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Effect of temperature on the uptake of copper by the brine shrimp, *Artemia franciscana*

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Abstract

The effect of temperature on the uptake of copper by the brine shrimp *Artemia franciscana* has been studied in chemically defined saltwater solutions. Animals were acclimated to temperatures ranging from 10°C to 35°C and exposed to copper at different temperatures within this range. The results show that within each temperature acclimation group, copper uptake increases with increasing temperature of exposure. Within each temperature acclimation group most of the variation can be related to the combined effect of temperature on the chemical speciation and diffusion rate of copper in the diffusion layer lining the exchange surfaces. The magnitude of the apparent activation energy for copper uptake indicates that it is a facilitated diffusion process. The effect of the temperature of exposure on copper uptake decreases with increasing temperature of acclimation. The temperature acclimation effect is such that, except for the lowest temperature of acclimation, exposure to the temperature of acclimation has no effect on copper uptake. This indicates that temperature acclimation involves physiological alterations that compensate for the effect of an increasing temperature of exposure on the transport of the free cupric ion across the solution–body interface.

Key words: Copper; Uptake; Temperature; Brine shrimp; *Artemia franciscana*

1. Introduction

The uptake and toxicity of metals in aquatic organisms strongly depends on the environmental conditions. Temperature is an important factor influencing both the chemistry of the environment and the physiology of aquatic organisms (Davies and Tribe, 1969; Stumm and Morgan, 1981; Cossins and Bowler, 1987). Most studies indicate that the uptake and toxicity of metals increases with temperature, but the relative importance of chemical and physiological processes are poorly understood

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(Cairns et al., 1975; Phillips, 1976; Cotter et al., 1982; McLusky et al., 1986; Nugegoda and Rainbow, 1987).

Changes in temperature have an important effect on the extent of metal complexation by altering the equilibrium position of reactions and the solubility of gases. The most important effects are changes in the hydrogen ion activity and total carbonate concentration. These alterations influence the extent of metal hydroxide and carbonate complexation which are controlling factors in copper speciation. (Baes and Mesmer, 1981; Turner and Whitfield, 1987; Byrne et al., 1988).

Most aquatic organisms are poikilothermic, that is, they are unable to control their body temperature so that their physiology is highly dependent on ambient temperature. Variations in temperature influence many different physiological processes, most notably the many catalytic systems involved in transport and metabolism. Within limits, a temperature increase accelerates most of these processes. However, given ample time, many aquatic organisms can compensate for temperature changes by appropriate changes in their physiological organisation. (Hazel and Prosser, 1974; Somero, 1978; Burton, 1986).

The most important process in the uptake of metals by aquatic organisms is the transport of the metal across the external membranes of the exchange surfaces. The organisation and functioning of these interfaces are strongly influenced by the temperature of the environment. Thus, the effect of temperature on metal uptake is complex and the combined result of physical, chemical and biological processes. To determine the relative importance of these different processes for the effect of temperature on the uptake of metals, we have studied the uptake of copper by the brine shrimp, *Artemia franciscana* under different temperature acclimation and exposure regimes.

2. Material and methods

2.1. Brine shrimp

Dried *Artemia franciscana* cysts from Great Salt Lake, Utah, USA were purchased from San Francisco Bay Brand, Newark, CA, USA. Cysts were hatched in a funnel-shaped plastic container filled with synthetic seawater (Wiegandt, Krefeld, Germany), and aerated from the bottom. The hatching suspension was illuminated by a fluorescent light tube. Hatching cyst density was 5 g l^{-1}. *Artemia* nauplii were harvested after 36 h. The larvae were grown from nauplii to adult in 150 l plastic rectangular air-water lift operated race-ways filled with synthetic seawater. Brine shrimp were fed with a suspension of the dried algae *Spirulina*. Animals reached maturity after 3 to 4 weeks and were used before they were 8 weeks old. The methods for intensive culturing of brine shrimp have been described by Sorgeloos et al. (1983).

2.2. Experimental procedures

The uptake of copper by brine shrimp was followed during 3 h of exposure to the metal in a static test system. Copper uptake is linear with time during the short

exposure period in the static system, displaying first-order uptake kinetics (Blust et al., 1988a). Experiments were conducted in thermostated rooms at six different temperatures, ranging from 10 ± 0.5 to 35 ± 0.5°C. Fifteen days before an experiment was run adult brine shrimp were collected from a batch culture for temperature acclimation. Animals were gradually acclimated to solutions of differing temperatures over a 5-day period and kept at the final temperature for 10 days (i.e. 10, 15, 20, 25, 30 and 35°C). On the last day of the acclimation period the animals were transferred for 1 h to a saltwater solution containing 1 mM 8-hydroxyquinoline-5-sulfonic acid. This strong metal ligand, which is not acutely toxic to brine shrimp, was used to remove metal bound to the external surfaces of the shrimp. For the remaining period the animals were kept in clean saltwater for defecation. Copper uptake by brine shrimp was measured for all 36 temperature of acclimation and exposure combinations possible. This experimental design allows the separation of short-term exposure and long-term acclimation effects of temperature on metal uptake.

The composition of 1 l of the chemically defined saltwater solution with a salinity of 3.5% was 23.50 g NaCl, 4.00 g Na_2SO_4, 0.680 g KCl, 0.196 g $NaHCO_3$, 1.470 g $CaCl_2 \cdot 2H_2O$, 10.78 g $MgCl_2 \cdot 6H_2O$ and 0.026 g H_3BO_3. The medium was prepared by dissolving the seven analytical grade products (Merck p.a.) in deionised water. A dispersion of 0.1 mmol l^{-1} manganese dioxide was added to the seawater to remove metals present in the analytical grade reagents. After an equilibration period of 24 h, the dispersion was filtered through a 0.2 μm membrane filter to remove the manganese dioxide from the solution (Van den Berg and Kramer, 1979). The pH of the solutions was adjusted with HCl or NaOH as required and the media were aerated to promote equilibration of gases with the atmosphere. The dissolved oxygen concentration, the total dissolved carbonate concentration, the hydrogen ion activity and the redox potential were measured to ensure that equilibrium conditions had been established. Dissolved oxygen was measured with a membrane covered amperometric electrode system (WTW OX191/EO90). Total dissolved carbon dioxide was measured with a gas sensing CO_2 electrode (Ingold 152323000), after acidification of the water sample (pH < 4.8) in a sealed measuring vessel. The hydrogen ion activity was measured with a glass electrode (Ingold 104573002). Redox potentials were measured with a wire-type platinum electrode (Ingold, 105003077).

Cupric nitrate was added to the test solutions from a 100 μmol l^{-1} cupric ion stock. In all series of experiments the total concentration of copper in test solutions was 5 μmol l^{-1}. Experiments were carried out in 0.5 l plastic beakers. Just before an experiment started about 50 animals were collected on a 250 μm screen, rinsed with clean medium and transferred to a beaker. After 180 min the beaker was emptied over a 250 μm screen and the collected animals transferred to a beaker with a saltwater solution containing 1 mM 8-hydroxyquinoline-5-sulfonic acid. One hour later the beaker was emptied over a 250 μm screen and the collected animals were rinsed with deionised water and divided into five plastic vials, dried for 24 h at 60°C and stored in a dessication box until analysed for copper. This procedure was repeated five times using animals from separate cultures and newly prepared media and reagents. The dissolved oxygen concentration, the total dissolved carbonate concentration, the hydrogen ion activity, the redox potential and the total dissolved copper concentration

were measured at the beginning and end of an experiment. Generally, all measured values remained within 10% of the initial values.

2.3. Chemical modelling

The equilibrium activities of the chemical species considered were calculated using the computer program SOLUTION (Blust et al., unpublished) an adaptation of the program COMPLEX (Ginzburg, 1976). This speciation model allows the calculation of the composition of solutions in equilibrium with gas and solid phases. A thermodynamic stability constant data base was built which is based on the data of Dickson and Whitfield (1981) for the major components, and the data of Smith and Martell (1976, 1989) and Martell and Smith (1982) for copper. For each ion-pair or complex considered, the stability constants listed for different ionic strengths were fitted to an interpolation function which has the form of an extended Debye–Hückel equation (Turner et al., 1981). Enthalpies to describe the effect of temperature on the equilibrium position of the reactions were taken from Byrne et al. (1988) and Smith and Martell (1989). The thermodynamics of the carbon dioxide system were described using the model of Whitfield and Turner (1986). The thermodynamic stability constants and the effect of temperature and ionic strength on the conditional stability constants of all copper species included in the model are given in Table 1. Case specific input comprises the total concentrations of the metals and ligands in the solution, the free hydrogen ion activity (pH), redox intensity (pe), temperature and the gas phases that are maintained in equilibrium with the solution. Results of the chemical speciation calculations are expressed on the molar concentration scale and multiplied by the appropriate activity coefficients to obtain species activities.

2.4. Metal analysis

Copper was measured by Graphite Furnace Atomic Absorption Spectrophotometry using a Perkin-Elmer 703 Spectrophotometer fitted with a Heated Graphite Atomiser HGA-500 and a deuterium arc background corrector. The method of sta-

Table 1
Thermodynamic stability constants and concentration products for copper species

Species	Log K	Log K' (10°C)	Log K' (20°C)	Log K' (30°C)
Cu^{2+}	–	–	–	–
$CuCl^{+}$	0.40	–0.10	–0.04	0.01
$CuSO_4^0$	2.35	1.24	1.29	1.35
$CuOH^{+}$	6.01	5.67	5.63	5.59
$Cu(OH)_2^0$	11.75	10.63	10.77	10.89
$CuCO_3^0$	6.74	5.61	5.69	5.76
$CuHCO_3^{+}$	12.25	10.70	10.70	10.70
$Cu(CO_3)_2^{2-}$	10.75	9.27	9.27	9.27

K thermodynamic stability constant; K'conditional stability constant at ionic strength 0.614 mol l^{-1}.

bilised temperature platform atomisation was used (Slavin et al., 1983). Biological material was dissolved with concentrated nitric acid in a microwave oven and diluted with deionised water to a 10% nitric acid solution. Saltwater solutions were diluted ten times with a 10% nitric acid solution to decrease the salinity and analysed against matrix matched calibration standards (Blust et al., 1988b).

2.5. Statistical analysis

All sets of data were tested for homoscedasticity by the log-ANOVA test for homogeneity of variances and for normality by the Kolmogorov–Smirnov test for goodness of fit. Analysis of variance, linear and non-linear regression methods were used for analysing the data. Significance levels of tests are indicated by asterisks according to the following probability ranges ($*0.05 \geq P > 0.01$, $**0.01 \geq P > 0.001$, $***P \leq 0.001$). Statistical methods are as outlined in Sokal and Rohlf, (1981) and Glantz and Slinker, (1990).

3. Results

3.1. Chemical speciation of copper in saltwater

The speciation of copper in an inorganic chemically defined saltwater solution changes with the hydrogen ion activity in the solution and is controlled by the concentration of hydroxide and carbonate. The ion product of water decreases strongly with temperature so that the hydrogen and hydroxide ion activity in the solution change with temperature. The solubility of carbon dioxide and the conditional stability constants for the two carbonic acid equilibria (i.e. HCO_3^- and H_2CO_3) decrease with temperature. In an open system these different processes result in a decrease in the total carbonate concentration and an increase in hydrogen ion activity. As such, a temperature change alters the speciation of copper by simultaneously shifting the equilibrium position of the different metal complexation reactions and the concentrations of the most important inorganic ligands. The effect of these different processes on the free cupric ion activity was calculated using the chemical speciation model SOLUTION and the results are summarised in Fig. 1a. Depending on the degree of phase equilibration the pH ranges from 7.8 to 8.2 in the temperature range 10–35°C. Over this range of temperatures and hydrogen ion activities, the free cupric ion activity varies between 0.043 and 0.237 nmol cm^{-3} for a solution containing 5 nmol cm^{-3} of copper.

3.2. Diffusion of copper in saltwater

Temperature has an important effect on the free diffusion of solutes. The Stokes–Einstein relation $D = RT/(6\pi nrN)$ provides an approximate expression for the diffusion coefficient (D) of a spherical solute if the hydrated radius (r) of the solute and the viscosity (n) of the solution are known (Robinson and Stokes, 1970). The equation

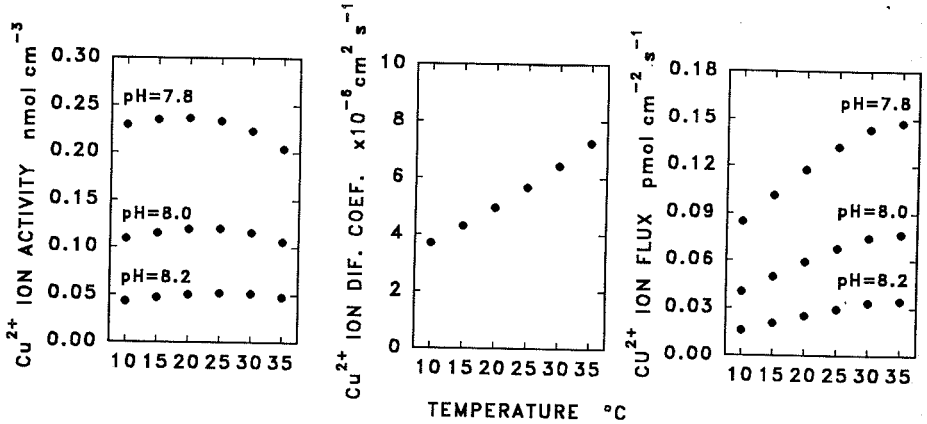

Fig. 1 (a) Effect of temperature on the activity of the free –cupric ion in the saltwater solution. (b) Effect of temperature on the diffusion rate of the free cupric ion in the saltwater solution. (c) Effect of temperature on the diffusional flux of the free cupric ion in the saltwater solution for a diffusion distance of 100 μm (Cu_{Total} = 5 μmol l^{-1}, sal = 3.5%).

predicts that the diffusion coefficient is inversely proportional to the solute radius and the viscosity of the solution. The hydrated radius can be obtained from the effective size of the solvated ion given by the molar hydrated volume of the solute sphere: $V = Nk(4\pi/3)r^3$ with N is Avogadro's number, k the solvent packing coefficient, and r the hydrated radius. The molar volume of the cupric ion with an hydration number of 10.3 is 147.8 cm^3 mol^{-1} for an infinite dilute aqueous solution of 25°C (Marcus, 1985). This results in an estimate for the radius of the hydrated ion of 404 pm for a water packing coefficient of k = 0.888. Given the effect of temperature on the viscosity of the saltwater solution and the radius of the solute, the diffusion coefficient of the cupric ion can be calculated in function of the temperature. The results of these calculations are summarised in Fig. 1b. Over the temperature range 10–35°C, the diffusion coefficient increases from 3.70 to 7.23 10^{-6} cm^2 s^{-1}.

3.3. *Diffusional flux of copper in saltwater*

The diffusional flux (J in mol cm^{-2} s^{-1}) is the amount of solute (mol) which crosses a plane of unit area (cm^2) perpendicular to the direction of flow in unit time (s). According to Fick's first law of diffusion for one-dimensional flux ($J = -D\,\Delta C/\Delta x$), the net rate of diffusion is proportional to the diffusion coefficient (D in cm^2 s^{-1}), the concentration difference (ΔC in mol cm^{-3}), and the diffusion distance (Δx in cm). When the solution is not ideal, such as seawater, the concentration difference must be expressed in terms of activity, i.e. the product of the concentration and the activity coefficient (Friedman, 1986). Hence, the effect of temperature on the initial flux of the cupric ion is given by the product of the activity in the water and the diffusion coefficient divided by the diffusion distance. Thus, flux is inversely proportional to the diffusion distance which is determined by the thickness of the unstirred layer adjacent to the exchange surface. The results of the calculations concerning the effect

of temperature on diffusional flux are summarised in Fig. 1c. Over the temperature range 10–35°C the diffusional flux of the cupric ion in the solution increases from 0.016 to 0.147 pmol cm^{-2} s^{-1} for an arbitrary diffusion distance of 100 μm. These results are further used to determine the effect of changes in the chemical speciation and diffusion of copper with temperature on the rate of copper uptake by brine shrimp.

3.4. *Uptake of copper by brine shrimp*

The effect of temperature on the uptake of copper by brine shrimp has been studied for six different temperature acclimation groups and six different temperature exposure groups. This experimental design makes it possible to separate the effect of temperature acclimation from the effect of temperature exposure on the uptake of copper by the organism. To minimise the effect of temperature on the speciation of copper, the hydrogen ion activity was kept constant in all treatment groups (pH 8.0 ± 0.1). The results summarised in Figs. 2a–f and 3a–f show that within each temperature acclimation group, copper uptake increases with increasing temperature of exposure. The effect of the temperature of exposure on copper uptake decreases

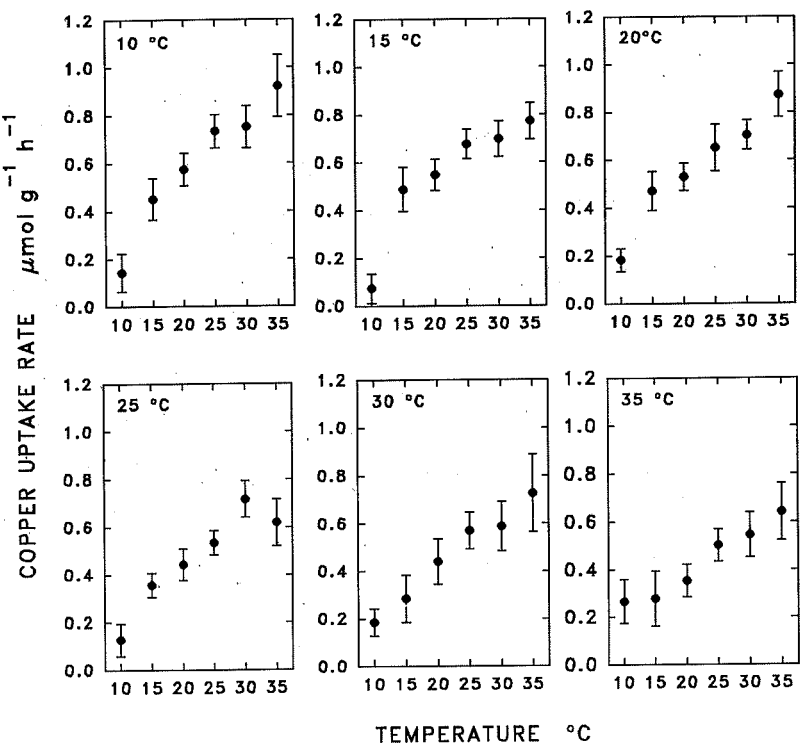

Fig. 2. Effect of the temperature of exposure on the rate of copper uptake by brine shrimp for the different temperature acclimation groups (Cu_{Total} = 5 μmol l^{-1}, sal = 3.5‰, pH = 8.0 ± 0.1). Means with standard deviations for five replicates are significantly different within groups (ANOVA: $p < 0.001$).

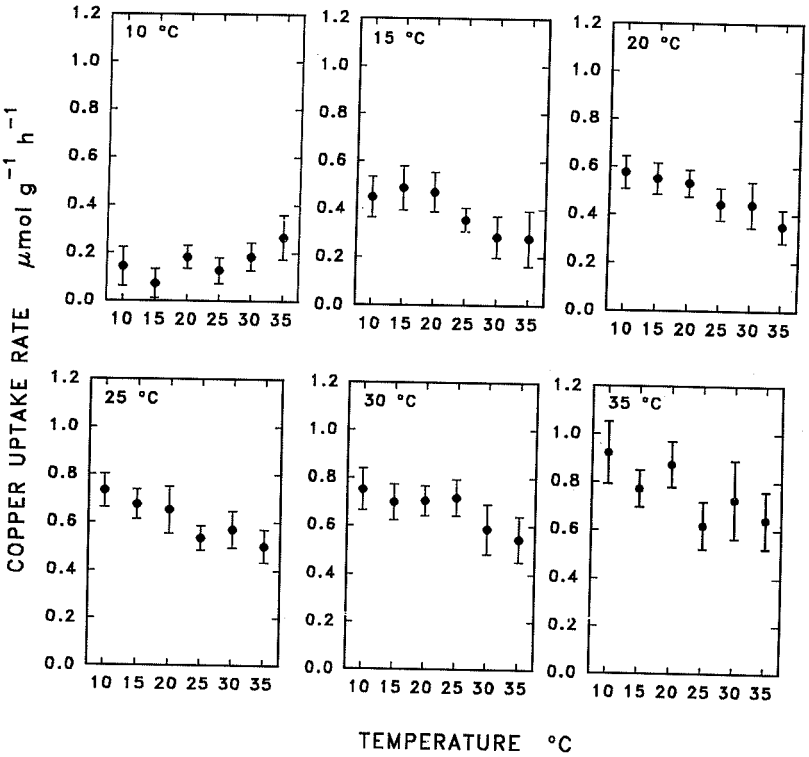

Fig. 3. Effect of the temperature of acclimation on the rate of copper uptake by brine shrimp for the different temperature exposure groups (Cu_{Total} = 5 μmol l^{-1}, sal = 3.5%, pH = 8.0 ± 0.1). Means with standard deviations for five replicates are significantly different within groups (ANOVA: $p < 0.01$).

with increasing temperature of acclimation. Thus, among the exposure groups, copper uptake decreases with increasing temperature of acclimation. The results of the analysis of variance for the effect of exposure and acclimation to the different temperatures on copper uptake are given in Table 2. Both the effects of the temperature of exposure and the temperature of acclimation on copper uptake by brine shrimp are highly significant.

Table 2
Two-way analysis of variance for the effect of the temperature of exposure and the temperature of acclimation on copper uptake by the brine shrimp (36 treatment groups with five replications)

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	F_s
Exposure	5	7.00	1.40	195.50***
Acclimation	5	0.66	0.13	18.38***
Interaction	25	0.58	0.023	3.25***
Error	144	1.03	0.0072	
Total	179	9.27		

To determine the relative importance of the different factors that contribute to the variation in copper uptake by the brine shrimp with temperature a non-linear regression model was constructed which considers the effects of physical, chemical and physiological processes on the rate of copper uptake. The mathematical description of the model has the form of a non-linear equation:

$$Cu_{uptake} = C_f * Cu_{act}^{2+} * Cu_{dif}^{2+} * T_{exp}^{k} * T_{acl}^{l}$$

The first factor in the regression model accounts for the effect of temperature on the activity of the free cupric ion (Cu_{act}^{2+}). The second factor accounts for the effect of temperature on the diffusion coefficient of the free cupric ion (Cu_{dif}^{2+}). The third factor accounts for the effect of the temperature of exposure on the rate of copper uptake (T_{exp}^{k}). The fourth factor accounts for the effect of the temperature of acclimation on the rate of copper uptake (T_{acl}^{l}). The two last factors are exponential, where k and l are temperature coefficients, which are negative when the rate of copper uptake decreases with temperature and positive when the rate of copper uptake increases with temperature. To relate the product of these four factors to copper uptake, it is necessary to introduce a coefficient of proportionality (C_f), which relates the activity of the cupric ion in the solution to the rate of copper uptake by the brine shrimp. The relative importance of the different factors was determined by the forward selection procedure, starting with a single factor and then add factors one at a time and evaluate the effect on the coefficient of determination of the regression model.

Table 3
Copper uptake rate in brine shrimp

Variable	B	SE	L_1	L_2
(1) $Cu_{uptake} = C_f * Cu_{act}^{2+}$ ($R^2 = 0.421$***, $n = 210$)				
C_f	4.478***	0.137	4.209	4.747
(2) $Cu_{uptake} = C_f * Cu_{act}^{2+} * Cu_{dif}^{2+}$ ($R^2 = 0.769$***, $n = 210$)				
C_f	0.863***	0.016	0.832	0.894
(3) $Cu_{uptake} = C_f * Cu_{act}^{2+} * Cu_{dif}^{2+} * T_{exp}^{k}$ ($R^2 = 0.840$***, $n = 210$)				
C_f	0.213***	0.036	0.142	0.284
Exponent (k)	0.434***	0.050	0.336	0.532
(4) $Cu_{uptake} = C_f * Cu_{act}^{2+} * Cu_{dif}^{2+} * T_{exp}^{k} * T_{acl}^{l}$ ($R^2 = 0.883$***, $n = 210$)				
C_f	0.455***	0.076	0.306	0.604
Exponent (k)	0.437***	0.043	0.353	0.521
Exponent (l)	−0.255***	0.029	0.198	0.312

Non-linear regression analysis of the pooled data: B partial regression coefficients; SE standard error of partial regression coefficients; L_1, L_2 confidence limits of partial regression coefficients. Free cupric ion activities in the solution are in μmol l^{-1}, copper uptake rates in brine shrimp are in μmol g^{-1} h^{-1}.

The results of the non-linear regression analysis are summarised in Table 3. Starting with the factor which accounts for the effect of the temperature of exposure on the activity of the free cupric ion in the solution explains 42.1% of the variation in copper uptake. Adding the factor which accounts for the biological effects of the temperature of exposure on the diffusion rate of the cupric ion in the solution explains 76.9% of the variation in copper uptake. Adding the factor which accounts for the biological effects of the temperature of exposure on the rate of copper uptake by brine shrimp explains 84.0% of the variation in copper uptake. The coefficient for the effect of the temperature of exposure on the rate of copper uptake is positive, which indicates that the rate of copper uptake increases with the temperature of exposure. Adding the factor which accounts for the biological effects of the temperature of acclimation on the rate of copper uptake by brine shrimp explains 88.9% of the variation in copper uptake. The coefficient for the effect of the temperature of acclimation on the rate of copper uptake is negative, which indicates that the rate of copper uptake decreases with the temperature of acclimation. Overall, the temperature acclimation effect is such that, except for the lowest temperature of acclimation, exposure to the temperature of acclimation has no effect on copper uptake. Thus, although copper uptake increases with the temperature of exposure this effect is largely set-off by the effect of the temperature of acclimation.

4. Discussion

The effect of temperature on biological processes is primarily caused by changes in reaction rates and/or changes in the position of equilibria. The temperature dependence of a reaction can be described by the Arrhenius equation: $k = A \exp[-E_a/(RT)]$, where k is the velocity constant of the reaction, A is a constant related to the collision frequency of molecules, E_a is the activation energy, R is the gas constant, and T the absolute temperature. A plot of the natural logarithm of the rate against reciprocal temperature yields a straight line with a slope of E_a/R (Alexandrov, 1977, Bowler and Fuller, 1987, Cossins and Bowler, 1987). The relation between reciprocal temperature of exposure and the natural logarithm of the rate of copper uptake is linear over the temperature range 15–35°C, in all but one case as shown in Fig. 4. The apparent activation energy for copper uptake depends on the temperature of acclimation but a general trend is not apparent as shown in Table 4. A number of plots show a break, with the activation energy larger at the lowest than at the higher temperatures. These breaks can be interpreted as transitions from one rate-limiting step to another, each with different activation energies, so that at low temperatures a reaction with a high activation energy is rate-limiting, whilst at higher temperatures a reaction with a lower activation energy is rate-limiting. The breaks in the curves disappear with increasing temperature of acclimation, indicating that the acclimation conditions alter the temperature response of the transport system.

The diffusion of the free cupric ion has a temperature dependence characterised by a low activation energy of 19 kJ K^{-1} mol^{-1}. A typical value for physical processes which are not very temperature sensitive. The activation energy for copper uptake by

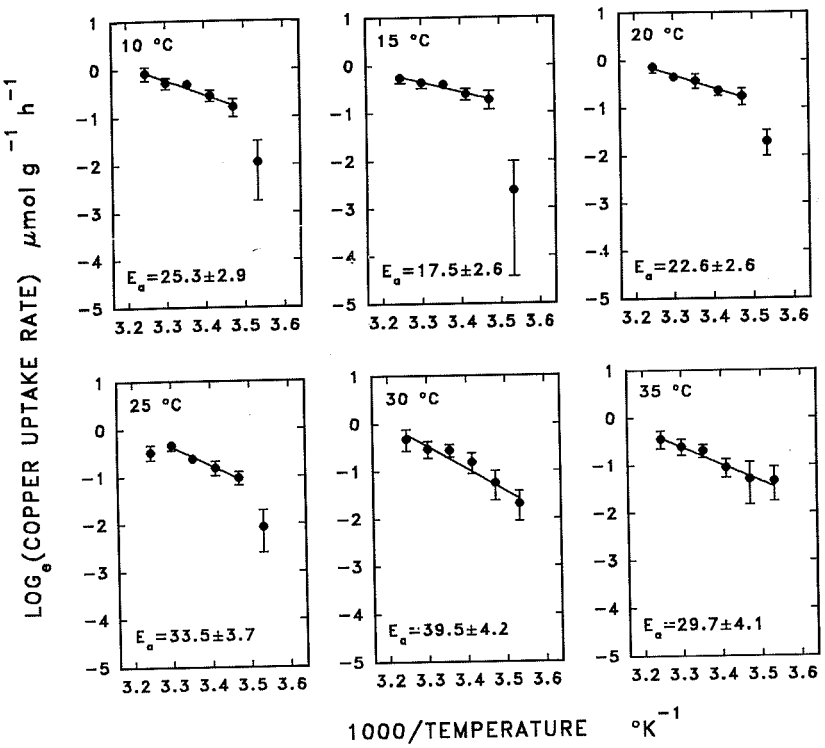

Fig. 4. Arrhenius plots for the effect of temperature of exposure on the rate of copper uptake by brine shrimp for the different temperature acclimation groups (Cu_{Total} = 5 µmol l^{-1}, sal = 3.5‰, pH = 8.0 ± 0.1). The fit of the regression line to the data is significant for all groups ($p < 0.001$), there are no significant deviations from regression ($p > 0.05$) and the slopes of the regression lines are significantly different ($p < 0.001$).

brine shrimp varies with the temperature of acclimation and ranges from 17.5 to 39.5 kJ K^{-1} mol^{-1}. The metabolic activity of brine shrimp has a temperature dependence characterised by a high activation energy of 49 kJ K^{-1} mol^{-1} at 25°C (Decleir et al, 1980). Thus, the apparent activation energy for the uptake of copper by brine shrimp is equal or higher than the activation energy for diffusion of the free cupric ion in the

Table 4
Apparent activation energy (E_a) for the effect of the temperature of exposure on the uptake of copper by brine shrimp

Acclimation	E_a (kJ mol^{-1})	SE	R^2	n
10°C	25.27***	2.91	0.766***	25
15°C	17.46***	2.61	0.661***	25
20°C	22.59***	2.59	0.768***	25
25°C	33.45***	3.69	0.821***	20
30°C	39.50***	4.21	0.759***	30
35°C	29.71***	4.14	0.648***	30

solution but considerably less than the activation energy for metabolic activity. Activation energies for the diffusion of chemical species in water are between 17 and 21 kJ mol^{-1}. Activation energies for passive and facilitated diffusion of chemical species across membranes are between 18–38 kJ mol^{-1}. These activation energies are generally larger than for diffusion in water which is due to the fact that the activation energy for movement across membranes includes the activation energy of both the membrane binding and membrane transport process (Stein; 1986, 1990; Hille, 1991). Diffusion processes that are of physiological significance take place over short distances, usually micrometers or less. Over longer distances transport takes place by other processes such as hydraulic flow or convection. Consequently, diffusional transport only assumes a significant role near membrane surfaces, where the presence of an unstirred layer may limit the rate of transfer of solutes to the membrane. The thickness of the unstirred layer depends on the physical structure of the exchange surface and may range from one to several hundreds of micrometers. Thus, if the metal is rapidly transported across the membranes of the exchange structures the rate of movement in the unstirred layers may represent the major barrier to the uptake of the metal (Barry and Diamond, 1984; Stein, 1990).

In each temperature exposure group copper uptake decreases with increasing temperature of acclimation. As a result there is no effect of temperature on the rate of copper uptake when the brine shrimp are acclimated to the temperature of exposure. Only for brine shrimp exposed to the lowest temperature of acclimation does the rate of copper uptake remain low. This shows that temperature acclimation influences copper uptake so that similar transport rates are maintained at different temperatures. Acclimation is not complete at the lowest temperature, which reflects the physiological inability to fully compensate for the effect of the temperature change.

The decreased rate of copper uptake at the lowest temperature may be the result of the effect of low temperatures on membrane transport processes. Membrane phospholipids are packed in a regular array, head-to-head, shoulder to shoulder, and tail-to-tail. Such arrays of phospholipids are crystalline but they can be ordered or disordered. The ordered bilayer melts as the temperature is raised through a critical temperature range over which the phase transition occurs. A protein (e.g. transport system) present in such a bilayer may well display quite different kinetic properties when it is surrounded by phospholipids in an ordered, in contrast to a fluid, state. As the temperature of a membrane is lowered, the membrane phospholipids undergo a thermal transition from a highly fluid state to a much more rigidly ordered state. (Overath and Thilo, 1978; Stein; 1986, 1990). This process often alters the functionality and temperature sensitivity of membrane embedded systems and may explain the break in the Arrhenius plots of the effect of temperature on copper uptake by brine shrimp.

In summary, it has been shown that the uptake of copper by brine shrimp increases with the temperature of exposure and decreases with the temperature of acclimation. The temperature of exposure effect can be related to the effect of temperature on the activity and diffusion rate of the cupric ion in the diffusion layer lining the exchange surfaces. The magnitude of the apparent activation energy for copper uptake indicates that it is a facilitated diffusion process. Temperature acclimation appears to

involve a number of physiological alterations which, within certain limits, compensate for the effect of an increasing temperature of exposure on the transport of copper across the exchange surfaces.

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LITHOSTRATIGRAPHY OF THE EGEM MEMBER (YPRESIAN) SOUTH-EAST OF THE GENT AGGLOMERATION (BELGIUM)

I. BOLLE & P. JACOBS[1]

For the construction of the southern part of the Ring-canal around Gent and the Merelbeke lock, an intense soil investigation programme was carried out in the 1940's, consisting of cable-tool drilling with disturbed and undisturbed sampling and of cone penetration tests. The results of these investigations showed the Egem Member (Yd of the geological map) between the Aalbeke (Yc) and the Merelbeke Member (Plm) to be characterized by an alternation of fine sandy and clayey sediment packages (1).

Between 1975 and 1990 a great number of deep cone penetration tests (up to 30 m) were carried out for the soil mechanical maps of the Gent region. Especially the cone resistance versus depth logs confirmed the alternation of the fine sandy and clayey units within the Egem Member (fig. 1). Laboratory analyses (grain-size e.g.) of both disturbed and undisturbed samples enabled the division of the Egem Member into three sandy and three clayey deposits (2, 3, 4).

Hydrogeological investigations in the 1980's with direct rotary drilling and geophysical borehole logging to characterize the different aquifers, confirmed the alternation of sandy and clayey layers within the Egem Member (5, 6). Especially resistivity and point-resistance logging showed very characteristic patterns (fig. 2).

Cross-sections, particle size distribution curves, plasticity index versus liquid limit diagrams, cone penetration tests and borehole logs led to the following division of the Egem Member southeast of Gent (from bottom to top):

- unit Yd1c: alternation of thin stiff clay layers and fine sandy clay layers (perhaps to be correlated with the Kortemark Member); thickness up to 15 m
- unit Yd2: very dense packed fine sand with glauconite; very high cone resistance values; thickness of about 5 m
- unit Yd3: 1 to 2 m thick stiff clay
- unit Yd4a: up to 10 or 15 m fine sand with glauconite and shells (Nummulites)
- unit Yd4b: sandstone layer of about 0,5 m thick
- unit Yd5: stiff clay to sandy clay; thickness about 3 m
- unit Yd6: fine sand with glauconite and shells, up to 10 m thick.

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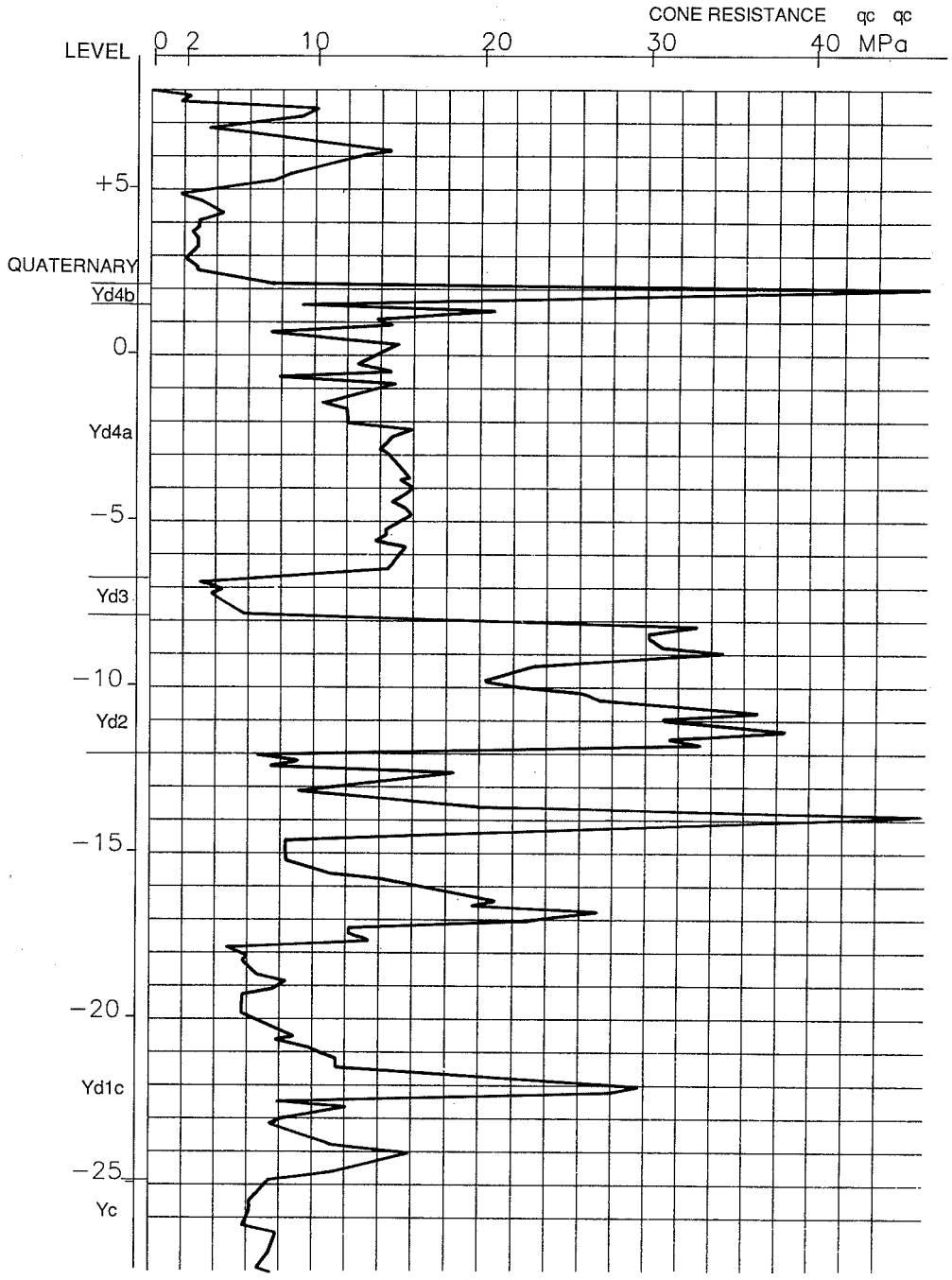

Figure 1. Cone penetration test in the Egem Member.

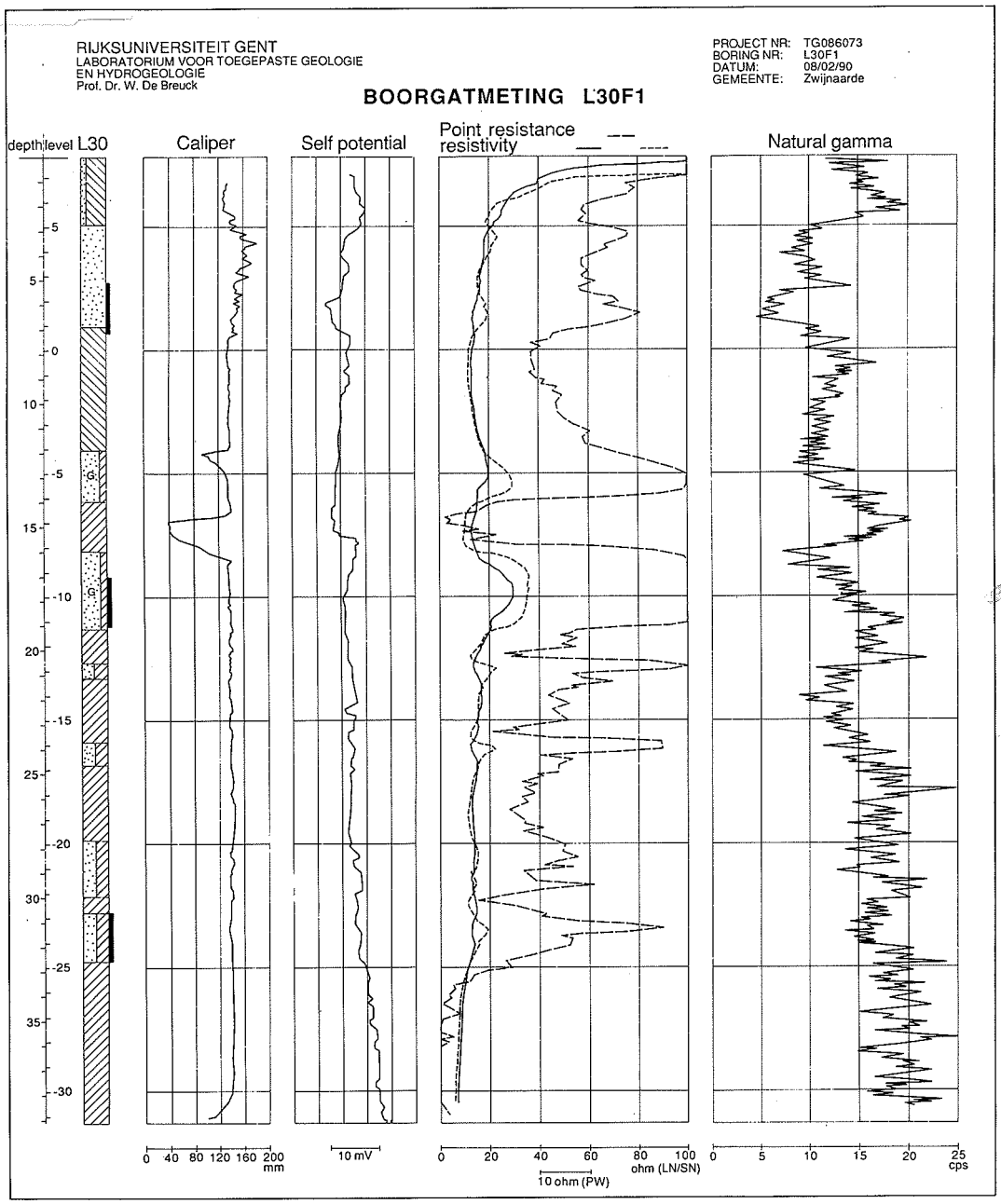

Figure 2. Borehole logs in the Egem Member.