Ecosystems & Ecophysiology – Lecture 12

Thermal tolerance

Objectives

1. Know the lethal temperature as that at which 50% of a sample of organisms survive (T_{L50}).

2. Describe how the lethal temperature changes with acclimation temperature, to give a measure of the thermal niche of an organism.

3. Describe the three mechanisms of freeze resistance: by osmotic lowering of the freezing point; supercooling; and antifreeze molecules.

4. Understand freeze tolerance as an alternative to freeze resistance, and the limitation to extracellular fluids.

5. Describe the possible and probable mechanisms of heat death in organisms, and the protective action of heat shock proteins (Hsp).

Thermal tolerance

■ If an organism cannot regulate its T_b, then it has two problems:
1. Lethal extreme T_bs
2. Disturbance caused by T_b changes between the extremes (Lecture 13)

Lethal temperature is defined as that at which 50% of organisms die: T_{L50}, similar to LD_{50} tests on drugs. Found from plot of survival on temperature

T_{L50} depends on exposure time, organisms may be killed by a long exposure to a temperature that they would survive for a short period, due to cumulative damage e.g. to enzymes

■ Upper T_{L50} of 4 species of barnacle decrease with longer exposure time, e.g. 35°C for 1 h but only 30°C for 6 h for *Balanus crenatus*

Use biologically relevant time – barnacles exposed at low tide for periods of several hours, when they are in danger of overheating

■ Prolonged exposure to less extreme temperatures usually increases tolerance, as organisms adjust to the new temperature. Acclimate = to experimental changes, acclimatise = to natural seasonal changes

High temperature survival curves for polychaete *Clymenella* acclimated to 5 or 15°C. Upper T_{L50} has increased by about 3°C in those acclimated to 15°C

■ Complete tolerance range – upper & lower T_{L50} for each acclimation temperature. Fundamental (rather than realised) thermal niche, breadth can be expressed as the area of each polygon ($^oC^2$)

■ Bullhead (*Ictalurus* catfish) has much broader thermal niche than salmon. Bullhead is eurythermal, salmon is stenothermal

Low temperatures

Many ectotherms adapted to survive & even function normally at low T_b. Some tropical species less tolerant of low T_b, especially aquatic species

Guppy *Lebistes* acclimated to 23°C has a lower T_{L50} of only 10°C. Similar in tropical cichlids including *Oreochromis niloticus* & *O. alcalicus* (also10°C)

Mechanisms of cold death at such moderate temperatures are poorly understood. Main danger from low temperature is freezing

Aquatic ectotherms experience only moderate low T_a as water freezes between 0 and –2°C (depending on salinity). High latent heat of fusion resists further temperature change

Terrestrial (& intertidal) ectotherms more susceptible to freezing as T_a can fall well below 0. Two main adaptations, freeze resistance & freeze tolerance

Freeze resistance

■ Organism does not freeze, and may be active at low temperature. Usually aquatic. 3 mechanisms:

1. Osmotic lowering of freezing point. Pure water freezes at 0°C. Freezing point (FP) is lowered by solutes, according to the equation **Δ FP = –1.86 O**. O is the osmolal concentration; sw has O = 1 so freezes at –1.86°C

FP depression is thus directly proportional to solute concentration. First mechanism of freeze resistance is just to increase body fluid concentration

Normal body fluid concentration of organisms gives protection against freezing in fw. Their FP is –0.6 to –0.7°C so they will freeze after all the surrounding water, & are protected by its latent heat of fusion

However, body fluids of marine organisms are typically less concentrated than sw, so they are in danger of freezing before the surrounding water

Some ectotherms accumulate high concentrations of specific solutes to lower their FP, typically:

1. Sugars – glucose, fructose, trehalose
2. Sugar alcohols – glycerol, sorbitol

These have low molecular weights so maximum osmotic effect & FP depression g^{-1} solute (depends on number of molecules, not mass)

■ Increased concentration of solutes in *Eurosta* fly larva at lower T_a. Glycogen is split to glycerol (first) then sorbitol, to lower the FP. Note glycogen shown as glucose units – concentration much lower as polymer

■ **2. Supercooling**. Pure water & solutions do not necessarily freeze at the FP calculated from their osmotic concentration. May remain liquid when cooled below the FP – the supercooled state, down to supercooling point SP

For this reason FP (liquid→solid) in practice is measured as the melting point MP (solid→liquid), as MP always corresponds to the osmotic concentration

Supercooling is general property of solutions & tissues. Solutes lower FP & also SP, but unclear whether solutes are actually involved in supercooling

Depends on the absence of ice crystals, which seed further ice growth. A supercooled liquid (or organism) will freeze if it comes into contact with ice

Examples of supercooling points (°C) & solute concentrations:

	SP	Solute	
Rana (frog)	–3.0	Glucose	0.41 M
Chrysemys (turtle)	–3.3	Amino acids	0.05 M
Trichiocampus (sawfly)	–8.6	Trehalose	9.0%
Rhabdophaga (midge)	–49.1	Glycerol	32.4%
Pytho (beetle)	–54.0	Glycerol, sugars	13.2%, 5.5%

Only moderate supercooling in the vertebrates, more in invertebrates.
No advantage in fw as body fluid FP is below that of water anyway

■ But useful for marine organisms, which would normally freeze at above –1.86°C. If SP is below –1.86°C, marine organisms will not freeze as along as they do not contact ice

Benthic fish in arctic fjords have FP of –0.7°C but live in permanently supercooled condition. No bottom ice so safe, with behaviour to avoid the surface. If tissues contact ice crystals they freeze immediately (& fatally)

There is some bottom ice in Antarctic. Anchor ice, small plates form on bottom down to 30 m, lift off when low density overcomes adhesion. Antarctic benthic fish therefore use a different strategy:

■ **3. Antifreeze molecules**. Antarctic fish show a difference between freezing & melting points. FP = –2.7°C, MP = –0.9°C. This FP low enough to give complete protection against freezing in sw

Similar difference between FP & MP in supercooling, but not supercooling as they do not freeze when contact ice. Have antifreeze molecules

These are non-colligative solutes, i.e. their action is not proportional to concentration, & much greater than other solutes (e.g. NaCl, glucose)

a) ■ Glycoproteins. Polymers of up to 50 tripeptide groups Alanine-Alanine-Threonine linked to carbohydrate, MW 2,600 to 33,000

b) Proteins. More variable structure, 3 general types. Sculpin fish *Myxocephalus* has α-helix with many alanine, MW 3,300-4,000

■ Both types work by having a high concentration of polar groups, strongly interact with ice crystals. Bind to ice nuclei & prevent further water molecules being added, stopping ice growth

Freeze tolerance
The other main strategy, organisms can freeze but survive. Must be inactive, typically terrestrial invertebrates dormant in winter

■ Tolerate freezing of part of the body water without tissue damage:

Frogs & turtles survive	35-50% body water frozen
Intertidal molluscs	54-76%
Insects	>90%

Survivable freezing is limited to the extracellular fluid. Cells become shrunk & distorted, but do not contain ice crystals. Animal dies if ice forms intracellularly as this disrupts membranes & organelles

■ As temperature drops & extracellular fluid freezes, the solutes in it are frozen out of the ice, concentrated in the unfrozen liquid

Lowers the FP of the remaining extracellular fluid, harder to freeze. Gives curve of increasing % of body water frozen with decreasing temperature

Increasing concentration of extracellular fluid also draws water from the cells by osmosis. So freeze-tolerant animals must also be very tolerant of dehydration – more than desert animals

■ Seasonal change of freeze-tolerance in molluscs is due to tolerance of tissue dehydration. 0°C acclimated mussels *Modiolus* tolerate an extra 6% of body water being frozen, giving another 2°C of freeze tolerance:

Acclimation temperature	Water frozen	Lethal temperature
23°C	35%	–7°C
0	41	–9

Can test this by acclimating the mussels to water of different salinity. Mussels from hypersaline water show greater freeze tolerance, as predicted:

Acclimation salinity	Lethal temperature
12	–5°C
34	–10
46	–12

High temperatures

■ Larvae of the chironomid *Polypedilum* live in shallow exposed pools on rocks in Uganda. Survive total dehydration, & heating to 102°C in this state

Fw crustacean *Triops* from Sudan aestivates in dry mud which can reach 80°C in the sun. In the lab they can survive heating to 99°C

Upper lethal temperatures of fish range from 43°C for the desert pupfish *Cypriodon*, in warm desert pools in the USA, down to only 6°C for the Antarctic fish *Trematomus*, lives at –1.86°C

■ In general the upper lethal temperature of a species is related to the maximum temperatures in the environment. Correlation for 19 spp of porcelain crabs *Petrolisthes*

This is an example of evolutionary (genetic) adaptation, not phenotypic change as in acclimation or acclimatisation

■ Also shown by position on shore in intertidal gastropods. Upper T_{L50} (°C):

Spray zone & upper intertidal	47-48.5
Middle intertidal	44-46
Lower intertidal	42-43
Infralittoral fringe	39

Aquatic organisms are often very stenothermal as they never experience high temperatures, so no selection pressure for resistance. Heat death from El Nino events in corals & kelp

Mechanisms of heat death

■ Less known about high temperature mortality than cold & freezing. Major cause in terrestrial animals is dehydration, especially where evaporative cooling used. Apart from water loss there are 5 suggested mechanisms:

1. Protein denaturation – permanent loss of function of enzymes. Possible where T_{L50} is 45-50^{o}C, but hardly for *Trematomus* at 6^{o}C

2. Thermal inactivation – reversible loss of enzyme activity faster than synthesis. May occur in some cases, but again not a general mechanism. Isolated enzymes of *Trematomus* increase activity to 30^{o}C

3. Failure of oxygen supply. Can be disproved by increasing the partial pressure of oxygen, has no effect on lethal temperatures

4. Failure of metabolic regulation. Consider the reaction pathways in the diagram. If C→D increases faster than B→C at higher temperature, then C will be depleted & C→E may not occur at all

Several hundred enzymes involved in metabolism, not all with the same Q_{10}, so a probable mechanism

5. Loss of membrane function. Cell membranes are lipid bilayer with attached proteins, held together by weak interactions of several types, all changed by temperature. Also a probable mechanism

Tolerance of high temperature

■ All organisms have molecular chaperones, proteins that allow other proteins to fold correctly in the cell, & restore shape when denatured

Some of these are heavily synthesised after heat stress, known as heat shock proteins (Hsp). Named by molecular weight, Hsp70 is 70,000 da. Look at effects on a denatured (unfolded) protein in vitro:

1. If Hsp70 + co-chaperone + ATP present, protein refolds to original state

2. If only Hsp70 or Hsp90, forms complex that prevents further unfolding, maintenance state, can re-fold later

3. If neither present, denatured protein can aggregate with other proteins, e.g. bovine serum albumin BSA in the lab. Refractory to re-folding, even when all other factors present

Hsp70 binds at hydrophobic sites of unfolded protein, shields them from interacting with other unfolded proteins. Releases protein when ATP split, allows it to re-fold

■ Heat shock response is increased synthesis of Hsp. Does not occur in Antarctic fish e.g. *Trematomus*, lost in 14 m years constant conditions

Bacteria synthesise Hsp within minutes of heat stress, multicellular organisms slower. Production of Hsp in gastropods, shocked by 2.5 h at 30^{o}C:

Tegula brunnea, sublittoral, usually at 10-18^{o}C, rarely > 25^{o}C
T. funebralis intertidal, often up to 32^{o}C

T. funebralis initiated production more quickly (significant increase *), maximum rate of synthesis in 1 h, while *T. brunnea* took > 10 h

Heat shock response of *T. funebralis* completed within a few h (not significantly different from initial **), while *T. brunnea* unable to complete response within one tidal cycle (took 30-50 h)