Ecosystems & Ecophysiology – Lecture 13

Thermal acclimation and adaptation

Objectives

1. Understand the compensation of R-T curves to temperature change, both phenotypic (acclimation) and evolutionary (adaptation).

2. Know that acclimatory changes in proteins are usually quantitative, apart from expression of isozyme variants in polyploid organisms.

3. Know that evolutionary changes in proteins are usually qualitative, of different allozymes.

4. Understand that enzymes have an optimum level of stability, and cannot function efficiently across a wide range of temperature.

5. Describe homeoviscosity of cell membranes by variation of the length and saturation of fatty acids in phospholipids.

Thermal acclimation and adaptation

■ If an organism cannot regulate its T_b, then it has two problems:
1. Lethal extreme T_bs (Lecture 12)
2. Changing physiological performance caused by T_b changes between the extremes. Now look at compensation for changes of T_b

Acclimation (experimental) & acclimatisation (natural) are phenotypic changes during the life of an individual. Physiological mechanisms similar to genetic adaptation of populations & species, over evolutionary time – include both

Consider a fish adapted to 5°C with an R-T curve along the solid blue line between the tolerance limits of 0-30°C, & a Q_{10} of 2

If the fish is moved to 25°C its upper T_{L50} might extend to 40°C. R-T curve would extend along the dashed line if no compensation of metabolism

Fish now has a much higher metabolic rate at the new T_a – position (2) at 25°C compared to position (1) at 5°C, 4 x higher for a Q_{10} of 2

If its energy intake does not increase by the same amount, there will be less energy available for growth or reproduction

If the fish acclimated perfectly to 25°C it would have the same rate at 25°C as it previously had at 5°C (position 3), with an R-T curve along the red line

The figure shows two possibilities – no compensation, or perfect compensation. Often partial compensation, R-T curve is between these

Can regard compensation as either horizontal or vertical shift of the R-T curve. Warm-adapted has R-T curve moved right or lowered, cold-adapted has R-T curve moved left or elevated

■ Compare R-T curves of fish from different areas. All have similar level of metabolic rate in nature, but at widely different T_b

Antarctic fish & tropical fish may be equally active, but at T_bs 25°C apart. This is evolutionary adaptation, involving genetic changes among many species

Same pattern for acclimatisation in temperate fish. Summer R-T curve shifted to right compared to winter. (The others are in non-seasonal environments)

Mechanisms of temperature compensation

■ Both proteins (enzymes) & lipids (membranes) are involved. Protein adaptation may be either:
1. Quantitative – more of the same enzymes
2. Qualitative – new enzyme variations

Proteins – Quantitative changes
Most cases of acclimation are of quantitative changes, especially of regulatory enzymes, i.e. ones that are rate-limiting to metabolic pathways

E.g. cytochrome oxidase activity in the goldfish. Fish from 15°C transferred to 25°C showed decreased activity after 30 days. Equivalent to lowering the R-T curve in warm acclimation

Other goldfish transferred to 5°C & enzyme activity increased after 30 days. Equivalent to elevating the R-T curve in cold acclimation

■ Quantitative strategy sometimes found in evolutionary adaptation. Two species of rockfish *Sebastes* from Pacific coast USA:

1. *S. auriculatus* in shallow water, above the seasonal thermocline in summer, at 12-22°C (note algae in photo)
2. *S. miniatus* in deep water (200-300m), below the seasonal thermocline, at 10-12°C

■ In freshly collected fish cytochrome oxidase activity is 70% higher in *S. miniatus* – elevated level in cold-adaptation. Also phenotypic changes in a cross-acclimation experiment with these species

S. miniatus kept at 20°C, & cytochrome oxidase activity decreased – lowered level in warm-acclimation. *S. auriculatus* kept at 10°C & activity increased – elevated level in cold-acclimation

So quantitative strategy is useful, especially over small temperature changes. But problem with larger temperature changes, as enzyme activity is usually low outside the optimum range

Little point in compensating with very large amounts of an enzyme that is very inefficient at the new temperature. In this case the qualitative strategy is better

Proteins – Qualitative changes
■ E.g. enzyme properties in the barracuda *Sphyraena*. 3 similar species along the Pacific coast of North & Central America:
S. argentea (USA) --- *S. lucasana* (Mexico) --- *S. ensis* (Central America)

■ Look at Michaelis-Menten constant (K_m), a measure of an enzyme's affinity for the substrate

Reaction velocity increases with increasing substrate concentration, to the maximum V_{max}. K_m is the substrate concentration at half V_{max}

It is thus an inverse measure of affinity for the substrate – a high K_m indicates low affinity (curve 3) & a low K_m indicates high affinity (curve 1)

K_m should be conserved within a narrow range, corresponding to physiological range of substrate concentration in the cells. Otherwise if:

(1) K_m too low – no reserve capacity in pathway. Affinity of the enzyme is too high, reaction is always near V_{max} in cellular conditions, no room to increase

(3) K_m too high – inefficient as capacity never used. Affinity of the enzyme is too low, reaction never near V_{max} even at highest cellular concentrations

■ Look at lactate dehydrogenase (LDH) in the barracuda. Variation of K_m with temperature for the 3 species – the normal T_b range is shown as the solid part of the line. K_m is concentration of substrate, in this case pyruvate

The 3 species have similar K_m at their respective T_b ranges, but their K_m values differ substantially at any single temperature

■ Shown in more detail in table. At 25°C, K_m & the rate of reaction k_{cat} are highest in the northern species & lowest in the southern species:

	S. argentea	*S. lucasana*	*S. ensis*
Distribution	North	Mid	South
K_m at 25°C (mM)	0.34	0.26	0.20
k_{cat} at 25°C (s^{-1})	890	730	660

But enzyme properties at the midrange T_b are very similar in the 3 species:

Mid range T_b (°C)	18	23	26
K_m at T_b (mM)	0.24	0.24	0.23
k_{cat} at T_b (s^{-1})	670	680	700

■ See the same pattern over a much wider temperature range in LDH from different groups of fish. All have a K_m of about 0.2 mM (pyruvate)

From –2°C in Antarctic notothenioid fish (e.g. *Trematomus*), to 35°C in the goby *Gillichthys seta*, lives in Californian tide pools with T_b up to 40°C

■ Another interesting example is conservation of acetylcholinesterase activity in fish. *Pagothenia* (Antarctic, –2°C), rainbow trout (temperate, 2°C) & ladyfish (tropical, 25°C) have similar K_m at typical T_b

But K_m increases greatly away from the typical T_b range, very low affinity for the substrate (in this case acetylcholine) & so low reaction velocity

Shows how poor quantitative strategy would be over larger temperature ranges – trout would need huge amounts of this enzyme at 25°C

■ Acetylcholinesterase is interesting because curves for trout acclimated to 18°C are very different from those acclimated to 2°C

In the trout acclimation is due to qualitative change, not quantitative change. Only a few known examples of qualitative changes during acclimation

The trout enzymes are isozymes, i.e. produced from multiple loci. Contrast to evolutionary changes which involve allozymes (at the same locus)

Most cases of qualitative changes in acclimation are of isozymes. In polyploid organisms that have multiple genetic copies available for modification. E.g. rainbow trout is tetraploid

Cost of qualitative adaptation

■ Enzymes have similar activity at the normal T_b of each species. There is a cost to this – enzymes are less active at low temperatures than they might otherwise be

E.g. Mg^{2+}/Ca^{2+} ATPase, an enzyme involved in muscle contraction. Activity (relative rate) measured in several species of fish at 0^oC – note very low activity in warm-water fish (*Dascyllus* – damselfish):

	Adaptation temperature (^oC)	Relative rate at 0^oC	ΔG^* (J mol^{-1})
Champsocephalus	–1 to 2	1.00	66.5
Notothenia	0 to 2	0.62	67.3
Cottus	3 to 12	0.45	68.1
Dascyllus	18 to 26	0.05	73.1

Also shows activation energy (ΔG^*) – the lower the value, the faster the reaction, as more molecules are above threshold for reaction

Why are the enzymes of warm water fish less active at low T_b ? Because this would limit their activity at higher T_b, i.e. at their typical T_b

■ Enzymes undergo conformation (shape) changes during catalysis, essential to their function. The old lock & key hypothesis is misleading, it implies rigid structural matching

Induced-fit hypothesis is better, like a glove on a hand, the enzyme wraps around the substrate into a final active form. E.g. binding of NADH and pyruvate to LDH

Conformational changes also occur during the reaction – enzyme must therefore be in a semistable state. These conformational changes involve making & breaking weak bonds, & thus energy changes

Extent of these energy changes determines the activation energy for the enzyme. There is an optimum level of stability for catalysis

Organisms from higher temperatures need more stable enzymes. Might in theory have more covalent bonding, e.g. disulphide bridges

But these would be too inflexible for induced fitting. No evidence for extra covalent bonding in proteins in organisms from hot environments. Instead, increased stability comes from more weak bonds

These weak bonds are harder to break at lower temperature. An enzyme adapted for optimum stability at high T_b thus becomes too stable at low T_b. It loses its ability to change shape, & thus its activity

So impossible for one enzyme to function efficiently over a wide range of temperatures, there must be qualitative changes for compensation

Lipids

■ Basic structure of cell membranes is a phospholipid bilayer with attached & embedded proteins. To be functional the membrane must also be in a semistable or liquid crystalline state

But the stability (viscosity) of membranes changes with temperature. Because the phospholipids are held together by the same weak bonds that determine the shape of enzymes

■ Membranes from organisms adapted to different temperatures should have similar fluidity at different T_bs. This is the concept of homeoviscosity

Fluidity of lipids depends on length of carbon chain & level of unsaturation of their fatty acids. Unsaturation = number of double bonds, saturated = 0

Melting point of fatty acids increases with chain length, shown in the saturated series lauric to arachidic C12 to C20. High M.P. = high stability, low fluidity

M.P. strongly decreases with number of double bonds, shown by C18 series stearic (saturated) to linolenic (3 double bonds)

Wide range of properties possible in similar molecules, compare arachidic (20:0) M.P. = 76°C with arachidonic (20:4) M.P. = –50°C

Melting points of fatty acids (°C) in relation to chain length : number of double bonds:

	Saturated	M.P.		Mostly unsaturated	M.P.
12:0	Lauric	44	18:0	Stearic	70
14:0	Myristic	54	18:1	Oleic	13
16:0	Palmitic	63	18:2	Linoleic	–5
18:0	Stearic	70	18:3	Linolenic	–11
20:0	Arachidic	76	20:4	Arachidonic	–50

Both effects are due to the interactions between fatty aid molecules in a solid, i.e. within the bilayer

■ Increasing chain length increases the length of the hydrophobic tails & so the cumulative strength of the bonds between them, so increases M.P.

Double bonds cause kinks in the chain, reduce the forces between neighbouring molecules & decrease the M.P., increasing fluidity

Homeoviscosity can therefore be achieved by including more unsaturated lipids in membranes at lower T_bs

■ Fatty acid composition (% unsaturated) of phospholipids from brains of fish from different temperatures:

	T_b (^oC)	Cholines	Ethanolamines
Arctic sculpin	0	63	79
Goldfish	5	60	75
Goldfish	25	55	66
Desert pupfish	34	50	64

For two classes of phospholipids (cholines & ethanolamines) more unsaturated) fatty acids at lower temperatures

Same pattern shown for both evolutionary adaptation (interspecific comparison) & acclimation (goldfish at 5 & 25^{o}C)

■ More recent study with more species of fish, from Antarctic (–2^{o}C) to tropics, shows linear increase in proportion of unsaturated fatty acids at lower adaptation temperatures