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Metabolic properties of Northern krill, *Meganyctiphanes norvegica*, from different climatic zones. II. Enzyme characteristics and activities

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Abstract Activities and characteristics of two metabolic key enzymes, citrate synthase (CS) and pyruvate kinase (PK), were studied in the Northern krill, *Meganyctiphanes norvegica*, with respect to adaptive properties under different thermal conditions. Krill were sampled during late winter/spring and summer from the constantly warm Ligurian Sea (12–13°C below the thermocline), the colder but also comparatively constant Clyde Sea (7–8°C), and the variable Kattegat (2–16°C). Both enzymes showed distinct tissue- and organ-specific activities, which were highest in the pleopods – the principal locomotive organs. The fourth and fifth abdominal segments, however, were used for routine investigation due to lowest variability. Specific activity of CS and PK did not differ between seasons in krill from the Kattegat or the Clyde Sea. In the Ligurian Sea, in contrast, specific CS activities were significantly lower during summer. Analysis of individual data illustrated a decrease of CS activity with size and an increase of PK activity with size. Taking these allometric effects into account, as emphasized by calculating the ratio between both enzymes, variation of CS and PK activities turned out to be solely dependent on body size, which differed between locations and seasons. Ligurian krill from the summer, however, were unique in that they showed a lower CS/PK ratio than would be predicted by the scaling effect. Thermal characteristics of each enzyme were similar between locations and seasons. During the winter, in Kattegat and Clyde Sea krill, K_m values (Michaelis–Menten constant) of CS towards acetyl-coenzyme A exhibited an almost constant level over the experimental temperature range of 4–16°C. During summer, however, K_m values were lower at 8°C in the Clyde Sea and at 12°C in the Kattegat. In Ligurian krill from the summer, K_m values were consistently lower than those of winter krill over the entire experimental temperature range. In conclusion, Kattegat and Clyde Sea krill show only minor adaptations to their respective thermal environments in terms of CS and PK characteristics. Ligurian krill, in contrast, exhibited decreased specific CS activity during summer, which might be compensated by elevated enzyme–substrate affinity as indicated by lower K_m values. Since temperature was constant during both seasons, this effect cannot be explained as a reaction to thermal conditions. Consequently, oligotrophic conditions in the Ligurian Sea during summer may entail a reduction in the somatic performance of krill, which is reflected by lower CS activity.

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Introduction

The Northern krill, *Meganyctiphanes norvegica*, is representative of a zooplankton species that is exposed to a variety of hydroclimatic conditions within its extraordinarily wide area of distribution along the northern and northeastern parts of the Atlantic Ocean and the adjacent seas (Mauchline 1960; Mauchline and Fischer 1969). An interdisciplinary European Union project was centered on *M. norvegica* as a model for studying the adaptive properties of zooplankton species to varying environmental conditions. In previous work (Saborowski et al., this issue), oxygen consumption and ammonia excretion at different temperatures were determined as a measure for overall metabolic rates. Respiration rates were similar between seasons in krill from the Clyde Sea and from the Kattegat, showing an exponential increase with temperature. Both populations exhibited the same level of respiration at the respective ambient temperatures

of 9°C in the Clyde Sea and 4–6°C in the Kattegat, which was considered to be an adaptation to the local ambient thermal conditions. Ligurian krill, however, failed to show such a relation. Although the metabolic performance of summer krill closely fit the scheme described above, krill from late winter/spring were characterized by significantly higher respiration rates, which indicated that respiratory adaptation in Ligurian krill was unique compared to krill from the other two locations, at higher latitudes. This feature was interpreted as a physiological and/or behavioral response to the very short reproductive period (Cuzin-Roudy and Buchholz 1999) associated with a short-term phytoplankton bloom in an otherwise oligotrophic environment, which is considered typical for the Ligurian Sea (Fabiano 1984; Goffart et al. 1995).

Accordingly, the question arises as to what extent the observed differences between sites relate to adaptive biochemical properties. Particularly, since adaptation of enzymatic turnover in key metabolic processes can be considered a powerful force in the regulation of overall metabolic performance. In this respect, we focussed our interest on pyruvate kinase, a key enzyme of the glycolytic pathway, and citrate synthase, a key enzyme of the Krebs cycle. Previous work by Vetter (1995) gave evidence for a rapid and effective compensation of the specific activity of citrate synthase in response to different maintenance temperatures.

The aim of the present study, as a consequence, was to investigate characteristics and specific activities of these key enzymes in relation to the specific thermal and ecological conditions at the three sites and to relate the enzyme characteristics to the respiratory properties presented previously.

Materials and methods

Samples

Northern krill , *Meganyctiphanes norvegica*, were caught with a MOCNESS (multiple opening–closing net and environmental sensing system, Wiebe et al. 1976) during each of six cruises with R.V. "Heincke" to the Ligurian Sea (Mediterranean), the Clyde Sea (Scotland) and the Danish Kattegat (Saborowski et al., this issue). The hauls were kept short (10–20 min). Krill were immediately separated from the catch, deep frozen at –80°C, and stored at this temperature until analysis in the home laboratory.

Tissue preparation

For routine analysis, the fifth and sixth segments were dissected from the abdomen of frozen animals. The pleopods were removed, and the remaining muscle tissue (10–50 mg, including the cuticle) was transferred into a reaction tube. The midgut gland and the cuticle of abdominal segments were also investigated. In order to compare enzyme activities between different segments, each of the six segments was dissected individually and separated from the pleopods. Enzyme analysis on pleopods was carried out on pooled samples of all ten pleopods of an individual.

Enzyme activities

The samples were homogenized in 1 ml of ice cold Tris/HCl-buffer (50 mmol l^{-1}, pH 7.5). Homogenization was performed with an ultrasonic cell disrupter (Branson sonifier B15, equipped with a microtip) for 3×5 s at 30% of maximal energy. During this process, the samples were maintained cooled in an ice/water bath to avoid denaturation due to temperature increase. The resulting homogenate was centrifuged at 15,000 *g* (4°C) for 15 min. The supernatant was used for the determination of citrate synthase and pyruvate kinase.

Citrate synthase (CS; EC 4.1.3.7) was determined after Stitt (1984). Standard assays were run with 520 µl of buffer (50 mmol l^{-1} Tris/HCl, supplemented with 100 mmol l^{-1} KCl and 1 mmol l^{-1} EDTA, adjusted to pH 7.5), 20 µl DTNBA (6.0 mmol l^{-1} buffer), 20 µl acetyl-coenzyme A (CoA) (6.0 mmol l^{-1} A. dest.) and 20 µl sample (supernatant). After 5 min of pre-incubation the reaction was initiated by addition of 20 µl oxaloacetate (12.0 mmol l^{-1} A. dest.) and monitored continuously at 405 nm. The standard assay temperature was 25°C.

Pyruvate kinase (PK; EC 2.7.1.40) was measured via the coupled reaction with LDH. Standard assays contained 500 µl buffer (50 mmol l^{-1} Tris/HCl, supplemented with 60 mmol l^{-1} KCl and 4 mmol l^{-1} $MgSO_4$, adjusted to pH 7.5), 20 µl NADH (7 mmol l^{-1} A. dest.), 20 µl PEP (16 mmol l^{-1}), 10 U LDH and 20 µl of sample. After 5 min of pre-incubation the reaction was started with the addition of 20 µl ADP (17 mmol l^{-1} A. dest.) and monitored at 340 nm at constant temperature.

Thermal profiles and thermal stability

The thermal profiles were determined for CS and for PK within the range of 4–60°C. Standard assays were run as described above. The reaction mixture was allowed to adjust to the experimental temperature for 5 min. Up to 30°C the reactions were started with oxaloacetate and ADP, respectively. At higher temperatures, the reaction was initiated by addition of the enzyme. Activities within the ambient temperature range were linearized by applying the Arrhenius equation, and the activation energy was calculated from the slope using the following formula:

$$\frac{\delta \ln A}{\delta T^{-1}} = -\frac{E_a}{R}$$

(the term A is a pre-exponential factor, T is the absolute temperature, E_a is the activation energy, and R is the gas constant).

For analysis of thermal stability, the samples were pre-incubated in a water bath for 5, 10, 20, 30, and 60 min at temperatures up to 50°C. Thereafter, the sample was assayed at 25°C as described above.

K_m values

The Michaelis–Menten constants (K_m) were measured for CS towards acetyl-CoA. The acetyl-CoA concentrations in the reaction mixture were 0, 2, 10, 20, 40, 100, and 200 µmol l^{-1}. Oxaloacetate concentration was always at saturating levels. Assays were carried out as described above with varying acetyl-CoA concentrations at each of the incubation temperatures of 4, 8, 12, 16, and 20°C.

Statistics

Enzyme activities were normalized to the fresh weight of the tissue sample and expressed as units per gram fresh weight. Statistical comparison of activities was performed with a *t*-test or an ANOVA followed by the Student–Newman–Keuls test. Data were presented as means (±standard deviation). The relation between specific enzyme activity and weight was expressed by linear regression.

Results

Organ specificity

The specific activity of CS was significantly highest in the pleopods (Table 1). CS activity in the muscle tissue was less than half in comparison. Midgut gland and cuticle had lowest activities. PK, in contrast, did not differ significantly between the pleopods and the muscle tissue. The PK activity of the cuticle was slightly lower. Lowest PK activity appeared in the midgut gland.

Specific CS activity increased almost continuously from the first towards the fifth abdominal segment, ranging between 4.6 and 11.2 U g^{-1} fresh wt (Fig. 1a). Activity was significantly decreased in the sixth segment (4.7 U g^{-1} fresh wt), but lowest in the telson and the uropods (2.1 U g^{-1} fresh wt). Again the pleopods showed by far the highest activity, which amounted to 29.4 U g^{-1} fresh wt. Specific PK activity increased in a similar way from 33.3 U g^{-1} fresh wt in the first segment towards 58.5 U g^{-1} fresh wt in the fifth segment (Fig. 1b). The sixth segment and the telson plus uropods (T + U) had the lowest activities of 24.8 and 7.2 U g^{-1} fresh wt, respectively. The activity within the pleopods was slightly higher compared to that in the fourth and fifth segments.

Allometric relation

The specific activities of CS and PK in the abdominal muscle samples from the fourth and fifth segments scattered largely. CS showed a statistically significant decrease with size (Fig. 2a). CS activities ranged between 6 and 19 U g^{-1} fresh wt, while sample size was between 17 and 63 mg. In contrast to CS, the specific activity of PK increased with size (Fig. 2b) and ranged between 32 and 118 U g^{-1} fresh wt.

CS/PK ratio

In order to emphasize the allometric effects on the enzyme activities from the different sampling sites, we calculated the ratio between CS and PK and plotted it against the average weight of the samples (Fig. 3). Clyde Sea krill were largest on average (48.3 and 41.3 mg, respectively), followed by Kattegat krill (33.4 and 30.5 mg). Ligurian krill samples were smallest (24.7 and 21.7 mg). This correlates closely with the length–weight measurements reported previously (Saborowski et al., this issue). The average CS/PK ratios of these samples ranged from 0.102 in Clyde Sea krill from the winter to 0.229 in Ligurian winter krill.

The samples from the Clyde Sea and the Kattegat as well as the winter/spring samples of the Ligurian Sea form a straight line with a negative slope ($r^2 = 0.994$). The Ligurian summer krill were distinct; these showed a very low CS/PK ratio of 0.126 at a low weight of 21.7 mg. These results clearly demonstrate that, with the exception of the latter, variation of enzyme activity in krill muscle is solely dependent on body size.

Table 1 *Meganyctiphanes norvegica*. Organ specificity of citrate synthase (*CS*) and pyruvate kinase (*PK*) in specimens from the Kattegat, sampled during Kattex II in March 1997 (means ± SD, $n = 6$–12)

	Muscle	Pleopods	Cuticle	Midgut gland
CS (U g^{-1} dry wt)	13.0 ± 1.5	35.5 ± 2.6	4.5 ± 0.5	9.7 ± 1.8
PK (U g^{-1} dry wt)	67.5 ± 10.7	72.1 ± 20.9	68.4 ± 10.1	4.8 ± 2.8

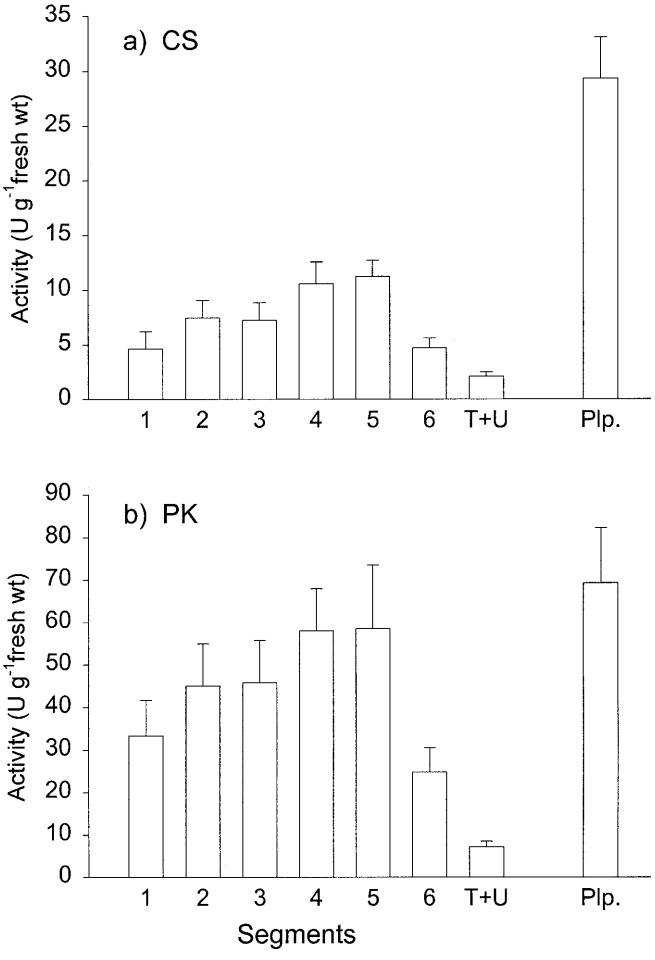

Fig. 1a, b *Meganyctiphanes norvegica*. Specific activity of: **a** citrate synthase (*CS*) and **b** pyruvate kinase (*PK*), in different abdominal segments, the telson plus uropods (*T + U*), and the pleopods (*Plp.*) of specimens sampled during Kattex II (March 1997) in the Kattegat

Temperature profiles

The thermal profiles of CS were similar for all locations and seasons, showing maximum activity at about 45°C

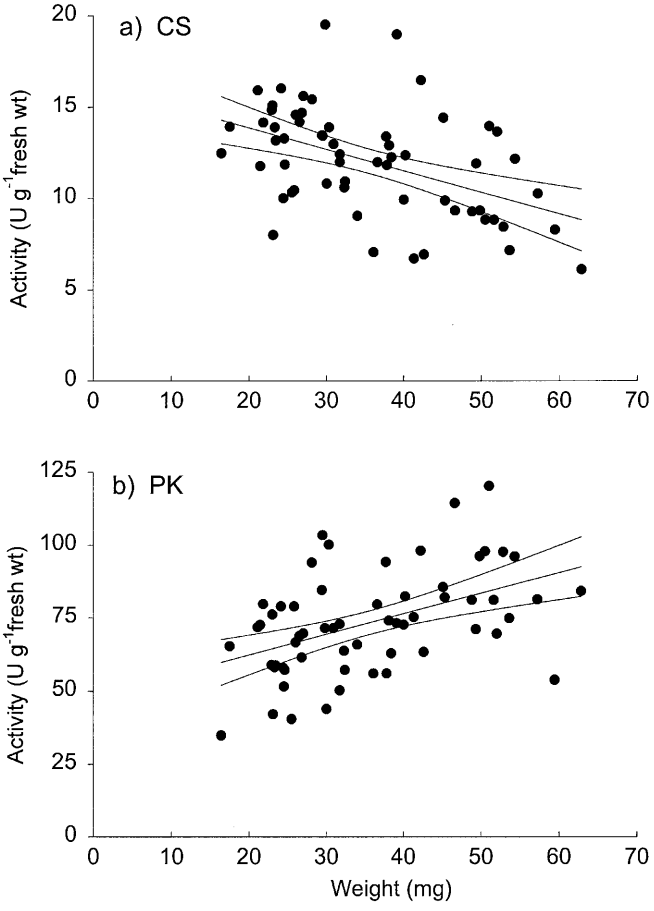

Fig. 2a, b *Meganyctiphanes norvegica*. Specific activity of: **a** citrate synthase (*CS*) and **b** pyruvate kinase (*PK*), from the fourth and fifth abdominal segments in relation to sample weight. Samples were obtained during Kattex II (March 1997) in the Kattegat. The regression lines and the 95% confidence intervals are shown

(Fig. 4, Table 2). At 4°C the activity amounted to only 20% of the maximum and reached 40% of the maximum at 20°C. A rapid loss of activity appeared at incubation temperatures above 45°C.

Also no differences in the thermal profiles of PK appeared between locations and seasons. However, some characteristics of the profile differed distinctly from those of CS. At 4°C PK had a very low activity of only 6% of the maximum and reached 23% of the maximum at 20°C (Fig. 4). A strong decrease of activity due to rapid thermal degradation appeared at temperatures >50°C.

Thermal stability

The thermal stability was investigated in the range of 0–40°C. At 0°C, CS activity was not affected for at least 60 min (Fig. 5a). Furthermore, incubation at 10°C and 20°C for 30 min had only minor effects on CS activity. However, incubation at 20°C for 60 min caused a distinct decrease of activity to 95% of the initial value. At an incubation temperature of 25°C, a significant

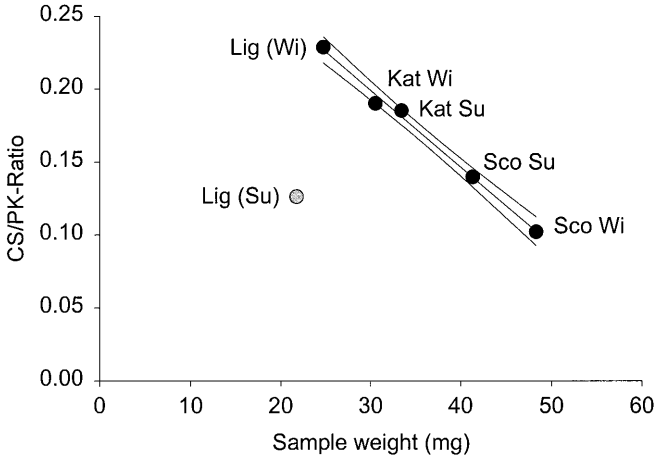

Fig. 3 Ratio of citrate synthase (*CS*) versus pyruvate kinase (*PK*) in relation to sample weight from the study sites Ligurian Sea (*Lig*), Kattegat (*Kat*), and Clyde Sea (*Sco*), during summer (*Su*) and winter (*Wi*) ($n = 10$–12)

decrease of activity was evident after approximately 10 min. A further increase of temperature accelerated enzyme denaturation. At 40°C, $<10\%$ of activity remained after 5 min of incubation.

PK was more stable than CS (Fig. 5b). On ice (0°C) activity remained unaffected for >4 h. Nor did incubation at 10°C for 60 min lead to a significant decrease. A distinct reduction to 85% of initial activity was observed after 30 min at 30°C. At 40°C, however, a strong reduction was evident already after 10 min of incubation. Nevertheless, after 60 min about 30% of activity still remained. Finally, at temperatures of 45°C and above, the enzyme was quickly destroyed and almost no activity was detected after 30 min of incubation.

Activation energy

The activation energy (E_a) of the CS-catalyzed reaction was calculated from the slope of the linearized temperature profiles after applying the Arrhenius equation. According to the temperature profiles, the slopes, and therefore the E_a values, were similar for all locations and seasons. The average activation energy amounted to 36.2 kJ mol^{-1} within the ambient temperature range of 4–20°C. For the PK reaction, the average activation energy was calculated as 60.2 ± 7.8 kJ mol^{-1} within the same temperature range.

Specific activities

The specific activities of CS ranged between 8.6 and 14.4 U g^{-1} fresh wt (Fig. 6a). Highest values appeared in Kattegat samples, and there were no differences between seasons. CS activities in Clyde Sea krill were lower than in Kattegat krill. Seasonal differences were not evident

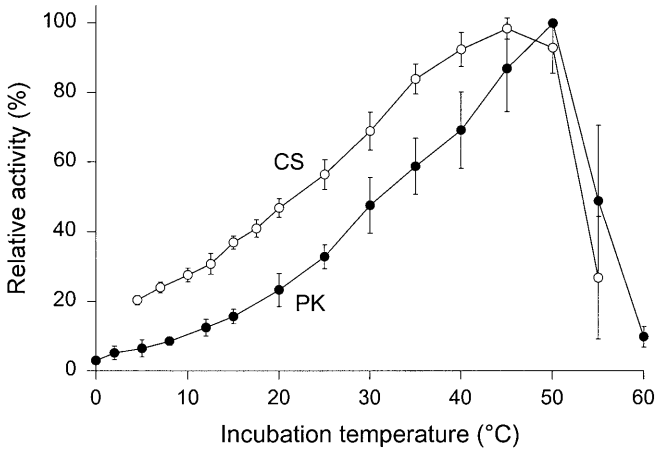

Fig. 4a, b *Meganyctiphanes norvegica*. Thermal activity profiles of: **a** citrate synthase (*CS*) and **b** pyruvate kinase (*PK*) averaged from all sampling locations (means ± SD, $n = 6$)

Table 2 *Meganyctiphanes norvegica*. Thermal characteristics of citrate synthase (*CS*) and pyruvate kinase (*PK*). Averaged values from all locations ($n = 12$)

Location	Abbrev.	Units	CS	PK
Max. of temp. profile	T_{max}	(°C)	45	50
Activation energy	E_a	($kJ \cdot mol^{-1}$)	36.2 ± 3.2	60.2 ± 7.8

here either. The most pronounced differences were found in Ligurian krill. While krill from the winter/spring had activities of 12.4 U g^{-1} fresh wt, summer krill showed significantly lower activities of 8.6 U g^{-1} fresh wt.

Specific activities of PK were generally higher than those of CS, ranging between 54.4 and 84.1 $U \cdot g^{-1}$ fresh wt (Fig. 6b). Again, no differences appeared between summer and winter samples from the Kattegat and the Clyde Sea. Seasonal differences appeared in Ligurian krill only. In contrast to CS, PK activities were higher in summer than in winter; these differences, however, were not statistically significant.

K_m values

The K_m values of CS against acetyl-CoA ranged between 14.8 and 28.4 μmol l^{-1} (Fig. 7). The winter values at all three sites were between 20 and 25 μmol l^{-1} and did not differ significantly. Also no significant change with temperature was evident at any site. In contrast, summer data showed significantly different patterns at each site. In Ligurian summer krill, K_m values were consistently lower (15.4–17.9 μmol l^{-1}) than those from winter/spring at each of the incubation temperatures. In Clyde Sea krill, K_m values decreased from 25.1 ± 4.2 μmol l^{-1} at 4°C to a minimum of 20.3 ± 1.9 μmol l^{-1} at 8°C and continuously increased again at higher incubation temperatures towards 28.4 ± 2.7 μmol l^{-1} at 20°C. A similar

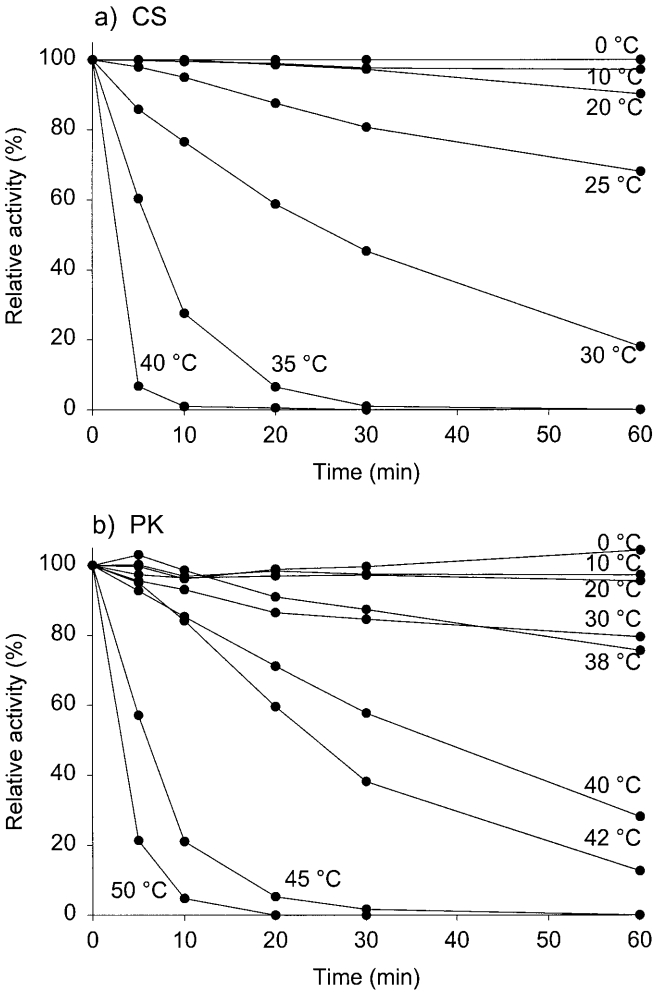

Fig. 5a, b *Meganyctiphanes norvegica*. Thermal stability of: **a** citrate synthase (*CS*) and **b** pyruvate kinase (*PK*), illustrated on samples from the Kattegat (Kattex II, March 1997). The pre-incubation temperatures are plotted close to the respective activity curve

V-shaped pattern appeared in the summer krill from the Kattegat. At 4°C the K_m value was 25.0 ± 1.7 μmol l^{-1} and decreased to a minimum of 17.8 ± 2.0 μmol l^{-1}, which, however, appeared at 12°C. Subsequently, K_m values increased at higher incubation temperatures towards 27.4 μmol l^{-1} at 20°C.

Discussion

Northern krill, *Meganyctiphanes norvegica*, are comparatively large planktonic crustaceans. The entire body would exceed the amount of tissue needed for enzymatic analysis by far. Furthermore, relatively often prominent but subtle parts of the body, such as the telson, the uropods, the thoracopods, or pleopods break off. The choice of suitable samples, however, is essential in comparative investigations to maintain a high quality standard, particularly in terms of reproducibility. Prior to routine analysis we screened some tissues and organs

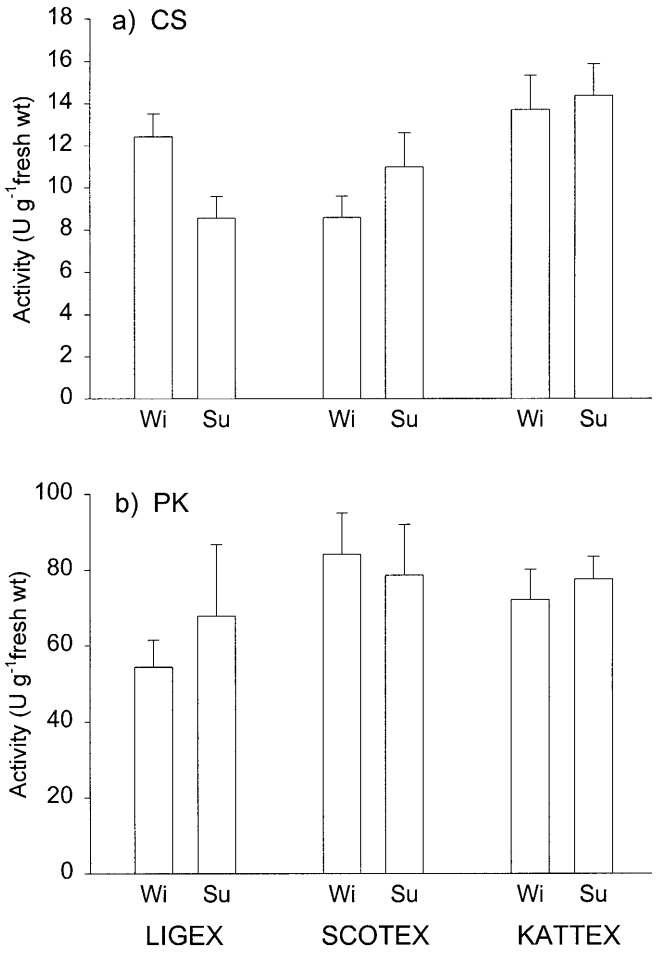

Fig. 6a, b *Meganyctiphanes norvegica*. Specific activity of: **a** citrate synthase (*CS*) and **b** pyruvate kinase (*PK*), in the abdominal muscle tissue (fourth and fifth segments) of specimens from all study sites, during summer (*Su*) and winter (*Wi*) (means ± SD, $n = 10–12$)

for practical suitability and reproducibility of enzyme activity. CS activity was variable when different organs were compared, but showed low variability within the same organs or tissues. The high CS activity and, thus, high aerobic potential in the pleopods is an indication of the high efficiency of the locomotive system and can be related to the extraordinary swimming performance of krill (Kils 1982).

In contrast to CS, PK activity was at equal levels in muscle, pleopods, and the cuticle. However, variability within the organs was higher than it was for CS. Both enzymes had lowest activities in the midgut gland. This screening clearly showed that variation of sample composition can have strong effects on activity data, particularly with regard to the absence or presence of pleopods, with high CS activity, or to the variable size of the midgut gland, with particularly low activity of both enzymes. In the latter case enzyme activity of total krill samples would be influenced by the nutritive state, which is closely linked to the size of the midgut gland. We obtained best reproducibility and easiest handling in the fourth and fifth abdominal segments, from which pleopods were severed but the cuticle remained. Control evaluations showed that the CS and PK activities in the cuticle were not affected by different moult stages.

The specific activities of both enzymes, CS as well as PK, showed distinct allometric relationships: CS activity decreased with size, while PK activity increased. This effect, often referred to as "metabolic scaling", has been intensively investigated in fishes (e.g. Somero and Childress 1985; Childress and Somero 1990) as well as in crustaceans (e.g. Houlihan et al. 1985; Berges et al. 1990; Berges and Ballantyne 1991). Although an exponential relation between enzyme activity and size should be expected, in our selected muscle samples the relation of both enzymes versus size was best described by a linear function. This can be explained by the comparatively narrow span of body sizes in the specimens taken from the field; neither extremely small nor extremely large animals were available, although these sizes might have better emphasized the exponential character of the scaling relation.

Effects of metabolic scaling can be explained by the anatomy and the fine structure of the complex abdominal muscle system (Daniel 1929). The "CS-rich" muscles of the pleopods originate from within the abdominal muscle system and, therefore, contribute to a specific share of CS activity in the abdomen. The overall amount of the "CS-rich" muscle tissue, however, is lower than the "CS-poor" tissue. Due to growth, the amount of tissue increases exponentially. However, because of the initial dissimilarity, "CS-rich" tissue increases less with size than the "CS-poor" tissue does. Simultaneously, the relative share of PK inevitably increases. With the progress of growth, and thus size, the span between both specific activities within the abdominal muscle system becomes larger and, accordingly, the calculated CS/PK ratio decreases with size.

The influence of the scaling effect on the enzyme activities of our samples became obvious when the average ratio between CS and PK was calculated and plotted against the average weight of the samples. Most data points shared the same regression line, which clearly demonstrated that the allometric effect was similar in those samples, irrespective of their geographical origin. Accordingly, the variation in activities must be seen as a result of scaling and thus solely dependent on the size of animals. An exception was observed among the Ligurian krill. While the winter data shared the characteristics of those of the Kattegat and Clyde Sea krill, which clearly fell on the common regression line, the summer value in Ligurian krill stood apart, showing a considerably lower ratio than the winter data at almost the same body size. The reason for the low CS/PK ratio might be a decreased amount of "CS-rich" muscles and/or a generally decreased amount of mitochondria within the muscle tissue. Peculiarly, this effect related to the season of lowest food availability. Therefore, it might reflect some kind of somatic reduction to save energy as a

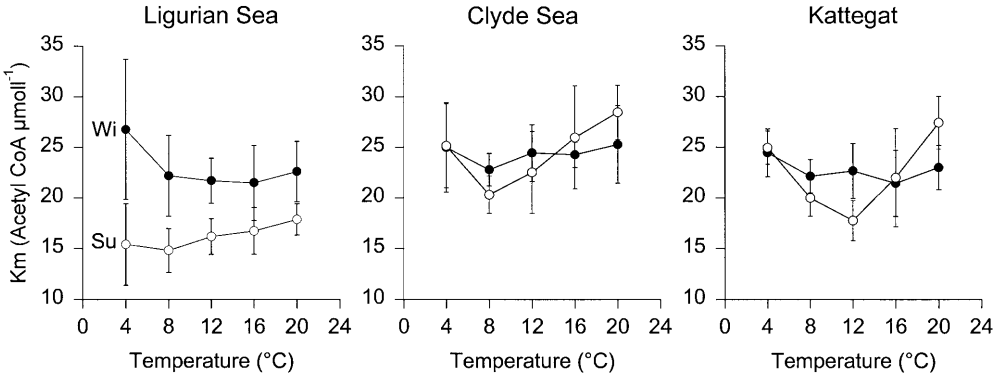

Fig. 7 *Meganyctiphanes norvegica*. K_m values of citrate synthase in relation to assay temperature (means ± SD, $n = 6$–12). *Open circles* represent summer data (*Su*); *filled circles* represent winter data (*Wi*)

strategy to cope with the food-limiting conditions of the Ligurian Sea during summer.

The thermal properties of CS and PK in the krill muscle did not reveal differences between locations or between seasons and, consequently, in this respect did not reflect adaptive characteristics related to the climatic gradient. The thermal profiles, moreover, reflected the combined thermodynamic properties and the heat resistance of the protein structure, which consisted of the exponential increase of activity with temperature, on the one hand, and the progressing denaturation of the protein at higher temperatures, on the other hand.

The uniform thermal activity profiles also resulted in similar Arrhenius plots and consequently gave similar activation energies of 36.2 kJ mol^{-1} for CS and 60.2 kJ mol^{-1} for PK. Compared to Vetter (1995), who investigated CS from Ligurian and Kattegat krill for thermal properties, the present activation energies for CS were lower, but, similarly, no variation between the locations was evident. In the case of pyruvate kinase, E_a values were consistently higher in the present study than those reported for *M. norvegica* by Vetter and Buchholz (1997). This can be explained by the different methods applied in the respective studies, e.g. use of selected tissue versus whole animals and use of crude extracts versus purified enzymes.

With regard to the effects of enzymatic compensation as a response to thermal changes, it seems comprehensible that specific activity normalized to body mass could serve as a suitable indicator for adaptive processes. Assuming a constant assay temperature, in the case of positive temperature compensation, specific activity is expected to be elevated in cold-adapted animals and low in warm-adapted ones, being adjusted to the same level when the animals were exposed to warm or cold ambient conditions. Corresponding observations have been reported and discussed by Vetter (1995) for the CS of Northern krill that were maintained in the laboratory for 11 days at different temperatures. Assuming such a regulatory capacity as a generalized adaptive feature in Northern krill, this should be even more pronounced with respect to the marked temperature differential between seasons. Equally, such a compensatory effect should also be expected as a response to the short-term temperature changes that krill experience during diel vertical migration. The present results, however, failed to support either of these assumptions. The seasonal comparisons resulted in only insignificant differences of CS activity in krill from the Kattegat and from the Clyde Sea. This was particularly unexpected in the Kattegat krill, because these animals encounter the greatest variations in temperature between seasons (> 10°C). Furthermore, no variation was found when Kattegat krill were taken from different thermal layers and were analyzed for enzyme activity (results not shown).

In contrast, Ligurian krill showed significantly higher activities of CS in winter/spring as compared to summer. Here, thermal conditions in the field were most uniform. Apart from short excursions into the warmer water strata in summer, krill spent most of the time in an isothermal environment at 12–13°C. Accordingly, other factors, such as the nutritive state, must be considered relevant in adaptive adjustments of enzyme activity.

K_m values were determined for CS towards acetyl-CoA in detail, but not against oxaloacetate. The latter determination showed K_m values which were more than five times higher than those towards acetyl-CoA. Because of this distinctly lower affinity between enzyme and substrate, a regulative capacity of this reaction step can be neglected. Furthermore, the K_m values of the allosteric PK were not considered in the present study, but were the subject of a separate investigation, due to their complex interactions with different effectors (Salomon et al. 2000).

K_m values of CS towards acetyl-CoA were similar at all locations during the winter. They exhibited the same level at all incubation temperatures between 4°C and 20°C, almost paralleling the *x*-axis. Furthermore, the K_m values were at a similar level of about 23 µmol l^{-1}. During winter, temperatures were almost constant (isothermal conditions) throughout the water column at each of the study sites. However, these were distinctly different, at 13°C in the Ligurian Sea, 7°C in the Clyde, and 4.5°C in the Kattegat.

Assuming compensatory adaptation to the ambient temperatures, a decreased K_m at each of the corresponding temperatures should be expected according to the reasoning of Baldwin and Hochachka (1970) and

Baldwin (1971). The patterns shown here, however, do not reflect such adaptation to a certain ambient temperature and indicate that during winter the kinetic properties of the CS reaction in Northern krill are not related to temperature. Furthermore, according to Hoffmann (1976), a broad plateau can be considered typical for eurythermal organisms. However, during winter each site exhibited the most thermally constant conditions.

During summer, however, the shape of the K_m-profiles changed significantly at all three sites. In the Ligurian Sea a parallel downward shift of the entire profile was apparent. K_m values were consistently lower at each of the incubation temperatures than during the winter, indicating a generally increased enzyme–substrate affinity. According to Hochachka and Somero (1984), the real adaptive requirement is not only the enzyme's characteristic but the correct pairing of K_m values with intracellular substrate concentrations. In this respect it might be suggested that the consistently lower K_m values of Ligurian summer krill correspond to a decrease of substrate concentration.

In contrast to the Ligurian krill, krill from the Clyde Sea and from the Kattegat showed a tendency to express V-shaped profiles, with minimum K_m values at 8°C and 12°C, respectively. This observation may relate to a seasonal adjustment of enzyme affinity in view of increased temperatures during summer at these two locations (sensus Hoffmann 1976), particularly in the upper third of the water column (thermal hydrography in Matthews et al. 1999). It can be argued that the enhancement of CS efficiency at higher temperatures may be beneficial in motility or feeding during excursions into the upper warm water layers.

In conclusion, with respect to adaptive enzymatic properties, seasonal changes in activity of CS were most pronounced in the Ligurian krill. During summer the specific activity of CS in the abdominal muscle was decreased which also was clearly demonstrated by the low CS/PK ratio. This may be considered as a response to limited food availability, followed by a certain degree of metabolic reduction. At the same time the K_m values of the CS were substantially decreased. This may be an adjustment of the enzyme to decreased substrate concentrations, but would also enhance the affinity between enzyme and substrate. The latter can be seen as a mechanism to, at least partly, compensate for the lower specific CS activity during summer. Temperature may not play a substantial role in this respect. This conclusion also applies to respiration measurements, whereby rates in the Ligurian Sea were strongly enhanced in comparison to low summer respiration, as a response to the short, highly productive phase during late winter and early spring (Saborowski et al., this issue).

In krill from the Clyde Sea and the Kattegat seasonal changes in specific activities and kinetic characteristics of CS were less distinct and possibly only related to some K_m adjustments due to thermal adaptation.

Most likely, irrespective of the hydroclimatic conditions, as exemplified by the three sites, enzymatic adaptation is more strongly influenced by trophic conditions and the nutritive state of the krill than by the thermal regime. This appears to be particularly so in the case of the Ligurian krill. This physiological distinction in terms of a strong trophic dependence may relate to the first report of genetic differentiation in *M. norvegica* by Zane et al. (2000), who showed that Ligurian krill are clearly distinct from all Atlantic populations tested, including krill from the Clyde Sea and the Kattegat.

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