Ecosystems & Ecophysiology – Lecture 17

Adaptation to extreme aquatic environments

Objectives

1. Describe osmoregulation by animals in the hypersaline environments of salt and soda lakes.

2. Understand how extreme halophytic archaea can tolerate hypersaline conditions.

3. Understand adaptations of respiratory pigments and metabolic pathways of aquatic animals to hypoxia and anoxia.

4. Describe metabolic pathways of bacteria for anaerobic respiration and for chemoautotrophy.

5. Know how animals exploit chemoautotrophic bacteria at hydrothermal vents and cold seeps.

Adaptation to extreme aquatic environments

■ Seen the major physical variables, finish with adaptations to extreme environments – hypersalinity & low oxygen

Hypersalinity
Environments more saline than sw, found in many saline & soda lakes, up to saturated NaCl (Great Salt Lake) or $MgCl_2$ (Dead Sea)

Tolerance limits of animals (osmolarity, sw = 1):

Cricoptopus	chironomid larva	1.5	
Coelops	fly larva	3	
Aedes	mosquito larva	4	
Artemia	crustacean	8.5	Great Salt Lake
Ephydra	brine fly larva	8.5	Great Salt Lake

■ Osmoregulation by animals similar to marine teleosts in sw. Body fluids hyposmotic to the medium, so tend to lose water. Replaced by drinking the medium & excess salts excreted

Artemia exceedingly euryhaline, NaCl from about 3 g l^{-1} (≈10% sw) to 300 g l^{-1} (8.5 x sw). Cannot tolerate fw

■ When in concentrated medium it drinks to replace water lost across cuticle, up to 7% body mass day^{-1}

Ions taken up across the gut, water follows by osmosis. Then salt secreted by salt glands on first 10 pairs of phyllopodia (seen beating in Practical 4)

■ Osmotic pressure of body fluids equivalent to 1-3% NaCl, even in medium of 30% NaCl (salinity of 300)

Gut fluid more concentrated, so water tends to be lost to it, as to the external medium. So active salt uptake, followed by water

■ Some mosquito larvae also in hypersaline water. *Aedes campestris* in alkaline soda lakes with high $NaHCO_3$ & pH > 10

Regulates over a range of OP of 500 x with only 2 x change in body fluid OP. Drinks in hypersaline conditions to replace water lost through cuticle

Excess salts excreted by Malpighian tubules & rectal glands. Balance: 8 mg larva drinks 2.4 μl water day^{-1} (37% of total body water)

Ingests 1.2 μmol Na^+ with water (125% of total body Na^+), + HCO_3^- & Cl^-. These salts excreted in 1.8 μl water day^{-1}. Excess 0.6 μl water day^{-1} covers that lost through cuticle

Anal papillae used for active uptake of ions in low salinity environments, as in *Culex* larvae. Disadvantage in hypersaline environment, 2/3 of total water loss is from them, as large surface area & thin cuticle

■ Most tolerant organisms to hypersalinity are the extreme halophytic bacteria & archaea, e.g *Halobacterium halobium* (Dead Sea). These are isosmotic to the medium, even at high osmotic pressure

Unlike multicellular organisms that accumulate organic solutes for osmoregulation, the microorganisms accumulate ions, especially K^+

Archaea have K^+ concentration 20-50 x that of normal cells, up to 5 M, varies to balance OP of medium. In fact 5 M KCl is insoluble & would precipitate out, needs anions other than Cl^- to balance charge

The anions are organic molecules similar to those used for osmotic effects (F.P. depression) in other organisms, but with sulphate, phosphate or other groups to give negative charge:

Glycerol → diglycerol phosphate (charge –1)
Trehalose → sulphotrehalose (charge –3)

■ Enzymes of extreme halophytes adapted to the high K^+, which would make those of other organisms rigid & inactive. Need semistable state to be functional. Plot of K_m for enzyme on salt concentration

Approximate position of line for *Pachygrapsus* crab enzyme (Lecture 16) shown in red, much more sensitive to high inorganic ions (Na^+ & K^+)

Proteins of extreme halophytes fold properly only with high K^+ present. Unique amino acid composition, high levels of acidic amino acids

Glutamate & aspartate make up 54% of amino acids, compared to 7% in *E. coli*. Acid –COOH side chains ionised to $–COO^-$ in cell. These would normally repel each other & make the protein too unstable

In cells of extreme halophytes the high K^+ neutralises the negative charges, allows enzymes to fold properly & have optimum stability

Low oxygen

■ Low O_2 saturation in water (hypoxia), obvious adaptation is to breathe at surface (Lecture 15). Alternative is to increase capacity for O_2 uptake

Respiratory pigments such as haemoglobin take up O_2 from water & deliver it to tissues (Life Processes Lecture 24). Usually thought of in the vertebrates, but also common in invertebrates from hypoxic environments

Oligochaete *Tubifex* & chironomid midge larvae have haemoglobin, tolerant of hypoxia & organic matter pollution, so low scores on water quality index

■ Animals can acclimate or adapt to hypoxia by changing amount or properties of the pigment. Compare polychaetes *Arenicola* from burrows in mud (Lecture 3), with predatory *Nephtys* on surface

■ Oxygen dissociation curves show the % saturation of haemoglobin with O_2 at different partial pressures of O_2. P_{50} is the pressure for 50% saturation

Arenicola is to the left, so it takes up O_2 at very low tensions from the water, transports to the tissues. P_{50} = 2 mmHg compared to 5 mmHg in *Nephtys*

Other animals that experience hypoxia have even lower P_{50}, 0.2 mmHg in the bivalve *Phacoides* & 0.1 mmHg in *Chironomus* larvae. In contrast mammals & birds have P_{50} of 30-50 mmHg

This is qualitative response in evolutionary adaptation, as with temperature adaptation of enzymes – a different molecule produced

■ Alternative is quanititative change, increase the amount of the same molecule. As with enzymes in thermal acclimation, this is usually associated with phenotypic change in individuals

Daphnia (planktonic crustacean) concentration of haemoglobin in blood increases in individuals kept at low O_2 tension. Proved that individuals with high haemoglobin concentration survive better than others in hypoxic water

Anoxia
Absence of oxygen. Organisms must use anaerobic metabolic pathways. Seen one example of these in medium-term exercise, formation of lactate to restore NAD^+ & allow glycolysis to continue

Also used in anoxia, for up to 40 hours by mangrove crabs *Uca.* Live in burrows when under water, not ventilated, survive by lactate production

Anaerobic metabolic pathways actually rather common, the first to evolve, Earth initially had an anoxic reducing atmosphere

These pathways all involve oxidation-reduction reactions, where reduced food molecules are oxidised, electrons passing to an electron acceptor

In aerobic respiration the final electron acceptor is O_2, which is reduced to H_2O. By electron transport chain in the mitochondria (Life Processes Lectures 19-21). Two alternatives to this:

■ **1. Fermentation**, where the final electron acceptor is an organic molecule, e.g. formation of lactate. The other familiar fermentation reaction produces ethanol, e.g. in yeast

Fermentation to ethanol also found in carp & goldfish, uniquely among vertebrates. Can survive periods in hypoxic water, ethanol excreted at gills

Characteristic of fermentation is low energy yield (of ATP), 2 ATP per glucose molecule from both lactate & ethanol pathways

ATP comes from glycolysis, formation of lactate or ethanol does not harvest more energy, just restores NAD^+ so glycolysis can continue

Contrast to aerobic respiration which produces 30 ATP per glucose molecule. Fermentation is inefficient as most of the energy of the glucose remains in the reduced organic molecule, lactate or ethanol – only partial oxidation

■ Animals enduring anoxia for long periods may have slightly more efficient fermentation pathways than to lactate or ethanol, e.g. in bivalve molluscs:

Glucose $\rightarrow$ octopine + 2 ATP
Glucose $\rightarrow$ succinate + 4 ATP
Glucose $\rightarrow$ propionate + 6 ATP
Aspartate $\rightarrow$ succinate + 1 ATP

Mussel *Mytilus* tolerates anoxia when valves closed at low tide. Has most efficient combination of fermentation of aspartate & glucose $\rightarrow$ propionate, gives 8 ATP per glucose + aspartate

■ **2. Anaerobic respiration**, where the final electron acceptor is an inorganic molecule other than O_2

Two levels of anaerobic respiration. Some pathways have similar yield of ATP to fermentation, e.g. oxidation using SO_4^{2-} by sulphur bacteria in mud:

$CH_2O + SO_4^{2-} + H^+ \rightarrow H_2S + HS^- + H_2O + CO_2$ Yield 6 ATP glucose^{-1}

Similar low yield from oxidation of organic molecules using CO_2 to methane (CH_4), by methanogenic microorganisms. Only partial oxidations, end products H_2S and CH_4 still have high free energy

One pathway of anaerobic respiration is much more efficient – denitrification. Uses an electron transport chain (with different cytochromes) & NO_3^- as the final electron acceptor, equivalent to aerobic respiration

High efficiency as final products (H_2O, CO_2 & N_2) have low free energy, so large change in free energy used to produce many ATP:

Aerobic respiration

Glucose + $O_2 \rightarrow CO_2 + H_2O$ $\Delta G = -2870$ kJ

Denitrification

Glucose + $NO_3^- + H^+ \rightarrow CO_2 + H_2O + N_2$ $\Delta G = -2670$ kJ

Chemoautotrophy

■ Related subject to anoxia is production of energy from reduced inorganic molecules by bacteria, e.g. at hydrothermal vents – chemoautotrophy

Photosynthetic autotrophy is use of light energy to fix CO_2 to organic molecules. Chemoautotrophy uses chemical energy (e.g. from H_2S) to fix CO_2

These pathways need O_2, occur at the interface between reducing conditions (vent water or deep mud) & oxidising conditions (ocean water or mud surface)

■ General pathway is reduced compound (H_2S, CH_4) + $O_2 \rightarrow$ oxidised compound (SO_4^{2-}, CO_2) + ATP. The ATP is then used to synthesise organic molecules using CO_2 as carbon source (i.e. fix CO_2)

Vent water has high H_2S, because SO_4^{2-} in sw has been reduced by geothermal processes as it percolates through rock above magma chamber

■ Chemoautotrophy at first looks like the reverse of anaerobic respiration, apparently magical as ATP produced in both directions. E.g. for sulphate/ sulphide reactions:

Anaerobic sulphate respiration

$$\text{Organic molecule} + SO_4^{2-} \rightarrow H_2S + CO_2 + \text{ATP}$$

Sulphide chemoautotrophy

$$\text{Organic molecule} \leftarrow \text{ATP} + SO_4^{2-} \leftarrow H_2S + O_2$$
$$+ CO_2$$

But not really. Key is that this anaerobic respiration (using SO_4^{2-}) of organic molecule is only partial oxidation, the product (H_2S) still has high energy

Chemoautotrophy fully oxidises the H_2S using O_2 to extract the remaining energy, & produce more ATP

■ Better think of as a sulphur cycle equivalent to Krebs cycle. Complete oxidation of organic molecule to CO_2 & H_2O, using O_2, restores SO_4^{2-} & produces ATP at two stages

■ Huge bacterial productivity where vent water with H_2S mixes with ocean water with O_2. Bacteria then consumed by animals (filter feeders or grazers of mats) or provide food as symbionts (in 10 animal phyla)

Animals orient themselves across the interface between anoxic & oxygenated water, or pump water across, or actively move between the two environments

Fast growth of vent animals based on this productivity, clams with symbionts grow 4 cm yr^{-1}, 22 cm after 6.5 years. Compared to typical deep sea clam takes 100 years to reach maturity at 0.8 cm

Tubeworm (Vestimentiferan) *Riftia* has highest linear growth of any marine invertebrate, grows to 2 m long & 2-3 cm diameter. Coelom contains large trophosome organs, with solid masses of symbiotic bacteria

Has haemoglobin in blood, transports both O_2 and H_2S to trophosome. These bind at two different sites – in other animals H_2S prevents the reversible binding of haemoglobin with O_2, so is a poison

■ Cold seep system similar, but uses CH_4 (of geological origin) instead of H_2S. Methanotrophic bacteria ("reverse" of methanogenic) use O_2 to oxidise CH_4 to CO_2 and produce ATP

Mussels *Bathymodiolus* have symbiotic methanotrophs in gills, can grow with CH_4 as the only energy & carbon source. Also symbionts in sponges, abundant at cold seeps

■ Chemoautotrophy also at whale carcases. Decaying oily tissues form reducing, sulphur-rich environment, persists for months or years

Anaerobic bacteria inside the bones decompose the high lipid content. Forms CH_4 & H_2S which diffuse out & are used by chemoautotrophic bacteria at interface with oxygenated water

■ Finally, return to anoxic mud (Lecture 3, slide 11). Redox potential discontinuity layer (RDL) is also an interface between anoxic & oxygenated environments. Redox potential changes from oxidising (+) to reducing (–)

Anaerobic decomposition below the RDL forms CH_4 and H_2S, which diffuse upwards. Chemoautotrophic bacteria in the RDL oxidise these & fix CO_2