Ecosystems & Ecophysiology – Lecture 16

Osmoregulation

Objectives

1. Understand the terms molarity, molality and osmolarity, and the characteristics of some electrolytic and non-electrolytic solutes.

2. Know that most marine invertebrates are isosmotic and osmoconformers, but ionic regulators.

3. Describe the mechanisms of osmoregulation in elasmobranchs, marine teleosts, and air-breathing marine vertebrates.

4. Understand the adaptations of halophytes to high salinity.

5. Describe osmoregulation of teleosts and invertebrates in freshwater.

Osmoregulation

■ The other major physical variable in aquatic systems is salinity, the level of dissolved solids – solutes. Organisms are also aqueous solutions, enclosed in more or less permeable membranes

Water tends to move across membranes towards more concentrated solution, by osmosis. Each solute also tends to diffuse down its concentration gradient

For optimum function organisms need to control both their water content (and so volume), and concentration & composition of solutes: osmoregulation

Concentration usually expressed in terms of mass in ecology, sw = 35 g l^{-1}. But solutes have physiological effects related to number of particles, not mass. So usually expressed in terms of moles in physiology

A molar solution has 1 mole solute l^{-1} of the final solution, = 1 M. Rather large unit for physiology, usually use mM

A molal solution has 1 mole solute kg water $^{-1}$, = 1 m. Only 1% different from molarity for sw. Thermodynamically more correct but harder to measure

Osmotic pressure (OP) determines water movement across membranes. For non-electrolytes = sum of the molarity (or molality) of the different solutes. Described as osmolarity (Osmoles l^{-1} or mOsm l^{-1}) or osmolality (kg^{-1})

Electrolytes – expect that NaCl would have twice the osmotic effect (e.g. FP depression) of glucose, as twice as many particles. Roughly the case – slide from Lecture 12

But not exactly, effect is lower by the osmotic coefficient ϕ = 0.91 for NaCl. As electrolytes have interactions between +ve and –ve ions & with H_2O

Varies among electrolytes & in mixtures, so osmolarity determined empirically (by FP) for each solution. Osmolarity of sw is about 1000 mOsm l^{-1}

Marine and estuarine invertebrates

■ Most marine invertebrates have body fluid osmotic pressure = sw, isosmotic with medium. Body fluid = extracellular (coelomic, haemolymph)

Marine bony fish (teleosts) have lower concentration than sw = hyposmotic. Freshwater fish have higher concentration than fw = hyperosmotic

If concentration of medium changes the OP of body fluids may change with it = osmoconformer. Or regulated at a constant level = osmoregulator. Graph is analogous to one for thermoregulation

■ Even if total concentration same as the medium, the ionic composition will differ – ionic regulation found to some extent in all organisms.

Ion concentrations in body fluids as % expected without regulation:

Ion	Echinoderm	*Aurelia*	*Mytilus*	*Carcinus*
Na^+	100	99	100	110
Mg^{2+}	98	97	97	34
Ca^{2+}	101	96	103	108
K^+	111	106	130	118
Cl^-	101	104	100	104
SO_4^{2-}	100	47	97	61

Echinoderms show no significant regulation of any ion in body fluids (but do show intracellular regulation)

Aurelia (jellyfish) regulates only SO_4^{2-}, for buoyancy – excludes heavy ion. *Mytilus* (mussel) has high K^+, *Carcinus* (crab) low Mg^{2+} and SO_4^{2-}

■ Most marine invertebrates can acclimate to brackish water (e.g. 80% sw), either as passive osmoconformers or active osmoregulators. Conformers become more dilute, but still show ionic regulation (*Mytilus*)

Regulators become hyperosmotic to the medium. Limits to tolerance, e.g. *Carcinus* can regulate down to 30% sw. *Eriocheir* (Chinese mitten crab) can extend into fw. See mechanisms of hyper-regulation in fw animals (end)

Hyporegulation (body fluids less concentrated than medium) rare in marine invertebrates, e.g. shrimp *Palaemonetes*, probably evolved from fw group. But common in marine teleosts (later)

■ Osmoconformers will show volume changes as water enters or leaves by osmosis, at least in the short term. In the long term volume may adjust towards original level as both ions & water move

E.g. sipunculid worm *Themiste* swells in 50% sw, shrinks in 140% sw. Partial regulation of volume after 100 h in 80% sw

■ Animals also need to regulate intracellular volume. Cells are isosmotic to body fluids, but different ion composition (higher K^+ & lower Na^+). If body fluid OP changes, this is matched by changes of organic molecules in cells

Concentration of free amino acids in cells increases during salinity stress. Amino acids regulated by changing level of protein degradation or synthesis

E.g. muscle cells of *Eriocheir* show transient shrinking when crab moved from fw→sw (% water decreases), but restored within 15 days as cell OP increases with amino acid accumulation

■ Organic molecules used inside cells as enzymes are sensitive to change in ions. K_m of crab (*Pachygrapsus*) enzyme strongly affected by Na^+ & K^+

Glycine has little effect – common at high concentration in cells of marine invertebrates. Barnacle muscle cells 70% of OP is from amino acids, > 50% from glycine

Note low effects of betaine & TMAO as well (later). Other organic molecules (lysine, arginine) have high effects, so only specific molecules used

Marine vertebrates

■ Body fluids of most vertebrates have less concentrated salts than sw. Osmotically 3 groups of marine vertebrates:

1. Isosmotic wth sw. Hagfish has high salt concentration, unique for vertebrates, ionic regulation only like marine invertebrates. Stenohaline

	Na^+ (mM)	Urea (mM)	mOsm l^{-1}	
Sea water	450	0	1000	
Hagfish	550	0	1150	sw
Dogfish	290	350	1000	sw
Coelacanth	200	350	950	sw
Crab-eating frog	250	350	830	brackish
Toadfish	160	0	390	sw
Goldfish	110	0	260	fw

Elasmobranchs (e.g. dogfish) have salts only 1/3 that of sw, but additional organic molecules (mostly urea) increase OP to be isosmotic with sw:

Blood urea 100 x concentration of mammals, toxic to other vertebrates at this level. High urea also in coelacanth (sw) & crab-eating frog *Rana cancrivora* (mangroves, brackish water)

■ These also have high levels of trimethylamine oxide, TMAO. Urea & TMAO have opposite effects on enzymes, e.g. data for bovine ribonuclease

Urea destabilises proteins, lowers the denaturation temperature. TMAO stabilises proteins, increases denaturation temperature. Urea & TMAO additive in combination, minimises effect on enzymes

Result is high OP for water balance, without damaging effects of high salt concentrations. Similar principle to intracellular amino acids in invertebrates

Elasmobranchs still need ionic regulation, salts enter down concentration gradient at gills, excreted in urine

■ 2. Hyposmotic – marine teleosts. Body fluids of teleosts only ¼ to 1/3 of sw concentration, in both sw species (toadfish) and fw species (goldfish)

■ Marine teleosts lose water by osmosis from gills, compensate by drinking sw. (Audi advert – should be marine species “drinking like fish”)

Need to eliminate the excess salts ingested. Fish kidney cannot form concentrated urine, but excretes divalent ions Mg^{2+} & SO_4^{2-}

Excess NaCl secreted at gills. Active transport of Cl^- by large chloride cells in gill epithelium, Na^+ follows passively

■ 3. Air-breathing marine vertebrates - mammals, birds & reptiles. Essentially still terrestrial for osmoregulation, isolated from sw as no gills

More problems than terrestrial as no fw to drink & high salts in food (algae & invertebrates isosmotic to sw)

Birds & reptiles have salt glands in the head, secrete hyperosmotic NaCl solution. Marine iguana has nasal salt gland, excretes salts from algae food

■ Marine mammals can form hyperosmotic urine, more concentrated than sw. Whale urine up to 820 mM Cl^- (sw = 530 mM)

So can eliminate excess salts from food or ingested sw. Compare whale & man drinking sw. Urine needed to eliminate the salt in 1000 ml sw:

	Volume (ml)	mM Cl^-	urine (ml)	mM Cl^-	Water (ml)
Human	1000	530	1350	400	–350
Whale	1000	530	650	820	+350

Whale has net gain of 1/3 litre water, human has net loss of 1/3 litre water. Human urine less concentrated than sw, so drinking it hastens death

Halophilic macrophytes

Halophytes similar situation to marine fish, tissue salt concentrations < sw. Must overcome problems of water & ions (& anoxia – Lecture 15)

1. Osmotic effects. Salinity reduces difference between soil water & root xylem, lowers the drive for water uptake. Need negative water potential to draw water into root from soil to supply transpiration

Halophytes have high internal solute concentration to maintain gradient of water potential. Saltmarsh perennial *Suaeda maritima* increases root tissue Na^+ from 14 to 270 mM as external Na^+ increases from 1 to 340 mM

But this has costs, need even more negative water potential to counteract gravity & draw water into leaves from roots. This limits the height of halophilic trees – mangroves maximum 10-15 m tall, any higher & leaves too stressed

2. Ionic effects – high ions can inhibit enzyme activity, as in animals. Plants can adapt to this, but only at the cost of limited growth rate

■ Saltmarsh *Juncus maritimus* has low growth across wide range of salinity, while *J. bufonius* from wet non-saline soils has high growth at low salinity, but falls to zero at higher salinity

Root water potential constant across salinity range in *J. maritimus*, high at low salinity, limits growth rate. Water potential varies substantially in *J. bufonius*, large rise at high salinity, no growth

Also volume regulation of cells by accumulation of organic solutes, & excretion of excess salts, as in animals

Halophytes accumulate Na^+ in vacuole by active transport, OP matched by organic solutes in cytoplasm. Many species use proline, forms 55% of amino acid pool, compared to 2-4% in other plants. Or betaine, similar to TMAO

■ Effects on enzyme activity of halophyte *Triglochin maritima*. High NaCl decreases activity, high proline has no effect

Excretion & abscission. Toxins accumulate around the plant if excreted by roots, so salts transported to shoots. Plants shed parts with high concentrations of toxins – older leaves have higher salt content

Also excretion by leaves. Salt excreting structures in leaf epidermis, salt washed off by rain & does not accumulate around plant

Halophyte *Halimione* has simple bladder hairs, bicellular trichomes. Distal cells with vacuole that accumulates salt, discharged when trichome brushed

Tamarix, mangroves & others have multicellular salt glands. Secretion has energy cost, seen as lower growth in concentrated salt solution

■ Organs are permanent, but secretion induced by high salt, flexible system to minimise cost of secretion, e.g. mangrove *Aegialitis*

Mangroves	Medium mM NaCl	Excretion nmol m^{-2} s^{-1}	Scale
Aegialitis	50	100	2% leaf content d^{-1}
Aegialitis	500	265	29% leaf content d^{-1}
Avicennia	field	120	
Grasses			
Diplachne	250	10	5 x uptake rate
Spartina alterniflora	690	41	

Avicennia leaves have salt glands on undersides, dense hair covering. Hair raises salt crystals from surface, prevents osmotic loss of water to crystals

■ Both adaptation to salinity and to anoxia have energy costs in mangroves, of salt secretion & aerencyma tissue respectively

Species cannot tolerate high levels of both stresses. Inundation (anoxia) toleration negatively correlated to salinity in Australian mangroves

Freshwater animals

■ Fresh water has 0.1-10 mOsm l^{-1}, << body fluids, animals hyperosmotic to the medium. Osmoregulation similar to good brackish water regulators

Body fluids regulated at different levels, but always hyperosmotic. *Anodonta* (fw mussel) 50 mOsm, *Eriocheir* 325 mOsm

■ Fw fish have similar body fluids to sw fish. Problem of osmotic water inflow at gills. Excess water excreted as dilute urine, up to 1/3 of body mass day^{-1}

Although dilute, urine is still a major loss of solutes, also lost by diffusion at gills. Need large intake of solutes, achieved by active transport inwards at gills

Fish mostly stenohaline, some species can migrate between fw & sw. These reverse the transport of ions at the gills, probably different populations of cells

■ Similar systems in invertebrates. Crayfish excretory system is antennary gland, secretes dilute urine. Primary (original) urine is isosmotic to body fluids

Then Na^+, K^+ & Cl^- withdrawn by active transport, in all parts including bladder, to give final dilute urine, equivalent to fish kidney

■ *Culex* mosquito larvae use anal papillae to take up ions from medium – hyporegulation in fw

Papillae larger in specimens acclimated to more dilute solutions. Equivalent to chloride cells in fish gills