PART I  General concepts
A. CLARKE

Temperature and energetics: an introduction to cold ocean physiology

Cold has long been regarded as inimicable to life. From the speculations of the earliest geographers and explorers to the azoic hypothesis of Edward Forbes, it was widely believed that the frozen polar regions and the cold, dark depths of the ocean would be essentially devoid of life. Even today, the richness of some polar marine invertebrate assemblages comes as a surprise to many. The tropics are still regarded as a less demanding environment, at least for marine organisms, and many palaeoecologists continue to refer to a cooling of the climate as ‘deterioration’ and a warming as ‘amelioration’.

The first physiologists to study polar marine organisms naturally turned their attention to the key areas of how teleost fish avoided freezing when living in waters significantly colder than the equilibrium freezing point of their body fluids, and how marine ectotherms managed to sustain metabolic activity at low temperatures (Scholander et al., 1953; Wohlschlag, 1960). In the marine environment, the avoidance of freezing is a peculiarly polar problem, and the essential mechanism was first elucidated in Antarctic fish by DeVries (DeVries & Wohlschlag, 1969; DeVries, 1971). Subsequent work on Arctic fish showed that most northern taxa utilised antifreeze proteins in contrast to the antifreeze glycoproteins of Antarctic notothenioids and the true cods (gadoids), thereby revealing an intriguing case of parallel evolution (Scott, Fletcher & Davies, 1986; Eastman, 1993).

In the terrestrial environment, exposure to freezing temperatures is a widespread environmental challenge. In contrast, for marine organisms freezing is essentially a problem only for polar teleost fish, intertidal organisms and those high latitude benthic invertebrates which become encased in anchor-ice. In the wider context of adaptation to temperature in general, freezing resistance therefore represents something of a special case in the marine environment. In this review, a general picture of temperature adaptation will be considered, attempting to place the polar regions in the context of the marine environment overall.

Temperature adaptation in marine organisms has been reviewed many times (Clarke, 1983, 1991; Hochachka & Somero, 1973, 1984; Cossins &
Bowler, 1987; Johnston, 1990). Instead of covering this ground again, an attempt will be made to identify the major constraints to physiological function in cold water, for it is through these constraints that temperature has its greatest impact on ecology and lifestyle.

The physical properties of cold seawater

Just as the ecology of any organism can be understood only in relation to those other organisms which share its environment, the physiology of that organism can only be understood in relation to its physical environment. The equations of state for seawater all contain a temperature term, and thus a change in environmental temperature brings with it concomitant changes in all other physical properties of seawater. Seawater at 0 °C and seawater at 30 °C vary in more than just temperature, and are quite different environments from the point of view of an organism attempting to make a living there (Clarke, 1983). In Table 1, a number of important environmental variables are compared for polar and tropical seawater. All show significant variation with temperature, and in all cases this variation is non-linear (although for some variables this non-linearity is not important over the range of temperatures of interest to physiologists).

The ecological and physiological implications of some of this variability have long been recognised. Thus the increased solubility of oxygen in cold seawater means that more oxygen can be carried in physical solution in the blood of polar ectotherms. As a result, there is a well-described tendency for a reduced haematocrit in polar fish (Kunzmann, 1991), which reaches its ultimate expression in the notothenioid ice-fish (Channichthyidae) (Macdonald & Wells, 1991). In icefish the genes for haemoglobin are not expressed (Cocca et al., 1995), and there are few circulating erythrocytes. Oxygen is carried to the tissues in physical solution and there are parallel adjustments in blood volume, heart size and circulatory architecture (Eastman, 1993). Interestingly, although the genes for myoglobin are also generally not expressed, a number of icefish have recently been shown to express myoglobin genes in heart tissue (Sidell and Vayda, this volume).

Although many species of ice-fish are sluggish and appear to have a rather sedentary lifestyle, it is likely that this reflects, at least in part, the ancestral niche of notothenioids as a taxon. Notothenioids evolved from a demersal form, which had secondarily lost a functional swimbladder (Eastman, 1993). In consequence, most notothenioids remain benthic or demersal in habit, although a number of species have subsequently evolved a semi-pelagic, fully pelagic or cryo-pelagic way of life, and these include at least one ice-fish (Champsocephalus gunnari). This species is partly demersal, but is also capable of moving into the water column to take planktonic prey such as
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Table 1. A comparison of the physical properties of seawater at 0 °C and 30 °C (based on Clarke, 1983)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value in seawater at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>poles (0 °C)</td>
</tr>
<tr>
<td>Thermodynamic temperature (K)</td>
<td>273.15</td>
</tr>
<tr>
<td>Acidity (log_{10}[H^+] = pH) (NB data for pure water)</td>
<td></td>
</tr>
<tr>
<td>Dynamic viscosity (mPa s)(^b)</td>
<td>1.8823</td>
</tr>
<tr>
<td>Density (g ml(^{-1}))(^c)</td>
<td>1.0273</td>
</tr>
<tr>
<td>Oxygen content (mol l(^{-1}))(^d)</td>
<td>8.05</td>
</tr>
<tr>
<td>Total carbon dioxide content (mol l(^{-1}))(^e)</td>
<td>644.9</td>
</tr>
<tr>
<td>Apparent solubility product for calcite (K(^{1/2})(_{calc}) × 10(^6))(^f)</td>
<td>0.711</td>
</tr>
</tbody>
</table>

Notes:
\(^a\) Data for pure water, since the pH of seawater is affected by many factors in addition to temperature.
\(^b\) Data for salinity 34 psu (from Table 25 in Riley & Skirrow, 1975).
\(^c\) Data for salinity 34 psu (from Table 2.1 in Riley & Chester, 1971).
\(^d\) Data for salinity 35 psu, relative to atmosphere of 20.95% O\(_2\), at pressure of 101.325 kPa and 100% relative humidity (from Table 6 in Riley & Skirrow, 1975).
\(^e\) Data for acidified seawater of chlorinity 19‰, atmospheric pressure of 101.325 kPa and 100% relative humidity (from Table 6.4 in Riley & Chester, 1971).
\(^f\) Data for seawater of chlorinity 19‰ (Edmund & Gieskes, 1970); data are approximate because of the problem of interference by other ionic species, especially Mg\(^2+\).

Euphausiids. It also lives in warmer waters where the benefits of enhanced oxygen solubility are not as marked as they are closer to the Antarctic continent. The haemoglobin-less condition does not therefore necessarily bring with it a concomitant inactive lifestyle, nor a limitation to the coldest waters for Champsocephalus esox is found north of the polar front (Gon & Heemstra, 1990).

Other aspects of the variation of seawater physics with temperature have been less well explored. For example, the viscosity of seawater is of fundamental significance, particularly to small organisms living at large Reynolds numbers. Recently, Podolsky and Emlet (1993) have shown that relatively
small temperature-related changes in viscosity can have significant effects on the energetics of echinoderm larvae. Nothing, however, appears to be known about the impact of temperature-mediated changes in viscosity on the energetics of sessile benthos that rely on ciliary mechanisms for feeding, or on the energetics of cold-water protozooplankton.

**Temperature and physiology**

Since all processes involving a change in free energy will be affected by temperature, it is to be expected that, in the absence of any evolutionary compensation, all physiological and biochemical processes will also show marked differences in rate between tropical and polar species. Thus for a typical enzyme-mediated reaction, a significant contribution to the free energy of activation for the reaction comes from the enthalpy of the reactants. At any given temperature, only a small fraction of the total population of molecules has sufficient energy to react, and this proportion can be estimated from statistical mechanics (specifically the Maxwell–Boltzmann distribution law). As temperature decreases, the proportion of molecules with sufficient kinetic energy to react also decreases, and the reaction rate slows accordingly; this relationship is effectively exponential.

Almost a century of physiological work has shown that, within an individual system (such as an individual organism, or isolated subsystem), this thermal dependency typically falls within narrow bounds, equivalent roughly to an increase in rate of $\times 2$ to $\times 3$ for each rise in temperature of 10 K (that is $Q_{10}=2$–3). It is striking that the temperature dependence of isolated systems, such as an enzyme reaction *in vitro*, and that of many processes in whole organisms are broadly the same. This suggests that evolution has resulted in an integrated physiology where most processes have a broadly similar relationship to temperature.

Extrapolation of the typical temperature dependency of physiological rates to the marine ecosystem as a whole would predict that physiological processes in tropical marine ectotherms at 30 °C will proceed at between 27 ($Q_{10}=3$) and 8 ($Q_{10}=2$) times the rate in polar species at 0 °C. Clearly, for some processes this would pose severe ecological difficulties: consider the fate of a fish whose escape response was curtailed by a factor as low as $\times 8$ when attempting to escape a predatory seal or seabird whose locomotor ability was maintained at a high level by endothermy.

**Compensation**

The simple observation that a rich and diverse marine flora and fauna can be found from the tropics to the poles indicates that some form of compensation has evolved to offset these direct temperature effects. Even if not
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Table 2. Kinetic parameters of enzymes isolated from three allopatric species of Pacific barracuda Sphyraena

<table>
<thead>
<tr>
<th>Species of Sphyraena</th>
<th>argentea</th>
<th>lucasana</th>
<th>ensis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kinetic parameters measured at 25°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$ (mM)</td>
<td>$0.34\pm0.03$</td>
<td>$0.26\pm0.02$</td>
<td>$0.20\pm0.02$</td>
</tr>
<tr>
<td>$k_{cat}$ (s$^{-1}$)</td>
<td>$893\pm54$</td>
<td>$730\pm37$</td>
<td>$658\pm19$</td>
</tr>
<tr>
<td><strong>Representative environmental temperature</strong></td>
<td>18</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td><strong>Kinetic parameters measured at representative environmental temperature</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$ (mM)</td>
<td>$0.24$</td>
<td>$0.24$</td>
<td>$0.24$</td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>$667$</td>
<td>$682$</td>
<td>$700$</td>
</tr>
</tbody>
</table>

Note:
Kinetic parameters are for isolated M$_4$ LDH operating in the direction of pyruvate reduction; $K_m$ is for pyruvate (mM). Data are presented for both 25°C and a temperature representative of the environment in which the fish lives ($K_m$, Michaelis–Menten constant; $k_{cat}$, turnover number).


‘perfect’ in theoretical terms, this compensation is clearly highly effective in ecological and evolutionary terms. A single, classical, example which demonstrates such compensation is the activity of M$_4$-LDH in related species of the Pacific barracuda Sphyraena (Graves & Somero, 1982).

The enzyme LDH catalyses the conversion of lactate to pyruvate (and vice versa) and is thereby critical in regulating the flux of the products of glycolysis into the Krebs cycle. Graves and Somero (1982) examined the thermodynamic properties of M$_4$-LDH isolated from four species of Sphyraena ranging from tropical to temperate waters in the eastern Pacific. Although enzymes isolated from each species showed typical thermal dependency in kinetic parameters, there was much less variation in these parameters when measured at the temperatures at which the fish lived (Table 2). This study exemplifies two important general conclusions. The first is that the enzymes isolated from organisms living at different temperatures themselves differ in some way, and the second is that these differences mean that the enzymes have broadly comparable activities at the temperatures at which the organisms actually live.

There are two important consequences which follow from this simple
Fig. 1. A conceptual model of compensation as homeostasis; evolution has modified cellular or organismal physiology such that the same process has the capacity to operate at broadly the same speed in organisms living at different temperatures. Note that this compensation plot has both bandwidth and slope. The bandwidth indicates that, at any given environmental temperature, there may be a range of rates in different organisms, perhaps associated with ecology or phylogenetic history. Perfect compensation across the ecological temperature range would be indicated by a slope of zero (that is, the same rate at all temperatures). The slight slope shown here indicates that underlying physical or physiological constraints may mean that compensation is not perfect. (Reproduced from Clarke, 1991.)

observation. The most important point is that it is unwise to extrapolate from experimental data obtained from one organism (say a eurythermal temperate marine ectotherm) to other organisms living elsewhere (say a polar or tropical species). Unfortunately, this is still done to predict the physiology of polar or tropical species, or to speculate on the likely effects of global warming.

The second consequence is a straightforward definition of temperature compensation, which is:

\[
\text{the maintenance of physiological rate in the face of temperature change}
\]

This definition of compensation is shown diagrammatically in Fig.1, and it applies equally to other forms of environmental challenge (such as pressure) as well as to temperature.

In the past few decades, many physiological processes in marine ectotherms have been studied to determine the extent to which such
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compensation has evolved, and the mechanisms by which it has been achieved. It is now clear that some processes have evolved almost perfect compensation (for example, microtubule assembly: Detrich & Overton, 1986; Detrich et al., 1989), others have achieved only partial compensation (for example, burst swimming performance in teleost fish: Johnston, Johnson & Battram, 1991) and some almost none at all (for example, in the speed of nervous conduction: Macdonald, 1981; Montgomery & Macdonald, 1984; or embryonic development: Bosch et al., 1987; Clarke, 1992). This poses the evolutionary question of whether there are particular constraints to temperature compensation, and what these might be.

Constraints to temperature adaptation

Viewed in evolutionary terms, an organism takes up material from its environment and manipulates this in such a way as to maximise its fitness (that is, to maximise the relative representation of its genes in the next generation). Since this process is fuelled by the oxidation of foodstuffs or reserves, the rate and efficiency of energetic pathways are critical to fitness.

An organism, however, contains a vast number of enzymes involved in a complex web of metabolic interactions. This complexity poses a problem in metabolic control for the organism, and an intellectual challenge to any physiologist attempting to understand how that organism can respond in an integrated way to a change in temperature. Traditionally, physiologists interested in temperature compensation have looked in detail at selected aspects of cellular or organismal operation. In this chapter, a different approach will be taken in that an attempt will be made to look at temperature compensation as a whole organism phenomenon, and to try to identify those aspects of physiology which appear to act as a constraint upon compensation. Rather than simply cataloguing known examples of temperature compensation, work will be within the conceptual framework first outlined by Hochachka and Somero (1973, 1984).

A conceptual framework for temperature compensation

Cellular physiology, indeed all life, depends absolutely on enzyme-mediated catalysis, and this catalysis is affected significantly by temperature. As discussed above, a typical enzyme reaction in a tropical marine invertebrate or fish will proceed at between about 10 and 30 times the rate in a polar relative, unless the organism does something about it. It is traditional amongst physiologists to concentrate on how organisms adjust to cope with the rate-depressing effect of low temperature rather than regarding a temperature-mediated increase in rate as a physiological problem. This reflects the long-held bias towards the tropics being ‘amenable’ or ‘easy’ places to live, and the poles as ‘challenging’ or ‘difficult’. 
Table 3. The major categories of compensatory response to a lowered cellular temperature

<table>
<thead>
<tr>
<th>Response</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative strategy</td>
<td>Increase the number of enzyme molecules to offset the reduced catalytic efficiency of an individual enzyme molecule at lower temperatures.</td>
</tr>
<tr>
<td>Qualitative strategy</td>
<td>A change in the type of enzyme(s) present. This could include a shift in the balance of alloenzymes with differing kinetic properties.</td>
</tr>
<tr>
<td>Modulation strategy</td>
<td>Modulation in the activity of pre-existing enzymes. This could include changes in enzyme–substrate binding kinetics with temperature, changes in cofactor binding kinetics, or modification of the immediate enzyme environment (membrane or cytosol).</td>
</tr>
</tbody>
</table>

Source: From Hochachka & Somero (1973).

Hochachka and Somero (1973) have classified the range of possible responses to the rate-depressing effects of low temperature into three broad categories. These are the quantitative strategy (more enzymes), the qualitative strategy (different enzymes) or modulation of the enzyme environment (Table 3). These strategies are not, of course, mutually exclusive.

Modulation of the enzyme environment

Modification of the enzyme environment offers the opportunity to modulate the effect of temperature on many enzyme systems at once. It would thus appear, \textit{a priori}, to be an effective way of simultaneously offsetting the impact of temperature and of maintaining the overall balance of metabolic pathways.

Many enzymes are membrane bound and it is now well established that the primary response of biological membranes to a change in temperature is an alteration in the fatty acid composition of the constituent phospholipids. There may also be changes in phospholipid head-group composition and phospholipid/cholesterol ratio. These changes serve to maintain the physical state of the membrane and the process is often referred to as homeoviscous adaptation (see Cossins, 1994 for a recent discussion). Most work in this area has been concerned with seasonal adjustments in eurythermal organisms. Unfortunately, most analyses of the lipid composition of polar organisms...
are not very helpful in attempting to understand evolutionary adjustments to membrane composition at low temperatures for they involve whole organism or whole tissue extracts which mix fatty acids from membranes with those from storage lipids. Nevertheless what evidence there is points to homeostatic changes in membrane composition having occurred during evolutionary adaptation to low temperatures.

For cytosolic enzymes, most attention has been directed at the pH of the intracellular environment. The ionic dissociation of pure water is temperature dependent, with \( \Delta pH/\Delta T \) being about 0.017. It is now well established that the pH of blood in marine ectotherms shows a similar temperature dependency, though offset to higher pH values to produce a higher relative alkalinity compared with pure water. This pH is actively regulated and it has been proposed that pH is adjusted to maintain the dissociation state of changed groups, and in particular the imidazole groups of histidyl residues (the alphastat hypothesis: Reeves, 1972, 1977). Regulation of alphastat and regulation of a constant relative alkalinity are different processes, but the two hypotheses would be difficult to distinguish by experiment.

There is evidence for the regulation of net protein change over a wide range of temperature in some organisms (for example the intracellular environment of skeletal muscle in the turtle *Pseudemys scripta* Malan, Wilson & Reeves, 1976). There is also widespread evidence for the maintenance of the net alkalinity of ectothermic blood across a range of environmental temperatures (Reeves, 1977; Clarke, 1987). This would suggest that a significant degree of compensation for temperature is achieved by a homeostatic adjustment of the intracellular ionic environment (see Pörtner et al., this volume), but firm evidence for this as a universal primary mechanism for temperature compensation is still lacking.

Changes in the number or type of enzymes

Changes in either the number or the type of enzymes (or perhaps both for these are not mutually exclusive processes) would appear to present a number of evolutionary difficulties. There is a limit to the solvent capacity of the cell and hence it seems intuitively unlikely that temperature compensation could be achieved by a simultaneous increase in the concentration of all cellular enzymes (to say nothing of substrates, cofactors, products and transcribed messages) without severe consequences for intracellular viscosity, diffusion, transport, and cellular energetics. In contrast, it would seem likely that, over evolutionary time, the process of natural selection will have driven changes in the nature of many enzymes and proteins. The key question here is the strength of selection, and this is likely to vary from enzyme to enzyme depending on its function and contribution to the overall fitness of the