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MiniReview

Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature

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Abstract

The effect of environmental temperature on the affinity of microorganisms for substrates is discussed in relation to measurements of affinity by either K_s values or specific affinity (a_A). It can be shown for psychrophiles, mesophiles and thermophiles that when a_A is used as the measure of affinity, affinity decreases consistently as temperature drops below the optimum temperature for growth. This effect may be because of stiffening of the lipids of the membrane below the temperature optimum, leading to decreased efficiency of transport proteins embedded in the membrane. The lower temperature limit for growth is, therefore, that temperature at which an organism is no longer able to supply the maintenance requirement of the growth rate-limiting nutrient because of loss of affinity for that substrate. This linking of temperature and affinity for substrates taken up by active transport (a temperature-modulated substrate affinity model) includes uptake of both organic and inorganic substrates. This effect of decreased substrate affinity at low temperature may have profound implications on the availability of substrates in the natural environment as environmental temperatures change. At temperatures below their optimum for growth microorganisms will become increasingly unable to sequester substrates from their environment because of lowered affinity, exacerbating the anyway near-starvation conditions in many natural environments.

Keywords: Low temperature; Specific affinity; Thermal adaptation; Membrane fluidity; Global warming

1. Cardinal temperatures for growth

Any species' response to temperature is characterised by a number of 'cardinal temperatures' [1] – upper and lower limits of temperature for growth – and an optimum temperature for growth at some point between these two extremes. The reasons for the upper limit of temperature are relatively well understood, imposed by increasing rates of denaturation of key cellular components as temperature rises: to the point that denaturation exceeds the rate at which they can be replaced, with consequent disruption of cellular function. The upper temperature limit is not immutable for a given species, some degree of adaptation being possible in terms of modifications of key structures such as membranes, and by production of heat shock proteins which protect cellular processes. However, the degree of adaptation of a given species to high temperature, indicated by changes in the maximum temperature for growth, is

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usually limited. The reasons for the lower temperature limit for growth are less clear, although there seems to be consensus that at the low temperature limit there is loss of membrane function. It has been proposed for a long time that uptake of key substrates is inhibited by low temperature because of effects on the cell membrane (e.g. [2]), but there has been little consistent evidence to support this case.

2. Adaptation of membranes to temperature

Much research has gone into examining how membrane structure and composition change with respect to temperature, both within a species and between species adapted to different temperature regimes, and a number of trends have emerged (for reviews see [3–5]). In microorganisms adapted to low temperature environments (psychrophiles and psychrotolerants) there tends to be an increased proportion of unsaturated membrane lipids, and a decreased proportion of branched chain lipids compared to species adapted to moderate (mesophiles) or high (thermophiles) ranges of temperature. Similar trends of change in membrane lipids can be observed within a single species when it is grown across its temperature range [6–9]. Furthermore, cold shock proteins may be produced when an organism is challenged by low temperature, some of which are enzymes such as desaturase enzymes associated with modification of the cell membrane in response to temperature [3,4]. Suutari and Laakso [9] found changes in the saturation index, mean chain length and the degree of branching in the membrane fatty acids of *Mycobacterium phlei* when it was subjected to temperature change.

Membranes are essentially colloidal solutions of phospholipids and proteins in a fluid (liquid crystalline) phase, and it is only in this fluid phase that they are biologically functional. As temperature decreases, membranes become increasingly viscous with decreasing membrane fluidity [10,11], and at some temperature will undergo a phase change to a gel ('solid') phase when biological function is lost [12]. The changes in membrane lipid composition in response to lowered environmental temperature, which are described above, have been suggested to result in maintenance of the cell membrane in a biologically functional fluid phase to as low a temperature as possible (homeoviscous adaptation [10]). Unsaturated lipids tend to have lower melting points than the equivalent saturated lipids, while branched lipids tend to have even higher melting points. Other things being equal, increased proportions of unsaturated lipids in membranes are suggested to reduce the temperature at which it undergoes a phase change, and therefore maintain membranes in a functional fluid condition to a lower temperature. In contrast, high proportions of saturated and branched lipids in membranes of thermophiles increase the phase change temperature of the membrane, conferring stability at high temperature but making the membrane 'stiff' and biologically nonfunctional at a comparatively high minimum temperature for growth. Proteins embedded in the membrane, including key respiratory and transport proteins, function only when the membrane is in the fluid phase, and cease activity on the phase change to the solid state [3,13]. Whether there is a progression of gradual change in membrane function as temperature decreases prior to the temperature of the phase change, or whether there is constant full function followed by complete cessation of activity at the phase change temperature, is poorly understood.

3. Nutrient limitation in natural environments, and 'affinity' for substrates

In virtually all natural environments vital resources such as energy substrates, and the substrates necessary for growth (nitrogenous compounds, phosphate, etc.), are present at very low, usually growth rate-limiting, concentrations (e.g. see [14]). Growth and survival depend, therefore, upon the ability of a species to sequester these sparse resources in extreme competition with other species competing for the same resources. If the uptake mechanism for a substrate depends upon passive diffusion then its rate of uptake is likely to be slow and influenced only by the concentration gradient across the membrane, although facilitated diffusion may increase the rate of passive uptake. Active uptake, though, depends upon the presence and activity of transporter pro-

teins in the membrane which can accumulate substrates against a concentration gradient. Continued transporter activity depends upon the membrane maintaining a charged energetic state required to power conformational changes which carry substrates across the membrane, and their activity can be affected by the fluidity of the membrane [15,16]. The efficiency of active uptake by a microorganism from its external environment of any substrate at low concentration depends upon the 'affinity' of the organism for that substrate. Affinity has been described most frequently for microorganisms by a Michaelis-Menten type of saturation curve relating growth rate to the concentration of the rate-limiting substrate (see [17]). Such a saturation curve is described by two 'constants', μ_{max}, the maximum specific growth rate, and K_s, the half-saturation constant (in the case of a specific enzyme reaction, V_{max} and K_m). In effect, it is the affinity of the active sites of the transport proteins in the cell membrane that influences K_s (or K_m), while the maximum capacity for the reaction, μ_{max} or V_{max}, is a function of the total number of active sites per unit biomass or per cell. Traditionally, the affinity of an organism for a substrate has been described by the value of K_s (or K_m), the half-saturation constant, but investigations designed to detect changes in the affinity of microorganisms for substrates by measuring solely changes in K_s (or K_m) values with temperature have generally failed to detect any consistent trends (e.g. [18,19]).

However, half-saturation constants alone are poor indicators of affinity for a substrate, particularly at the low substrate concentrations relevant to microorganisms in their natural environments. Fig. 1 illustrates two growth curves for organisms with the same K_s values but different μ_{max} values. Clearly, at low substrate concentrations the ability of one organism to outcompete the other for substrate is a function not just of K_s but of both μ_{max} and K_s. In essence, it is the initial slope of the curve, at very low substrate concentrations typical of many natural environments (when substrate concentration $\ll K_s$), which dictates which organism wins out in terms of the rate at which it sequesters the substrate and grows, and this slope is approximated by μ_{max}/K_s. Button and coworkers [17,20,21] have termed this function the specific affinity (a_A), and have argued that it is a much more robust measure than K_s of the

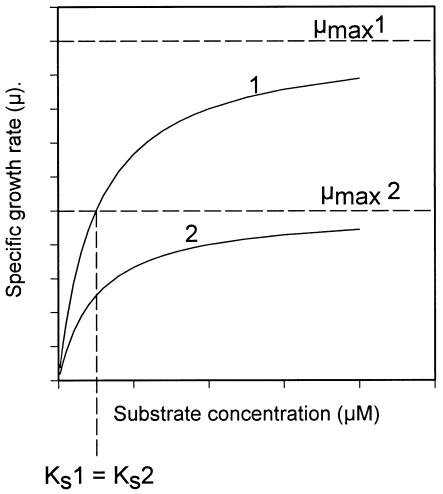

Fig. 1. Plots of Michaelis-Menten kinetics for growth of two bacteria with different μ_{max} but the same K_s.

affinity of an organism for a substrate, and is, furthermore, independent of the actual mechanism of uptake. The specific growth rate, μ, is related to the extracellular concentration of growth rate-limiting substrate S through the ability to sequester substrate as described by the specific affinity for the substrate, a_A, and the efficiency of conversion of that substrate to cell material (the cell yield per mol of the substrate, Y_s) by

$$\mu = a_A Y_s S \quad (1)$$

(see [17,21]).

There are comparatively few data sets in the literature which include both μ_{max} and K_s at different temperatures. For those data which are available, when we examine the effect of temperature on the affinity for substrates, using a_A as a measure of 'affinity', consistent trends start to be seen where they were not seen solely with K_s [22]. Fig. 2 shows trends with temperature of a_A for growth on glucose by psychrotolerant and mesophilic bacteria. The range of temperatures over which each bacterium grows and the actual values of a_A at a given temperature differ for each organism, reflecting their different broad physiological adaptation with respect to the range of temperature required for their growth. However, in all cases there was a consistent trend of decrease of a_A as temperature decreased below

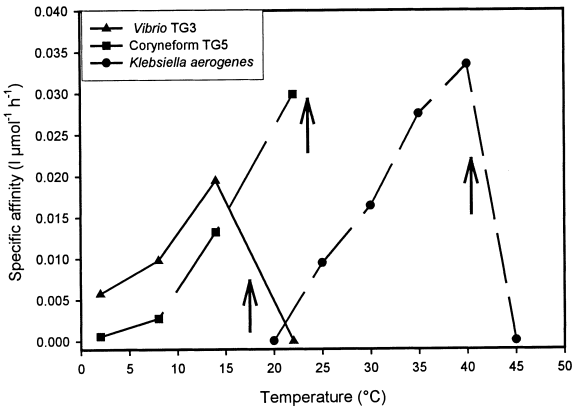

Fig. 2. Plots of specific affinity for glucose versus temperature for a psychrophilic *Vibrio* TG3, a psychrotolerant coryneform TG5 and a mesophilic *Klebsiella aerogenes* (data for *K. aerogenes* [23]; and for the psychrotolerants [18], with additional data courtesy of the authors). Arrows indicate the optimum temperature for growth of each bacterium.

the optimum temperature for growth, indicating a consistent decrease in the affinity of the bacteria for substrate as temperature declined, whether they were psychrophiles or mesophiles. In a study with carbon-limited chemostats, Herbert and Bell [24] examined growth of the psychrophilic *Vibrio* AF1 on a range of seven different substrates, measuring both μ_{max} and K_s at different temperatures up to its optimum at 17°C. Growth on sucrose, lactose, galactose, mannose, ribose and xylose all showed increases in specific affinity up to the optimum growth temperature, although on glucose the result was equivocal. The small number of studies available which have measured both K_s and μ_{max} at different temperatures support, therefore, the general paradigm that when environmental temperature decreases below the optimum for growth there is consistently decreased affinity for substrates when affinity is measured by μ_{max}/K_s.

The trend of decreased affinity with lowered temperature applies not just to uptake of organic substrates (electron donors). We have shown (Table 1) with nitrate-limited anaerobic chemostats that affinity for nitrate by nitrate-respiring bacteria (i.e. for uptake of electron acceptor) also decreases with temperature [25].

4. Evidence of effect of temperature on affinity for substrates?

What evidence is there from the natural environment that uptake affinity for substrates is indeed inhibited by decreased temperature? In a key paper, Pomeroy et al. [26] showed that in Arctic seawater off Newfoundland (in situ temperature −1°C) the respiratory rate of the bacterial community was inhibited at the in situ temperature but was stimulated either by higher temperature or by higher concentrations of added substrates (glucose or proteose peptone). Wiebe et al. [27] hypothesised that there was an interaction between temperature and substrate requirement such that higher substrate concentrations were necessary at temperatures near the lower temperature limit of a species. Wiebe et al. [28] showed that even mesophilic marine bacteria, isolated from the southeastern subtropical shelf waters of the USA, were inhibited by lack of substrate at temperatures near their minimum for growth, despite the minimum temperature being comparatively high,

Table 1
Changes with temperature in specific affinity (1 μmol^{-1} h^{-1}) for nitrate by nitrate-respiring bacteria in anaerobic N-limited chemostats ([25] and Lloyd and Nedwell, unpublished data)

Substrate	Organism (optimum temperature for growth)	Specific affinity for nitrate (1 μmol^{-1} h^{-1}) at a temperature (°C) of			
		5	10	15	20
Glucose	*Klebsiella pneumoniae* (28°C)		0.00118	0.00488	0.013
	Aeromonas sp. (14°C)		0.0033	0.0015	0.00006
	Klebsiella oxytoca (28°C)		0.00011		0.00059
	Citrobacter sp. (19°C)	0.00003			0.00004
Acetate	*Klebsiella oxytoca*		0.00014		0.00021
	Citrobacter sp.	0.00012			0.00021

near 10°C. Growth rates were increased either by a rise in temperature or by addition of substrates. These workers concluded that there was an enhanced requirement for substrate near their lower temperature limit for growth of both mesophilic and psychrotolerant bacteria. This is entirely consistent with the proposed model of decreased affinity for substrates by these bacterial communities at low temperature.

5. Effect of low temperature on uptake of inorganic substrates

The above arguments on how low temperature may influence uptake of organic substrates also apply to uptake of at least some inorganic substrates. It has been argued previously that low temperature influences the ability of algae to take up nitrate [29], but investigation of that ability has usually been restricted to measurements of K_s values for nitrate at different temperatures. As with uptake of organic substrates, there has been no apparent consistent pattern of variation of K_s with temperature. For example, Mechling and Kilham [19] examined uptake of silicate by algae in fed batch culture experiments, and of nine algal species for which there were data in their paper, three showed a decrease of K_m with low temperature, three showed no effect, and three others showed increased values of K_m. My group's recent work with both bacteria and algae [30,31] has shown that when affinity for nitrate is measured by specific affinity, rather than just by K_s, there was a consistent trend of decreased $a_{A(nitrate)}$ with lowered temperature (Fig. 3). This holds true for all physiological types (psychrophiles, mesophiles, thermophiles). In contrast, specific affinity for ammonia ($a_{A(amm)}$) showed much less response to temperature. It has been demonstrated also for higher plant roots that nitrate uptake is affected much more by temperature than is ammonium uptake (e.g. [32,33]), so it appears that the differing effects of temperature on uptake of both nitrate and ammonium are consistent across bacteria, algae and higher plants. This is not surprising as the biochemical requirements to assimilate nitrate and ammonium are identical in all organisms assimilating them, and evolutionarily these mechanisms are therefore likely to be strongly conserved.

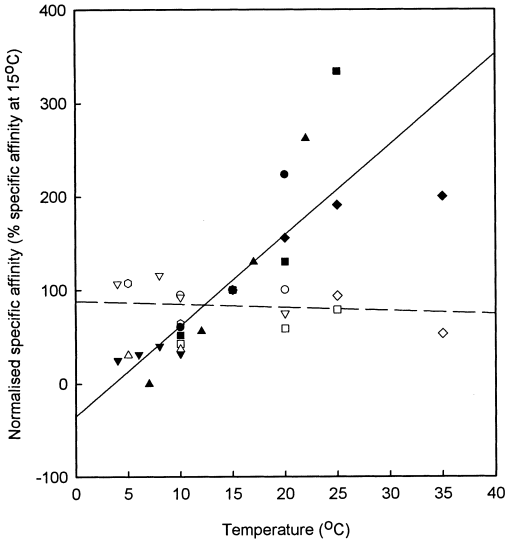

Fig. 3. Proportional change of specific affinity with temperature, normalised to 15°C, for nitrate (closed symbols) and ammonia (open symbols) with five bacteria and two algae [30,31]. Bacteria: (○) *Klebsiella oxytoca*, (▽) *Vibrio logei*, (◇) *E. coli*, (△) *Hydrogenophaga pseudoflava*, (hexagon) *Brevibacterium* sp. Algae: (□) *Dunaliella tertiolecta*, (▲) *Chaetoceros curvisetum*. (*H. pseudoflava* could not grow on nitrate, and *C. curvisetum* was not grown on ammonium.) A third alga, a psychrophilic *Chaetoceros* sp., showed the same trends with temperature on nitrate and ammonium but did not grow as high at 15°C, and is not shown in this figure. Similarly, a thermophilic *Bacillus stearothermophilus* showed similar trends with temperature, but did not grow at 15°C.

The lesser effect of temperature on ammonium uptake may be consistent with at least some passive transport of NH_3 across the membrane contributing to total ammonium uptake. Passive transport is not affected by decreased fluidity of the membrane at low temperature.

6. Reason for change in specific affinity for substrates with lowered temperature

The relationship between specific growth rate and substrate concentration is shown in Eq. 1, where sequestration of the external substrate S is determined by specific affinity a_A and efficiency of use of the sequestered substrate by Y. Changes in growth rate with temperature may be the result of changes in

either of these terms. The first is the ability of a cell to sequester substrate from the environment, which might be regulated by the affinity of the transport proteins in the membrane for a substrate changing with temperature. Secondly, there may be changes with temperature in the efficiency of a downstream, intracellular enzyme which regulates the assimilation of the substrate, and hence of the cell yield. In a steady-state situation (such as in our chemostat experiments) the changes in a_A with temperature must reflect changes in the uptake of the growth rate-limiting nutrient, not changes in a downstream assimilatory function. If a downstream enzyme, rather than uptake rate, regulated growth rate (i.e. was less than substrate uptake rate) there would be intracellular accumulation of the substrate, i.e. non-steady state. The reported changes in a_A with temperature therefore indicate an effect on the uptake of substrate across the membrane, not of response of downstream assimilatory enzymes to temperature change which might become apparent as changes in Y. There is no evidence in the literature that cell yields show any consistent trends of change with temperature. Furthermore, it is difficult to envisage that all of the different intracellular assimilatory enzymes required for the myriad of different substrates available in the environment would react identically to lowered temperature. It is much more likely that the common factor influencing the response to lowered temperature is the effect of temperature on the membrane itself, particularly as the decrease of specific affinity with lowered temperature appears to be associated particularly with substrates taken up by active transport (see later).

Such a trend of decreased affinity with temperature might be expected if the effect of decreasing temperature was to decrease the fluidity of the cell membrane, despite variations in the membrane lipids which adapt for different ranges of temperature in the different physiological types of bacteria (see Section 2 above). Decreases in membrane fluidity (making it 'stiffer') reduce the efficiency of transport proteins and enzymes in the membrane (e.g. [16,34]). The consistent decrease of a_A with lowered temperature provides the first strong evidence that temperature reduced below a species' optimum does decrease substrate uptake; and furthermore suggests that the lower limit of temperature for growth may be determined by the lowest temperature at which a species can maintain membrane fluidity and active transport across the membrane. Presumably, the minimum temperature for growth is that temperature at which the affinity of the transporter proteins for sequestration of those growth rate-limiting substrates taken up by active transport is so low that it is no longer able to meet the minimum maintenance requirements of the cell for that substrate, and it will then die. If the energy source is the growth rate-limiting substrate, this may be associated with loss of the ability, because of a consequent low energy charge within the cell, to maintain the transporter molecules in a charged state capable of taking up substrates. The minimum temperature for growth will vary greatly with different physiological types (psychrophiles to thermophiles), reflecting the broad range of temperature over which their membrane lipid composition adapts their membranes to be in a fluid state, but in all types it seems to be loss of efficient substrate sequestration that imposes the lower temperature limit.

Furthermore, there seems to be progressively decreased specific affinity for substrate with decreasing temperature below the growth optimum, rather than constant affinity across the growth temperature range with a sudden decrease near the minimum temperature for growth. This trend of steadily decreased affinity over a broad temperature range presumably reflects progressively decreasing functional efficiency of membrane transport as temperature declines, before uptake and growth finally becomes completely inhibited at the lower temperature limit. It does not indicate a sudden change of affinity near the minimum temperature that might be associated with a sudden phase change of the membrane.

It is possible to measure membrane fluidity with probes such as 1,6-diphenyl-1,3,5-hexatriene which are fluorescent only in a lipid phase, such as in the membrane. Usually the fluidity of the membrane is measured as anisotropy by fluorescence polarisation (e.g. [35,36]), which decreases as membrane fluidity increases. Where such fluorescent probes have been used with membrane preparations of bacteria there has been no evidence of sharp phase transitions with temperature (e.g. [36]), but rather a continuous change of membrane fluidity. This broad change seems to be associated with the diversity of lipids

in bacterial membranes, also interacting with the proteins embedded in the membrane. Fig. 4 illustrates changes of anisotropy with temperature measured in whole cells of *Escherichia coli* and a psychrophilic bacterium JC/CPS/2/5 [37]. There appears to be consistent trends of increased membrane fluidity over a broad range of temperature, rather than sharp phase transitions, reflecting the consistent trends of change of specific affinity with temperature measured in other bacteria. Bacterial auxotrophs with a less diverse range of membrane lipids tend to have sharper phase change temperatures [38].

7. Ecological implications of the interaction between temperature and affinity for substrates

Our observations of the relationship between temperature and specific affinity for organic and inorganic substrates provide a mechanism to explain the effects of substrate addition at low temperature on natural communities of marine bacteria [26–28]. The implication is that any substrate which is taken up by some form of active transport is likely to become increasingly less available as temperature decreases because the ability of an organism to sequester the substrate declines at low temperature. I have termed this a 'temperature-modulated substrate affinity' mechanism. That is, there is an interaction between specific affinity for a substrate and temperature, both influencing the kinetics of substrate uptake as temperature changes, and hence influencing bacterial growth and competition.

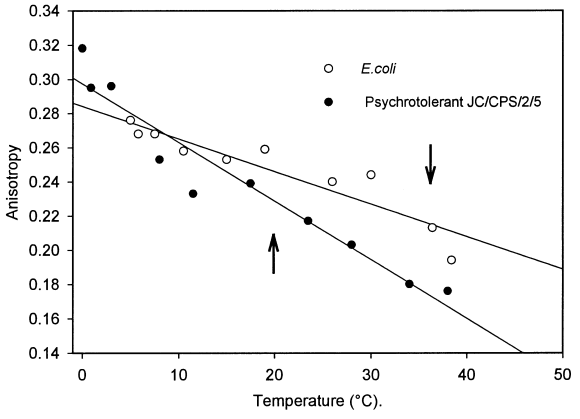

Fig. 4. Thermal dependence of 1,6-diphenyl-1,3,5-hexatriene anisotropy measured by fluorescence polarisation in intact cells of *E. coli* and a psychrotolerant JC/CPS/2/5.

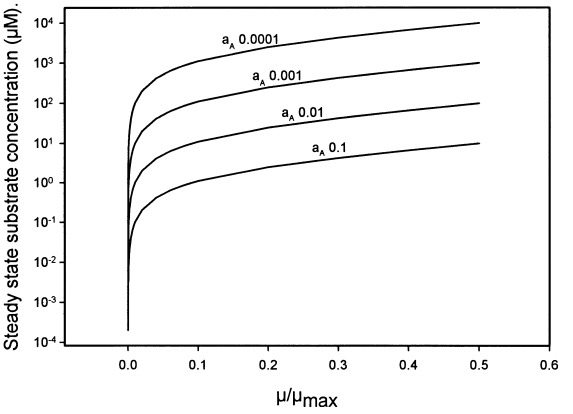

Fig. 5. Change of steady-state substrate concentrations with proportional growth rate (μ as a proportion of μ_{max}) at different specific affinity values for the substrate.

The minimum concentration at which an organism can remove substrate from its environment is a function of its affinity for the substrate. In general therefore, progressively larger pools of unavailable residual substrate will remain in an environment as temperature decreases, because the decreasing affinity of the organism(s) for the substrate at low temperature prevents any further uptake. We can derive a relationship between a_A, and growth rate, μ, as a proportion of the maximum specific growth rate, μ_m, which illustrates their effect on steady-state substrate concentration S_s.

$$K_s = S_s \frac{(\mu_{max} - D)}{D}$$

$$a_A = \mu_{max} / K_s$$

$$S_s = \frac{1}{a_A} \frac{\mu_{max} \cdot \mu}{(\mu_{max} - \mu)}$$

Fig. 5 illustrates the steady-state concentrations of substrate at different values of a_A and growth rate, for growth rates $< 0.5\ \mu_{max}$ where a_A is applicable. It is clear that at the low growth rates typical of most natural environments the steady-state substrate concentration is a function of both the affinity of the organism for the substrate and the growth rate of the organism. The steady-state substrate concentra-

tion is inversely related to a_A but directly related to growth rate. As a_A is also directly related to temperature, a corollary is that at low temperature the presence of even a relatively high concentration of substrate in the environment does not imply that the organisms present are not substrate-limited. Limiting nutrient concentrations may become much higher at the lower end of the temperature range of a species than at the high temperature end because of lower affinity at low temperature. Furthermore, inhibition of growth at low temperature because of low affinity may be reversed if higher concentrations of substrate are added to overcome the limitation on substrate availability by low affinity, as shown by Pomeroy et al. [26] and Wiebe et al. [27,28]. This emphasises the synergy between temperature and substrate concentration in controlling the availability of a substrate at low temperature. In effect, for any organotrophic microorganism, which in the vast majority of natural environments anyway exist under conditions of low substrate availability and severe energy limitation, low temperature exacerbates starvation because of decreased affinity for substrates, even when what may be considerable concentrations of substrates remain present in the environment. Any species which has a higher affinity than another for a substrate at a given low temperature will be able to sequester substrate more effectively, will tend to outcompete the other at low temperature, and therefore will be selected for by low temperature.

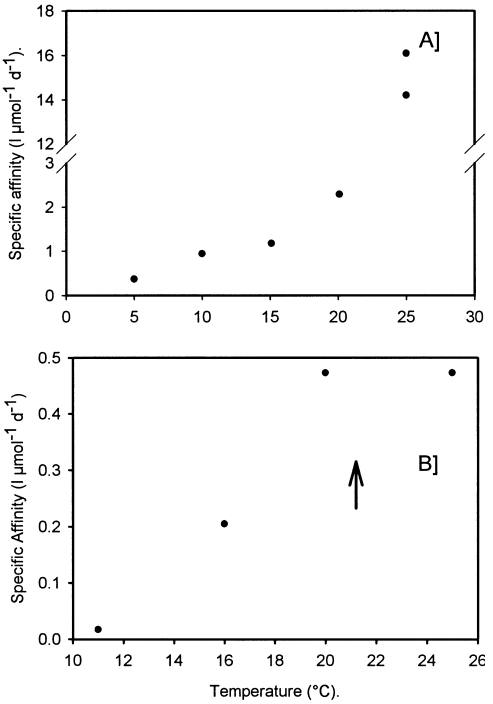

Fig. 6. Examples of the effect of temperature on specific affinity reported in the literature for (A) phosphorus in P-limited chemostats of the green unicellular alga *Scenedesmus quadricauda* [46] and (B) nitrate in N-limited chemostat cultures of *Scenedesmus* sp. [47]. Specific affinities have been calculated from values of μ_{max} and K_s reported at different temperatures. Arrow indicates optimum temperature for growth.

Any effect of low temperature on uptake of inorganic substrates may also have profound effects on primary production, and we would predict that, other things being equal, sequestration of any inorganic substrate taken up by membrane associated active transport would be inhibited at low temperature. Specific affinity for nitrate in a range of bacteria and algae decreases along with environmental temperature (Q_{10} about 3) but specific affinity for ammonium does not change significantly with temperature (Q_{10} about 1) [30,31]. This lower effect of temperature on uptake of ammonium compared to nitrate uptake may be because of passive uptake of at least some ammonia, but the difference will have profound effects on which source of nitrogen is utilised at low temperature. In the Southern Ocean during most of the summer growth season primary production is commonly supported by assimilation of ammonium rather than nitrate, despite the usually much higher concentrations of the latter [39,40]. The f ratios for nitrogen uptake are typically < 0.5, indicating predominant use of ammonium rather than nitrate. These observations are consistent with an affinity for nitrate significantly decreased by low temperature, to the extent that even relatively high concentrations of nitrate remain unavailable in the environment at low temperature. Assimilation of nitrate by algae may also be synergistically influenced by environmental factors other than temperature such as Fe availability [41], particularly as assimilation of nitrate requires more Fe than does assimilation of ammonium [42,43]. However, other things being equal, lowered temperature will exacerbate the difficulty of algae to sequester nitrate. Such decreased affinity at low temperature is less significant for ammonium uptake, and ammonium can there-

fore be sequestered to a much lower concentration than nitrate even at low temperature. Raven et al. [44] have pointed out that on a global basis more than half of the combined nitrogen assimilated during primary production on land and in the sea is ammonium, despite its generally lower concentration than nitrate in the oceans.

Although there is little published data on the uptake of other inorganic nutrients, a study of the effect of temperature on uptake of silicate by the ice alga *Pseudonitzschia seriata* [45] which measured both μ_{max} and K_s showed that (except for one anomalous point at 0°C) $a_{A(Si)}$ increased with temperature up to the alga's optimum temperature. Data for the green alga *Scenedesmus* (Fig. 6) revealed that, when replotted as a_A values, affinity for both phosphate [46] and nitrate [47] decreased with lowered temperature. Broecker and Peng [48] have argued also that phosphate uptake must be inhibited by low temperature in polar seas.

In conclusion, it seems that as environmental temperature drops below the optimum growth temperature for a species, there is reduction in its affinity for any substrates which are taken up by active transport processes. The most likely explanation of this phenomenon is that reduction in the fluidity of the membrane influences transporter molecules embedded in the membrane, presumably because 'stiffening' of the membrane by lowered temperature reduces transport protein efficiency. This reduced efficiency is exhibited as a progressive loss of affinity for the substrate as temperature decreases below the species' optimum temperature for growth. In effect, the minimum threshold concentration to which the substrate can be sequestered from the environment rises with decreasing temperature, leaving an increasing concentration of inaccessible substrate, exacerbating the tendency toward 'starvation' at low temperature.

8. Global implications

This physiological phenomenon may have profound effects on global ecology. Polar oceans at temperatures of −1 to 2–3°C have microbial communities, both bacterial and algal, which are physiologically stressed in the sense that the populations' environmental temperature is well below the optimum temperature for growth. The majority of the microbial community is of psychrotolerant types able to grow at 0°C but with optimum temperatures >20°C; while even the small proportion of obligate psychrophiles have optimum temperatures greater than the environmental temperature. Therefore, small changes in oceanic temperatures may have significant effects upon the affinity of these microorganisms for substrates, and hence on the effective size of the pools of nutrients taken up by active transport. Even slight oceanic warming during the interglacials would result in increased affinity by algae and bacteria for those substrates (nitrate, phosphate, silicate) taken up by active transport, and effectively increase the sizes of the available pools of these nutrients in the oceans. Nitrate has a much shorter residence time in the oceans (10^3–10^4 years) than does phosphorus and is therefore more likely to show glacial-interglacial variations [49]. This increase in available nutrients with higher temperature would be predicted to increase oceanic primary production and CO_2 drawdown during the interglacials. Such a scenario is consistent with the data from the profiles of $\delta^{13}C$ isotopic ratios in benthic foraminiferan tests in Southern Ocean sediment cores which suggest increased interglacial oceanic production, data which have hitherto not been explained [48]. However, it contradicts data from ice-cores which indicate increased CO_2 concentrations during interglacials. The phenomenon may also explain apparent decreases in water column denitrification rates during glacial periods [50]. As noted above, affinity for nitrate by denitrifying bacteria also decreases with temperature [24].

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