cell so that the concentration inside the cell is higher than that outside the cell (Brown, 1978). This is usually associated with the effective exclusion of the cell’s major external solute, which is most likely NaCl. When a cell is in a dilute environment its intracellular solutes (salts, nucleotides, proteins and intermediary metabolites) contribute to the cell’s “water status”, or osmosis. On the other hand, when increases in desiccation occur one single substance predominantly emerges to act as the osmoregulator. Examples of such osmoregulators are α-galactosylglycerol, cyclohexanetetrol, aspartate, glutamate, γ-aminobutyrate and proline, as well as miscellaneous polyols (Brown, 1978).
Different terms used

Marine eukaryotic unicellular algae mainly counter-balance the **extracellular osmolality** ($\pi_e$), the osmotic pressure outside the cell, with the **intracellular osmolality** ($\pi_i$), the osmotic pressure inside the cell. This is carried out by the complex interaction of synthesizing organic solutes, accumulating amounts of $K^+$, and by the partial exclusion of amounts of $Na^+$ (Dickson & Kirst, 1986, 1987). Rhodes (1987) found that the flux redistributions occurring in metabolism during adverse salinity conditions should be regarded as a complex function of sensitivity coefficients to all of the metabolic steps involved.

The **osmotic pressure** is dependent on the amount of free solute molecules in the solution, and in theory can be calculated from the known solution composition, or by use of an osmometer (Walsby, 1982). The osmotic pressure is defined as: $\pi = (\pi_i - y \pi_e) / (1 - y)$ where $\pi_i$ is the internal osmotic pressure, $\pi_e$ is the external osmotic pressure, $\pi$ is the osmotic pressure of the total (cellular) suspension, and $y$ is the total water present outside of the cell (Walsby, 1982).

**Turgor pressure** can be described as the hydrostatic pressure difference of the cell between the internal and external media. In order to measure it a pressure probe is needed (Walsby, 1982). Miniature pressure probes have been developed by plant physiologists that connect to pressure transducers, however, they cannot be used with cells less than 20 $\mu$m in diameter and the direct turgor pressure in prokaryotes can only be measured by using their gas vesicles if they have them (Walsby, 1982). Turgor pressure, the cell's volumetric elastic modulus (defined below) and cell surface hydraulic conductivity (permeability), are important contributors to the study and understanding of cell volume changes. These are brought about by increasing, or decreasing the solute concentrations as well as the relative cell volume which is the ratio of the total cell volume under salinity stress to the initial cell water volume.

The ‘volumetric elastic modulus’ is defined as: $\varepsilon = (\Delta P / \Delta V) V_o$. Where $\varepsilon$ is the volumetric elastic modulus relationship between the pressure change, $\Delta P$, and the relative volume change $\Delta V / V_o$. The cellular membrane hydraulic conductivity (membrane permeability) is defined as: $L_p = \ln 2 / \pi \cdot V / \pi V_o (\varepsilon + \pi)$, where $L_p$ is the cellular membrane hydraulic conductivity (the water flow rate through the cell surface under a pressure differential). $V$ is the cell volume, $A$ is the cell surface area through which water is driven and $\tau$ is the half time in which the equilibrium is restored and $\pi$ is the osmotic pressure (Walsby, 1982).
In plant cells the volumetric elastic modulus ($\varepsilon$) is dependent on volume and pressure. There are two important contradictory aspects of water relations and turgor pressure, namely the **half-time** ($\tau$) or rate of the cells’ response to osmotic stress and the internal **water content regulation**. This can also be explained by describing the half-time of the rapid phase of turgor pressure by not only being controlled by the cells’ geometry and their membranes’ hydraulic conductivity, but also by the cell walls’ elastic properties: $\tau = \ln 2 \cdot V/A \cdot L_p (\varepsilon + \pi_t)$ (Zimmermann, 1978). In this sense the half-time of the turgor regulation or the water exchange ($\tau$) for single cells is explained for the exponential water exchange. This equation is based upon the concept of water transport across the membranes’ barrier in the terms of the irreversible processes’ thermodynamics (see Zimmermann, 1978).

Not only do low volumetric elastic modulus ($\varepsilon$) values contribute to the cells’ ability to resist short-term fluctuations in the environment’s salinity, high $\varepsilon$ values may be of ecological and physiological advantage (Zimmermann, 1978). It may be of grand ecological significance for some plants that high $\varepsilon$ values ensure the relative water content change in a cell whereas the concentration is much smaller in cells with low $\varepsilon$ values in response to osmotic stress, particularly in plants growing in the earth’s arid zones.

**Inorganic and organic osmolytes**

Salinity changes affect organisms by osmotic stress and ionic stress, and induce a change of cellular ionic ratios (Kirst, 1980). The physiological processes used to tolerate these salinity changes are referred to as “osmoregulation”, osmotic acclimation, or “turgor regulation” (Kirst, 1989). Inorganic ion concentrations, or **inorganic osmolytes**, change in response to salinity stresses after the turgor pressure and water fluxes initiate the osmoregulatory processes. Kirst (1977) suggested that the inorganic ions $K^+$, $Na^+$ and $Cl^-$ act to “bridge the concentration gap” by immediately responding to osmotic stress. They do this by increasing or decreasing, depending on the ion, to maintain the cell’s normal osmotic pressure. After changes in the inorganic osmolyte concentration occur, as a result of salinity stress, organic osmolytes (organic molecules involved in the process of osmoregulation) begin to accumulate.

It has been discovered by many investigators (reviewed in Rudulier et al., 1984) that a
group of organic molecules (glycine betaine, proline betaine, proline, and others) behave as osmoprotectants. These molecules accumulate in cells in response to osmotic stress. They protect the cells from damage by cellular dehydration by balancing the cytoplasmic osmotic strength with the environment. Several of these molecules have also been discovered to behave as osmoprotectants for other organisms, such as bacteria (Rudulier et al., 1984). In addition to acting as osmotic balancing agents these osmoprotectants are very likely to interact with the cells' crucial macromolecules to aid in modulating the cellular biological activity (Rudulier et al., 1984). For example, Yancey et al. (1982) proposed that osmoprotectants probably act as compatible solutes (they protect the enzyme activity at low water availability levels) as well as stabilizing protein structure and function. The simplest explanation for how osmoprotectants work, is that they enable the cell to balance its cytoplasmic osmotic strength to that of its environment, preventing a net water loss. However evidence suggests that osmoprotectants fulfill other functions such as influencing protein stability and structure (Rudulier, 1984).

**Ionic relations**

The main ions involved in the ionic relations during osmoregulation are K⁺, Na⁺, Cl⁻ and sulfate (Kirst, 1989). The concentrations of other ions, such as Mg²⁺ and Ca²⁺, are not influenced to any great extent, but they may act as a kind of messenger (Kauss, 1987; Tazawa et al., 1987; found in Kirst, 1989). An apparent trend in the three main classes of marine macroalgae, (Chlorophyceae, Phaeophyceae, and Rhodophyceae) is that the Cl⁻ concentration change usually parallels the salinity fluctuation (Kirst, 1989).

There are three general types of species in respect to their K⁺ and Na⁺ relation: species with (1) a high Na⁺ content which increases with Cl⁻ under hyper osmotic stress whereas K⁺ is practically unaffected, (2) K⁺ as the major cation and a low Na⁺ concentration that increases steeply to exceed the K⁺ level under extreme salinity stress, and (3) both cations in approximately equal amounts (Kirst, 1989).

In marine microalgae that have a high cytoplasm to vacuole volume ratio the cytoplasm is typically (but not always) high in K⁺ and low in both Na⁺ and Cl⁻ (Kirst, 1977). However, it is difficult to determine the absolute ion content in microalgae. The absolute ion content may vary depending on the methods used to wash the algal pellets free of adhering medium, in
estimating the cell volume, and in correcting for the free space between the packed cells (Kirst, 1989). Species, such as *Dunaliella*, have Na⁺ concentrations that have been found to range from 7 mM to 800 mM in cells grown in 1.5 M NaCl, which is extremely saline (Ginsberg, 1981, found in Kirst, 1989).

In algae the ion concentrations are regulated by ion-selective carriers, which are driven by their membrane potential and other mechanisms such as ‘facilitated diffusion via ion-selective channels’, are also involved during the quick changes and in the recovery of the ionic composition (Kirst, 1989). The membrane potential in most freshwater algae, as well as in higher plants, is generated by an active H⁺ pump (Gutnecht et al., 1978; Raven, 1985; Walker, 1980; found in Kirst, 1989). However, in marine algae such membrane potentials and active ion-pumps, that are probably involved in creating the potential difference, are much more complex (Kirst, 1989).

Some generalizations which can be made from the results of Gutnecht & Dainty (1968) are that: all cells pump out Na⁺ and most actively absorb Cl⁻ and K⁺; the cytoplasm and vacuoles are electronegative in regard to sea water; both cytoplasm and “sap” are high in K⁺, low in Na⁺ and the “sap” is high in Cl⁻; and most ion influxes and effluxes are light stimulated and depressed by the dark, anaerobiosis and inhibitors.

**Organic relations**

Not only are ions accumulated and excreted in algae as a response to salinity changes but certain organic solutes of low-molecular-weight are as well (see Kirst, 1989). There are many cases in which these are the same compounds that are the organism’s main photosynthetic products. However there are differences in the compound preference between different taxonomic classes (Table 1).

These organic osmolytes have roughly an equal osmotic potential in molal solutions (about -2.0 to -2.5 MPa; Kirst, 1989). There is a big difference in the energy costs as well as the required amounts of carbon and nitrogen needed to achieve these potentials (Kirst, 1989). The double function of organic osmolytes, that they act not only as osmolytes but also as “compatible solutes”, may explain how these compounds are actually an advantage (Kirst, 1989). These solutes are termed “compatible solutes” because they have a protective function for the cell’s
enzymatic activities in concentrated saline conditions. Proline and glycerol were found to be the most effective organic osmolytes that were tested for their protective capacity (Kirst, 1989). However, it is not the case that all organic osmolytes are suitable as compatible solutes (Kirst, 1989). For example, sucrose can act as an osmoregulator but it doesn’t protect the enzyme activity very well in concentrated conditions, thus it does not function well as a compatible solute.

Table 1. A review of the organic osmolytes that are found in marine algae, summarized from Kirst 1989.

<table>
<thead>
<tr>
<th>Organic Osmolytes (Compatible Solutes)</th>
<th>Taxonomic Class (species)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polyols</strong></td>
<td></td>
</tr>
<tr>
<td>glycerol</td>
<td><em>Dunaliella</em>; <em>Phaeophyceae; Prasinophyceae</em></td>
</tr>
<tr>
<td>mannitol</td>
<td>most diatoms; <em>Chlorophyceae</em>;</td>
</tr>
<tr>
<td>sucrose</td>
<td><em>Chlorophyceae</em>; <em>Charophyceae</em></td>
</tr>
<tr>
<td>floridoside; digeneaside</td>
<td><em>Rhodophyceae</em></td>
</tr>
<tr>
<td>isofloridoside</td>
<td>Poterioochromonas malhamensis</td>
</tr>
<tr>
<td><strong>Quaternary Ammonium Derivatives</strong></td>
<td></td>
</tr>
<tr>
<td>glycine betaine</td>
<td>Tetraselmis (Platymonas); <em>Chaetomorpha capillaris</em>; <em>Cladophora rupestris</em>; important in higher plants</td>
</tr>
<tr>
<td><strong>Tertiary Sulphonium Compounds</strong></td>
<td></td>
</tr>
<tr>
<td>β-(dimethylsulphonio)-propionate (DMSP)</td>
<td>phytoplankton; <em>Chlorophyceae</em>; <em>Rhodophyceae</em> (traces)</td>
</tr>
</tbody>
</table>

**Osmolyte concentration change coordination**

The changes in the concentrations of the ionic and the organic osmolytes throughout the osmotic adjustment period are coordinated (Kirst, 1989). It is generally known that the ionic osmolyte contents change before those of the organic osmolytes. As a steady state is reached, the ion concentrations again decrease in synchrony with the compatible solutes that have accumulated (Kirst, 1989). For example, *Dunaliella parva* responds to hyperosmotic shocks with
a rapid increase in Na⁺ concentrations, partially balanced with Cl⁻, for the first 20-60 minutes following the shock. This is followed by a decrease in Na⁺ concentrations while mannitol and glycerol reach their maximum concentrations after 60-90 minutes following the shock (Kirst, 1989). Changes in inorganic and organic osmolyte composition under constant salinity have also been observed in some algae, depending on the stages of development and in response to seasonal variations (Kirst, 1989).

**Compatible Solutes**

Growth of unicellular organisms requires them to exist in a liquid suspension or liquid/gas (solid) interface because they must obtain nutrients directly from solution (Brown, 1978). The result of taking nutrients from solution is that the micro-organisms' water interactions are determined by the concentration of solutes in the aqueous solution in which they grow (Brown, 1978). Most organisms, multicellular as well as micro-organisms, can rarely tolerate conditions that are highly concentrated (roughly 0.3 M NaCl or 0.5 M sucrose). However, there are some micro-organisms (i.e. algae, bacteria, yeasts, moulds) that can tolerate very concentrated conditions. Halophilic algae, which are best represented by some species of the genus *Dunaliella*, tolerate saturated NaCl and require minimal salt concentrations (Brown, 1978).

The micro-organisms that grow in concentrated environments have a comparably low water interior and usually have the turgor of flexible cells (Brown, 1978). It is important that the cell's enzyme complement be functional when in a saline environment. This can be achieved by producing enzymes inherently resistant to inhibition or an intracellular environment that is not excessively inhibitory (Brown, 1978). Halophilic bacteria use both of these mechanisms, but eukaryotes solely depend on a modification of their interiors which they accomplish by the accumulation of a "compatible solute". Ideally compatible solutes should be non-inhibitory (binding less to the enzymes than more inhibitory solutes) nor should they inactivate or "denature" an enzyme over a long time period (Brown, 1978).

The non-inhibitory nature of compatible solutes can be caused by their low affinity for the enzyme proteins (Brown, 1978). Affinity (and hence inhibition) of the normal cellular solutes for enzymes is related to physico-chemical properties suggesting that hydrophobic interactions are at least partially responsible for the normal binding that quickly occurs under salinity stress.
(Brown, 1978). Hydrophobic interactions may explain the specific action of high concentration levels of salts on bacterial enzymes (Lanyi, 1974; found in Brown, 1978). However, stereochemical factors have not yet been fully evaluated (Brown, 1978).

Accumulation of polyhydric alcohols (where the polyol is oftentimes glycerol) is used by salt-tolerant eukaryotes as a response to water stress where such polyols confer an incredible degree of enzyme protection in concentrated environments. It is possible that salt-tolerant species are dependent on different central metabolic pathways as compared with non-tolerant species and that the enzymes that catalyze these pathways are also different. This does not seem to be the case with Dunaliella. As Brown (1978) found: “The major consequence of the metabolic differences seems to lie in the polyol production. There is no evidence of any such difference between halophilic and non-halophilic species of the alga, Dunaliella.”

It should be noted that polyols, and glycerol in particular, have the ability to preserve enzyme function (by not excessively inhibiting nor inactivating them) when the levels of water availability are low due to high salinity. In fact studies on glycerol clearly emphasize that glycerol neither excessively inhibits, nor inactivates enzymes, and it may even contribute to the protein molecular stabilization (Brown, 1978). Glycerol’s overall protective nature has also been illustrated by both its experimental and natural use as an “antifreezing agent” in protecting blood cells, spermatozoa, insects as well as fish (Schmidt-Nielsen, 1975).

All in all compatible solute accumulation is essential for micro-organism growth in saline environments in order to protect the cells’ enzymatic activities. A compatible solute may function as an osmoregulator to respond to water stress and as an enzyme protector in highly concentrated environments (Brown, 1978). There are several kinds of solutes that carry out this function when under slight to moderate stress, however, under severe conditions eukaryotes resort to polyhydric alcohols and turn to glycerol. Compatible solutes rarely activate enzymes. At most they function as poor inhibitors, although this is not in conflict with their protective role. In the absence of a compatible solute, other substances more inhibitory in nature, would be present at higher concentrations (Brown, 1978).

**Osmolyte Evolution**

There are two properties that distinguish compatible solutes from noncompatible solutes.
Firstly, some perturbing solutes specifically interact with ligands (ie. cofactors, modulators and substrates) and with active sites, which therefore disturb the functioning of the cell’s enzymes (Yancey et al., 1982). Secondly, compatible and perturbing solutes have an effect on the solubility hydration and the charge interactions of different protein groups. For example, amino acid side chains and peptide backbone groups can be different and such structural effects can be translated into functional manifestations (Yancey et al., 1982). Osmolytes may, in theory, affect the charged, polar or nonpolar groups of proteins in such a fashion that may increase or decrease their affinities to other inter- or intramolecular moieties (Yancey et al., 1982).

Phylogenetically diverse organisms (ie. bacteria, unicellular algae, vascular plants, invertebrates and vertebrates; Table 2) all utilize a small organic osmolyte family which leads one to assume that there are strong selective pressures associated with this system and that it is an example of convergent evolution (Yancey et al., 1982).

Table 2. A distribution of various osmolyte systems. Taken from Yancey et al., 1982.

<table>
<thead>
<tr>
<th>Osmolyte system (occurrences)</th>
<th>Principal osmolytes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Polyhydric alcohols-polyols</strong></td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Glucosylglycerol</td>
</tr>
<tr>
<td><em>Synechococcus sp.</em></td>
<td>Arabitol</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Arabitol, glycerol, mannitol</td>
</tr>
<tr>
<td><em>Lichens</em></td>
<td>Mannitol</td>
</tr>
<tr>
<td><em>Unicellular algae</em></td>
<td>Glycerol</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>Sucrose</td>
</tr>
<tr>
<td><em>Ochromonas mimuhamersis</em></td>
<td>Isottritol</td>
</tr>
<tr>
<td><em>Marine</em></td>
<td></td>
</tr>
<tr>
<td><em>Galeocerda polyastigma</em></td>
<td>Glycerol, sorbitol</td>
</tr>
<tr>
<td><em>Ephestia kuehniella</em></td>
<td>Glycerol</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>Glycerol, trehalose</td>
</tr>
<tr>
<td><strong>B. Amino acids and amino acid derivatives</strong></td>
<td></td>
</tr>
<tr>
<td><em>Betabacteria</em></td>
<td>Glyceric acid, proline</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>Glyceric acid, proline</td>
</tr>
<tr>
<td><em>Sporosarcina lacuna</em></td>
<td>γ-Aminobutyric acid, proline</td>
</tr>
<tr>
<td><em>Methanobrevibacter methanoplanis</em></td>
<td>Glycine, alanine, proline</td>
</tr>
<tr>
<td><em>Uncultured sp</em></td>
<td>Betaine</td>
</tr>
<tr>
<td><em>Anoplospora sp</em></td>
<td>Betaine</td>
</tr>
<tr>
<td><em>Aspergillus sp</em></td>
<td>Proline</td>
</tr>
<tr>
<td><em>Morphothecium nodosum</em></td>
<td>Proline</td>
</tr>
<tr>
<td><em>Invertebrates</em></td>
<td></td>
</tr>
<tr>
<td><em>All phylog of marine invertebrates</em></td>
<td>Various amino acids</td>
</tr>
<tr>
<td>(see examples in Table 1)</td>
<td></td>
</tr>
<tr>
<td><strong>C. Urea and methanofuran</strong></td>
<td></td>
</tr>
<tr>
<td><em>Carangidae</em></td>
<td></td>
</tr>
<tr>
<td><em>拱asparaginase</em></td>
<td></td>
</tr>
<tr>
<td><em>Melobesia</em></td>
<td></td>
</tr>
<tr>
<td><em>Mesoplancichthys</em></td>
<td></td>
</tr>
<tr>
<td><em>Coelacanth</em> (Lutjanus chalumnae)</td>
<td></td>
</tr>
<tr>
<td><em>Molluscs</em></td>
<td></td>
</tr>
<tr>
<td><em>Brachidactylus</em></td>
<td></td>
</tr>
<tr>
<td><em>Lophiidae</em></td>
<td></td>
</tr>
<tr>
<td><em>Amphibia</em></td>
<td></td>
</tr>
<tr>
<td><em>Bufo melanostictus</em></td>
<td></td>
</tr>
<tr>
<td><strong>D. Urea and methanofuran</strong></td>
<td></td>
</tr>
<tr>
<td><em>Archaeobacteria</em></td>
<td></td>
</tr>
<tr>
<td><em>Halobacterium salinarum</em></td>
<td>K⁺</td>
</tr>
</tbody>
</table>
Yancey et al. (1982) recognized that only a few organic osmolyte classes have been adopted by phylogenetically diverse organisms and that they reflect two main universal phenomena. The first phenomena is that the set of physico-chemical interactions, ubiquitously present between the water solutes and macromolecules, determine which different solutes are compatible with the structure and function of the macromolecules. The second phenomena is termed “genetic simplicity” which means that by using systems of compatible solutes proteins are capable of functioning in the presence of varying solute concentrations with the avoidance the modification of proteins.

**Osmotic acclimation**

Algae respond to salinity changes in a well-organized *biphasic fashion* beginning with quick changes in *turgor pressure* in cells with walls (walled cells) or in changes in volume in wall-less cells, as a result of *water fluxes* into, or out of the organism after the appearance of an *osmotic gradient*. The next step involves osmotic adjustment where the cellular osmolyte concentrations change to reach a new steady state. Both of the steps involved in this osmotic adjustment perform different functions in a *feedback loop*. Salinity changes are recognized by turgor pressure, or a volume sensing detector, which in turn triggers an effector to readjust the volume, or turgor pressure by use of several reactions. It is this adjustment that is regulated by the feedback mechanism in between the detector and the effector (Kirst, 1989).

According to Zimmerman (1978) there are several important phases or steps that occur throughout the processes of osmotic adjustment. The first is that there is an immediate instantaneous change in volume due almost exclusively to the flow of water into, or out of the cell. This is dependent on the direction of the osmotic stress. In this phase the intracellular ‘osmotically active particle’ concentration changes by shrinking or swelling. The solute transport across the cell’s membrane plays only a minor role in this phase. This is because the cell membrane’s water permeability, or hydraulic conductivity, is greater by several orders of magnitude than both the solute permeability of the cell’s membrane and the metabolic turnover rate of the cell’s osmotically active solutes.

The half-time of the water exchange and the cell’s volume changes are determined by the cell’s geometry, its elastic properties as well as the cell membrane’s hydraulic conductivity
(permeability). This is an idealized view of the biphasic kinetics of the cell's volume regulation in response to osmotic stress. However, differences may be found to deviate from this model. *Dunaliella parva*, for instance, was found to show changes in the volume regulation between hypertonic and hypotonic stress (Rabinowitch et al., 1975). Therefore, variations in this osmotic adjustment process may be found not only in different organisms but within the same organism.

**Turgor Sensing**

Determination of the sensing mechanism for turgor pressure has led to several theories on the physiological mechanisms governing salinity tolerance (reviewed in Kirst, 1989). Some of these theories are: that anisotropic changes caused in the membrane are initiated by tension, that there is an electrochemical compression of the cellular membrane, and that there are stretch-activated ion channels (Kirst, 1989). All of these models describe a transformation in the ion transport of the cell caused by a triggering of an external stimulus from salinity changes whereby a disturbance in the membrane structure is involved.

In the 'anisotropic membrane change model' a pressure gradient is thought to create the membrane's anisotropic changes (membrane changes having different values for one or more properties, i.e. compressibility or refractive index, when these are measured along different axes), and the sensor is located in the plasmalemma because it is unlikely that pressure gradients exist between the cell's organelles and cytoplasm and within the cytoplasm (Bisson & Gutnecht, 1980; found in Kirst, 1989). In the turgor transduction process the turgor pressure causes the plasmalemma to press against the cell wall and into gaps between cellulose microfibrils (Kirst, 1989). This causes an asymmetrical curvature in the lipid bilayer of the membrane and a shift in the lipid distribution, especially in the membrane proteins. It is this shift which affects the active ion transport that in turn changes the cell's membrane permeability, or releases some kind of messenger which activates the enzyme systems that are involved in osmotic adaptation (Kirst, 1989).

In the 'electro-mechanical model' the membrane is regarded as an electrical capacitor that is filled up with an elastic, dielectric material whose elastic properties act to counterbalance the compressive mechanical forces that are derived from both the turgor pressure, and the electrical compressive forces that arise from the membrane potential (Kirst, 1989). In order to sense the
turgor pressure changes, the membrane thickness must first change as a result of the alteration in the mechanical compression. As a result, the transport throughout the whole membrane is affected (Kirst, 1989). This occurs by intrinsic electric field changes or from the changes in the active pumps or the channels. In short, salinity changes cause a turgor pressure change that alters the thickness of the membrane. This change in membrane thickness controls the rate of translocation of the mobile charges through the membrane, which link the regulatory processes to the biophysical processes of the cell (Kirst, 1989).

The importance of the concept of the electromechanical model is that the mechanical forces produced by pressure gradients or by absolute pressure, caused by salinity changes, can affect the membrane mechanics in two ways. Firstly, by directly compressing the membrane, or its perpendicular parts, secondly, by the stretching of the cell membrane, which only occurs in the presence of pressure gradients (Zimmermann, 1978). The pressure gradients in walled cells is controlled by the cell walls’ elastic properties, which are coupled to the cell’s membrane (Zimmermann et al., 1977).

This model postulates that the geometric dimensional changes present in the membrane, or its parts that may be involved in the turgor sensing, are transformed into intrinsic electrical field distributional changes within the membrane. It further proposes that due to similarities in the biochemical and biophysical properties, between walled and wall-less cells during osmotic adjustment, the same beginning transformational step is involved in the regulatory response. This therefore fulfills a major criterion for the pressure transducer mechanism within the membrane (Zimmermann, 1978).

The ‘stretch-activated ion channels model’ suggests that stretch-activated ion channels are somehow involved with the mechanoreception and osmoreception of the cell and therefore also the volume regulation (Ubl et al., 1987, found in Kirst, 1989). The channel opening frequency increases when an increasing force is applied to the membrane (Kirst, 1989). This theory treats the mechanoreceptor-operated ion channels as a model for mechanical signal transductions in plant cells (Edwards & Pickard, 1987) and can be visualized as a part of some turgor-sensing mechanism. In this mechanism the channel proteins that are distributed throughout the membrane are connected with “spectrin-like linkers” which are inelastic filaments (Guharay & Sachs, 1984) that are parallel to the membrane’s inner surface. When a change in the membrane’s tension occurs these filaments tug at their attachment sites with the channel proteins.
'resulting in an opening or closure of the conduit' (Kirst, 1989). The filament network which is connected by these channel proteins operates as an amplifier and gathers force from the membrane’s large area (Kirst, 1989). An applied version of this model with the osmotic adjustment in algae is as follows (see Kirst, 1989): (1) the distortion in the plasmalemma causes the Ca\(^{2+}\) channels (see Kirst, 1989) to open, resulting in an influx of Ca\(^{2+}\), this causes the cytosolic Ca\(^{2+}\) to rise which has an effect on the transport functions as well as the metabolism, or (2) the distortion in the plasmalemma opens the Cl\(^{-}\) channels causing a change in the cellular Cl\(^{-}\) levels and/or a change in the electrical properties which in turn has an effect on the transport.

Several theories of how algal cells respond to increases in salinity have been reviewed by Kirst (1990). Cowan et al. (1992) proposed a ‘cascade theory’ from studies on Dunaliella salina, based on the ‘stretch activated ion channels model’, where there is a distortion of the plasmalemma triggering a change in the pH which causes a redistribution of abscisic acid inhibiting H\(^{+}\)-ATPase (found in Kirst, 1989). This increases the abscisic acid levels and opens the Ca\(^{2+}\) channels leading to an influx of Ca\(^{2+}\) and a rise in cytosolic Ca\(^{2+}\) which again heightens abscisic acid levels in an effect on metabolism and enzyme activity. Cowan et al. (1992) concluded that Dunaliella salina respond to stress by magnifying glycerol production, accumulating β-carotene and enhancing abscisic acid metabolism. Therefore the cellular responses are regulatory depending on a variety of mechanisms perhaps linked to abscisic acid balance modifications (Cowan et al., 1992; found in Kirst, 1989).

The regulation of turgor pressure is known to fail in algae that are exposed to hyposaline media (Kirst, 1989). The reason for this is not yet fully understood. In estuarine species that have been studied the internal osmotic potential adjustment is accomplished by changes in the cells’ volume and by control of the internal osmolytes. One difference in estuarine and marine species, dependent on which species, is that the cell walls are thinner and that they have a low elastic modulus giving them a greater ability to swell or shrink (Kirst, 1989). This response is swift, and allows the organism to survive the rapid tidal salinity fluctuations. The ionic concentration changes in estuarine algae are very fast effector mechanisms and contribute in a larger proportion to the regulation than the organic solutes do (Kirst, 1989; also see ‘Inorganic osmolytes’ and ‘Organic osmolytes’ sections). These concentration changes have been suggested to reduce the energy costs of the regulation of the turgor pressure (Kirst & Bisson, 1979; Reed & Barron, 1989; found in Kirst, 1989).
Water Fluxes

In the first phase of osmotic adjustment the water fluxes occur with a half-time (see ‘Different terms used’ section) in microalgae ranging from 5-10 sec and lasting anywhere from minutes to hours in macroalgae (Kirst, 1989). The water flux under hypoosmotic conditions is at least transiently alleviated and the processes, of changing the cellular concentrations of osmolytes, do not act under immediate metabolic control (Kirst, 1989). Acting as a passive “osmometer”, however, these processes do depend on certain physico-chemical properties of the cell-wall-membrane complex like water permeability, hydraulic conductivity and elasticity (Kirst, 1989).

Compartmentation/ Vacuoles

The permeability coefficients of the cell’s plasmalemma may be an important issue involved in the osmotic regulation of both the inorganic and organic osmolytes involved. Compartmentation of the osmolytes into cytoplasmic organelles may be necessary in organisms containing multiple compatible solutes (Settler & Greenway, 1983), or may even help regulate the internal organization of the enzymes that either regulate the synthesis, or degradation of these osmolytes (Frank & Wegmann, 1974; Grimmler & Lotter, 1982).

According to the research of Heywood (1978) several algae and protozoa possess these osmoregulatory organelles, that are referred to as contractile vacuoles, which expel a hypotonic solution out of the cell to compensate for the water influx due to osmosis from the external medium. ‘Although contractile vacuoles are present in some marine organisms (Dodge, 1973; Lloyd, 1928, found in Heywood, 1978), they are usually absent from organisms inhabiting marine or brackish water’ (Heywood, 1978). Further evidence indicates that the contractile vacuole is a permanent organelle possessing its own specialized membrane, whereby a hydrophylic substance of the Golgi apparatus attracts water into the vesicles of the Golgi, supplementary vacuoles and the contractile vacuole. In this way the increased intracellular water from osmosis sequestered into the contractile vacuole is expelled from the cell (Heywood, 1978).

The possession of a contractile vacuole is associated with an absence, or minor rigidity
of a cell wall (Hartog, 1889; found in Heywood, 1978). When there is a slight presence of a cell wall, or an irregular cell wall, maintaining the cellular equilibrium may be dependent on the expulsion of water (Lloyd, 1928; found in Heywood, 1978). The contractile vacuole has a 'bristle coat structure' extending from the external membrane into the cytoplasm which may provide additional strength allowing it to withstand pressure changes that occur during the contraction of the vacuole cycle (Heywood, 1978). Prusch and Dunham (1970) found, in the contractile vacuoles of *Amoeba proteus*, a microscopic, freshwater protozoan, that in order for contraction of the vacuoles to take place, adenosine triphosphate (ATP) and Mg²⁺ were needed, suggesting that the systolic force is generated by the vacuole itself.

**Specific osmolytes in specific algae**

It is known that marine algae and higher plants accumulate organic solutes in response to changes in salinity. These include quaternary ammonium compounds (QAC's), β-dimethylsulphoniopropionate (DMSP), amino acids and their derivatives, heterosides, saccharides and polyhydric alcohols (Dickson & Kirst, 1987). Onium solutes such as the quaternary ammonium and tertiary sulphonium compounds are very likely involved in osmotically adapting eukaryotic unicellular algae to environments which are saline (Dickson & Kirst, 1986).

Dickson & Kirst (1986) found that the QAC’s glycine betaine, homarine (N-methyl picolinic acid betaine), and the tertiary sulphonium compound DMSP are all involved in *Platymonas subcordiformis* osmoregulation. Therefore, there may be multiple osmolytes working together in one single organism to protect it from the effects of external salinity changes. Some marine microalgae have even been found to regulate their free amino acids in order to respond to steady state salinities (Gilles & Pequeux, 1977; Liu & Hellebust, 1976) which appear to regain control under changes in intracellular osmotic stress in a wide range of marine algae (Hellebust, 1976; Flowers et al., 1977; Bisson and Kirst, 1979).

For example, *Ulva lactuca*, a macroscopic green alga inhabiting intertidal zones in estuaries and rocky shores, is affected by rapid salinity changes caused by tidal interactions, evaporation and rainfall (Dickson et al., 1980). In the studies of Dickson et al. (1980) on the effects of hypo- and hyper-saline stress on various levels of inorganic and organic solutes it was
found that the K⁺, Na⁺ and Cl⁻ tissue concentration levels dropped under hyperosmotic stress. It
was further found that Na⁺ increased transiently and K⁺ and Cl⁻ accumulated under hyperosmotic
stress. In these studies it was also discovered that the DMSP tissue content responded to salinity
changes, that free sugars and amino acids (proline) did not seem to be important in osmotic
changes and that tertiary sulphonium dipolar ions play an analogous role with glycine betaine
in some algae.

Laimins et al. (1981) proposed that a membrane-bound osmosensing protein in
Escherichia coli regulates the potassium transport genes as a function of osmotic strength.
Furthermore, in E. coli, there is a glycine betaine uptake system that can be used for studies of
membrane regulation functions in association with osmotic tolerance (Rudulier, 1984). The
major osmoprotectants of E. coli are betaine, proline and glutamate, the most important being
glycine betaine, because of its dipolar characteristics and high solubility in H₂O (Chambers et
al., 1987).

Dimethylthetin is chemically related to the naturally occurring DMSP found in marine
algae. It differs from glycine betaine by the substitution of a positively charged dimethyl moiety
in place of the quaternary nitrogen group (Chambers et al., 1987). In studies on E. coli Chambers
et al. (1987) found that the bacterial cells do not distinguish between betaines which contain a
sulphur or nitrogen group, both compounds (dimethylletin and betaine) supported their growth
in hypertonic NaCl equally well. They hypothesized that dimethylthetin may even be useful as
a probe in studying betaine storage and comparing its medium excretion and uptake. Research
on E. coli, such as this, may lead to new insights into the knowledge of how these molecules are
used in osmotic adjustment processes in marine microalgae as well.

**Glycerol**

Glycerol synthesis is used by freshwater as well as marine microalgae in response to
increases in salinity. One particular microalgae that responds to salinity stress is the genus
Dunaliella sp., which possess the ability to adapt to changes in salinity by the synthesis or the
elimination of glycerol (León & Galván, 1995). Glycerol accumulation and excretion, as a means
of osmotic adjustment, is also used by Chlamydomonas reinhardtii, a freshwater green alga
(León & Galván, 1995). The difference between the halotolerant Dunaliella species and the
freshwater *Chlamydomonas* sp., is that *Dunaliella* retains the glycerol it produces so when there is a high enough intracellular glycerol concentration for osmotic balance, no further glycerol synthesis is needed. A continuous glycerol production is needed in *Chlamydomonas* in order to maintain an equilibrium of the synthesis and excretion of cytoplasmic glycerol (León & Galván, 1994).

When algae of the genera *Dunaliella* and *Asteromona* were grown at salt concentrations of 0.5 M to above 5 M glycerol was demonstrated to be the major internal solute osmoregulator (Wegmann et al., 1980). The internal glycerol concentration in these algae was shown to be proportional to the growth medium salt concentration when it reached values greater than 4 M. *Dunaliella* have a unique property enabling them to maintain high intracellular to medium glycerol concentration gradients, which allows them to grow in high saline medias with little glycerol (Wegmann et al., 1980). Wegmann (1971) demonstrated that this accumulation of glycerol depends on the water potential and not on the NaCl found in the medium. This is similar to the responses of iso-osmolar mannitol or sucrose. What is interesting about the intracellular glycerol content in *Dunaliella* and *Asteromona* is that it has been found to be highly temperature-dependent (Wegmann et al., 1980). ‘The underlying mechanism may involve a temperature-dependent conformational transition of a component of the cellular membrane which is essential for glycerol impermeability’ (Wegmann et al., 1980).

*Dunaliella parva* is a green halophilic algae which accumulates large intracellular glycerol amounts as a way to osmoregulate (Ben-Amotz & Avron, 1973). Its osmoregulation was found to be dependent upon the formation and degradation of its intracellular glycerol. Their observations suggested that the major function of glycerol in *D. parva* is to maintain osmotic balance since glycerol is not excreted into the medium. They further concluded that glycerol formation and degradation are not light dependent, but because glycerol in *Dunaliella* is a photosynthetic product two metabolic pathways may be responsible for the formation of glycerol: ‘one using a photosynthetic product and the other via the metabolic degradation of starch, the storage product in *Dunaliella*’ (Ben-Amotz & Avron, 1973).
Glycerol’s Enzymatic Protection

Dunaliella salina is a halotolerant unicellular green alga which responds to osmotic stress by the regulation of a carbon flux between starch synthesis in the chloroplast and glycerol production in the cytoplasm (Bental et al., 1990). Following the results of D. salina by Chitlaru & Pick (1991) it was suggested that phosphofructokinase may be an important juncture enzyme in glycerol production regulation and that the pentose-phosphate pathway plays a principal role in conserving the oxidation-reduction balance throughout the synthesis of glycerol.

It was further proposed that glycerol is produced by the pentose phosphate pathway with the use of glyceraldehyde’s phosphate as an intermediate in the glycolytic pathway (Figure 1). This schematic theory proposed that glycerol is first produced from starch which is activated by phosphofructokinase stimulation and plausibly also from starch phosphorylase. Secondly, that the glucose to glycerol carbon flow involves the operation of two distinct metabolic pathways that are located inside the chloroplast. The first metabolic pathway is that the glycolitic pathway produces glycerol phosphate as NADH and ATP are consumed. The second metabolic pathway is that the pentose phosphate pathway supplements the deficiency of reducing equivalents. In this second metabolic pathway lost ATP is replenished, at least partially, by photosynthesis, respiration and glyceraldehydes phosphate conversion to pyruvate. Thirdly, the transport of glycerol phosphate out of the chloroplast occurs by the phosphate translocator as an exchange for a phosphate group (P_i) and then is hydrolyzed by glycerol phosphatase within the cytoplasm (Figure 1).

Belmans & Van Laere (1986) found that when Dunaliella tertiolecta cells were exposed to a hyperosmotic shock, they immediately began to synthesize glycerol. This glycerol production was associated with an increase in the enzyme glycerol-3-phosphate, which suggested an increase in in vivo glycerol-3-phosphate dehydrogenase activity, because the amounts of fructose 1-6-biphosphate and triose-phosphate remained constant, or decreased throughout this period. It was suggested that this increased activity in glycerol-3-phosphate is most likely not due to covalent modification, but to the concentration changes or to the compartmentation of the glycerol-3-phosphate dehydrogenase effectors.
The synthesis of glycerol after exposure to hyperosmotic shock does not occur so simply. The following series of events was described which may alter the cellular volume and the protein content that might lead to a change in the glycerol-3-phosphate content. It was proposed that ATP and changes in cellular pH associated with the hyperosmotic shock, were the candidates most likely to stimulate the formation of glycerol-3-phosphate (Belmause & Van Laere, 1986). The Na\(^+\)/H\(^+\) antiport system described by Katz et al., (1986) may, after a hyperosmotic shock, have an induction both on the direct cytoplasmic acidification and on decreasing the ATP content by H\(^+\)-ATPase activation. Dihydroxyacetone-phosphate reductase changes in their dissociation-association (Marengo et al., 1985), that are induced by cellular volume changes and protein concentration after a hyperosmotic shock, may also play a contributing role to this phenomenon.
Furthermore, it was found in two *Dunaliella* species, that glycerol concentrations of close to 4 M do not affect the enzyme glucose-6-phosphate, whereas KCl and NaCl have deleterious effects on this enzyme (Yancy et al., 1982). This clearly supports the theory that glycerol acts as a compatible solute since the glycerol concentrations protect this enzyme whereas KCl and NaCl do just the opposite.

**Proline and glycine betaine**

The accumulation of proline in plant cells as a response to hyperosmotic stress is a widely known phenomenon. There are two possible main roles for the accumulation of proline in response to hyperosmotic stress. The first is proline's ability to function as an osmotic regulator and its relationship to restore a non-stress volume and turgor pressure. The second is related to the regulation of the cell's water structure that protects the cell against the reduction of cytoplasmic constituent hydration which is induced by high levels of salt stress (Schobert, 1980).

One unicellular green flagellate, *Chlamydomonas reinhardii* (Chlorophyta), has a very close relationship to the halotolerant algae *Carteria* and *Dunaliella* (Reynoso & De Gamboa, 1982). *C. reinhardii* is a freshwater algae. It was discovered that there is a linear relationship between the induction of halotolerance and the proline concentration in the medium. Results of Reynoso and De Gamboa (1982) demonstrated that adding proline to the medium induces salt tolerance and also suggested that proline plays an active role in the cell's intracellular processes that are connected with the tolerance of salt.

Dickson & Kirst (1987) also found that the unicellular marine algae *Phaeodactylum tricornutum*, *Cyclotella cryptica*, *Cyclotella meneghiniana* and *Porphyridium aerugineum*, all synthesize and accumulate proline and glycine betaine as a response to NaCl concentration increases, whereas *C. cryptica* and *C. meneghiniana* synthesize and accumulate homarine (N-methyl picolinic acid betaine) as well. *Phaeodactylum tricornutum* and *P. aerugineum* also synthesized intracellular glycerol to respond to increased salinities. The only algae that synthesized DMSP was *P. tricornutum*. The quantity of the DMSP was dependent on the amount of NaCl that was in the medium. Furthermore, the intracellular K⁺ concentrations were three to six times higher than Na⁺, thus media salinity increases led to cellular K⁺ accumulation and uptake, and a small cellular Na⁺ and Cl⁻ uptake whereas there was a loss of the intracellular NO₃⁻.
This supports the fact that the osmotic regulation in marine microalgae is a complex interaction utilizing both inorganic as well as organic compounds and that the organic compounds each organism uses may be species specific.

**β-dimethylsulphoniopropionate (DMSP)**

β-dimethylsulphoniopropionate (DMSP) is a tertiary sulphonium compound and occurs in several marine macroalgae (Challenger, 1959) as well as in several marine microalgae (Ackman et al, 1966). DMSP is involved in marine macroalgae osmotic acclimation (Dickson et al., 1980; Reed 1983). It is a tertiary sulphonium compound (Table I) which is analogous to quaternary ammonium compounds such as glycine betaine and proline which are widespread through marine organisms (Dacey & Wakeham, 1986).

Dickson and Kirst (1986) discovered that DMSP, glycine betaine and homarine, which are all quaternary ammonium compounds, are involved in the osmotic acclimation of the marine microalgae, *Platymonas subcordiformis*. They proposed, based on their calculations of the inorganic osmolytes, that an evaluation should be made on the integrated activities of both inorganic and organic solutes on the regulation of cell volume. Their research indicates a need to access the role of both quaternary ammonium and sulphonium compounds for the adaptation of eukaryotic microalgae to the saline environment (Dickson & Kirst, 1986).

In studies on the osmotic responses in the green intertidal algae, *Ulva lactuca*, the only organic compound that responded to hyper- and hypooxic and steady-state salinity stress was DMSP, suggesting that it is involved in osmoregulation and acts as a cytoplasmic compatible solute (Dickson et al., 1982). It was further found that tissue K⁺, Na⁺ and Cl⁻ concentrations decreased under hypooxic conditions while a stable increase in K⁺ and Cl⁻ with a transient Na⁺ increase, was seen under hypooxic stress.

Recently Karsten et al. (1991) discovered that the DMSP concentrations of an Atlantic green algae correlated with the light factor. Further studies on the green macroalgae *Ullothrix implexa, Acrosiphonia arcta* and *Ulva rigida* proved that the DMSP content had a direct correlation with the 'light factor' where the algal DMSP level rose with increasing light irradiance (Karsten et al. 1991). They suggested that the possibility exists that each species may require a certain light level in order to obtain optimal DMSP biosynthesis.
Glycine betaine is known to be the nitrogen analog of DMSP and is also known to be involved in the osmoregulation of some plant cells (Wyn Jones & Storey, 1981). One interesting find by Turner et al. (1988) was that the coccolithophore, *Emiliania huxleyi*, produced less internal DMSP when placed in a medium that was nitrogen supplemented. When nitrate was added to a culture deprived of nitrogen, the cellular DMSP content decreased within a 24 hour period. This supports the speculation that when in a nitrogen-limited environment, algae may actually increase their limited ability to produce glycine betaine and therefore synthesize DMSP as a result (Andreae, 1986).

**Salt tolerant algae**

The term “halophil” is the one most commonly used by microbiologists to describe micro-organisms that have enhanced salt tolerance, including salt-tolerant unicellular algae. There are some algae that thrive in saturated saline environments. These include the algal genus *Dunaliella* (phylum Chlorophyta, Order Volvocales) and some species within the genus *Chlamydomonas*, within the same order (Brown, 1978). Ecological studies suggest that members of the Chlamydomonas genus are in general less tolerant than those of *Dunaliella* (Brown, 1978). For example species of *Dunaliella* are the only algae that can be observed in the Great Salt Lake (Utah, USA) in times of full saturation (Brock. 1975).

One characteristic that should be remembered when comparing the salt tolerance and relations of algae, especially those within the same genus, is the salt optima position for their growth rate (Fig. 2; Brown. 1978). As can be seen in Figure 2, the halophilic species, *Dunaliella viridis*, has its optimum close to the bottom range of the species *Dunaliella tertiolecta*. It can be seen by their growth range that they show a salt-tolerance through each of their curves instead of a salt requirement.
Cyanobacteria are blue-green algae that occur in aquatic habitats, variable in their ionic composition and salinity, in freshwater, brackish, marine and hypersaline environments (Fogg et al., 1973; Carr and Whitton, 1982). Studies by Mackay et al. (1984) classified Cyanobacteria into three physiological groups based on their organic osmotica, which is coupled to their upper limits of salt tolerance. The “marine” group accumulated glucosyl-glycerol under NaCl stress, the “freshwater” group consisted of simple sugars, instead of the heteroside glucosylglycerol, under osmotic stress and the most “halotolerant” group contained quaternary nitrogen compounds as their organic osmotica. Mackay et al. (1984) found a trend that led towards glucosyl-glycerol production in the marine isolates and to a sucrose/trehalose accumulation in the freshwater forms. Mackay et al. (1984) also claimed to have found no “absolute” differences between the cyanobacteria from the differing habitats.

The data accumulated by Reed et al. (1984) suggested that the production of glucosyl-glycerol is not unique to marine cyanobacteria. It was also suggested that some strains may accumulate betaines as well in addition to accumulating sucrose or trehalose. The carbohydrate content by itself may not allow a distinction to be made between the “hypersaline” strains and the normal strains. Reed and Stewart (1985) re-evaluated these findings and came up with the
following conclusions: there are three broad but overlapping categories of cyanobacteria which are characterized by (a) disaccharide accumulation in the “least halotolerant”, (b) heterosides in the “intermediate halotolerant”, or (c) betaines in “most halotolerant”. They also found that there is no absolute correlation between the accumulation profile of organic solutes and their habitats, the accumulation of glucosyl-glycerol and growth in media that are unique to “marine” cyanobacteria, and that instead of halotolerance being linked to habitat, it is linked to the organic osmotica compatibility.

Some exceptional cases

There are some other exceptional cases of marine algae osmotically adapting to very harsh conditions such as in the Great Salt Lake and in the Polar regions. The algae that live in the Great Salt Lake are interesting. The lake is eight times saltier than the oceans yet the microalgae *Dunaliella salina* and *Dunaliella viridis* manage to flourish there creating magnificent red and green algal blooms in the summer. The red colourations predominate in the northern arm of the lake and the green colourations predominate in the southern arm (Zahl, 1967).

According to Brock (1975), *Dunaliella* are not optimally adapted to the conditions of the Great Salt Lake. They maintain their populations at such high salinities because they have no competition with other algae. His studies revealed that *Dunaliella* is present virtually throughout the Great Salt Lake but that there are marked differences in the population density. This may be correlated with grazing animals, the brine shrimp, *Artemia salina* in particular. He further suggested that because it has been impossible for *Dunaliella* to optimally adapt to saturated salt through evolutionary processes, no other algae, blue-green or eucaryotic, seem to be able to compete with *Dunaliella* at such high salinities.

Another interesting case is algae that have osmotically adapted to the ice-cold Polar regions. The data of Karsten et al. (1992) support the idea that DMSP-biosynthesis is light dependent. It also acts as an “antifreezing” agent since low water temperatures stimulated the DMSP content in several species of Antarctic green macro algae. It was therefore apparent in these plants that DMSP acts as a cryoprotectant. This data demonstrated that *Ulothrix implexa*, *Ulothrix subflaccida*, *Enteromorpha bulbosa* and *Arcosiphonia arcta* all synthesize and
accumulate high DMSP concentrations in the light under hypersaline conditions. The capacity to produce DMSP under hypersaline conditions in the dark was extremely low in all species.

Algae in the upper littoral zone undergo a combination of both osmotic and freezing stresses in the course of the tidal fluctuations and the changing seasons due to the mixture of seawater with freshwater that originates from the thawing sea-ice (Karsten et al., 1990). Since these species grow in the littoral zone they are exposed to large variations in their external osmotic pressure as a result of the tidal fluctuations and changes in the weather conditions. The isolated Antarctic species, *U. implexa*, *U. subflaccida*, *E. bulbosa*, and *A. arcta*, accumulated DMSP linearly with salinity increases (Karsten et al., 1992). This capability declined on the other hand with *L. viridis*, which is a Subantarctic/cold temperate species, and *B. minima*, which is a temperate species (Karsten et al., 1990). This indicates that there is a negative correlation between the capacity to synthesize DMSP in extreme hypersaline conditions and in the polar and temperate region temperature regime. It is reasonable to assume that the biosynthesis of DMSP in salt-stressed algae is controlled by enzymes that are “light-dependent” instead of by ATP and NADPH which are formed photosynthetically (Karsten et al., 1990).

Thus, aside from being exposed to large salinity variations, Antarctic algae in the intertidal zone are also exposed to a large temperature range. The work of Karsten et al. (1992) clearly demonstrated that DMSP accumulation is positively influenced by low water temperatures. This supports the assumption that the temperature regime of Antarctica intensifies an increase in DMSP in salt-stressed algae. The higher DMSP levels in the Antarctic species at 0°C in comparison to those at 10°C led Karsten et al. (1992) to assume that DMSP functions not only as an osmoprotectant but also as a cryoprotectant. Supposably DMSP acts as an “antifreeze” by keeping the cytoplasm liquid below temperatures of 0°C. ‘Freezing of the cytoplasm prevents any enzymatic activities of the cell, but if the cytoplasm is kept liquid below 0°C by high concentrations of anti-freeze compounds metabolism is possible. Cryoprotectants are also known to protect proteins directly by molecular interactions from damage during freeze-thawing (Carpenter and Crowe, 1988)” (Karsten et al., 1992).
Metabolic Pathways

The study of several metabolic pathways and how they are regulated, have mostly been investigated under salinity stress in unicellular algal species. The understanding of the biosynthetic regulations of marine macroalgae's organic components is scanty, as is the overall knowledge of the regulation of the formation and degradation of these compounds. There is even less knowledge over the molecular biology of these processes. Therefore, it may be interesting to look at other organisms and what is known about their osmotic regulation as a comparison, such as the osm genes found in Escherichia coli which control proline and betaine production for protecting the cells from dehydration (Redulier et al., 1984).

The following is a summary of mechanisms that probably participate in regulating the size of the osmolyte pool during osmotic adjustments (Kirst, 1989).

(A) Hyperosmotic stress:

1. De novo synthesis of enzymes.
2. Activation of enzymes involved in synthesis of the osmolyte.
3. Reduced degradation or metabolism.
4. Remobilization of reserve products in light and dark.
5. Redistribution within cellular compartments.

(B) Hypoosmotic stress:

1. Transfer into polymeric reserve products.
2. Inhibition of enzymes involved in synthesis.
4. Release of organic osmolytes into the medium: emergency reaction observed in micro- and macroalgae.
Ecological implications of osmoregulation

The use of osmolytes is complex, their functions include osmoprotection, internal enzymatic function protection (compatible solutes) and cryoprotection (antifreeze) in ultra-cold environments. The following Table (3) is a summarized scheme of the processes and properties involved in the ecology of osmoregulation in marine algae, their characteristics and the possible relevance these may lead to under their natural conditions.

Table 3. A summary of the ecological importance and the characteristics of the discussed mechanisms and their involved features in the process and functioning of osmotic adjustment. (Kirst, 1989).

<table>
<thead>
<tr>
<th>Processes and properties</th>
<th>Characteristics</th>
<th>Possible relevance under natural conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Water flux</td>
<td>Very fast; passive; inherent result of any change in salinity</td>
<td>May be a sufficient response to balance small osmotic gradients; shock experiences</td>
</tr>
<tr>
<td>2. Ion transport</td>
<td>Fast; low energy cost; selective uptake or release; vacuole and partially cytoplasmic</td>
<td>Major short-term response in intertidal zones and estuaries</td>
</tr>
<tr>
<td>3. Organic osmolytes</td>
<td>Slow; high energy costs; mobility in cytoplasm</td>
<td>Long-term adjustment to extreme osmotic stresses; seasonal changes in lagoon; desiccation in supralittoral habitats (salt marshes and rock pools); ice algae; too slow for tidal zones</td>
</tr>
<tr>
<td>a. synthesis or degradation</td>
<td>Slow; high energy costs; possibly in several cellular compartments</td>
<td>As above (3)</td>
</tr>
<tr>
<td>b. accumulation of several osmolytes</td>
<td>Slow; high energy costs; compounds accumulated due to developmental stages</td>
<td>Hyperosmotic shocks</td>
</tr>
<tr>
<td>c. buffer capacity of high contents of organics</td>
<td>Compounds accumulated due to developmental stages</td>
<td>Supralittoral, tidal zones and estuaries</td>
</tr>
<tr>
<td>4. Morphological and anatomical features</td>
<td>Passive; energy costs during construction</td>
<td>Supralittoral, tidal zones and estuaries</td>
</tr>
<tr>
<td>a. buffer effects; large vacuoles; massive thalli</td>
<td>Passive; energy costs during construction</td>
<td>Supralittoral, tidal zones and estuaries</td>
</tr>
<tr>
<td>b. cell walls; ion exchange capacity</td>
<td>Passive; energy costs during construction</td>
<td>Supralittoral, tidal zones and estuaries</td>
</tr>
<tr>
<td>c. elastic properties of cell walls</td>
<td>Passive; energy costs during construction</td>
<td>Supralittoral, tidal zones and estuaries</td>
</tr>
<tr>
<td>5. Endurance of salinity fluctuations</td>
<td>Passive; partial incomplete turgor pressure regulation</td>
<td>Intertidal zones; time limited</td>
</tr>
<tr>
<td>6. Life cycles</td>
<td>Gametophyte and sporophyte differ in resistance; dominant stages (gametophytes, spores)</td>
<td>Survival of extreme conditions; ice algae</td>
</tr>
<tr>
<td>7. Retention of seawater between tufts and thallus layers</td>
<td>Passive; depends on population density</td>
<td>Intertidal zones</td>
</tr>
</tbody>
</table>
Conclusions

In order to maintain turgor pressure and/or the ion concentration when faced with environmental salinity perturbations, it is required that an adjustment be made to the ion transport rates and/or the biochemical reactions that comprise the components of the more basic homeostasis process. Osmotic adjustment depends on the membrane potential. This can be derived from the fact that both turgor pressure and electrical fields produce stressful conditions leading to a compression in the membrane. The turgor pressure is controlled by the concentration of specific ions and this control should also be dependent on their contribution to the osmoregulation.

The biphasic osmoregulatory response to environmental salinity stress is quite similar in both walled and wall-less cells. On the macroscopic scale the turgor pressure is the regulative parameter in walled cells and the volume is the regulating parameter in wall-less cells. The switch between these forms of regulation is continuous and can be determined by the extension of the volumetric elastic modulus of the cell wall. This in turn depends on the turgor pressure and the cell’s volume and is an extremely complex parameter.

It may be of interest in further research to have more studies invested in the volume kinetics of cells in response to osmotic stress, as well as possible mechanisms involved in turgor pressure sensing based on properties of the electromechanical model. Investing more effort and time into the understanding of both of these problems would significantly increase our knowledge of how the specific properties of osmotic adjustment play different roles and how they all contribute to an overall immense scheme of the process of osmoregulation in all organisms.

Contractile vacuole complexes are also used by cells lacking continuous cell walls to dispose of huge water influxes. In the situation that a large water influx arises between a cell, its internal solutes and environment, contractile vacuoles enable the cell to discharge of the excess internal water. This occurs by a contraction cycle made up of the diastolic phase, or filling-up, and the systolic phase, or expulsion.

Once the cell’s osmotic adjustment processes have been ‘turned on’ by the turgor pressure and the cells volume/water flux, the cells respond by producing intracellular physico-chemical changes, utilizing both inorganic osmolytes (in the beginning) and organic osmolytes (later on). It is the use of these osmolytes that enables the cells to maintain an osmotic balance with the drastic extracellular salinity changes. Therefore, marine microalgae osmotically acclimate to salinity changes by first sensing the change in the osmotic gradient by changes in turgor pressure or water flux. Next, these algae adjust themselves via inorganic (Na⁺, K⁺ and Cl⁻) and then with inorganic osmolytes (polyols, quaternary ammonium derivatives or sulphonium compounds). These inorganic and organic osmolyte changes are coordinated. They synchronize with each other so that when the increase in inorganic osmolytes reach steady state they decrease in synchrony with an increase in the organic osmolytes. Furthermore, multiple osmolytes may work together within one organism to protect it from the adverse salinity stresses.

Compatible solutes, or organic osmolytes, accumulate in micro-organisms in low water availability environments. These molecules are termed “compatible solutes” because they function as both an osmoregulator and an enzyme protector. Marine microalgae share the use of these compatible solutes with phylogenetically diverse organisms. This leads to the assumption that strong selective pressures are associated with their use and that this is an example of
convergent evolution. Some compatible solutes (i.e., glycerol and DMSP) have also been shown to correlate with the light factor. This indicates that they are light dependent. However, it has been suggested that the specific light level required may be different for each species.

Glycerol is used as a compatible solute in many marine organisms. Two metabolic pathways are plausibly responsible for glycerol formation, namely that glycerol is used as a photosynthetic product and that it is used in the metabolic degradation of starch. It is also known to play a major role in enzyme protection. Proline and glycine betaine are also compatible solutes of marine microalgae. These two solutes may work together, as in the case of Phaeodactylum tricornutum, Cyclotella cryptica, Cyclotella meneghiniana and Porphyridium aerugineum, or individually, such as in Chlamydomonas reinhardtii. which only synthesizes proline under salinity stress. β-dimethylsulphoniopropionate (DMSP) is the tertiary sulfonium analog to the quaternary ammonium compounds proline and glycine betaine and acts as an osmoprotector in marine microalgae. Algae in the Polar regions have learned to utilize the osmolyte DMSP as both an osmoregulator and a cryoprotectant (an antifreezing agent). In this manner, they are capable of tolerating both extreme salinity and temperature fluctuations.

Salt-tolerant algae are termed “halophils”. Members of the genus Dunaliella and some species within the genus Chlamydomonas are salt tolerant. Their growth rate in relation to their salt optima does not necessarily depend on their salt-tolerance but does depend on their salt requirement. Thus, they can grow in salt environments that fulfill their salt-requirements. In the case of the algae inhabiting the Great Salt Lake, Dunaliella salina and Dunaliella parva, they flourish in extreme salinities that are eight times that of the oceans. They survive these extreme conditions not because they have evolved special salt tolerance mechanisms, but because there is no competition from other algae.

The functioning of osmotic adjustment in marine microalgae is a complex process. Hyperosmotic stress and hypoosmotic stress are the metabolic pathways utilized in order to undergo the osmotic adjustment processes. These both include the formation and degradation of compounds that control and protect the biosynthetic regulations. The specific processes and properties, and their specific characteristics of the acclimation to salinity stress play very specific roles in the process of osmotic adjustment in marine microalgae. Some of these specific roles and their ecological implications are understood. However, studies on many more species and how they respond to salinity stresses are needed to gain a complete understanding of the osmotic adjustment in marine microalgae.
Reference List


The osmotic adjustment of marine microalgae

and the complex role osmolytes play in this process

Thesis of Liz Kendall
Supervisor: Dr. Gieskes
Department of Marine Biology,
The University of Groningen
April 1996
Summary:

Algae inhabit a wide variety of both marine and freshwater habitats. These habitats differ in regard to various factors such as chemical composition, the organisms that live there, the light which may radiate into that particular area, the temperature of the sites depending on where the environment is located, just to name a few. One factor that varies from environment to environment is the salinity. This paper will look at the mechanisms utilized by marine algae to cope with the changes in salinity content in their habitats and most importantly how they use different osmolytes to carry out this process.

Marine algae “osmotically adjust” themselves to external salinity changes, in a biphasic manner. Firstly, this includes changes in turgor pressure or large internal water fluxes in response to osmotic gradients. Secondly, an internally regulated osmotic adjustment occurs with the use of both inorganic and organic osmolytes.

Compatible solutes are ions and molecules used by many organisms to osmotically adjust and they play a double role in the process of osmotic adjustment. They act as osmolytes and also protect the cellular enzymatic activities under salinity stress. They are called “compatible solutes” because they protect the cellular enzymatic activity. The main compatible solutes are polyols (including amino acids, carbohydrates and sugars), quaternary ammonium derivatives or tertiary sulphonium compounds. Certain species and taxonomic classes use specific compatible solutes and some even use combinations of them.

Glycerol is an important compatible solute because it plays a major role with glycolysis and interactions with enzymes of the Krebs cycle. β-dimethylsulphoniopropionate (DMSP) is a compatible solute that is produced in large quantities by marine microalgae as an osmoregulator. This compatible solute has also been suggested to be a cryoprotectant in marine microalgae that inhabit the cold waters of the polar region. Therefore, microalgae utilize DMSP for both osmoregulation and as a cryoprotectant.

Some algae, e.g. the genus Dunaliella and some Chlamydomonas species, have an enhanced tolerance to salt. These salt-tolerant algae are referred to as halophils and their salt optima in relation to their growth rate differs from the normal salt tolerant species. They are salt-tolerant because they have an extreme tolerance of low water availability. Special types of marine microalgae with a similar salinity tolerance inhabit the Great Salt Lake.

There are two main metabolic pathways that marine algae utilize in order to osmotically adjust under salinity stress. The first metabolic pathway is termed hyperosmotic stress by which an abundance of enzymes is synthesized and then osmolyes are produced, where the cellular metabolism is reduced, reserve products are remobilized and redistributed within the compartments of the cell. The second metabolic pathway is termed hypoosmotic stress in which polymeric reserve products are utilized, synthesis of enzymes is inhibited, degradative pathways are inhibited and organic osmolytes are released into the medium to protect the cell from rupturing by increased osmotic stress. These pathways function together in synchrony to enable marine micro-algae to survive in their environment.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Different terms used</td>
<td>3</td>
</tr>
<tr>
<td>Inorganic and organic osmolytes</td>
<td>4</td>
</tr>
<tr>
<td>Ionic relations</td>
<td>5</td>
</tr>
<tr>
<td>Organic relations</td>
<td>6</td>
</tr>
<tr>
<td>Osmolyte concentration change coordination</td>
<td>7</td>
</tr>
<tr>
<td>Compatible solutes</td>
<td>8</td>
</tr>
<tr>
<td>Osmolyte evolution</td>
<td>9</td>
</tr>
<tr>
<td>Osmotic acclimation</td>
<td>11</td>
</tr>
<tr>
<td>Turgor sensing</td>
<td>12</td>
</tr>
<tr>
<td>Water fluxes</td>
<td>15</td>
</tr>
<tr>
<td>Compartmentation/Vacuoles</td>
<td>15</td>
</tr>
<tr>
<td>Specific osmolytes in specific algae</td>
<td>16</td>
</tr>
<tr>
<td>Glycerol</td>
<td>17</td>
</tr>
<tr>
<td>Glycerol's enzymatic protection</td>
<td>19</td>
</tr>
<tr>
<td>Proline and glycine betaine</td>
<td>21</td>
</tr>
<tr>
<td>β-dimethylsulphoniopropionate (DMSP)</td>
<td>22</td>
</tr>
<tr>
<td>Salt tolerant algae</td>
<td>23</td>
</tr>
<tr>
<td>Some exceptional cases</td>
<td>25</td>
</tr>
<tr>
<td>Metabolic pathways</td>
<td>27</td>
</tr>
<tr>
<td>Ecological implications of osmoregulation</td>
<td>28</td>
</tr>
<tr>
<td>Conclusions</td>
<td>29</td>
</tr>
<tr>
<td>Reference List</td>
<td>31</td>
</tr>
</tbody>
</table>
Introduction:

Regulation of cellular internal ionic composition is a physico-chemical process necessary for the survival of a variety of organisms. This process, which is commonly referred to as osmotic adjustment (Reed & Barron, 1983), has evolved in many organisms in highly specialized ways. The internal and external ionic composition of cells is related to the cells' osmotic pressure and turgor pressure and can be measured in a variety of ways including calculations of the extracellular space and tissue composition (reviewed in Dainty, 1963, 1976; found in Zimmermann, 1978), electrical (electromechanical) potential measurements, as well as measurements of the cellular dimensions and morphology (reviewed in Zimmermann & Steudle, 1977; found in Zimmermann, 1978).

In order for marine microalgae to survive in their natural environment they must constantly adjust themselves physically to their external world. This thesis focuses on how osmotically adjustment occurs specifically in marine microalgae. A brief description of the theoretical mechanisms microalgae utilize in order to osmotically adjust to external salinity changes as well as the inorganic and organic osmoregulators or “osmolytes” will also be covered. The goal of this study is to gain a basic understanding of the complex mechanisms and theories governing osmotic adjustment in marine microalgae.

The main factors that contribute to marine algal growth and distribution are temperature, light, water movements, nutrients, but also salinity (Soeder & Stengel, 1974). Temperature is important in the determination of general geographical distributions, growth rates and the metabolic physiology of algae. Marine algae are dependent on light for photosynthesis and photoautotrophic growth. These processes, however, are sensitive to temperature and light intensities which can stimulate or inhibit metabolism and growth.

Biochemical reactions of cells are carried out with nutrient components in an internal water-solvent medium (Walsby, 1982). Cell water compositions are therefore important in allowing the cell to carry out the biochemical reactions continuously occurring. Furthermore, the cell’s water volume is important in determining pH values, osmotic pressure, ionic strength, the accumulation of other solutes and the cell’s membrane potential.

Cells lacking continuous cell walls (wall-less cells) utilize contractile vacuole complexes
in order to get rid of excess fluid arising from an osmotic influx of water (Hausmann and Patterson, 1984). Such an influx arises when an osmotic gradient exists between a cell, high in internal solutes, and an external environment which is lower. This is the normal situation in freshwater environments, but it is also found in marine waters. This causes the cell to swell and ultimately rupture (Kirst, 1980). Vacuole contents are discharged at a specific spot on the cell surface and the contraction cycle of the vacuole is the sum of the filling (diastole) and expulsion (systole) of the contents of the vacuole (Kirst, 1980).

Many organisms have evolved sophisticated mechanisms that balance their osmotic strength with that of their environment. Although a large number of organic molecules could fulfill this function, an organism may fill this need by using only a few of such compounds (Rudulier, 1984). Compatible solutes accumulate intracellularly when in the presence of high levels of extracellular solutes and are known to protect cells from dehydration (Chambers et al., 1987).

A cell will do one of the following things if subjected to water stress when exposed to a concentrated solution with a low water availability: either thermodynamically equilibrate with the solution, for example by losing water, or it will suffer a temporary water loss, but invest energy into accumulating a solute to create a concentration thermodynamically equal in measure with the external water activity (Brown, 1978), where the water activity roughly correlates negatively with the salinity concentration. Such a solute can be a retained metabolite or a substance accumulated extracellularly where the solute accumulation lowers the intracellular water activity (increases the concentration) so that entering water obtains thermodynamic equivalence with the external medium (Brown, 1978).

In the process of osmoregulation the main criterion is that the osmoregulator is retained or “pooled” within the cell so that the concentration inside the cell is higher than that outside the cell (Brown, 1978). This is usually associated with the effective exclusion of the cell’s major external solute, which is most likely NaCl. When a cell is in a dilute environment its intracellular solutes (salts, nucleotides, proteins and intermediary metabolites) contribute to the cell’s “water status”, or osmosis. On the other hand, when increases in desiccation occur one single substance predominantly emerges to act as the osmoregulator. Examples of such osmoregulators are α-galactosylglycerol, cyclohexanetetrol, aspartate, glutamate, γ-aminobutyrate and proline, as well as miscellaneous polyols (Brown, 1978).
Different terms used

Marine eukaryotic unicellular algae mainly counter-balance the **extracellular osmolality** \((\pi_e)\), the osmotic pressure outside the cell, with the **intracellular osmolality** \((\pi_i)\), the osmotic pressure inside the cell. This is carried out by the complex interaction of synthesizing organic solutes, accumulating amounts of \(K^+\), and by the partial exclusion of amounts of \(Na^+\) (Dickson & Kirst, 1986, 1987). Rhodes (1987) found that the flux redistributions occurring in metabolism during adverse salinity conditions should be regarded as a complex function of sensitivity coefficients to all of the metabolic steps involved.

The **osmotic pressure** is dependent on the amount of free solute molecules in the solution, and in theory can be calculated from the known solution composition, or by use of an osmometer (Walsby, 1982). The osmotic pressure is defined as: \(\pi = \frac{(\pi_i - y \pi_e)}{1-y}\) where \(\pi_i\) is the internal osmotic pressure, \(\pi_e\) is the external osmotic pressure, \(\pi_t\) is the osmotic pressure of the total (cellular) suspension, and \(y\) is the total water present outside of the cell (Walsby, 1982).

**Turgor pressure** can be described as the hydrostatic pressure difference of the cell between the internal and external media. In order to measure it a pressure probe is needed (Walsby, 1982). Miniature pressure probes have been developed by plant physiologists that connect to pressure transducers, however, they cannot be used with cells less than 20 \(\mu\)m in diameter and the direct turgor pressure in prokaryotes can only be measured by using their gas vesicles if they have them (Walsby, 1982). Turgor pressure, the cell’s volumetric elastic modulus (defined below) and cell surface hydraulic conductivity (permeability), are important contributors to the study and understanding of cell volume changes. These are brought about by increasing, or decreasing the solute concentrations as well as the relative cell volume which is the ratio of the total cell volume under salinity stress to the initial cell water volume.

The ‘volumetric elastic modulus’ is defined as: \(\varepsilon = (\Delta P/\Delta V) \pi_e\). Where \(\varepsilon\) is the volumetric elastic modulus relationship between the pressure change, \(\Delta P\), and the relative volume change \(\Delta V/V_e\). The cellular membrane hydraulic conductivity (membrane permeability) is defined as: \(L_p = \ln 2/\tau \pi V/A1/(\varepsilon + \pi)\), where \(L_p\) is the cellular membrane hydraulic conductivity (the water flow rate through the cell surface under a pressure differential). \(V\) is the cell volume, \(A\) is the cell surface area through which water is driven and \(\tau\) is the half time in which the equilibrium is restored and \(\pi\) is the osmotic pressure (Walsby, 1982).
In plant cells the volumetric elastic modulus ($\varepsilon$) is dependent on volume and pressure. There are two important contradictory aspects of water relations and turgor pressure, namely the **half-time** ($\tau$) or rate of the cells’ response to osmotic stress and the internal **water content regulation**. This can also be explained by describing the half-time of the rapid phase of turgor pressure by not only being controlled by the cells’ geometry and their membranes’ hydraulic conductivity, but also by the cell walls’ elastic properties: $\tau = \ln 2 \cdot V / A \cdot L_p (\varepsilon + \pi_i)$ (Zimmermann, 1978). In this sense the half-time of the turgor regulation or the water exchange ($\tau$) for single cells is explained for the exponential water exchange. This equation is based upon the concept of water transport across the membranes’ barrier in the terms of the irreversible processes’ thermodynamics (see Zimmermann, 1978).

Not only do low volumetric elastic modulus ($\varepsilon$) values contribute to the cells’ ability to resist short-term fluctuations in the environment’s salinity, high $\varepsilon$ values may be of ecological and physiological advantage (Zimmermann, 1978). It may be of grand ecological significance for some plants that high $\varepsilon$ values ensure the relative water content change in a cell whereas the concentration is much smaller in cells with low $\varepsilon$ values in response to osmotic stress, particularly in plants growing in the earth’s arid zones.

**Inorganic and organic osmolytes**

Salinity changes affect organisms by osmotic stress and ionic stress, and induce a change of cellular ionic ratios (Kirst, 1980). The physiological processes used to tolerate these salinity changes are referred to as “osmoregulation”, osmotic acclimation, or “turgor regulation” (Kirst, 1989). Inorganic ion concentrations, or **inorganic osmolytes**, change in response to salinity stresses after the turgor pressure and water fluxes initiate the osmoregulatory processes. Kirst (1977) suggested that the inorganic ions $K^+$, $Na^+$ and $Cl^-$ act to “bridge the concentration gap” by immediately responding to osmotic stress. They do this by increasing or decreasing, depending on the ion, to maintain the cell’s normal osmotic pressure. After changes in the inorganic osmolyte concentration occur, as a result of salinity stress, organic osmolytes (organic molecules involved in the process of osmoregulation) begin to accumulate.

It has been discovered by many investigators (reviewed in Rudulier et al., 1984) that a
group of organic molecules (glycine betaine, proline betaine, proline, and others) behave as **osmoprotectants**. These molecules accumulate in cells in response to osmotic stress. They protect the cells from damage by cellular dehydration by balancing the cytoplasmic osmotic strength with the environment. Several of these molecules have also been discovered to behave as osmoprotectants for other organisms, such as bacteria (Rudulier et al., 1984). In addition to acting as osmotic balancing agents, these osmoprotectants are very likely to interact with the cells' crucial macromolecules to aid in modulating the cellular biological activity (Rudulier et al., 1984). For example, Yancey et al. (1982) proposed that osmoprotectants probably act as compatible solutes (they protect the enzyme activity at low water availability levels) as well as stabilizing protein structure and function. The simplest explanation for how osmoprotectants work is that they enable the cell to balance its cytoplasmic osmotic strength to that of its environment, preventing a net water loss. However, evidence suggests that osmoprotectants fulfill other functions such as influencing protein stability and structure (Rudulier, 1984).

**Ionic relations**

The main ions involved in the ionic relations during osmoregulation are K⁺, Na⁺, Cl⁻, and sulfate (Kirst, 1989). The concentrations of other ions, such as Mg²⁺ and Ca²⁺, are not influenced to any great extent, but they may act as a kind of messenger (Kauss, 1987; Tazawa et al., 1987; found in Kirst, 1989). An apparent trend in the three main classes of marine macroalgae (Chlorophyceae, Phaeophyceae, and Rhodophyceae) is that the Cl⁻ concentration change usually parallels the salinity fluctuation (Kirst, 1989).

There are three general types of species in respect to their K⁺ and Na⁺ relation: species with (1) a high Na⁺ content which increases with Cl⁻ under hyperosmotic stress whereas K⁺ is practically unaffected, (2) K⁺ as the major cation and a low Na⁺ concentration that increases steeply to exceed the K⁺ level under extreme salinity stress, and (3) both cations in approximately equal amounts (Kirst, 1989).

In marine microalgae that have a high cytoplasm to vacuole volume ratio the cytoplasm is typically (but not always) high in K⁺ and low in both Na⁺ and Cl⁻ (Kirst, 1977). However, it is difficult to determine the absolute ion content in microalgae. The absolute ion content may vary depending on the methods used to wash the algal pellets free of adhering medium, in
estimating the cell volume, and in correcting for the free space between the packed cells (Kirst, 1989). Species, such as Dunaliella, have Na⁺ concentrations that have been found to range from 7 mM to 800 mM in cells grown in 1.5 M NaCl, which is extremely saline (Ginsberg, 1981; found in Kirst, 1989).

In algae the ion concentrations are regulated by ion-selective carriers, which are driven by their membrane potential and other mechanisms such as ‘facilitated diffusion via ion-selective channels’, are also involved during the quick changes and in the recovery of the ionic composition (Kirst, 1989). The membrane potential in most freshwater algae, as well as in higher plants, is generated by an active H⁺ pump (Gutnecht et al., 1978; Raven, 1985; Walker, 1980; found in Kirst, 1989). However, in marine algae such membrane potentials and active ion-pumps, that are probably involved in creating the potential difference, are much more complex (Kirst, 1989).

Some generalizations which can be made from the results of Gutnecht & Dainty (1968) are that: all cells pump out Na⁺ and most actively absorb Cl⁻ and K⁺; the cytoplasm and vacuoles are electronegative in regard to sea water; both cytoplasm and “sap” are high in K⁺, low in Na⁺ and the “sap” is high in Cl⁻; and most ion influxes and effluxes are light stimulated and depressed by the dark, anaerobiosis and inhibitors.

**Organic relations**

Not only are ions accumulated and excreted in algae as a response to salinity changes but certain organic solutes of low-molecular-weight are as well (see Kirst, 1989). There are many cases in which these are the same compounds that are the organism’s main photosynthetic products. However there are differences in the compound preference between different taxonomic classes (Table 1).

These organic osmolytes have roughly an equal osmotic potential in molal solutions (about -2.0 to -2.5 MPa; Kirst, 1989). There is a big difference in the energy costs as well as the required amounts of carbon and nitrogen needed to achieve these potentials (Kirst, 1989). The double function of organic osmolytes, that they act not only as osmolytes but also as “compatible solutes”, may explain how these compounds are actually an advantage (Kirst, 1989). These solutes are termed “compatible solutes” because they have a protective function for the cell’s
enzymatic activities in concentrated saline conditions. Proline and glycerol were found to be the most effective organic osmolytes that were tested for their protective capacity (Kirst, 1989). However, it is not the case that all organic osmolytes are suitable as compatible solutes (Kirst, 1989). For example, sucrose can act as an osmoregulator but it doesn’t protect the enzyme activity very well in concentrated conditions, thus it does not function well as a compatible solute.

Table 1. A review of the organic osmolytes that are found in marine algae, summarized from Kirst 1989.

<table>
<thead>
<tr>
<th>Organic Osmolytes (Compatible Solutes)</th>
<th>Taxonomic Class (species)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polyols</strong></td>
<td></td>
</tr>
<tr>
<td>glycerol</td>
<td><em>Dunaliella; Phaeophyceae; Prasinophyceae</em></td>
</tr>
<tr>
<td>mannitol</td>
<td>most diatoms; Chlorophyceae;</td>
</tr>
<tr>
<td>sucrose</td>
<td>Chlorophyceae; Charophyceae</td>
</tr>
<tr>
<td>floridoside; digeneaside</td>
<td>Rhodophyceae</td>
</tr>
<tr>
<td>isofloridoside</td>
<td><em>Poterioochromonas malhamensis</em></td>
</tr>
<tr>
<td><strong>Quaternary Ammonium Derivatives</strong></td>
<td></td>
</tr>
<tr>
<td>glycine betaine</td>
<td><em>Tetraselmis (Platymonas); Chaetomorpha capillaris; Cladophora rupestris; important in higher plants</em></td>
</tr>
<tr>
<td><strong>Tertiary Sulphonium Compounds</strong></td>
<td></td>
</tr>
<tr>
<td>β-(dimethylsulphonio)-propionate (DMSP)</td>
<td>phytoplankton; Chlorophyceae; Rhodophyceae; Phaeophyceae (traces)</td>
</tr>
</tbody>
</table>

**Osmolyte concentration change coordination**

The changes in the concentrations of the ionic and the organic osmolytes throughout the osmotic adjustment period are coordinated (Kirst, 1989). It is generally known that the ionic osmolyte contents change before those of the organic osmolytes. As a steady state is reached, the ion concentrations again decrease in synchrony with the compatible solutes that have accumulated (Kirst, 1989). For example, *Dunaliella parva* responds to hyperosmotic shocks with
a rapid increase in Na\(^+\) concentrations, partially balanced with Cl\(^-\), for the first 20-60 minutes following the shock. This is followed by a decrease in Na\(^+\) concentrations while mannitol and glycerol reach their maximum concentrations after 60-90 minutes following the shock (Kirst, 1989). Changes in inorganic and organic osmolyte composition under constant salinity have also been observed in some algae, depending on the stages of development and in response to seasonal variations (Kirst, 1989).

**Compatible Solutes**

Growth of unicellular organisms requires them to exist in a liquid suspension or liquid/gas (solid) interface because they must obtain nutrients directly from solution (Brown, 1978). The result of taking nutrients from solution is that the micro-organisms’ water interactions are determined by the concentration of solutes in the aqueous solution in which they grow (Brown, 1978). Most organisms, multicellular as well as micro-organisms, can rarely tolerate conditions that are highly concentrated (roughly 0.3 M NaCl or 0.5 M sucrose). However, there are some micro-organisms (ie. algae, bacteria, yeasts, moulds) that can tolerate very concentrated conditions. Halophilic algae, which are best represented by some species of the genus *Dunaliella*, tolerate saturated NaCl and require minimal salt concentrations (Brown, 1978).

The micro-organisms that grow in concentrated environments have a comparably low water interior and usually have the turgor of flexible cells (Brown, 1978). It is important that the cell’s enzyme complement be functional when in a saline environment. This can be achieved by producing enzymes inherently resistant to inhibition or an intracellular environment that is not excessively inhibitory (Brown, 1978). Halophilic bacteria use both of these mechanisms, but eukaryotes solely depend on a modification of their interiors which they accomplish by the accumulation of a “compatible solute”. Ideally compatible solutes should be non-inhibitory (binding less to the enzymes than more inhibitory solutes) nor should they inactivate or “denature” an enzyme over a long time period (Brown, 1978).

The non-inhibitory nature of compatible solutes can be caused by their low affinity for the enzyme proteins (Brown, 1978). Affinity (and hence inhibition) of the normal cellular solutes for enzymes is related to physico-chemical properties suggesting that hydrophobic interactions are at least partially responsible for the normal binding that quickly occurs under salinity stress.
Hydrophobic interactions may explain the specific action of high concentration levels of salts on bacterial enzymes (Lanyi, 1974; found in Brown, 1978). However, stereochemical factors have not yet been fully evaluated (Brown, 1978).

Accumulation of polyhydric alcohols (where the polyol is oftentimes glycerol) is used by salt-tolerant eukaryotes as a response to water stress where such polyols confer an incredible degree of enzyme protection in concentrated environments. It is possible that salt-tolerant species are dependent on different central metabolic pathways as compared with non-tolerant species and that the enzymes that catalyze these pathways are also different. This does not seem to be the case with Dunaliella. As Brown (1978) found: “The major consequence of the metabolic differences seems to lie in the polyol production. There is no evidence of any such difference between halophilic and non-halophilic species of the alga, Dunaliella.”

It should be noted that polyols, and glycerol in particular, have the ability to preserve enzyme function (by not excessively inhibiting nor inactivating them) when the levels of water availability are low due to high salinity. In fact studies on glycerol clearly emphasize that glycerol neither excessively inhibits, nor inactivates enzymes, and it may even contribute to the protein molecular stabilization (Brown, 1978). Glycerol’s overall protective nature has also been illustrated by both its experimental and natural use as an “antifreezing agent” in protecting blood cells, spermatozoa, insects as well as fish (Schmidt-Nielsen, 1975).

All in all compatible solute accumulation is essential for micro-organism growth in saline environments in order to protect the cells’ enzymatic activities. A compatible solute may function as an osmoregulator to respond to water stress and as an enzyme protector in highly concentrated environments (Brown, 1978). There are several kinds of solutes that carry out this function when under slight to moderate stress, however, under severe conditions eukaryotes resort to polyhydric alcohols and turn to glycerol. Compatible solutes rarely activate enzymes. At most they function as poor inhibitors, although this is not in conflict with their protective role. In the absence of a compatible solute, other substances more inhibitory in nature, would be present at higher concentrations (Brown, 1978).

Osmolyte Evolution

There are two properties that distinguish compatible solutes from noncompatible solutes.
Firstly, some perturbing solutes specifically interact with ligands (i.e. cofactors, modulators and substrates) and with active sites, which therefore disturb the functioning of the cell's enzymes (Yancey et al., 1982). Secondly, compatible and perturbing solutes have an effect on the solubility hydration and the charge interactions of different protein groups. For example, amino acid side chains and peptide backbone groups can be different and such structural effects can be translated into functional manifestations (Yancey et al., 1982). Osmolytes may, in theory, affect the charged, polar or nonpolar groups of proteins in such a fashion that may increase or decrease their affinities to other inter- or intramolecular moieties (Yancey et al., 1982).

Phylogenetically diverse organisms (i.e. bacteria, unicellular algae, vascular plants, invertebrates and vertebrates; Table 2) all utilize a small organic osmolyte family which leads one to assume that there are strong selective pressures associated with this system and that it is an example of convergent evolution (Yancey et al., 1982).

Table 2. A distribution of various osmolyte systems. Taken from Yancey et al., 1982.

<table>
<thead>
<tr>
<th>Osmolyte system (occurrences)</th>
<th>Principal osmolytes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Polyhydric alcohols-polyols</strong></td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Glucose/glycerol</td>
</tr>
<tr>
<td>Synechococcus sp.</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
</tr>
<tr>
<td>Sectococcus prattenii</td>
<td></td>
</tr>
<tr>
<td>Asteromyces rudolphii</td>
<td></td>
</tr>
<tr>
<td>Lichens</td>
<td></td>
</tr>
<tr>
<td>L aromatic acid</td>
<td></td>
</tr>
<tr>
<td>Uncellular algae</td>
<td></td>
</tr>
<tr>
<td>Dunaliella spp.</td>
<td></td>
</tr>
<tr>
<td>Chlorella pyrenoidosa</td>
<td></td>
</tr>
<tr>
<td>Ochromonas maritime</td>
<td></td>
</tr>
<tr>
<td>Meloidella alga</td>
<td></td>
</tr>
<tr>
<td>Fucus spp.</td>
<td></td>
</tr>
<tr>
<td>Vascular plants</td>
<td></td>
</tr>
<tr>
<td>Glaucus minutum L.</td>
<td></td>
</tr>
<tr>
<td>Insects (freeze-tolerant or -resistant)</td>
<td></td>
</tr>
<tr>
<td>Euryarchaeota solubilis (Euglena)</td>
<td></td>
</tr>
<tr>
<td>Proacteylophilus</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td></td>
</tr>
<tr>
<td><strong>B. Amino acids and amino acid derivatives</strong></td>
<td></td>
</tr>
<tr>
<td>Estuaries</td>
<td></td>
</tr>
<tr>
<td>Kribbeella spp.</td>
<td></td>
</tr>
<tr>
<td>Salmoneella enteritidis</td>
<td></td>
</tr>
<tr>
<td>Synechococcus lacisii</td>
<td></td>
</tr>
<tr>
<td>Prokaryotes</td>
<td></td>
</tr>
<tr>
<td>Micrococcus acidus</td>
<td></td>
</tr>
<tr>
<td>Vascular plants</td>
<td></td>
</tr>
<tr>
<td>Saprolegia spp</td>
<td></td>
</tr>
<tr>
<td>Amylophila spp</td>
<td></td>
</tr>
<tr>
<td>Aper belongi</td>
<td></td>
</tr>
<tr>
<td>Mastigophorobacter novihominis</td>
<td></td>
</tr>
<tr>
<td>Vertebrates</td>
<td></td>
</tr>
<tr>
<td>Hyla versicolor</td>
<td></td>
</tr>
<tr>
<td><strong>C. Urea and methylamines</strong></td>
<td></td>
</tr>
<tr>
<td>Cardioglyphic fishes (e.g. morarch and melo)</td>
<td>marine and estuarine)</td>
</tr>
<tr>
<td>see examples in Table 1</td>
<td></td>
</tr>
<tr>
<td>Coelacanth (L. atalanta)</td>
<td></td>
</tr>
<tr>
<td><strong>D. Urea: estivating forms</strong></td>
<td></td>
</tr>
<tr>
<td>Molluscs</td>
<td></td>
</tr>
<tr>
<td>Balamus brydei</td>
<td></td>
</tr>
<tr>
<td>Lungfishes: African and South American</td>
<td></td>
</tr>
<tr>
<td>Amphibians</td>
<td></td>
</tr>
<tr>
<td>Steganopus eurich (spadefoot toad)</td>
<td></td>
</tr>
<tr>
<td>Archaelaia</td>
<td></td>
</tr>
<tr>
<td>Halobacterium sp.</td>
<td></td>
</tr>
<tr>
<td><strong>E. Inorganic ions</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K⁺</td>
</tr>
</tbody>
</table>

10
Yancey et al. (1982) recognized that only a few organic osmolyte classes have been adopted by phylogenetically diverse organisms and that they reflect two main universal phenomena. The first phenomena is that the set of physico-chemical interactions, ubiquitously present between the water solutes and macromolecules, determine which different solutes are compatible with the structure and function of the macromolecules. The second phenomena is termed "genetic simplicity" which means that by using systems of compatible solutes proteins are capable of functioning in the presence of varying solute concentrations with the avoidance the modification of proteins.

**Osmotic acclimation**

Algae respond to salinity changes in a well-organized biphasic fashion beginning with quick changes in turgor pressure in cells with walls (walled cells) or in changes in volume in wall-less cells, as a result of water fluxes into, or out of the organism after the appearance of an osmotic gradient. The next step involves osmotic adjustment where the cellular osmolyte concentrations change to reach a new steady state. Both of the steps involved in this osmotic adjustment perform different functions in a feedback loop. Salinity changes are recognized by turgor pressure, or a volume sensing detector, which in turn triggers an effector to readjust the volume, or turgor pressure by use of several reactions. It is this adjustment that is regulated by the feedback mechanism in between the detector and the effector (Kirst, 1989).

According to Zimmerman (1978) there are several important phases or steps that occur throughout the processes of osmotic adjustment. The first is that there is an immediate instantaneous change in volume due almost exclusively to the flow of water into, or out of the cell. This is dependent on the direction of the osmotic stress. In this phase the intracellular "osmotically active particle" concentration changes by shrinking or swelling. The solute transport across the cell’s membrane plays only a minor role in this phase. This is because the cell membrane’s water permeability, or hydraulic conductivity, is greater by several orders of magnitude than both the solute permeability of the cell’s membrane and the metabolic turnover rate of the cell’s osmotically active solutes.

The half-time of the water exchange and the cell’s volume changes are determined by the cell’s geometry, its elastic properties as well as the cell membrane’s hydraulic conductivity
(permeability). This is an idealized view of the biphasic kinetics of the cell’s volume regulation in response to osmotic stress. However, differences may be found to deviate from this model. *Dunaliella parva*, for instance, was found to show changes in the volume regulation between hypertonic and hypotonic stress (Rabinowitch et al., 1975). Therefore, variations in this osmotic adjustment process may be found not only in different organisms but within the same organism.

**Turgor Sensing**

Determination of the sensing mechanism for turgor pressure has led to several theories on the physiological mechanisms governing salinity tolerance (reviewed in Kirst, 1989). Some of these theories are: that anisotropic changes caused in the membrane are initiated by tension, that there is an electrochemical compression of the cellular membrane, and that there are stretch-activated ion channels (Kirst, 1989). All of these models describe a transformation in the ion transport of the cell caused by a triggering of an external stimulus from salinity changes whereby a disturbance in the membrane structure is involved.

In the ‘anisotropic membrane change model’ a pressure gradient is thought to create the membrane’s anisotropic changes (membrane changes having different values for one or more properties, i.e. compressibility or refractive index, when these are measured along different axes), and the sensor is located in the plasmalemma because it is unlikely that pressure gradients exist between the cell’s organelles and cytoplasm and within the cytoplasm (Bisson & Gutnecht, 1980; found in Kirst, 1989). In the turgor transduction process the turgor pressure causes the plasmalemma to press against the cell wall and into gaps between cellulose microfibrils (Kirst, 1989). This causes an asymmetrical curvature in the lipid bilayer of the membrane and a shift in the lipid distribution, especially in the membrane proteins. It is this shift which affects the active ion transport that in turn changes the cell’s membrane permeability, or releases some kind of messenger which activates the enzyme systems that are involved in osmotic adaptation (Kirst, 1989).

In the ‘electro-mechanical model’ the membrane is regarded as an electrical capacitor that is filled up with an elastic, dielectric material whose elastic properties act to counterbalance the compressive mechanical forces that are derived from both the turgor pressure, and the electrical compressive forces that arise from the membrane potential (Kirst, 1989). In order to sense the
turgor pressure changes, the membrane thickness must first change as a result of the alteration in the mechanical compression. As a result, the transport throughout the whole membrane is affected (Kirst, 1989). This occurs by intrinsic electric field changes or from the changes in the active pumps or the channels. In short, salinity changes cause a turgor pressure change that alters the thickness of the membrane. This change in membrane thickness controls the rate of translocation of the mobile charges through the membrane, which link the regulatory processes to the biophysical processes of the cell (Kirst, 1989).

The importance of the concept of the electromechanical model is that the mechanical forces produced by pressure gradients or by absolute pressure, caused by salinity changes, can affect the membrane mechanics in two ways. Firstly, by directly compressing the membrane, or its perpendicular parts, secondly, by the stretching of the cell membrane, which only occurs in the presence of pressure gradients (Zimmermann, 1978). The pressure gradients in walled cells is controlled by the cell walls’ elastic properties, which are coupled to the cell’s membrane (Zimmermann et al., 1977).

This model postulates that the geometric dimensional changes present in the membrane, or its parts that may be involved in the turgor sensing, are transformed into intrinsic electrical field distributional changes within the membrane. It further proposes that due to similarities in the biochemical and biophysical properties, between walled and wall-less cells during osmotic adjustment, the same beginning transformational step is involved in the regulatory response. This therefore fulfills a major criterion for the pressure transducer mechanism within the membrane (Zimmermann, 1978).

The ‘stretch-activated ion channels model’ suggests that stretch-activated ion channels are somehow involved with the mechanoreception and osmoreception of the cell and therefore also the volume regulation (Ubl et al., 1987, found in Kirst, 1989). The channel opening frequency increases when an increasing force is applied to the membrane (Kirst, 1989). This theory treats the mechanoreceptor-operated ion channels as a model for mechanical signal transductions in plant cells (Edwards & Pickard, 1987) and can be visualized as a part of some turgor-sensing mechanism. In this mechanism the channel proteins that are distributed throughout the membrane are connected with “spectrin-like linkers” which are inelastic filaments (Guharay & Sachs, 1984) that are parallel to the membrane’s inner surface. When a change in the membrane’s tension occurs these filaments tug at their attachment sites with the channel proteins.
resulting in an opening or closure of the conduit’ (Kirst, 1989). The filament network which is connected by these channel proteins operates as an amplifier and gathers force from the membrane’s large area (Kirst, 1989). An applied version of this model with the osmotic adjustment in algae is as follows (see Kirst, 1989): (1) the distortion in the plasmalemma causes the Ca\textsuperscript{2+} channels (see Kirst, 1989) to open, resulting in an influx of Ca\textsuperscript{2+}, this causes the cytosolic Ca\textsuperscript{2+} to rise which has an effect on the transport functions as well as the metabolism, or (2) the distortion in the plasmalemma opens the Cl\textsuperscript{-} channels causing a change in the cellular Cl\textsuperscript{-} levels and/or a change in the electrical properties which in turn has an effect on the transport.

Several theories of how algal cells respond to increases in salinity have been reviewed by Kirst (1990). Cowan et al. (1992) proposed a ‘cascade theory’ from studies on Dunaliella salina, based on the ‘stretch activated ion channels model’, where there is a distortion of the plasmalemma triggering a change in the pH which causes a redistribution of abscisic acid inhibiting H\textsuperscript{+}-ATPase (found in Kirst, 1989). This increases the abscisic acid levels and opens the Ca\textsuperscript{2+} channels leading to an influx of Ca\textsuperscript{2+} and a rise in cytosolic Ca\textsuperscript{2+} which again heightens abscisic acid levels in an effect on metabolism and enzyme activity. Cowan et al. (1992) concluded that Dunaliella salina respond to stress by magnifying glycerol production, accumulating β-carotene and enhancing abscisic acid metabolism. Therefore the cellular responses are regulatory depending on a variety of mechanisms perhaps linked to abscisic acid balance modifications (Cowan et al., 1992; found in Kirst, 1989).

The regulation of turgor pressure is known to fail in algae that are exposed to hyposaline media (Kirst, 1989). The reason for this is not yet fully understood. In estuarine species that have been studied the internal osmotic potential adjustment is accomplished by changes in the cells’ volume and by control of the internal osmolytes. One difference in estuarine and marine species, dependent on which species, is that the cell walls are thinner and that they have a low elastic modulus giving them a greater ability to swell or shrink (Kirst, 1989). This response is swift, and allows the organism to survive the rapid tidal salinity fluctuations. The ionic concentration changes in estuarine algae are very fast effector mechanisms and contribute in a larger proportion to the regulation than the organic solutes do (Kirst, 1989; also see ‘Inorganic osmolytes’ and ‘Organic osmolytes’ sections). These concentration changes have been suggested to reduce the energy costs of the regulation of the turgor pressure (Kirst & Bisson, 1979; Reed & Barron, 1989; found in Kirst, 1989).
Water Fluxes

In the first phase of osmotic adjustment the water fluxes occur with a half-time (see ‘Different terms used’ section) in microalgae ranging from 5-10 sec and lasting anywhere from minutes to hours in macroalgae (Kirst, 1989). The water flux under hypoosmotic conditions is at least transiently alleviated and the processes, of changing the cellular concentrations of osmolytes, do not act under immediate metabolic control (Kirst, 1989). Acting as a passive “osmometer”, however, these processes do depend on certain physico-chemical properties of the cell-wall-membrane complex like water permeability, hydraulic conductivity and elasticity (Kirst, 1989).

Compartmentation/ Vacuoles

The permeability coefficients of the cell’s plasmalemma may be an important issue involved in the osmotic regulation of both the inorganic and organic osmolytes involved. Compartmentation of the osmolytes into cytoplasmic organelles may be necessary in organisms containing multiple compatible solutes (Settler & Greenway, 1983), or may even help regulate the internal organization of the enzymes that either regulate the synthesis, or degradation of these osmolytes (Frank & Wegmann, 1974; Grimmler & Lotter, 1982).

According to the research of Heywood (1978) several algae and protozoa possess these osmoregulatory organelles, that are referred to as contractile vacuoles, which expel a hypotonic solution out of the cell to compensate for the water influx due to osmosis from the external medium. ‘Although contractile vacuoles are present in some marine organisms (Dodge, 1973; Lloyd, 1928, found in Heywood, 1978), they are usually absent from organisms inhabiting marine or brackish water’ (Heywood, 1978). Further evidence indicates that the contractile vacuole is a permanent organelle possessing its own specialized membrane, whereby a hydrophylic substance of the Golgi apparatus attracts water into the vesicles of the Golgi, supplementary vacuoles and the contractile vacuole. In this way the increased intracellular water from osmosis sequestered into the contractile vacuole is expelled from the cell (Heywood, 1978).

The possession of a contractile vacuole is associated with an absence, or minor rigidity
of a cell wall (Hartog, 1889; found in Heywood, 1978). When there is a slight presence of a cell wall, or an irregular cell wall, maintaining the cellular equilibrium may be dependent on the expulsion of water (Lloyd, 1928; found in Heywood, 1978). The contractile vacuole has a 'bristle coat structure' extending from the external membrane into the cytoplasm which may provide additional strength allowing it to withstand pressure changes that occur during the contraction of the vacuole cycle (Heywood, 1978). Prusch and Dunham (1970) found, in the contractile vacuoles of *Amoeba proteus*, a microscopic, freshwater protozoan, that in order for contraction of the vacuoles to take place, adenosine triphosphate (ATP) and Mg²⁺ were needed, suggesting that the systolic force is generated by the vacuole itself.

**Specific osmolytes in specific algae**

It is known that marine algae and higher plants accumulate organic solutes in response to changes in salinity. These include quaternary ammonium compounds (QAC’s), β-dimethylsulphonopropionate (DMSP), amino acids and their derivatives, heterosides, saccharides and polyhydric alcohols (Dickson & Kirst, 1987). Onium solutes such as the quaternary ammonium and tertiary sulphonium compounds are very likely involved in osmotically adapting eukaryotic unicellular algae to environments which are saline (Dickson & Kirst, 1986).

Dickson & Kirst (1986) found that the QAC’s glycine betaine, homarine (N-methyl picolinic acid betaine), and the tertiary sulphonium compound DMSP are all involved in *Platymonas subcordiformis* osmoregulation. Therefore, there may be multiple osmolytes working together in one single organism to protect it from the effects of external salinity changes. Some marine microalgae have even been found to regulate their free amino acids in order to respond to steady state salinities (Gilles & Pequeux, 1977; Liu & Hellebust, 1976) which appear to regain control under changes in intracellular osmotic stress in a wide range of marine algae (Hellebust, 1976; Flowers et al., 1977; Bisson and Kirst, 1979).

For example, *Ulva lactuca*, a macroscopic green alga inhabiting intertidal zones in estuaries and rocky shores, is affected by rapid salinity changes caused by tidal interactions, evaporation and rainfall (Dickson et al., 1980). In the studies of Dickson et al. (1980) on the effects of hypo- and hyper-saline stress on various levels of inorganic and organic solutes it was
found that the K⁺, Na⁺ and Cl⁻ tissue concentration levels dropped under hyperosmotic stress. It was further found that Na⁺ increased transiently and K⁺ and Cl⁻ accumulated under hyperosmotic stress. In these studies it was also discovered that the DMSP tissue content responded to salinity changes, that free sugars and amino acids (proline) did not seem to be important in osmotic changes and that tertiary sulphonium dipolar ions play an analogous role with glycine betaine in some algae.

Laimins et al. (1981) proposed that a membrane-bound osmosensing protein in *Escherichia coli* regulates the potassium transport genes as a function of osmotic strength. Furthermore, in *E. coli*, there is a glycine betaine uptake system that can be used for studies of membrane regulation functions in association with osmotic tolerance (Rudulier, 1984). The major osmoprotectants of *E. coli* are betaine, proline and glutamate, the most important being glycine betaine, because of its dipolar characteristics and high solubility in H₂O (Chambers et al., 1987).

Dimethylthetin is chemically related to the naturally occurring DMSP found in marine algae. It differs from glycine betaine by the substitution of a positively charged dimethyl moiety in place of the quaternary nitrogen group (Chambers et al., 1987). In studies on *E. coli* Chambers et al. (1987) found that the bacterial cells do not distinguish between betaines which contain a sulphur or nitrogen group, both compounds (dimethyletin and betaine) supported their growth in hypertonic NaCl equally well. They hypothesized that dimethylthetin may even be useful as a probe in studying betaine storage and comparing its medium excretion and uptake. Research on *E. coli*, such as this, may lead to new insights into the knowledge of how these molecules are used in osmotic adjustment processes in marine microalgae as well.

**Glycerol**

Glycerol synthesis is used by freshwater as well as marine microalgae in response to increases in salinity. One particular microalgae that responds to salinity stress is the genus *Dunaliella* sp., which possess the ability to adapt to changes in salinity by the synthesis or the elimination of glycerol (León & Galván, 1995). Glycerol accumulation and excretion, as a means of osmotic adjustment, is also used by *Chlamydomonas reinhardtii*, a freshwater green alga (León & Galván, 1995). The difference between the halotolerant *Dunaliella* species and the
freshwater *Chlamydomonas* sp., is that *Dunaliella* retains the glycerol it produces so when there is a high enough intracellular glycerol concentration for osmotic balance, no further glycerol synthesis is needed. A continuous glycerol production is needed in *Chlamydomonas* in order to maintain an equilibrium of the synthesis and excretion of cytoplasmic glycerol (León & Galván, 1994).

When algae of the genera *Dunaliella* and *Asteromona* were grown at salt concentrations of 0.5 M to above 5 M glycerol was demonstrated to be the major internal solute osmoregulator (Wegmann et al., 1980). The internal glycerol concentration in these algae was shown to be proportional to the growth medium salt concentration when it reached values greater than 4 M. *Dunaliella* have a unique property enabling them to maintain high intracellular to medium glycerol concentration gradients, which allows them to grow in high saline medias with little glycerol (Wegmann et al., 1980). Wegmann (1971) demonstrated that this accumulation of glycerol depends on the water potential and not on the NaCl found in the medium. This is similar to the responses of iso-osmolal mannitol or sucrose. What is interesting about the intracellular glycerol content in *Dunaliella* and *Asteromona* is that it has been found to be highly temperature-dependent (Wegmann et al., 1980). ‘The underlying mechanism may involve a temperature-dependent conformational transition of a component of the cellular membrane which is essential for glycerol impermeability’ (Wegmann et al., 1980).

*Dunaliella parva* is a green halophilic algae which accumulates large intracellular glycerol amounts as a way to osmoregulate (Ben-Amotz & Avron, 1973). Its osmoregulation was found to be dependent upon the formation and degradation of its intracellular glycerol. Their observations suggested that the major function of glycerol in *D. parva* is to maintain osmotic balance since glycerol is not excreted into the medium. They further concluded that glycerol formation and degradation are not light dependent, but because glycerol in *Dunaliella* is a photosynthetic product two metabolic pathways may be responsible for the formation of glycerol: ‘one using a photosynthetic product and the other via the metabolic degradation of starch, the storage product in *Dunaliella*’ (Ben-Amotz & Avron, 1973).
**Glycerol’s Enzymatic Protection**

*Dunaliello salina* is a halotolerant unicellular green alga which responds to osmotic stress by the regulation of a carbon flux between starch synthesis in the chloroplast and glycerol production in the cytoplasm (Bental et al., 1990). Following the results of *D. salina* by Chitlaru & Pick (1991) it was suggested that phosphofructokinase may be an important juncture enzyme in glycerol production regulation and that the pentose-phosphate pathway plays a principal role in conserving the oxidation-reduction balance throughout the synthesis of glycerol.

It was further proposed that glycerol is produced by the pentose phosphate pathway with the use of glyceraldehyde’s phosphate as an intermediate in the glycolytic pathway (Figure 1). This schematic theory proposed that glycerol is first produced from starch which is activated by phosphofructokinase stimulation and plausibly also from starch phosphorylase. Secondly, that the glucose to glycerol carbon flow involves the operation of two distinct metabolic pathways that are located inside the chloroplast. The first metabolic pathway is that the glycolytic pathway produces glycerol phosphate as NADH and ATP are consumed. The second metabolic pathway is that the pentose phosphate pathway supplements the deficiency of reducing equivalents. In this second metabolic pathway lost ATP is replenished, at least partially, by photosynthesis, respiration and glyceraldehydes phosphate conversion to pyruvate. Thirdly, the transport of glycerol phosphate out of the chloroplast occurs by the phosphate translocator as an exchange for a phosphate group (P_i) and then is hydrolyzed by glycerol phosphatase within the cytoplasm (Figure 1).

Belmans & Van Laere (1986) found that when *Dunaliella tertiolecta* cells were exposed to a hyperosmotic shock, they immediately began to synthesize glycerol. This glycerol production was associated with an increase in the enzyme glycerol-3-phosphate, which suggested an increase in in vivo glycerol-3-phosphate dehydrogenase activity, because the amounts of fructose 1-6-biphosphate and triose-phosphate remained constant, or decreased throughout this period. It was suggested that this increased activity in glycerol-3-phosphate is most likely not due to covalent modification, but to the concentration changes or to the compartmentation of the glycerol-3-phosphate dehydrogenase effectors.
The synthesis of glycerol after exposure to hyperosmotic shock does not occur so simply. The following series of events was described which may alter the cellular volume and the protein content that might lead to a change in the glycerol-3-phosphate content. It was proposed that ATP and changes in cellular pH associated with the hyperosmotic shock, were the candidates most likely to stimulate the formation of glycerol-3-phosphate (Belmause & Van Laere, 1986). The Na\(^+/\)H\(^+\) antiport system described by Katz et al., (1986) may, after a hyperosmotic shock, have an induction both on the direct cytoplasmic acidification and on decreasing the ATP content by H\(^+\)-ATPase activation. Dihydroxyacetone-phosphate reductase changes in their dissociation-association (Marengo et al., 1985), that are induced by cellular volume changes and protein concentration after a hyperosmotic shock, may also play a contributing role to this phenomenon.
Furthermore, it was found in two *Dunaliella* species, that glycerol concentrations of close to 4 M do not affect the enzyme glucose-6-phosphate, whereas KCl and NaCl have deleterious effects on this enzyme (Yancv et al., 1982). This clearly supports the theory that glycerol acts as a compatible solute since the glycerol concentrations protect this enzyme whereas KCl and NaCl do just the opposite.

**Proline and glycine betaine**

The accumulation of proline in plant cells as a response to hyperosmotic stress is a widely known phenomenon. There are two possible main roles for the accumulation of proline in response to hyperosmotic stress. The first is proline’s ability to function as an osmotic regulator and its relationship to restore a non-stress volume and turgor pressure. The second is related to the regulation of the cell’s water structure that protects the cell against the reduction of cytoplasmic constituent hydration which is induced by high levels of salt stress (Schobert, 1980).

One unicellular green flagellate, *Chlamydomonas reinhardii* (Chlorophyta), has a very close relationship to the halotolerant algae *Carteria* and *Dunaliella* (Reynoso & De Gamboa, 1982). *C. Reinhardii* is a freshwater algae. It was discovered that there is a linear relationship between the induction of halotolerance and the proline concentration in the medium. Results of Reynoso and De Gamboa (1982) demonstrated that adding proline to the medium induces salt tolerance and also suggested that proline plays an active role in the cell’s intracellular processes that are connected with the tolerance of salt.

Dickson & Kirst (1987) also found that the unicellular marine algae *Phaeodactylum tricornutum*, *Cyclotella cryptica*, *Cyclotella meneghiniana* and *Porphyridium aerugineum*, all synthesize and accumulate proline and glycine betaine as a response to NaCl concentration increases, whereas *C. cryptica* and *C. meneghiniana* synthesize and accumulate homarine (N-methyl picolinic acid betaine) as well. *Phaeodactylum tricornutum* and *P. aerugineum* also synthesized intracellular glycerol to respond to increased salinities. The only algae that synthesized DMSP was *P. tricornutum*. The quantity of the DMSP was dependent on the amount of NaCl that was in the medium. Furthermore, the intracellular K⁺ concentrations were three to six times higher than Na⁺, thus media salinity increases led to cellular K⁺ accumulation and uptake, and a small cellular Na⁺ and Cl⁻ uptake whereas there was a loss of the intracellular NO₃⁻.
This supports the fact that the osmotic regulation in marine microalgae is a complex interaction utilizing both inorganic as well as organic compounds and that the organic compounds each organism uses may be species specific.

**β-dimethyl sulphoniopropionate (DMSP)**

β-dimethyl sulphoniopropionate (DMSP) is a tertiary sulphonium compound and occurs in several marine macroalgae (Challenger, 1959) as well as in several marine microalgae (Ackman et al., 1966). DMSP is involved in marine macroalgae osmotic acclimation (Dickson et al., 1980; Reed, 1983). It is a tertiary sulfonium compound (Table 1) which is analogous to quaternary ammonium compounds such as glycine betaine and proline which are widespread through marine organisms (Dacey & Wakeham, 1986).

Dickson and Kirst (1986) discovered that DMSP, glycine betaine and homarine, which are all quaternary ammonium compounds, are involved in the osmotic acclimation of the marine microalgae, *Platymonas subcordiformis*. They proposed, based on their calculations of the inorganic osmolytes, that an evaluation should be made on the integrated activities of both inorganic and organic solutes on the regulation of cell volume. Their research indicates a need to access the role of both quaternary ammonium and sulphonium compounds for the adaptation of eukaryotic microalgae to the saline environment (Dickson & Kirst, 1986).

In studies on the osmotic responses in the green intertidal algae, *Ulva lactuca*, the only organic compound that responded to hyper- and hypoosmotic and steady-state salinity stress was DMSP, suggesting that it is involved in osmoregulation and acts as a cytoplasmic compatible solute (Dickson et al., 1982). It was further found that tissue K⁺, Na⁺ and Cl⁻ concentrations decreased under hypoosmotic conditions while a stable increase in K⁺ and Cl⁻ with a transient Na⁺ increase, was seen under hyperosmotic stress.

Recently Karsten et al. (1991) discovered that the DMSP concentrations of an Atlantic green algae correlated with the light factor. Further studies on the green macroalgae *Ulothrix implexa*, *Acrosiphonia arcta* and *Ulva rigida* proved that the DMSP content had a direct correlation with the ‘light factor’ where the algal DMSP level rose with increasing light irradiance (Karsten et al. 1991). They suggested that the possibility exists that each species may require a certain light level in order to obtain optimal DMSP biosynthesis.
Glycine betaine is known to be the nitrogen analog of DMSP and is also known to be involved in the osmoregulation of some plant cells (Wyn Jones & Storey, 1981). One interesting find by Turner et al. (1988) was that the coccolithophore, *Emiliania huxleyi*, produced less internal DMSP when placed in a medium that was nitrogen supplemented. When nitrate was added to a culture deprived of nitrogen, the cellular DMSP content decreased within a 24 hour period. This supports the speculation that when in a nitrogen-limited environment, algae may actually increase their limited ability to produce glycine betaine and therefore synthesize DMSP as a result (Andreae, 1986).

**Salt tolerant algae**

The term "halophil" is the one most commonly used by microbiologists to describe micro-organisms that have enhanced salt tolerance, including salt-tolerant unicellular algae. There are some algae that thrive in saturated saline environments. These include the algal genus *Dunaliella* (phylum Chlorophyta, Order Volvocales) and some species within the genus *Chlamydomonas*, within the same order (Brown, 1978). Ecological studies suggest that members of the Chlamydomonas genus are in general less tolerant than those of *Dunaliella* (Brown, 1978). For example species of *Dunaliella* are the only algae that can be observed in the Great Salt Lake (Utah, USA) in times of full saturation (Brock, 1975).

One characteristic that should be remembered when comparing the salt tolerance and relations of alga, especially those within the same genus, is the salt optima position for their growth rate (Fig. 2; Brown, 1978). As can be seen in Figure 2, the halophilic species, *Dunaliella viridis*, has its optimum close to the bottom range of the species *Dunaliella tertiolecta*. It can be seen by their growth range that they show a salt-tolerance through each of their curves instead of a salt requirement.
Figure 2. *Dunaliella tertiolecta* (▲) and *Dunaliella viridis* (■) exponential growth rates as compared to increases in sodium chloride (NaCl). Taken from Brown, 1978.

Cyanobacteria are blue-green algae that occur in aquatic habitats, variable in their ionic composition and salinity, in freshwater, brackish, marine and hypersaline environments (Fogg et al., 1973; Carr and Whitton, 1982). Studies by Mackay et al. (1984) classified Cyanobacteria into three physiological groups based on their organic osmotica, which is coupled to their upper limits of salt tolerance. The “marine” group accumulated glucosyl-glycerol under NaCl stress, the “freshwater” group consisted of simple sugars, instead of the heteroside glucosylglycerol, under osmotic stress and the most “halotolerant” group contained quaternary nitrogen compounds as their organic osmotica. Mackay et al. (1984) found a trend that led towards glucosyl-glycerol production in the marine isolates and to a sucrose/trehalose accumulation in the freshwater forms. Mackay et al. (1984) also claimed to have found no “absolute” differences between the cyanobacteria from the differing habitats.

The data accumulated by Reed et al. (1984) suggested that the production of glucosyl-glycerol is not unique to marine cyanobacteria. It was also suggested that some strains may accumulate betaines as well in addition to accumulating sucrose or trehalose. The carbohydrate content by itself may not allow a distinction to be made between the “hypersaline” strains and the normal strains. Reed and Stewart (1985) re-evaluated these findings and came up with the
following conclusions: there are three broad but overlapping categories of cyanobacteria which are characterized by (a) disaccharide accumulation in the “least halotolerant”, (b) heterosides in the “intermediate halotolerant”, or (c) betaines in the “most halotolerant”. They also found that there is no absolute correlation between the accumulation profile of organic solutes and their habitats, the accumulation of glucosyl-glycerol and growth in media that are unique to “marine” cyanobacteria, and that instead of halotolerance being linked to habitat, it is linked to the organic osmotica compatibility.

**Some exceptional cases**

There are some other exceptional cases of marine algae osmotically adapting to very harsh conditions such as in the Great Salt Lake and in the Polar regions. The algae that live in the Great Salt Lake are interesting. The lake is eight times saltier than the oceans yet the microalgae *Dunaliella salina* and *Dunaliella viridis* manage to flourish there creating magnificent red and green algal blooms in the summer. The red colourations predominate in the northern arm of the lake and the green colourations predominate in the southern arm (Zahl, 1967).

According to Brock (1975), *Dunaliella* are not optimally adapted to the conditions of the Great Salt Lake. They maintain their populations at such high salinities because they have no competition with other algae. His studies revealed that *Dunaliella* is present virtually throughout the Great Salt Lake but that there are marked differences in the population density. This may be correlated with grazing animals, the brine shrimp, *Artemia salina* in particular. He further suggested that because it has been impossible for *Dunaliella* to optimally adapt to saturated salt through evolutionary processes, no other algae, blue-green or eucaryotic, seem to be able to compete with *Dunaliella* at such high salinities.

Another interesting case is algae that have osmotically adapted to the ice-cold Polar regions. The data of Karsten et al. (1992) support the idea that DMSP-biosynthesis is light dependent. It also acts as an “antifreezing” agent since low water temperatures stimulated the DMSP content in several species of Antarctic green macro algae. It was therefore apparent in these plants that DMSP acts as a cryoprotectant. This data demonstrated that *Ulothrix impexa*, *Ulothrix subflaccida*, *Enteromorpha bulbosa* and *Arcosiphonia arcta* all synthesize and
accumulate high DMSP concentrations in the light under hypersaline conditions. The capacity to produce DMSP under hypersaline conditions in the dark was extremely low in all species.

Algae in the upper littoral zone undergo a combination of both osmotic and freezing stresses in the course of the tidal fluctuations and the changing seasons due to the mixture of seawater with freshwater that originates from the thawing sea-ice (Karsten et al., 1990). Since these species grow in the littoral zone they are exposed to large variations in their external osmotic pressure as a result of the tidal fluctuations and changes in the weather conditions. The isolated Antarctic species, *U. implexa, U. subflaccida, E. bulbosa,* and *A. arcta,* accumulated DMSP linearly with salinity increases (Karsten et al., 1992). This capability declined on the other hand with *Utera,* which is a Subantarctic/cold temperate species, and *Blidingia minima,* which is a temperate species (Karsten et al., 1990). This indicates that there is a negative correlation between the capacity to synthesize DMSP in extreme hypersaline conditions and in the polar and temperate region temperature regime. It is reasonable to assume that the biosynthesis of DMSP in salt-stressed algae is controlled by enzymes that are “light-dependent” instead of by ATP and NADPH which are formed photosynthetically (Karsten et al., 1990).

Thus, aside from being exposed to large salinity variations, Antarctic algae in the intertidal zone are also exposed to a large temperature range. The work of Karsten et al. (1992) clearly demonstrated that DMSP accumulation is positively influenced by low water temperatures. This supports the assumption that the temperature regime of Antarctica intensifies an increase in DMSP in salt-stressed algae. The higher DMSP levels in the Antarctic species at 0°C in comparison to those at 10°C led Karsten et al. (1992) to assume that DMSP functions not only as an osmoprotectant but also as a cryoprotectant. Supposably DMSP acts as an “antifreeze” by keeping the cytoplasm liquid below temperatures of 0°C. ‘Freezing of the cytoplasm prevents any enzymatic activities of the cell, but if the cytoplasm is kept liquid below 0°C by high concentrations of anti-freeze compounds metabolism is possible. Cryoprotectants are also known to protect proteins directly by molecular interactions from damage during freeze-thawing (Carpenter and Crowe, 1988)” (Karsten et al., 1992).
Metabolic Pathways

The study of several metabolic pathways and how they are regulated, have mostly been investigated under salinity stress in unicellular algal species. The understanding of the biosynthetic regulations of marine macroalgae’s organic components is scanty, as is the overall knowledge of the regulation of the formation and degradation of these compounds. There is even less knowledge over the molecular biology of these processes. Therefore, it may be interesting to look at other organisms and what is known about their osmotic regulation as a comparison, such as the osm genes found in Escherichia coli which control proline and betaine production for protecting the cells from dehydration (Redulier et al., 1984).

The following is a summary of mechanisms that probably participate in regulating the size of the osmolyte pool during osmotic adjustments (Kirst, 1989).

(A) Hyperosmotic stress:

1. De novo synthesis of enzymes.
2. Activation of enzymes involved in synthesis of the osmolyte.
3. Reduced degradation or metabolism.
4. Remobilization of reserve products in light and dark.
5. Redistribution within cellular compartments.

(B) Hypoosmotic stress:

1. Transfer into polymeric reserve products.
2. Inhibition of enzymes involved in synthesis.
4. Release of organic osmolytes into the medium: emergency reaction observed in micro- and macroalgae.
Ecological implications of osmoregulation

The use of osmolytes is complex, their functions include osmoprotection, internal enzymatic function protection (compatible solutes) and cryoprotection (antifreeze) in ultra-cold environments. The following Table (3) is a summarized scheme of the processes and properties involved in the ecology of osmoregulation in marine algae, their characteristics and the possible relevance these may lead to under their natural conditions.

Table 3. A summary of the ecological importance and the characteristics of the discussed mechanisms and their involved features in the process and functioning of osmotic adjustment. (Kirst, 1989).

<table>
<thead>
<tr>
<th>Processes and properties</th>
<th>Characteristics</th>
<th>Possible relevance under natural conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Water flux</td>
<td>very fast, passive; inherent result of any change in salinity</td>
<td>may be a sufficient response to balance small osmotic gradients; shock experiences</td>
</tr>
<tr>
<td>2. Ion transport</td>
<td>fast, low energy cost; selective uptake or release; vacuole and partially cytoplasm</td>
<td>major short-term response in intertidal zones and estuaries</td>
</tr>
<tr>
<td>3. Organic osmolytes</td>
<td>slow; high energy costs; mobility in cytoplasm</td>
<td>long-term adjustment to extreme osmotic stresses; seasonal changes in lagoons; desiccation in supralittoral habitats (salt marshes and rock pools); ice algae; too slow for tidal zones</td>
</tr>
<tr>
<td>a. synthesis or degradation</td>
<td>slow; high energy costs; possibly in several cell compaments</td>
<td>as above (3a)</td>
</tr>
<tr>
<td>b. accumulation of several osmolytes</td>
<td>slow; high energy costs; compounds accumulated due to developmental stages</td>
<td>hyperosmotic shocks</td>
</tr>
<tr>
<td>c. buffer capacity of high contents of organics</td>
<td>hyperosmotic shock</td>
<td></td>
</tr>
<tr>
<td>4. Morphological and anatomical features</td>
<td>passive, energy cost during construction</td>
<td>supralittoral, tidal zones and estuaries</td>
</tr>
<tr>
<td>a. buffer effects: large vacuoles; massive thalli</td>
<td>passive; partial (incomplete) tissue pressure regulation</td>
<td>intertidal zones; time limited</td>
</tr>
<tr>
<td>b. cell walls: ion exchange capacity</td>
<td>gametophyte and sporophyte differ in resistance; dormant stages (zygote, spores)</td>
<td>survival of extreme conditions; ice algae</td>
</tr>
<tr>
<td>c. elastic properties of cell walls</td>
<td>passive; depends on population density</td>
<td>intertidal zones</td>
</tr>
</tbody>
</table>

28
Conclusions

In order to maintain turgor pressure and/or the ion concentration when faced with environmental salinity perturbations, it is required that an adjustment be made to the ion transport rates and/or the biochemical reactions that comprise the components of the more basic homeostasis process. Osmotic adjustment depends on the membrane potential. This can be derived from the fact that both turgor pressure and electrical fields produce stressful conditions leading to a compression in the membrane. The turgor pressure is controlled by the concentration of specific ions and this control should also be dependent on their contribution to the osmoregulation.

The biphasic osmoregulatory response to environmental salinity stress is quite similar in both walled and wall-less cells. On the macroscopic scale the turgor pressure is the regulating parameter in walled cells and the volume is the regulating parameter in wall-less cells. The switch between these forms of regulation is continuous and can be determined by the extension of the volumetric elastic modulus of the cell wall. This in turn depends on the turgor pressure and the cell’s volume and is an extremely complex parameter.

It may be of interest in further research to have more studies invested in the volume kinetics of cells in response to osmotic stress, as well as possible mechanisms involved in turgor pressure sensing based on properties of the electromechanical model. Investing more effort and time into the understanding of both of these problems would significantly increase our knowledge of how the specific properties of osmotic adjustment play different roles and how they all contribute to an overall immense scheme of the process of osmoregulation in all organisms.

Contractile vacuole complexes are also used by cells lacking continuous cell walls to dispose of huge water influxes. In the situation that a large water influx arises between a cell, its internal solutes and environment, contractile vacuoles enable the cell to discharge the excess internal water. This occurs by a contraction cycle made up of the diastolic phase, or filling-up, and the systolic phase, or expulsion.

Once the cell’s osmotic adjustment processes have been ‘turned on’ by the turgor pressure and the cells volume/water flux, the cells respond by producing intracellular physico-chemical changes, utilizing both inorganic osmolytes (in the beginning) and organic osmolytes (later on). It is the use of these osmolytes that enables the cells to maintain an osmotic balance with the drastic extracellular salinity changes. Therefore, marine microalgae osmotically acclimate to salinity changes by first sensing the change in the osmotic gradient by changes in turgor pressure or water flux. Next, these algae adjust themselves via inorganic (Na⁺, K⁺ and Cl⁻) and then with inorganic osmolytes (polyols, quaternary ammonium derivatives or sulphonium compounds). These inorganic and organic osmolyte changes are coordinated. They synchronize with each other so that when the increase in inorganic osmolytes reach steady state they decrease in synchrony with an increase in the organic osmolytes. Furthermore, multiple osmolytes may work together within one organism to protect it from the adverse salinity stresses.

Compatible solutes, or organic osmolytes, accumulate in micro-organisms in low water availability environments. These molecules are termed “compatible solutes” because they function as both an osmoregulator and an enzyme protector. Marine microalgae share the use of these compatible solutes with phylogenetically diverse organisms. This leads to the assumption that strong selective pressures are associated with their use and that this is an example of
convergent evolution. Some compatible solutes (ie. glycerol and DMSP) have also been shown to correlate with the light factor. This indicates that they are light dependent. However, it has been suggested that the specific light level required may be different for each species.

Glycerol is used as a compatible solute in many marine organisms. Two metabolic pathways are plausibly responsible for glycerol formation, namely that glycerol is used as a photosynthetic product and that it is used in the metabolic degradation of starch. It is also known to play a major role in enzyme protection. Proline and glycine betaine are also compatible solutes of marine microalgae. These two solutes may work together, as in the case of *Phaeodactylum tricornutum*, *Cyclotella cryptica*, *Cyclotella meneghiniana* and *Porphyridium aerugineum*, or individually, such as in *Chlamydomonas reinhardtii*. which only synthesizes proline under salinity stress. β-dimethylsulphoniopropionate (DMSP) is the tertiary sulfonium analog to the quaternary ammonium compounds proline and glycine betaine and acts as an osmoprotector in marine microalgae. Algae in the Polar regions have learned to utilize the osmolyte DMSP as both an osmoregulator and a cryoprotectant (an antifreezing agent). In this manner, they are capable of tolerating both extreme salinity and temperature fluctuations.

Salt-tolerant algae are termed “halophils”. Members of the genus *Dunaliella* and some species within the genus *Chlamydomonas* are salt tolerant. Their growth rate in relation to their salt optima does not necessarily depend on their salt-tolerance but does depend on their salt requirement. Thus, they can grow in salt environments that fulfill their salt-requirements. In the case of the algae inhabiting the Great Salt Lake, *Dunaliella salina* and *Dunaliella parva*, they flourish in extreme salinities that are eight times that of the oceans. They survive these extreme conditions not because they have evolved special salt tolerance mechanisms, but because there is no competition from other algae.

The functioning of osmotic adjustment in marine microalgae is a complex process. Hyperosmotic stress and hypoosmotic stress are the metabolic pathways utilized in order to undergo the osmotic adjustment processes. These both include the formation and degradation of compounds that control and protect the biosynthetic regulations. The specific processes and properties, and their specific characteristics of the acclimation to salinity stress play very specific roles in the process of osmotic adjustment in marine microalgae. Some of these specific roles and their ecological implications are understood. However, studies on many more species and how they respond to salinity stresses are needed to gain a complete understanding of the osmotic adjustment in marine microalgae.
Reference List


