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DESICCATION STRESS

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The threat of desiccation for organisms inhabiting the intertidal zone occurs during emersion at low tides or when organisms are positioned in the high intertidal zone, where wetting occurs primarily by spring tides, storm waves, and spray. Drying due to evaporative water loss is the most common mechanism for dehydration, although during winter in northern temperate regions freezing can also occur, which reduces the liquid water in extracellular fluids and can lead to intracellular dehydration in multicellular organisms. Freezing tolerance has been reported and characterized for a number of intertidal invertebrates, including gastropods such as an air-breathing snail and a periwinkle, and bivalve genera including the common and ribbed mussels.

BIOLOGICAL RESPONSES TO THE THREAT OF DESICCATION

Behavioral defenses against stress and injury from the loss of water from cells, tissues, or body fluids are seen across all organisms resident in the rocky intertidal zone: microorganisms, animals, and plants. For organisms that are highly mobile, the first response to water stress is generally behavioral: to leave the area or to seek microhabitats that afford some degree of protection against dehydration (Figs. 1, 2). Such refugia include crevasses in the substratum, animals aggregated into clumps, cover and shade underneath macroalgae, and accumulated organic detritus. Other, less mobile organisms (Figs. 1, 2) restrict

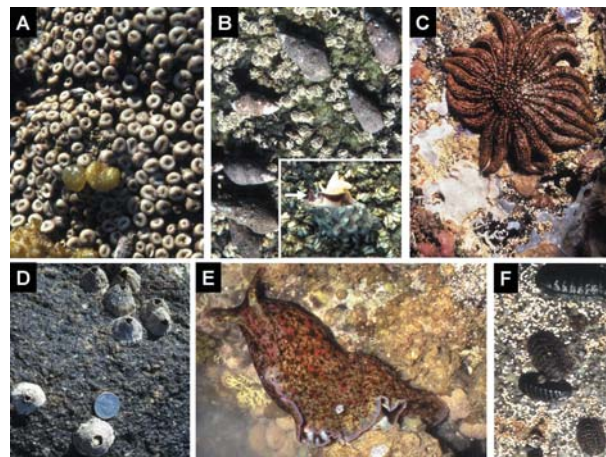


FIGURE 1 Selected inhabitants of the intertidal zone in the northern Gulf of California, where tidal amplitudes are among the largest in the world (6–9 m of vertical displacement). (A) The colonial zoanthid anemone, *Palythoa ignota*, tightly constricted during emersion and pictured with the brown bubble gum alga *Colpomenia*; (B) a field of acorn barnacles, *Chthamalus*, occupied by the prosobranch snails *Cerithium* and (inset) *Acanthina angelica*, whose apertural spine (arrow) is used to pry open opercular plates of barnacles on which it feeds; (C) the gulf sun star, *Heliaster kubiniji*, tightly adhered to rock surface and with restricted locomotion during emersion; (D) the thatched barnacle, *Tetraclita stalactifera*, from the higher mid intertidal zone; (E) the sea hare, *Aplysia californica*, trapped at low tide in a rock crevasse; and (F) the green chiton *Chiton virgulatus*. The behavioral and physiological mechanisms possessed by these organisms for avoiding or tolerating water loss during emersion are varied (see text). Photographs by.

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various activities such as filter feeding and irrigation of respiratory epithelia (e.g., ectoprocts, barnacles, bivalves), constrict to reduce surface area and attach gravel/shell debris to body wall (sea anemones), and adhere more tightly to the rocky substratum (e.g., chitons, limpets, snails)—all behaviors that retard water loss. Behavioral responses to emersion may also include the synchronization of gamete release or hatching of embryos.

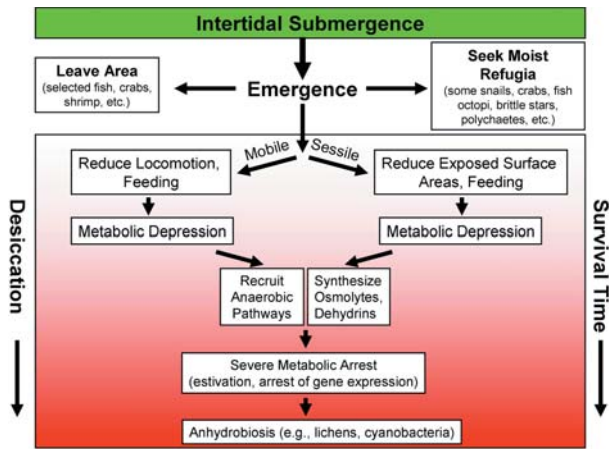


FIGURE 2 Metabolic depression is a natural consequence of assuming postures that restrict water loss; reduction in gas exchange across respiratory surfaces promotes a decrease in energy metabolism and, commonly, a greater reliance on anaerobic metabolism. In more extreme cases, states of estivation (snails) and anhydrobiosis (lichens, cyanobacteria) can be entered.

Physiological and biochemical features important for resisting or tolerating water loss are numerous in organisms of the intertidal zone. These include (a) the deployment of desiccation-resistant egg cases for embryonic development, (b) reduction in water permeabilities of body coverings and epithelia (with the unavoidable disruption of gas exchange), (c) accumulation of metabolic end products as a result of concomitant oxygen limitation, (d) short-term, facultative depression in metabolic and developmental rates, (e) maintenance of intracellular osmolytes for water retention and macromolecular protection at low-water activities, (f) differential gene expression for the production of protective macromolecules, and, in extreme cases, (g) global downregulation of gene expression and metabolism during estivation and anhydrobiosis. Energy conservation, as in options (d) and (g), is critical during extended periods when nutrient acquisition is offset by the potential for severe water loss.

DEPRESSION OF METABOLISM

Water loss during emersion is increased by active processes such as locomotion, feeding, and ventilation of respiratory surfaces. Sessile organisms, which often dominate significant areas of the intertidal zone, respond to desiccation stress by reducing the exposed surface areas across which water loss takes place. Thus, when these processes and surface areas are restricted, overall metabolism is commonly depressed, either by active downregulation or “automatically” by impeded gas exchange (e.g., collapsed gills). Different strategies in response to aerial exposure and water loss can be distinguished across species.

Depending on the species, some sea anemones store significant seawater within the body cavity (coelenteron) during emersion. Intertidal anemones become quiescent during exposure, and overall metabolism declines as a function of time in air. For intertidally acclimatized individuals of some species, aerobic metabolic pathways (ones requiring oxygen) predominate, as evidenced by the lack of an oxygen debt upon reimmersion and by the observation that rates of respiration and heat release by the animals are consistent with the complete conversion of energy fuels to carbon dioxide and water. However, subtidally acclimatized specimens rely partly on anaerobic pathways (those that do not require oxygen) as length of experimental emersion is extended. Anaerobic pathways are apparently recruited in certain other anemones as well during aerial exposure. End products, such as the amino acids alanine and glutamate, have been measured.

A rich literature exists concerning physiological responses of intertidal molluscs to emersion. For example, the common mussel withstands the physical, chemical, and biotic factors during low tide by trapping water in the mantle cavity and tightly closing their valves to restrict water loss. Long-term closure is accomplished using catch muscles, which substantially reduces the energetic cost of keeping the valves tightly closed. When valves are closed, gas exchange is greatly depressed, oxygen supply limited, and metabolism downregulated; the mussel relies generally on anaerobic ATP generation during these periods. The oxygen debt that builds up during the anaerobic phase is paid off by aerobic respiration on the next incoming tide. Water loss from body tissues is minimal during air exposure if valves are kept closed. However, in some individuals of the common mussel, and more typically in other species such as the common cockle and the ribbed mussel, the phenomenon of “gaping” of valves can occur during emersion, which allows for a greater rate of oxygen consumption with the concomitant tradeoff of greater tissue desiccation.

Snails that live above the high-tide mark in the intertidal zone greatly minimize locomotion and feeding during emersion, and as a result, evaporative water loss from tissues is reduced. Nevertheless, water loss can reach 30% or more after many hours of air exposure at elevated temperatures. In some species, entry into a state of estivation is a common occurrence, which involves withdrawing the body deeply into the shell, occluding the shell aperture, and even cementing with mucus the edge of the aperture to the substratum. Estivation in snails is commonly associated with major metabolic depression. In nonestivating snails in the upper intertidal, desiccation is normally

associated with varying degrees of metabolic depression, either active or passive. Compared with other intertidal gastropods with shells, limpets exhibit much greater water losses during emersion, apparently in part because of the wide aperture of their dish-shaped shells. Aerobic metabolism accounts for the vast majority of overall ATP turnover during emersion in snails. Although anaerobic metabolism and accumulation of end products (alanine and the organic acid succinate) have been documented in at least one gastropod species during emersion, the contribution to overall energy production is very low. Many species of gastropods are reported not to recruit anaerobic pathways at all during air exposure.

Opisthobranch gastropods (sea slugs, sea hares, nudibranchs) can become stranded occasionally during low tide in the intertidal zone and exposed to air. Some sea hares (Fig. 1E) tolerate emersion for many hours, during which time some loss of body water occurs and oxygen uptake drops dramatically compared to the aquatic value. Evidence indicates the metabolic depression is accompanied by anaerobic energy production; whole-body lactate and alanine are elevated severalfold.

As early as the 1950s, studies on crustacea underscored that intertidal barnacles ceased filter feeding and became quiescent upon emersion, in contrast to subtidal barnacles, which remained active and were more quickly desiccated. Aerobic metabolism in barnacles is the primary energy source, even though anaerobic production of lactate occurs to a limited extent. Similarly, intertidal crabs often show a depressed aerobic metabolism soon after exposure that is thought to arise from impeded gas exchange due to clumping of gills, absence of ventilation, and depleted oxygen in unventilated water surrounding the gills. The presence or absence of lactate accumulation varies across species.

Most intertidal fishes do not emerge voluntarily, in contrast to marine amphibious fishes such as mudskippers and rockskippers. Consequently, the duration of air exposure is very limited in intertidal fishes, and thus desiccation is an infrequent concern. Still, it has been established that numerous families of intertidal fishes, including sculpins, gobies, pricklebacks, gunnels, and sea chubs have the capacity for aerial gas exchange. In species for which data exist, anaerobic pathways are not recruited to supplement routine metabolism during brief bouts of emergence.

Water loss during emersion can be very fast among species of intertidal seaweeds, ranging from 10% to over 90% loss in water content after only a few hours under moderately desiccating conditions. Tolerance to

water loss is key to survival of seaweeds in the intertidal zone, rather than the possession of efficient mechanisms for the prevention of water loss. Species with high rates of water loss also exhibit large inhibitions of photosynthesis and dark (mitochondrial) respiration. The degree of water loss is a better predictor of metabolic depression than is the height in the intertidal zone at which various species reside. Similarly, within a given species (as for selected brown algae), vertical distribution in the intertidal zone is not tightly correlated with photosynthetic rates during air exposure. Desiccation as a result of emersion has also been shown to retard embryonic development in at least one intertidal alga.

Very high in the intertidal zone (supralittoral fringe), one can find blackish lichens and cyanobacteria that are quite tolerant to the dry environmental conditions that exist, where seawater spray can often be the only mechanism for hydration. Some cyanobacteria are embedded in a gelatinous mass that helps retard water loss and can produce a stress protein that is similar to plant dehydrins (see the next section). Other cyanobacteria experience massive water loss and tolerate such air-dried states for prolonged periods. The severe desiccation observed for lichens over extended periods (days, weeks) is an example of anhydrobiosis. Lichens can survive multiple cycles of anhydrobiosis. Other anhydrobiotic organisms, as well as species experiencing milder forms of water stress, are afforded protection by small organic solutes and protective proteins found intracellularly.

ORGANIC OSMOLYTES AND PROTECTIVE MACROMOLECULES DURING DESICCATION

When cellular water is lost as a result of desiccation or some other form of water stress (freezing, fluctuating salinity), intracellular osmolytes become concentrated. The beneficial effect of intracellular osmolytes is to retard water loss from the cell. While some contribution to the intracellular osmotic pressure of cells comes from inorganic ions, a substantial fraction of the osmotically active solutes are organic osmolytes. The term “compatible solute” was originally coined in 1972 by A. D. Brown and colleagues and applied to small carbohydrates termed polyols (i.e., polyhydric alcohols). When accumulated to high levels, these compounds do not disturb macromolecular structure and function, yet they serve to provide osmotic balance with the external environment. In addition to organic osmolytes that can be considered nonperturbing or compatible, other organic osmolytes can actually stabilize macromolecules during water stress. Protective effects of osmolytes have been documented for proteins

and for phospholipids that form membrane bilayers. An evaluation of the dominant types of organic osmolyte systems and their evolution later suggested that a common feature of many osmolytes was that they exerted influences on macromolecules by acting on the solvent properties of water. It is appropriate to note that in the case of anhydrobiosis, disaccharides such as trehalose are technically not serving the role of an osmolyte, because virtually all cellular water is lost in this state. However, trehalose is considered an osmolyte for other nonanhydrobiotic species. Table 1 lists some representative organic osmolytes that are found in various groups of organisms inhabiting the intertidal zone. The presence of organic osmolytes across these diverse organisms reflects the common threat of water stress that is faced.

TABLE 1
Representative Organic Osmolytes Accumulated in Various Groups of Intertidal Organisms.

Group of Organisms	Class of Osmolytes	Osmolytes
	Polyhydric alcohols	
Cyanobacteria		Glucosylglycerol
Lichens		Mannosidomannitol
Multicellular brown algae		Mannitol
	Amino acids and derivatives	
Invertebrates (all phyla)		Various amino acids*
Mollusca, Cnidaria		Octopine
	Methylamines	
Invertebrates (multiple phyla)		Glycine betaine, proline betaine, trimethylamine oxide

*Amino acids commonly accumulated as osmolytes: alanine, β -alanine, serine, glycine, taurine, proline.

Late embryogenesis abundant (LEA) proteins were first identified in land plants, and their expression is associated with desiccation tolerance in seeds and anhydrobiotic plants. One family of proteins within this group is dehydrins, which are thought to confer dehydration tolerance in plants. The precise mechanism by which dehydrins act is unclear. As discussed in the preceding section, brown algae display remarkable tolerances to water loss. Some of these algae constitutively express proteins that are related to dehydrins, which can be specific for certain embryonic stages and certain species. It has not been demonstrated whether these dehydrin-like proteins protect the algae against intertidal desiccation during emergence. Upon osmotic challenge, dehydrin-like proteins are also inducible in certain cyanobacteria represented in the intertidal zone.

APPLICATION OF FINDINGS TO CELL STABILIZATION

The comparative physiology of animals inhabiting the intertidal zone and other environments in which water stress is frequent has led to the appreciation of mechanisms, such as those summarized in the preceding section, that can limit damage incurred during desiccation. One approach that appears to improve survivorship during desiccation stress is the accumulation of low-molecular-weight carbohydrates. Trehalose is a prime example of a sugar utilized by a broad range of species that naturally cope with periods of severe water loss. The lessons learned from organisms that are naturally desiccation-tolerant are being applied to cell stabilization problems in the biomedical field. The ultimate goal is to prepare desiccated human cells for storage at ambient temperature. Trehalose has been shown to be effective in improving desiccation tolerance for a variety of macromolecular assemblies and cells including liposomes, enzymes, retroviruses, platelets, fibroblasts, hematopoietic stem cells, and macrophages (Fig. 3). For biostabilization of cells, trehalose has its greatest impact when present on both surfaces of the membrane. To succeed in preparing dried cells that retain high viability upon rehydration would offer tremendous economic and practical advantages over traditional cryopreservation protocols.

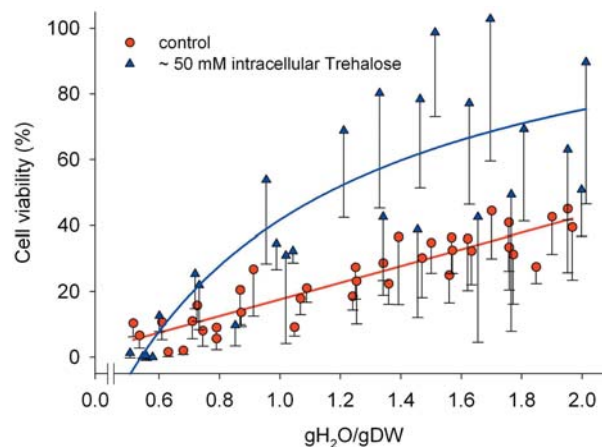


FIGURE 3 Naturally occurring osmolytes promote cell stabilization during drying. Loading an organic osmolyte such as the disaccharide trehalose into mammalian cells increases survival post-drying. Mouse macrophage cells were air dried to a range of final water contents in an intracellular-like medium containing 250 mM trehalose. Upon rehydration, cells that were permeabilized in order to load approximately 50 mM intracellular trehalose showed higher survival at all water contents, compared to control cells that were not loaded with trehalose. Values are means \pm SD ($n = 10$). Modified from Elliot *et al.* (2006).

SEE ALSO THE FOLLOWING ARTICLES

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DIFFUSION

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Diffusion is the name given to the process in which cumulative random motions result in the net movement of material. Diffusion theory has been used to describe the movement of a variety of properties, including heat through walls, molecules through membranes, eddies throughout the ocean, and bacteria swimming around a leaky cell.

MOLECULAR DIFFUSION

Life is full of random motions that combine to form predictable patterns, particularly when large numbers of objects are involved. One of the best known patterns is molecular diffusion, the net movement of molecules in air or water as a result of random thermal motions. The biological importance of molecular diffusion to marine systems results from the need of aquatic organisms to gather those molecules needed for life from solution and to be rid of those that are toxic. Because water currents are damped down to nothing next to a surface, the last

micrometer that must be traversed to get to the surface, at least, is controlled by the random process of molecular diffusion.

Diffusion Coefficient

Molecular diffusion results from thermal vibrations pushing at random on molecules, propelling them along, and opposing retardation provided by viscosity, slowing them down (Fig. 1). The cumulative effect of these movement spurts is to move a molecule along a random walk. With all molecules subjected to their own random walks, there is a net movement of molecules called diffusion. The rate of diffusion is described using a diffusion coefficient D with units of length²/time: cm² s⁻¹ or m² s⁻¹. Typical values are those for O₂ molecules in water: 1.0×10^{-5} cm² s⁻¹ at 0°C increasing to 2.7×10^{-5} cm² s⁻¹ at 30°C. In air, values are about ten-thousandfold larger: 0.18 cm² s⁻¹ at 0°C and increasing only slightly to 0.21 cm² s⁻¹ at 30°C. Much of the difference in the temperature effect between the atmosphere and water results from how temperature changes the viscosities of water and air.

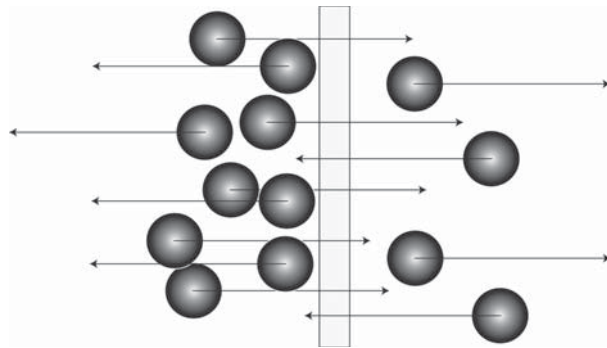


FIGURE 1 If there are more molecules on one side of a surface, then there will be an average movement of molecules to the region of lower concentration even though each molecule has an equal chance of moving toward or away from the surface. In this case, there is a net movement of two molecules to the right.

Dimensional Analysis

A rough idea of the distances and times involved in a diffusive process can be gained by using the dimensions of the process as a guide. In a given time t , there is only one way to calculate the distance L that diffusion will spread a substance without invoking other processes described by their own dimensional constants: $L \sim \sqrt{Dt}$. The square root implies that diffusion is very rapid for small distances and slow for larger ones: in 1 s, a patch of O₂ molecules in water will spread 30 μm, 300 μm = 0.3 mm in 100s, 0.9 cm