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HAEMOLYMPH Mg^{2+} REGULATION IN DECAPOD CRUSTACEANS: PHYSIOLOGICAL CORRELATES AND ECOLOGICAL CONSEQUENCES IN POLAR AREAS

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Summary

Reptant decapod crustaceans are almost absent from the Southern Ocean south of the Antarctic Convergence. We tested the hypothesis that this may be due to the reduced ability of this group to regulate Mg^{2+} levels in the haemolymph ($[Mg^{2+}]_{HL}$). Mg^{2+} acts as an anaesthetic in marine invertebrates and its level is higher in Reptantia (crabs such as *Cancer* spp., *Chionoecetes* spp., *Maja* spp., 30–50 mmol l^{-1}) than in Natantia (prawns such as *Pandalus* spp., *Palaemon* spp., *Crangon* spp., 5–12 mmol l^{-1}).

We varied $[Mg^{2+}]_{HL}$ in three species of reptant decapod crustaceans, *Carcinus maenas*, *Hyas araneus* and *Eurypodius latreillei*, and investigated heart rate, the rate of oxygen consumption and levels of spontaneous and forced activity at different temperatures. The rate of oxygen consumption and heart rate increased significantly with reduction in $[Mg^{2+}]_{HL}$ over the entire temperature range investigated in *E. latreillei*. In *H. araneus*, an increase in metabolic and heart rates compared with control values was found only at temperatures below 2 °C. Forced and spontaneous activity levels increased significantly in the group of $[Mg^{2+}]_{HL}$-reduced animals below 0 °C, at which control animals were mostly inactive. At a reduced $[Mg^{2+}]_{HL}$ of 5–12 mmol l^{-1}, which is the $[Mg^{2+}]_{HL}$ of caridean shrimps in the Southern Ocean, Q_{10} and activation energy were reduced for all these variables and extended the temperature range over which physiological functions were maintained.

We suggest that the high $[Mg^{2+}]_{HL}$ in Reptantia causes relaxation of the animals and reduces their scope for activity, especially at temperatures below 0 °C. The hypothesis that the synergistic effects of high $[Mg^{2+}]_{HL}$ and low temperature probably prevented the Reptantia from recolonizing the permanently cold water of polar areas is discussed.

Key words: anaesthetic, *Carcinus maenas*, *Chorismus antarcticus*, Crustacea, Decapoda, *Eurypodius latreillei*, heart rate, *Hyas araneus*, Mg^{2+}, *Notocrangon antarcticus*, oxygen consumption, Q_{10}, relaxation, scope for activity, thermal limit.

Introduction

Crustacean haemolymph is more or less isoionic to sea water except for the levels of Mg^{2+} (Robertson, 1960; Mantel and Farmer, 1983). Shrimps regulate haemolymph levels of Mg^{2+} ($[Mg^{2+}]_{HL}$) at 5–12 mmol l^{-1}, far below the Mg^{2+} concentration of sea water (53 mmol l^{-1}). In contrast, most Reptantia (Brachyura, Anomura, Palinura, Astacidea) show higher values of $[Mg^{2+}]_{HL}$ of between 30 and 50 mmol l^{-1} (Walters and Uglow, 1981; Tentori and Lockwood, 1990; present study). This difference could be the result of different Mg^{2+} excretion systems. While the ablation of the eyestalk of *Palaemon serratus* has a significant effect on the regulation of Mg^{2+} levels (Franklin et al., 1978), this is not the case in *Cancer magister* (Holliday, 1980). Holliday (1980) concludes that there is a 'fundamental difference in the nature of control of magnesium' between the two groups of crustaceans. Since then, some studies have addressed the neuroendocrine control of Mg^{2+} regulation (McNamara et al., 1990, 1991); however, the mechanisms of Mg^{2+} excretion and their differences between the two groups remain unexplored.

Mg^{2+} is an effective anaesthetic in marine invertebrates (Pantin, 1946), and $[Mg^{2+}]_{HL}$ is correlated with the level of activity in different species of decapod crustaceans (Robertson, 1953; Walters and Uglow, 1981) and in the amphipod *Talitrus saltator* (Spicer et al., 1994). Similarly, activity and Mg^{2+} levels appear to be correlated in the snail *Helix pomatia* (Spicer et al., 1994) and even in a variety of vertebrates despite low blood Mg^{2+} contents (Kayser, 1961). In almost all these species, low activity levels in general, or seasonal decreases in activity (e.g. during hibernation), are correlated with elevated $[Mg^{2+}]_{HL}$ (or extracellular Mg^{2+} concentration, $[Mg^{2+}]_e$) while higher activity levels are correlated with lower levels of $[Mg^{2+}]_{HL}$ (or $[Mg^{2+}]_e$). Accordingly, Robertson (1953)

characterized decapod species such as *Dromia vulgaris*, *Lithodes maja* and *Maja squinado*, with a $[Mg^{2+}]_{HL}$ of approximately 50 mmol l^{-1}, as 'living in a semi-narcotized state'.

These characteristics of $[Mg^{2+}]_{HL}$ regulation have stimulated us to consider its potential influence on the geographical distribution of decapod crustaceans at low temperature. This group occurs in nearly every aquatic habitat of the world. The group Reptantia, however, is largely absent from the Southern Ocean, south of the Antarctic Convergence (Yaldwyn, 1965). In contrast, the Natantia (Caridea, Penaeidea) are represented by 24 different species, 12 pelagic and 12 benthic (Arntz and Gorny, 1991; Arntz et al., 1994; Gorny, 1999), and some of the latter reach densities of 1–5 specimens m^{-2} (Gutt et al., 1991).

There is evidence that the effect of different anaesthetics, such as halothane, enflurane and ethanol, is enhanced at low temperatures (McKenzie et al., 1992). The same has been reported for the effects of Mg^{2+} in the water flea *Daphnia magna* (Lagerspetz and Tiiska, 1996). The combined effects of high $[Mg^{2+}]_{HL}$ and constantly low water temperature below 0 °C could have prevented the Reptantia from colonizing the shelf areas around the Antarctic continent. Similary, these relationships may explain why reptant decapods are also absent from areas with extremely low water temperatures in the high Arctic.

The present study was designed to investigate the physiological functions affected by Mg^{2+} at different temperatures in various species of crab. *Eurypodius latreillei* (Guerin) and *Hyas araneus* (L.) are two reptant species that live close to the low-temperature limit of this group. *E. latreillei* is, together with *Peltarion spinosulum* (White), the southernmost reptant representative (Gorny, 1999; Gorny and Frederich, 1998). *H. araneus* lives between the temperate zone of the North Sea and the Arctic waters of the Barents and Kara Seas (Christiansen, 1969). *Carcinus maenas* (L.) was included as an active reptant species that regulates $[Mg^{2+}]_{HL}$ far below the seawater concentration but still at levels higher than those of most shrimps. For an analysis of the limiting effects of Mg^{2+}, we studied the rate of oxygen consumption, heart rate, spontaneous activity and the capacity to react to experimental stimulation at different temperatures and Mg^{2+} levels.

Materials and methods

Animals

Adult *Hyas araneus* and *Carcinus maenas* were obtained from the Biologische Anstalt Helgoland, Germany. *Eurypodius latreillei* were collected in the Magellan Strait with Agassiz trawls in November 1994 during a cruise of RV *Victor Hensen* (Arntz et al., 1996) and then transferred to the Alfred-Wegener-Institute, Bremerhaven, Germany. In November 1997, further animals were collected by SCUBA divers in Bahía Laredo near Punta Arenas, Chile. Experiments were carried out at the Alfred-Wegener-Institute and the Instituto de la Patagonia, Universidad de Magallanes in Punta Arenas. The animals were held in large aerated aquaria with recirculating natural sea water at 5 °C for at least 2 weeks prior to the start of the experiments. They were fed twice a week with pieces of *Mytilus* sp. or *Crangon crangon*. Only animals in the intermoult stage, indicated by a hard exoskeleton, were used.

During cruise ANT XV/3 of RV *Polarstern* between January and March 1998, the Antarctic caridean shrimps *Chorismus antarcticus* (Pfeffer) and *Notocrangon antarcticus* (Pfeffer) were collected from bottom trawls in the Weddell Sea (37°38.0′S, 22°14.6′W, depth 437 m) and close to the Antarctic Peninsula (62°16.9′S, 58°42.3′W, depth 375 m) to obtain samples for an analysis of $[Mg^{2+}]_{HL}$.

Mg^{2+} incubation

Since *H. araneus* and *E. latreillei* are only very poor Mg^{2+} regulators ($[Mg^{2+}]_{HL}$ is 36–48 mmol l^{-1} in *E. latreillei* and 39–51 mmol l^{-1} in *H. araneus*), it is easy to modify $[Mg^{2+}]_{HL}$ by changing the Mg^{2+} content of the artificial sea water used for incubations (Aquarium Systems, Sarrebourg, France; ion composition in mmol l^{-1}: Na^+, 487; K^+, 10; Ca^{2+}, 10; Cl^-, 490; SO_4^{2-}, 27; Mg^{2+}, 6–12 added as $MgCl_2$, pH 8.0). Despite regulating $[Mg^{2+}]_{HL}$ at 12–20 mmol l^{-1}, *C. maenas* also reduces $[Mg^{2+}]_{HL}$ during incubation in Mg^{2+}-reduced sea water.

Haemolymph samples from the animals were usually obtained by inserting a syringe needle into the articular membrane at the coxa of the last walking leg. For repeated sampling, haemolymph was taken through a small hole drilled in the carapace above the heart. The hole was covered with a latex dam to prevent loss of haemolymph. Samples of approximately 30 µl were taken at regular intervals. In an initial experiment, eight specimens of *H. araneus* were incubated in artificial sea water containing 6 mmol l^{-1} Mg^{2+}. $[Mg^{2+}]_{HL}$ was estimated using a photometric assay (Merckotest Magnesium, Merck, Darmstadt, Germany). After 3 days of incubation, $[Mg^{2+}]_{HL}$ remained constant at 8 mmol l^{-1}. In further experiments, animals were incubated for at least 3 days before starting the experiments.

Rates of oxygen consumption

The rate of oxygen consumption was measured according to the method of De Wachter et al. (1997) using a polarographic oxygen electrode (Eschweiler, Kiel, Germany) in a flow-through respirometer. The respirometer was filled with filtered (pore size 0.45 µm) natural (53 mmol l^{-1} Mg^{2+}) sea water for control animals or with filtered artificial (6 mmol l^{-1} Mg^{2+}) sea water for $[Mg^{2+}]_{HL}$-reduced specimens. Temperature was decreased in steps of approximately 2 °C per night. All animals were allowed more than 12 h to adapt. Rates of oxygen consumption were recorded for more than 90 min per animal. The temperature range investigated was between 8.1 °C and 0 °C for *E. latreillei* and between 9 °C and −1.5 °C for *H. araneus*. *E. latreillei* started to die at 0 °C. P_{O_2} in the outflowing water from the respirometer chambers never dropped below 16 kPa. Mean rates of oxygen consumption were calculated as nmol O_2 min^{-1} g^{-1} fresh mass.

Heart rate

Heart rate was recorded using the non-invasive photoplethysmograph technique introduced by Depledge (1984). The photosensor (isiTEC, Bremerhaven, Germany) was fixed to the carapace of the crabs using cyanoacrylate glue and dental periphery wax, and connected to a computer system (MacLab, AD Instruments, Australia). The animals were kept in darkened aquaria for 24 h at 8 °C. This acclimation period was sufficient for resting heart rates to be achieved. Control animals were incubated in natural sea water ([Mg^{2+}] 53 mmol l^{-1}), while a second group of crabs was incubated in Mg^{2+}-reduced sea water ([Mg^{2+}] 12 and 6 mmol l^{-1}). In some experiments, the temperature was lowered in a stepwise manner to 7.2, 5.0, 2.0, 0 and −1.2 °C. Heart rate was evaluated over a period of 2 h, approximately 12 h after the new temperature had been reached. In other experiments, the temperature was decreased continuously over 9 h from 8 °C to −1.5 °C, and heart rate was monitored over the entire period. In all species, absolute values and percentage changes in heart rate were compared for the different temperature regimes.

Spontaneous activity

Measurements of the spontaneous activity of *E. latreillei* were performed following the protocol of Foyle et al. (1989) using a low-light-intensity video camera mounted above three walking tanks and connected to a time-lapse video recorder. The incubation water had Mg^{2+} concentrations of 50, 30 and 15 mmol l^{-1} and the temperatures investigated were 10.0, 2.5, 1.0 and −1.0 °C. A grid of 4 cm×4 cm (the mean carapace length of the animals was approximately 4 cm) was painted on the bottom of the tanks (60 cm×40 cm), and the walking distance was determined by counting the number of grid squares passed by the crabs over a 24 h period at each combination of temperature and Mg^{2+} concentration.

Reaction to experimental stimulation

To investigate the capacity of the animals to respond to experimental stimulation, *H. araneus* were held in 25 l aquaria (a maximum of four animals per aquarium). The two different temperature regimes investigated were the same as during heart rate analysis. For each combination of experimental temperature and Mg^{2+} level, the animals were turned upside-down, and the time (reaction time) to return to the upright position was monitored. Most of the crabs turned back by rolling over their pleon, but some rolled over their rostrum. Because the latter movements occurred much more slowly, only occasionally and were not temperature-dependent, they were excluded from the calculations.

Statistical analyses

Statistical significance was tested at $P<0.05$ and $P<0.01$ using analysis of covariance (ANCOVA, SuperAnova, Abacus Concepts 1991) or analysis of variance (ANOVA, SuperAnova, Abacus Concepts 1991). Values are presented as means ± standard deviation (S.D.). Discontinuities in the temperature-dependence of the variables investigated were tested using Arrhenius analysis and a Q-BASIC program for the identification of critical points (Yeager and Ultsch, 1989).

Results

$[Mg^{2+}]_{HL}$ of Antarctic shrimps

Notocrangon antarcticus and *Chorismus antarcticus* are strong $[Mg^{2+}]_{HL}$ regulators. The $[Mg^{2+}]_{HL}$ of *Notocrangon antarcticus* is 8.0±2.0 mmol l^{-1} (N=33) and that of *Chorismus antarcticus* is 7.9±2.5 mmol l^{-1} (N=17). There were no significant differences between the populations in the Weddell Sea (bottom water temperature −1.6 °C, salinity 34.4 ‰) and those close to the Antarctic Peninsula (bottom water temperature +0.1 °C, salinity 34.5 ‰).

Rates of oxygen consumption

In neither of the species investigated was the rate of oxygen consumption constant over time. Phases of very low oxygen demand alternated with peaks of very high oxygen consumption. An example is shown for *H. araneus* in Fig. 1; *E. latreillei* exhibited the same pattern. However, periods of 90 min proved to be long enough for reproducible estimates of mean values which correspond to routine metabolic rates. Standard metabolic rates (SMRs) were not calculated because decapods exhibit phases of apnoea and bradycardia like intermittent breathers. Metabolic rates during apnoeic periods are below SMR. In consequence, calculations of SMR were not possible.

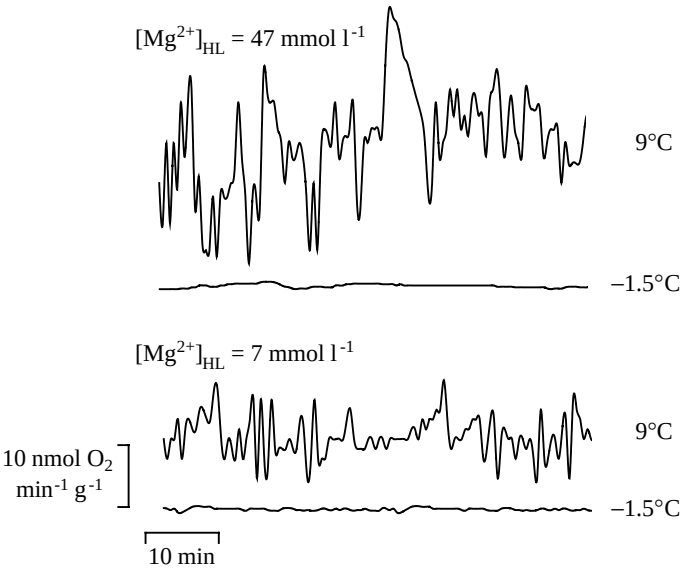

Fig. 1. Typical oxygen consumption recordings for two specimens of *Hyas araneus* with different haemolymph Mg^{2+} concentrations ($[Mg^{2+}]_{HL}$) at 9 and −1.5 °C. The variability in the rate of oxygen consumption is much more pronounced at 9 °C than at −1.5 °C. Measurements conducted with $[Mg^{2+}]_{HL}$-reduced animals at 9 °C revealed smaller fluctuations compared with control animals at 9 °C. The same effect can be seen in *Eurypodius latreillei*, but is not shown here.

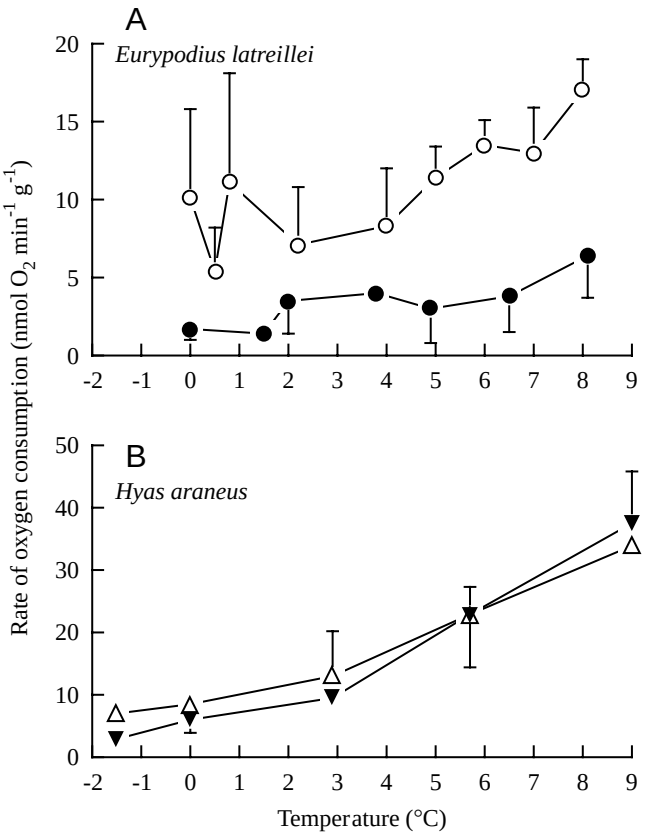

Fig. 2. Rates of oxygen consumption of *Eurypodius latreillei* (A) and *Hyas araneus* (B) at different temperatures and haemolymph Mg^{2+} concentrations ($[Mg^{2+}]_{HL}$). (A) While the oxygen demand of control animals ($[Mg^{2+}]_{HL}$=45 mmol l^{-1}, filled symbols) in *E. latreillei* decreases with decreasing temperature with a Q_{10} of 5.1 for the whole temperature range, $[Mg^{2+}]_{HL}$-reduced animals (8 mmol l^{-1}, open symbols) show a Q_{10} of 4.6. The rate of oxygen consumption of $[Mg^{2+}]_{HL}$-reduced animals is significantly elevated (ANCOVA, P<0.01) at all temperatures investigated. (B) The trend for an increase in the rate of oxygen consumption in *H. araneus* at low $[Mg^{2+}]_{HL}$ (open symbols) was insignificant (ANOVA); however, the significant difference in slopes (ANCOVA, P<0.05, $\log_e$-transformed data, not shown) between these two groups (normal $[Mg^{2+}]_{HL}$ and reduced $[Mg^{2+}]_{HL}$) indicates a reduction in thermal sensitivity at low $[Mg^{2+}]_{HL}$. Values are means ± S.D., N=5.

The rate of oxygen consumption in control specimens of *E. latreillei* ($[Mg^{2+}]_{HL}$=45 mmol l^{-1}) was 6.4±2.7 nmol O_2 min^{-1} g^{-1} (N=5) at 8.1 °C (Fig. 2A). A decrease in temperature resulted in a reducion in the rate of oxygen consumption with a Q_{10} value of 5.1 between 0 and 8.1 °C. A comparison of the data over this short period of temperature acclimation (12 h) with data from animals that had been incubated for 2 weeks revealed no difference. Obviously, incubation for at least 12 h at each temperature was sufficient and no over- or undershoot reaction to temperature change was visible.

$[Mg^{2+}]_{HL}$-reduced ($[Mg^{2+}]_{HL}$=8 mmol l^{-1}) animals had a significantly higher oxygen demand at all temperatures investigated (ANCOVA, P<0.01). This increase was 2.7-fold at 8 °C, reaching 17.0±2.0 nmol O_2 min^{-1} g^{-1}, and 3.2-fold at 0.5 °C, reaching 5.4±2.8 nmol O_2 min^{-1} g^{-1} (N=5). The change in the rate of oxygen consumption with temperature showed a Q_{10} of 4.6 between 0.5 and 8.1 °C. The values at 0.8 °C and 0 °C may indicate a rise in the rate of oxygen consumption, but the change was non-significant as a result of the large inter-individual variability.

The pattern of oxygen consumption changes in *H. araneus* was different from that in *E. latreillei* (Fig. 2B). Control animals showed an oxygen demand of 37.5± 8.3 nmol O_2 min^{-1} g^{-1} (N=5) at 9 °C. The oxygen demand decreased to 2.9±0.5 nmol O_2 min^{-1} g^{-1} at −1.5 °C (for Q_{10} values see Table 1). The rate of oxygen consumption of animals with reduced $[Mg^{2+}]_{HL}$ was significantly different in slope (ANCOVA, P<0.05) from that of control animals in $\log_e$-transformed data (not shown). This transformation was necessary for an equal distribution of residuals, as required for this statistical test. Animals from both $[Mg^{2+}]_{HL}$ treatments showed more variability in rates of oxygen consumption at higher temperatures (Fig. 1). Peaks of very high oxygen consumption alternated with periods during which the rate of oxygen consumption dropped to very low values at temperatures between 3 and 9 °C. At temperatures between 0 and −1.5 °C, these changes in rates of oxygen consumption disappeared, and the animals showed a more or less constant level of oxygen demand, occasionally interrupted by small peaks of spontaneous higher demand. Variability in rates of oxygen consumption was small among animals from the two $[Mg^{2+}]_{HL}$ groups. Different metabolic rates were measured at −1.5 °C depending on $[Mg^{2+}]_{HL}$ (2.9±0.5 nmol O_2 min^{-1} g^{-1} at 47 mmol l^{-1} $[Mg^{2+}]_{HL}$ and 7.0±1.2 nmol O_2 min^{-1} g^{-1} at 7 mmol l^{-1} $[Mg^{2+}]_{HL}$).

Heart rate

While heart rate fell almost linearly in control animals of all species between 7 and −1 °C (Fig. 3), the reduction in heart rate in $[Mg^{2+}]_{HL}$-reduced animals differed among species.

In *E. latreillei* at 36 mmol l^{-1} $[Mg^{2+}]_{HL}$, heart rate dropped from 87.6±8.8 beats min^{-1} at 8 °C to 13.0±7.1 beats min^{-1} (N=16) at −1.7 °C. $[Mg^{2+}]_{HL}$-reduced animals ($[Mg^{2+}]_{HL}$=8 mmol l^{-1}) showed significantly (ANCOVA, P<0.01) elevated heart rates at both these temperatures, with a decrease from 98.3±3.1 beats min^{-1} (N=18) at 8 °C to 22.7±8.5 beats min^{-1} at −1.7 °C. Heart rates of crabs are influenced by a variety of factors such as size, age, sex and moulting stage (Maynard, 1960; Ahsanullah and Newell, 1971; McMahon and Burnett, 1990), which may explain the high variability between individuals. To correct for this variability and to investigate whether a clear pattern of thermal sensitivity prevails, control values were normalized to 100 % (Fig. 4). The percentage change in heart rate was similar in both $[Mg^{2+}]_{HL}$ groups in *E. latreillei* (Fig. 4A), but differed from that of the other two species (Fig. 4B,C).

Heart rates in control *H. araneus* ($[Mg^{2+}]_{HL}$=50 mmol l^{-1}) reached levels between 1 and 15 beats min^{-1} (N=6) at subzero temperatures (Fig. 3B). In $[Mg^{2+}]_{HL}$-reduced animals ($[Mg^{2+}]_{HL}$=15 mmol l^{-1}), a decrease could only be observed

Table 1. *Q_{10} values, Arrhenius activation energies (E_A) and the ratio E_A control [Mg^{2+}]$_{HL}$/E_A reduced [Mg^{2+}]$_{HL}$) for rate of oxygen consumption and heart rate in* Eurypodius latreillei *and* Hyas araneus *and for heart rate only in* Carcinus maenas

	Q_{10}		E_A (kJ mol^{-1})		
	Control	Reduced [Mg^{2+}]$_{HL}$	Control	Reduced [Mg^{2+}]$_{HL}$	E_A ratio
Eurypodius latreillei					
Heart rate	7.1	4.5	124.1	95.2	1.3
Rate of oxygen consumption	5.1	4.6	103.8	97.4	1.1
Hyas araneus					
Heart rate	6.0	1.7	113.3	33.6	3.4
Rate of oxygen consumption	11.4	4.5	154.7	95.6	1.6
Carcinus maenas					
Heart rate	19.0	1.8	186.0	37.1	5.0

E_A=2.3$\boldsymbol{R}T_1T_2\log Q_{10}/10$ and has units of kJ mol^{-1}, where $\boldsymbol{R}$ is the gas constant and T_1 and T_2 are absolute temperatures.
Data at all temperatures were used in the calculation of mean E_A and Q_{10} (see figures).
[Mg^{2+}]$_{HL}$, haemolymph Mg^{2+} concentration.

between 7 and 2 °C; heart rate remained constant and never decreased below 20 beats min^{-1} at lower temperatures, with a significant (ANCOVA, P<0.05) difference between the two [Mg^{2+}]$_{HL}$ groups. Fig. 4B confirms the difference in thermal sensitivity of the two groups of animals.

Heart rates in control *C. maenas* ([Mg^{2+}]$_{HL}$=16 mmol l^{-1}, N=8) fell from 45.9±13.6 beats min^{-1} at 6.4 °C to 5.5±1.9 beats min^{-1} at –0.8 °C (Fig. 3C). A reduction in [Mg^{2+}]$_{HL}$ led to a significant (ANCOVA, P<0.05) reduction in the slope. Heart rate in [Mg^{2+}]$_{HL}$-reduced animals (N=8) fell from 30.3±19.5 beats min^{-1} at 6.4 °C to 15.2±4.5 beats min^{-1} at –0.8 °C. Again, the data presented in Fig. 4C confirm the change in thermal sensitivity in response to a reduction in [Mg^{2+}]$_{HL}$.

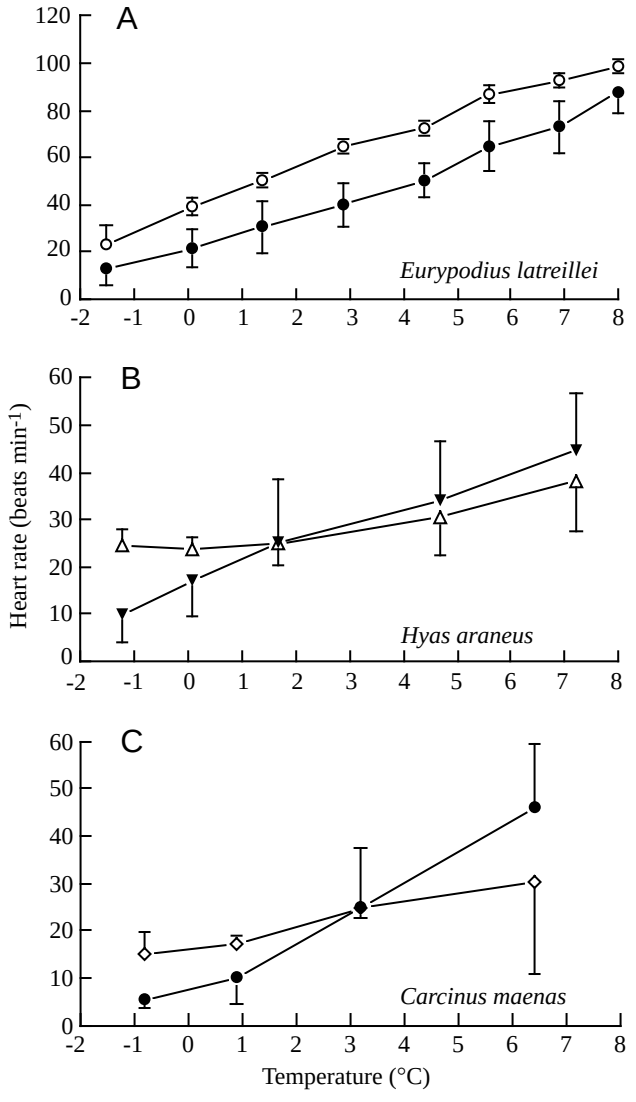

Fig. 3. Heart rate of *Eurypodius latreillei* (A), *Hyas araneus* (B) and *Carcinus maenas* (C) at different temperatures and haemolymph Mg^{2+} concentrations ([Mg^{2+}]$_{HL}$) (controls, filled symbols; [Mg^{2+}]$_{HL}$-reduced animals, open symbols). (A) Comparable with oxygen consumption data, there is a significant (ANCOVA, P<0.01) parallel shift in heart rate towards higher values over the whole temperature range in [Mg^{2+}]$_{HL}$-reduced *E. latreillei* compared with control animals. (B) [Mg^{2+}]$_{HL}$-reduced *H. araneus* exhibit elevated heart rates compared with control animals only at temperatures of 0 and –1.4 °C. The slope is significantly different between the two groups of animals (ANCOVA, P<0.05). (C) Heart rate in control *C. maenas* decreases nearly linearly with decreasing temperature, although [Mg^{2+}]$_{HL}$ is already low (16 mmol l^{-1}) compared with that of other Brachyura. As for *H. araneus*, a further reduction of [Mg^{2+}]$_{HL}$ to 8 mmol l^{-1} results in a reduced thermal sensitivity, the slope being significantly different from that of controls (ANCOVA, P<0.05). Values are means ± S.D., N=8.

Arrhenius analyses of rates of oxygen consumption and heart rate

Arrhenius analyses of rates of oxygen consumption and heart rate data for all species investigated revealed no discontinuity in the temperature-dependence of these two variables. This indicates a continuous slowing of metabolic rate with decreasing temperature. The Arrhenius activation energy (E_A) of physiological processes in [Mg^{2+}]$_{HL}$-reduced

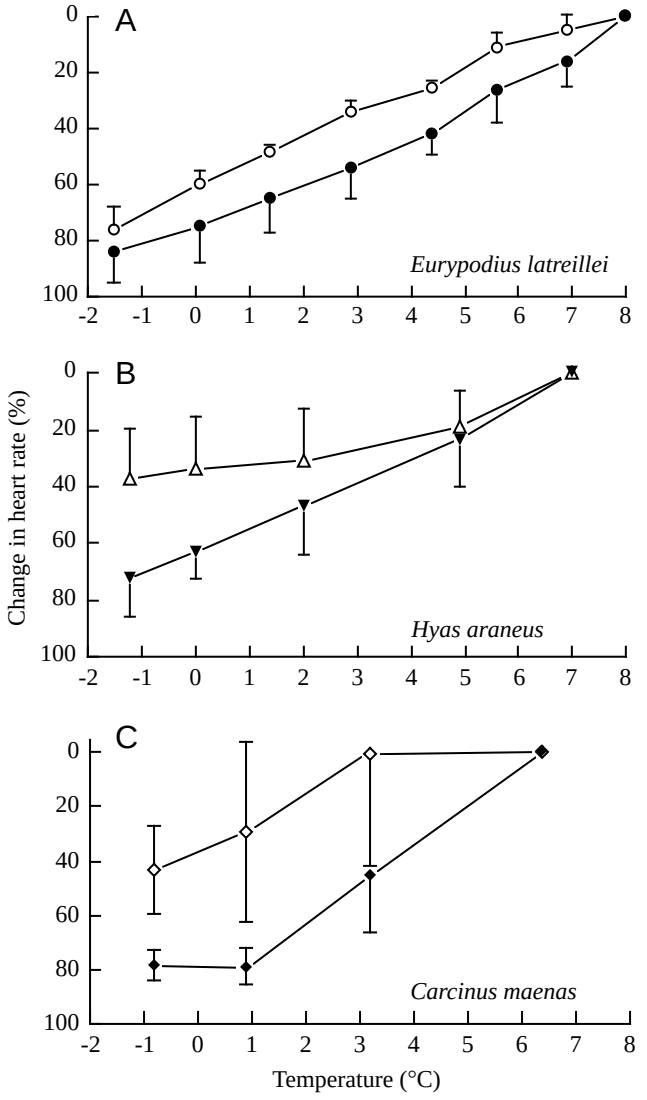

Fig. 4. Percentage change in heart rate in *Eurypodius latreillei* (A), *Hyas araneus* (B) and *Carcinus maenas* (C) at different temperatures and haemolymph Mg^{2+} concentrations ($[Mg^{2+}]_{HL}$) (controls, filled symbols; $[Mg^{2+}]_{HL}$-reduced animals, open symbols). (A) The slope of change in heart rate in *E. latreillei* is the same at both levels of $[Mg^{2+}]_{HL}$ and is linear over the range of temperatures investigated. (B) While *H. araneus* reduces its heart rate linearly with temperature at control $[Mg^{2+}]_{HL}$, the slope becomes less steep below 2 °C in $[Mg^{2+}]_{HL}$-reduced animals. (C) In control *C. maenas*, heart rate falls between 6 and 1 °C. At lower temperatures, no further reduction could be observed. Heart rate remains high in $[Mg^{2+}]_{HL}$-reduced animals. Values are means ± S.D., *N*=8.

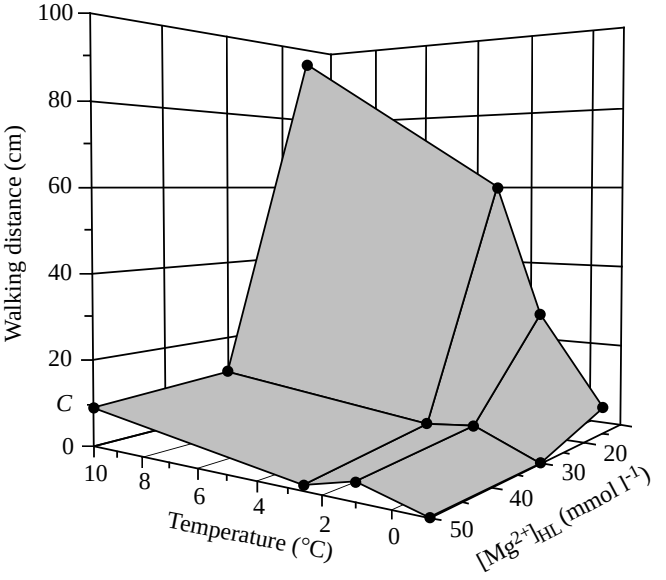

Fig. 5. The distance covered in 1 h by spontaneous movements in *Eurypodius latreillei* at different temperatures and haemolymph Mg^{2+} concentrations ($[Mg^{2+}]_{HL}$). No major difference could be seen between control animals (*C*) ($[Mg^{2+}]_{HL}$=50 mmol l^{-1}) and animals at slightly reduced $[Mg^{2+}]_{HL}$ ($[Mg^{2+}]_{HL}$=30 mmol l^{-1}). Animals with $[Mg^{2+}]_{HL}$ reduced to almost shrimp-like levels ($[Mg^{2+}]_{HL}$=15 mmol l^{-1}) were much more active at all temperatures investigated and remained active at subzero temperatures in contrast to the other two groups.

animals was between 1.1- and fivefold lower than in control animals (Table 1), indicating a reduction in thermal sensitivity for all variables investigated at low $[Mg^{2+}]_{HL}$.

Spontaneous activity

A reduction in $[Mg^{2+}]_{HL}$ had a pronounced effect on spontaneous activity. This was characterized by long periods of inactivity interrupted by bursts of movements. On average, control *E. latreillei* moved 10 times further in 1 h at 10 °C than at 2.5 °C (Fig. 5). Between 2.5 and 1 °C, there was a small increase in walking distance. Walking distance was much higher at low $[Mg^{2+}]_{HL}$ ($[Mg^{2+}]_{HL}$=15 mmol l^{-1}), with a 10-fold increase at 10 °C and at 2.5 °C compared with control animals. At −1 °C, the walking distance in this group was still comparable with that of control animals at +1 °C. There was no major difference between control animals and those with slightly reduced $[Mg^{2+}]_{HL}$ ($[Mg^{2+}]_{HL}$=30 mmol l^{-1}).

Reaction to experimental stimulation

No difference in reaction time was observed between animals cooled in a stepwise manner over several days and those cooled within 1 day, so the data from both experiments were pooled (Fig. 6). A temperature decrement from 6.8 to 2 °C had no significant effect on reaction time in control *H. araneus*. ($[Mg^{2+}]_{HL}$=38.8 mmol l^{-1}; mean reaction time 6.2±0.9 s, *N*=12). The reaction time increased to 18.5±13.6 s at −2 °C. The standard deviation increased at subzero temperatures because some animals still reacted quite quickly while others needed more than 1 min to return to the upright position. Two significantly different linear regressions resulted, with a slope of 0.5 s $°C^{-1}$ between 6.8 and 0.3 °C and of 4.6 s $°C^{-1}$ between 0.3 and −2 °C. The regressions intersect at 0.3 °C and 7.8 s reaction time.

$[Mg^{2+}]_{HL}$-reduced ($[Mg^{2+}]$=9.5 mmol l^{-1}) crabs had a mean reaction time of 6.5±1.5 s calculated for all temperatures

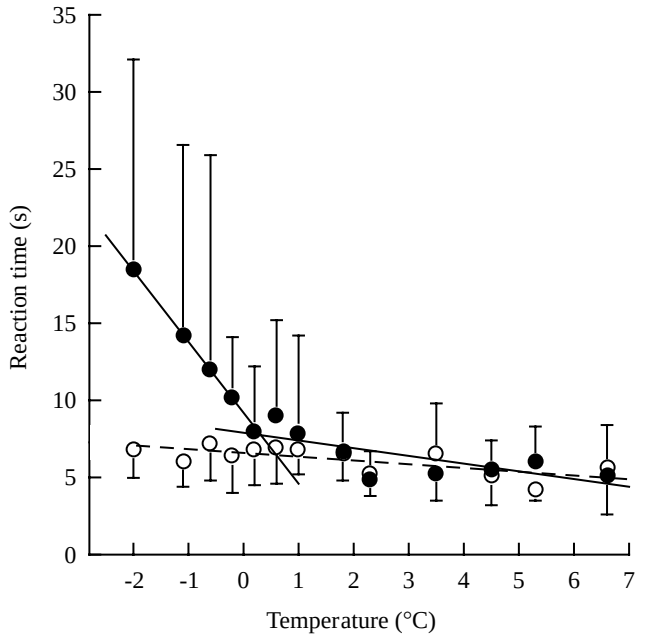

Fig. 6. Reaction time after experimental stimulation in *Hyas araneus* at different temperatures and haemolymph Mg^{2+} concentrations ($[Mg^{2+}]_{HL}$). Control animals (filled circles) took 6.2 s to react above 2 °C and reacted more slowly at lower temperatures. At −2 °C, they needed 18.5 s to return to the upright position. Two significantly different linear regressions (solid lines) (r^2=0.7206, P<0.05 above 2 °C; r^2=0.9995, P<0.01 below 2 °C) were fitted using a Q-BASIC program to identify critical points (Yeager and Ultsch, 1989). The two regressions intersect at 0.3 °C. $[Mg^{2+}]_{HL}$-reduced animals (open circles) exhibited a mean reaction time of 6.5 s at all temperatures investigated. Values are means ± S.D., N=12.

investigated. It was not possible to fit two different regressions or to identify a critical temperature. The slope of the regression line through all the data points for this group of crabs was 0.2 s °C^{-1}, again reflecting a reduction in thermal sensitivity of $[Mg^{2+}]_{HL}$-reduced animals.

Discussion

Reptant decapods were abundant in the Antarctic during the Cretaceous period, as shown by the fossil record (Feldmann and Zinsmeister, 1984a,b). Feldmann (1986) even describes the origin of two reptant decapod species during the late Cretaceous and Eocene periods in the southern circum-Pacific region and their dispersal from there to lower latitudes. The reptant decapod fauna was clearly not extinct at the Cretaceous–Tertiary boundary (Feldmann and Tshudy, 1989), and the youngest fossil of the Reptantia in Antarctica is *Antarctidromia inflata*, which was found on King George Island and corresponds to the lower Miocene (20 million years ago) (Förster et al., 1985). The loss of the reptant decapod fauna in the Antarctic coincides with the cooling of the Southern Ocean during the Tertiary period, when the water temperature dropped from 15–20 °C to the present level of +2 to −1.8 °C. Clarke (1990) stated that this geologically rapid temperature change is equivalent to a shift of 0.003 °C every 1000 years and that it could not represent ‘an evolutionary challenge to marine faunas’. Considering the lower costs of basal metabolism at cold temperatures resulting from the Q_{10} effect, living in cold waters should even be advantageous (Clarke, 1980) and ‘cold water *per se* cannot be the explanation’ for the absence of the Reptantia in the high Antarctic (Clarke and Crame, 1989).

Brey et al. (1996) suggested that the stenobathic benthic fauna, among them the Reptantia, became extinct during glaciation of the Antarctic shelf and that only eurybathic species such as Natantia were able to survive on the continental slope. A recolonisation of the shelf should have been easier and faster for eurybathic species. In contrast, species that survived only north of the Polar Front had to cross the deep-sea trench in the Drake Passage and the circum-Antarctic current, both of which developed after Gondwana broke away and South America gradually separated from Antarctica in the Oligocene period approximately 25–30 million years ago (Clarke, 1990). Another hypothesis suggests that the different modes of reproduction of the two groups may play a role in this context (Reptantia are brooders, whereas many Natantia are broadcasters). This hypothesis has been reviewed elsewhere (Arntz et al., 1994) and also provides no commonly accepted explanation.

Dell (1972) assumes that a colonisation of Antarctica is still possible today *via* the islands of the Scotia Arc. Also, the three findings of Reptantia in the Antarctic (*Paralomis spectabilis*, Birsthein and Vinogradov, 1967; *Stereomastis suhmi*, larvae only, Tiefenbacher, 1994; *Lithodes murrayi*, Klages et al., 1995) show that Reptantia are able to cross successfully both the Drake Passage and the circum-Antarctic current and to live in the Antarctic in an ecological niche that is usually occupied by isopods and amphipods (Clarke and Crame, 1989; Brandt, 1991). However, it is obvious that Reptantia in the Antarctic were found at water temperatures above 0 °C (*P. spectabilis*, +1.1 °C; *S. suhmi*, not reported; *L. murrayi*, +1.8 °C). The total absence of Reptantia in the Antarctic is probably restricted to areas with temperatures below 0 °C. This is especially the case in the well-investigated eastern Weddell Sea shelf, which is characterized by very low constant temperatures of −1.8 °C (Seabrooke et al., 1971; Arntz et al., 1992) and a well-documented fauna of caridean decapods (for a review, see Gorny, 1999).

The distribution of reptant and natant decapods in the Arctic is not as distinctly different as in the Antarctic. Reptant decapods (e.g. *Hyas araneus*, *Chionoecetes opilio*) are found in the far North around 0 °C. Nonetheless, they are absent from areas reaching water temperatures of −1 °C and below. These areas are not as clearly geographically separated as in the Antarctic. Comparable with the Antarctic, the natant shrimps are clearly the more abundant decapods in the extremely cold regions of the Arctic (Squires, 1990). Moreover, it can be inferred that the deep-sea trench and the circum-Antarctic current around Antarctica emphasize the separation of these groups more than in the Arctic.

Our experiments suggest that $[Mg^{2+}]_{HL}$ may be an important variable in explaining the different thermal sensitivities of Natantia and Reptantia. Experimental reduction of $[Mg^{2+}]_{HL}$ in Reptantia to natantian levels significantly reduces (ANOVA, $P<0.05$) the Arrhenius activation energy of all variables investigated in $[Mg^{2+}]_{HL}$-reduced compared with control animals. Arrhenius analysis revealed no discontinuity in the temperature-dependence of any of the variables investigated at lower levels of $[Mg^{2+}]_{HL}$. A $[Mg^{2+}]$ threshold is also not evident from this analysis. Lowered Q_{10} and E_A values, however, reflect the reduced thermal sensitivity of $[Mg^{2+}]_{HL}$-reduced animals.

A phenomenon observed in the three reptant species studied is the change in variability of heart rate and the rate of oxygen consumption with decreasing $[Mg^{2+}]_{HL}$. While unstressed crabs show a wide range of heart rates (e.g. Depledge, 1977; Cumberlidge and Uglow, 1977; Booth et al., 1982; Aagaard, 1996; M. Frederich, personal observation) and large fluctuations in rates of oxygen consumption (Fig. 1), this variability is minimized when the animals are stressed by handling, temperature changes or hypoxia (M. Frederich, personal observation; B. De Wachter, personal communication). Animals with a low $[Mg^{2+}]_{HL}$ usually exhibit a smaller variability in the two parameters, but the range remained greater at higher temperatures than at temperatures around or below 0 °C. This indicates that reduced $[Mg^{2+}]_{HL}$ may cause the excitability of the organism to be elevated. Nevertheless, *H. araneus* survived for 4 months in artificial sea water with 6 mmol l^{-1} Mg^{2+}, indicating that the animals are able to tolerate low $[Mg^{2+}]_{HL}$ for extended periods. However, the long-term consequences of reduction in $[Mg^{2+}]_{HL}$, including the effects on of reproduction and growth, may only be detectable during long-term captivity in Mg^{2+}-reduced sea water.

The temperature-dependence of the effects of reduction in $[Mg^{2+}]$ is different in the three investigated species. At all temperatures, heart rates, rates of oxygen consumption and spontaneous activity of *E. latreillei* exhibited a shift to higher values at low $[Mg^{2+}]_{HL}$, whereas an increase in the rate of oxygen consumption and heart rate and a reduction in reaction time to experimental stimulation was found in *H. araneus* at low and subzero temperatures. The same pattern could be seen in the heart rate of *C. maenas*. Results for this species are surprising because *C. maenas* is one of the strongest $[Mg^{2+}]_{HL}$ regulators among brachyuran crabs. This may have led to the low thermal sensitivity of the variables investigated compared with other Brachyura. A reduction in $[Mg^{2+}]_{HL}$ reduces thermal sensitivity even further. Additional investigations are required to identify the mechanisms involved in these inter-specific differences.

A non-quantified observation of spontaneous activity is reported by Holliday (1980). He describes *Cancer magister* held in Mg^{2+}-free sea water as 'easily excited and aggressive'. In our study on *H. araneus*, the decrease in $[Mg^{2+}]_{HL}$ did not result in a linear increase in levels of spontaneous activity. The difference in walking distance between control animals and those subjected to a moderate reduction in $[Mg^{2+}]_{HL}$ ($[Mg^{2+}]_{HL}$=30 mmol l^{-1}) was very small. Only a reduction in $[Mg^{2+}]_{HL}$ to 12 mmol l^{-1} led to a significant increase in levels of spontaneous activity. Furthermore, *H. araneus* and *E. latreillei* exhibit a rather large variability of $[Mg^{2+}]_{HL}$ with no detectable influence on activity or other variables. $[Mg^{2+}]_{HL}$ varied between 39 and 51 mmol l^{-1} in *H. araneus*, between 36 and 48 mmol l^{-1} in *E. latreillei* and between 12 and 20 mmol l^{-1} in *C. maenas*. The Antarctic shrimps investigated, *Notocrangon antarcticus* and *Chorismus antarcticus*, varied in their $[Mg^{2+}]_{HL}$ between 5.5 and 11.6 mmol l^{-1} and between 5.0 and 12.3 mmol l^{-1}, respectively. A threshold value may exist in the range between 30 and 12 mmol l^{-1} that enables the animals to increase their levels of spontaneous activity. Sartoris and Pörtner (1997) identified a $[Mg^{2+}]_{HL}$ threshold of 15 mmol l^{-1} in *Crangon crangon* above which intracellular pH was protected from temperature changes. However, we are far from being able to explain the origin or usefulness of such a threshold level.

A variety of studies have shown a correlation between salinity and the rate of oxygen consumption and between salinity and heart rate in crabs. Taylor (1977), Jury et al. (1994) and McGaw and Reiber (1998) reported an increase in heart rate and in rates of oxygen uptake at low salinities for *Carcinus maenas* and *Homarus americanus*. Bearing in mind that Mg^{2+} levels are reduced at low salinity, it would be interesting to investigate the role of Mg^{2+} in this case.

The narcotising effect of Mg^{2+} derives mainly from its inhibitory effect on synaptic transmitter release at the neuromuscular junction (Katz, 1936; Del Castillo and Katz, 1954). An increase in Mg^{2+} level decreases the average number of transmitter quanta released per nerve stimulus (Wernig, 1972) by a competitive inhibition of Ca^{2+} binding (Dudel et al., 1982). A narcotising effect should therefore be seen first at the neuromuscular level, which is associated with activity. Furthermore, Howarth and Levi (1998) have shown an inhibition of contractile activity in rabbit myocytes at elevated intracellular $[Mg^{2+}]$. Obviously, Mg^{2+} acts as a relaxant rather than as an anaesthetic.

During relaxation of its muscles, an animal is still able to maintain its standard metabolic rate, but its scope for activity, defined by Bennett (1978) as the difference between minimum and maximum rates of oxygen consumption in fish, is minimized. In Fig. 7, the difference between mean and maximum heart rates was calculated as a measure of the scope for activity from data provided by Walters and Uglow (1981) and collected in this study. In crabs, it is preferable to calculate scope for activity as the difference between mean resting (instead of minimum) and maximum values because of frequent periods of apnoea and bradycardia or acardia. A significant linear correlation results, indicating that the scope for activity increases with decreasing $[Mg^{2+}]_{HL}$. The animal at the top of this activity scale is *Carcinus maenas*, which exhibits a high capacity for regulation of $[Mg^{2+}]$ among brachyuran crabs but still responds to a further reduction in $[Mg^{2+}]_{HL}$ with an increase in levels of activity.

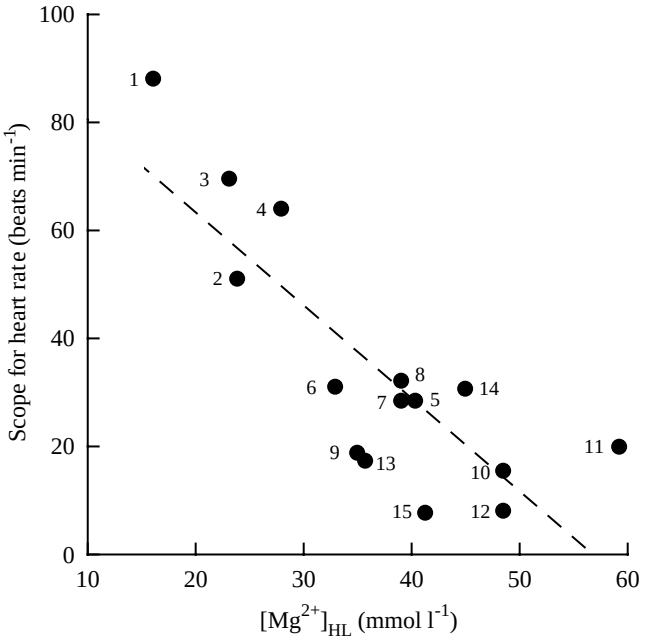

Fig. 7. Scope for heart rate, calculated as the difference between resting and maximum heart rates, for 15 different decapod species at 10±1.5 °C as a measure of the scope for activity in relation to haemolymph Mg^{2+} concentration ($[Mg^{2+}]_{HL}$). The broken line indicates a significant linear correlation (r^2=0.817, P<0.01). 1, *Carcinus maenas*; 2, *Nephrops norvegicus*; 3, *Pagurus bernhardus*; 4, *Macropipus tuberculatus*; 5, *Polybius henslowii*; 6, *Cancer pagurus*; 7, *Geryon tridens*; 8, *Maja squinado*; 9, *Corystes cassivelaunus*; 10, *Lithodes maja*; 11, *Atelecyclus rotundatus*; 12, *Munida bamffica*; 13, *Eurypodius latreillei*; 14, *Hyas araneus*; 15, *Peltarion spinosulum*. Species 1–12, data from Walters and Uglow (1981); species 13 and 14 (present study); species 15, M. Frederich (unpublished data).

For different marine invertebrate species from temperate zones, low critical temperatures (T_c) were identified at values above freezing. T_c is characterized by the onset of anaerobic metabolism. Survival below the T_c is not possible unless an adaptional shift in this threshold temperature occurs (Zielinski and Pörtner, 1996; De Wachter et al., 1997; Sommer et al., 1997; Pörtner et al., 1998). Lactate, which is the main end product of anaerobic metabolism in decapod crustaceans (Gäde, 1983; Spicer and McMahon, 1990), was not found to accumulate significantly in the haemolymph after lowering the temperature to −1.9 °C for 2 h in *H. araneus* and *E. latreillei* (M. Frederich, unpublished data). Accordingly, the data in that study indicate that no such critical temperature exists for the two species investigated. However, a critical temperature could clearly be identified in the temperate to warm-water species *Maja squinado*. Moreover, our data show that temperature limitation may set in above the anaerobic threshold when the aerobic scope for activity is reduced owing to an insufficient capacity for ventilation and circulation (M. Frederich and H.-O. Pörtner, in preparation). These thermal thresholds are shifted to lower values by a reduction in $[Mg^{2+}]_{HL}$. As a corollary, low temperature and high $[Mg^{2+}]_{HL}$ may synergistically reduce the scope for activity and limit the ecological tolerance range and colonization of cold waters. In the cold-water crabs *H. araneus* and *E. latreillei*, Mg^{2+} anaesthesia may actually counteract the transition to anaerobiosis (Sartoris and Pörtner, 1997) since both species reduce their metabolic rate at subzero temperatures to very low levels and are able to survive under these conditions with reduced activity. Nonetheless, aerobic scope and the level of activity seem to be reduced in response to high $[Mg^{2+}]_{HL}$ which, we suggest, is a factor as crucial as temperature.

If an enhanced scope for activity reflects the advantage of low $[Mg^{2+}]_{HL}$ at low temperatures, why do all Reptantia not develop a mechanism of regulating $[Mg^{2+}]_{HL}$ as effectively as shrimps? Brachyura are the youngest group among Reptantia, which appeared in the late Jurassic and radiated during the Cretaceous (Schram, 1982), and they have the poorest ability to regulate $[Mg^{2+}]_{HL}$. Given that all other decapod groups contain stronger $[Mg^{2+}]_{HL}$ regulators, the ancestor of the poorly regulating Brachyura should also have had low $[Mg^{2+}]_{HL}$ levels. Therefore, it must have been advantageous to reduce $[Mg^{2+}]_{HL}$ regulation. Bearing in mind that the reduction in $[Mg^{2+}]_{HL}$ leads to a higher scope for activity, especially below 0 °C, and that ion regulation requires energy, this energy would be wasted without a positive effect above 0 °C. The water temperature in most areas is above 0 °C, and there was a minimum polar temperature of 0 °C (Barron, 1992) during the radiation of the Brachyura in the warm Cretaceous period. This may explain why Reptantia with a higher capacity for $[Mg^{2+}]_{HL}$ regulation evolved so rarely. In those cases where a low $[Mg^{2+}]_{HL}$ prevails, the possibility should be investigated that a high degree of eurythermality may require $[Mg^{2+}]_{HL}$ to be low, since a reduction in $[Mg^{2+}]_{HL}$ reduces thermal sensitivity. This is conceivable for *C. maenas*, which is exposed to large temperature fluctuations in the intertidal zone, and possibly also for other Reptantia found in temperate waters. In cold waters, the capacity of reptantians to regulate $[Mg^{2+}]_{HL}$ may not be sufficient to downregulate $[Mg^{2+}]_{HL}$. As a consequence, the much more active natant decapods, isopods and amphipods, all with low $[Mg^{2+}]_{HL}$, will succeed in competition for ecological niches in polar areas at the expense of the higher costs of regulating $[Mg^{2+}]_{HL}$.

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