

# Metabolic and enzymatic adaptations in northern krill, *Meganyctiphanes norvegica*, and Antarctic krill, *Euphausia superba*

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**Abstract:** The Antarctic krill, *Euphausia superba*, is restricted to the Antarctic Ocean. The northern krill, *Meganyctiphanes norvegica*, is extremely widely distributed from the arctic North Atlantic to the warm Mediterranean. Respiration measurements showed no seasonal differences in rates determined in krill from the thermally stable Clyde Sea (Scotland) and the cooler but variable Danish Kattegat. In the warm Ligurian Sea, where temperatures are stable, krill showed higher rates in April than in September, indicating reactions to the short but intensive productive season. Krill can passively benefit from enhancements of overall metabolism when ascending into upper, warmer water strata during their pronounced diel vertical migration. Michaelis–Menten constants ( $K_m$ ) of citrate synthase (CS) were compared. In terms of respiration and enzyme regulation, krill from the Ligurian Sea stand apart: temperature and nutrition appear to be of different influence, relatable to genetic differentiation in the species. In contrast,  $K_m$  of CS in *E. superba* is temperature independent, highlighting the species' stenothermal physiology. A basal level of activity of digestive enzymes ensures immediate utilization of patchy food sources. Specific induction, including that of chitinases, indicating omnivory in both species, underlines krill's exceptional capacity to adapt to highly variable trophic environments. Processes of moult, growth, and reproduction are locally and seasonally adjusted.

**Résumé :** Le krill antarctique, *Euphausia superba*, n'habite que l'Océan antarctique, alors que le krill nordique, *Meganyctiphanes norvegica*, possède une répartition géographique très étendue qui va de la région arctique de l'Atlantique Nord aux eaux chaudes de la Méditerranée. Les taux respiratoires n'accusent pas de variation saisonnière chez le krill de la mer de Clyde en Écosse, un milieu à forte stabilité thermique, ni chez celui du Cattégat danois, une mer plus froide et plus changeante. Dans la mer Ligurienne, où les températures sont chaudes et stables, le krill a des taux respiratoires plus élevés en avril qu'en septembre, en réaction à la courte mais intense saison de production. Le krill peut bénéficier passivement d'un accroissement général de son métabolisme lorsqu'il se déplace vers les couches d'eau supérieures plus chaudes durant sa migration verticale quotidienne marquée. Nous avons comparé les constantes de Michaelis–Menten ( $K_m$ ) de la citrate synthase (CS). En ce qui a trait à la respiration et la régulation enzymatique, le krill de la mer Ligurienne fait bande à part: la température et la nutrition semblent y avoir une influence particulière, à cause d'une différenciation génétique chez l'espèce. En revanche, la  $K_m$  de la citrate synthase est indépendante de la température chez *E. superba*, une caractéristique physiologique d'une espèce sténotherme. Le maintien d'un niveau d'activité de base chez les enzymes digestives permet l'utilisation immédiate de ressources alimentaires à répartition contagieuse. L'induction spécifique, entre autres celle des chitinases, qui indique l'omnivorie chez les deux espèces, illustre l'extraordinaire capacité du krill à s'adapter à des environnements trophiques extrêmement variables. Les mécanismes de la mue, de la croissance et de la reproduction s'adaptent aux conditions locales et saisonnières.

[Traduit par la Rédaction]

## Introduction

The northern krill, *Meganyctiphanes norvegica*, attains the largest body size among the euphausiid species of the northern hemisphere. Its distribution covers an exceptionally wide longitudinal and latitudinal range in North Atlantic waters, spanning from the Gulf of St. Lawrence to the Aegean Sea and from the Barents Sea to the upwelling zones of the Cape

Verde Islands (Mauchline and Fisher 1969). Accordingly, this krill species is ideally suited for a model study in climatic adaptation.

Based on earlier work on local populations of *M. norvegica*, comparative investigations in the framework of the Third Marine Science and Technology Programme of the European Union were conducted in three areas during 1996–1999 (Buchholz et al. 1998). The Ligurian Sea in the warm and constant northern Mediterranean Sea and the Clyde Sea, which reflects cool and constant Atlantic conditions, were contrasted by the Danish Kattegat where waters of the North and Baltic seas meet with consequently variable temperatures.

This study concentrates on results of the project and earlier literature in terms of ecophysiological climatic adaptation, traced by respiration measurements, and the characteristics of key metabolic and digestive enzymes. These are discussed

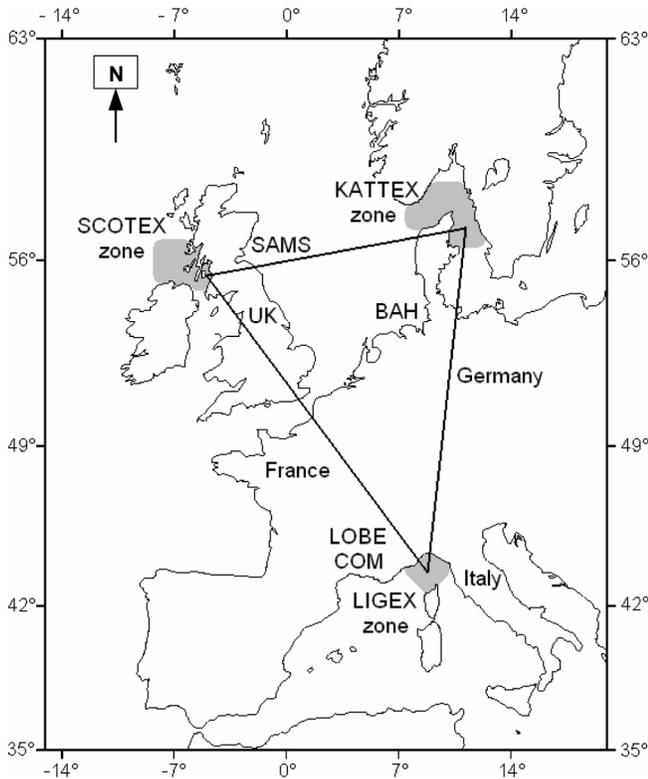
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**Table 1.** Locations and sampling periods.

Location	Cruise	Season	Position	Max. depth (m)	Sampling period
Ligurian Sea	Ligex I	Winter–spring	43°00'N, 07°30'E	2000	1–29 April 1996
	Ligex II	Summer–fall			5–25 Sept. 1997
Clyde Sea	Scotex I	Summer–fall	55°40'N, 05°00'W	150	2–11 July 1996
	Scotex II	Winter–spring			15–28. Feb. 1997
Kattegat	Kattex I	Summer–fall	57°18'N, 11°25'E	131	14–29 July 1996
	Kattex II	Winter–spring			4–19 March 1997

**Fig. 1.** Climatic triangle. Source: PEP database (physiological ecology of a pelagic crustacean).

in the light of the multidisciplinary approach on population dynamics and behaviour (Guérin-Ancey et al. 1998; Tarling et al. 1998b, 2000) and on adaptive processes linked to nutrition (Lass et al. 2000; Virtue et al. 2000), reproduction (Cuzin-Roudy and Buchholz 1999; Cuzin-Roudy 2000), and lipid metabolism (Mayzaud et al. 1999), including the first attempt to differentiate a possible genetic separation of these populations (Zane et al. 2000).

*Meganyctiphanes norvegica* is an exceptionally strong vertical migrator. Accordingly, when krill energetics are discussed, these have to be related to different regimes with a spring amplitude of diel vertical migration (DVM) of 800 m in the deep Mediterranean (Velsch 1997) as well as less than 100 m at the shallower northern locations (Tarling et al. 1998a). Different effects of the local seasonal trophic regimes are also taken into account where applicable.

In contrast with *M. norvegica*, the Antarctic krill, *Euphausia superba*, is strictly bound to polar waters. With respect to adaptive features of respiration and enzymes, some comparisons will be drawn with the largest and most numerous euphausiid on the basis of recent work, particu-

larly under seasonal aspects. Finally, some open questions in krill ecophysiology will be highlighted and possible ways to find answers to these by multidisciplinary approaches.

## Euphausiid metabolism

### *Meganyctiphanes norvegica* in the climatic triangle: locations, hydrography, and trophic conditions

#### Locations

For the biological comparison, locations (see Fig. 1) were chosen where abundant swarms of krill had been regularly encountered and studied previously: the Arran Deep in the Clyde Sea, western Scotland (Mauchline 1960), the Läsö Deep in the Danish Kattegat (Buchholz and Boysen-Ennen 1988), and the frontal system in the Ligurian Sea, which was located at the beginning of the cruises by conductivity–temperature–depth (CTD) on a section between Nice and Calvi (Corse) (Labat and Cuzin-Roudy 1996).

The cruises (Table 1) were conducted onboard the German RV *Heincke*, a 1990-built, 1000-t, multipurpose ship. The CTD, an acoustic doppler current profiler (ADCP, 150 kHz), a dual-frequency sonar (38 and 120 kHz), and a multi-opening/closing net system with an environmental sensing system (MOCNESS, 1 m<sup>2</sup>, 300- and 2000- $\mu$ m mesh) were regularly deployed and maintenance experiments with live krill performed in parallel.

#### Hydroclimatic features

A review of the situation encountered during at least one cruise in winter and summer to the Clyde Sea and the Kattegat is given in Matthews et al. (1999). Here, the measurements made during the cruises are related to general oceanographic features of the areas. Another covering the Ligurian Sea is currently being prepared. In the present paper, typical thermal profiles from the climatic triangle are presented as a background for biological adaptation (Fig. 2).

The subsurface and deep waters (>2000 m) of the Ligurian Sea are constantly at 12–13°C during summer and winter, while only a thin surface layer (about 30 m) increases in temperature during summer (up to 24°C). During vertical migration, krill enter the surface layers only for short periods but generally remain below a sharp thermocline at about 30 m (Tarling et al. 1999a).

The Scottish Firth of Clyde is colder than the Ligurian Sea. In the Arran Deep, winter temperatures were constantly at 6–8°C throughout the water column. During summer, a slight stratification appeared with warmer surface waters of up to 12°C. However, krill were again exposed to comparatively constant diel and annual temperatures.

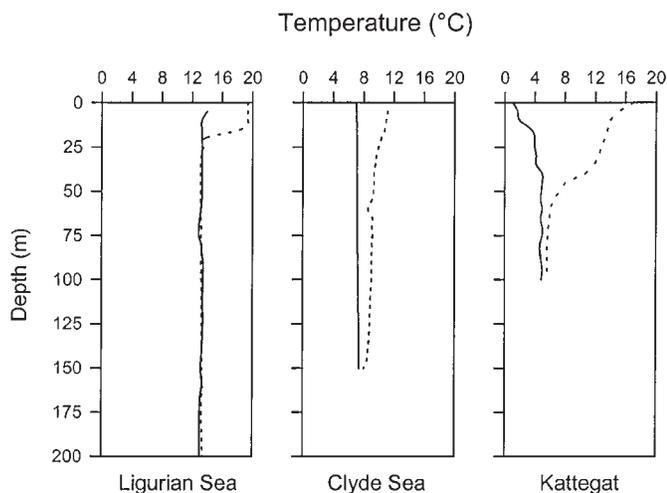
In sharp contrast with these conditions, the Danish Kattegat is characterized by high thermal variability. The

**Table 2.** Chlorophyll *a* (Chl *a*) concentrations integrated over a 100-m water column (Chl *a* determined on filters by fluorometry) and energy values of krill stomach contents (converted from carbon, hydrogen, and nitrogen analysis after Salonen et al. (1976);  $n = 6\text{--}12$  krill).

Season	Ligurian Sea		Clyde Sea		Kattegat	
	Chl <i>a</i> ( $\mu\text{g}\cdot\text{m}^{-2}$ )	Energy (J·individual <sup>-1</sup> )	Chl <i>a</i> ( $\mu\text{g}\cdot\text{m}^{-2}$ )	Energy (J·individual <sup>-1</sup> )	Chl <i>a</i> ( $\mu\text{g}\cdot\text{m}^{-2}$ )	Energy (J·individual <sup>-1</sup> )
Winter–spring	38–181 (Apr.)	2±1	10–33 (Feb.)	3±2	21–96 (Mar.)	2±1
Summer–fall	27–31 (Sept.)	1±1	317–422 (July)	5±4	24–32 (July)	2±1

**Note:** Source: P. Mayzaud (Laboratoire d'Océanographie Biochimique et d'Ecologie, Observatoire Océanologique, B.P. 28, 06230 Villefranche-sur-Mer, France, personal communication) and R. Saborowski (PEP database, personal communication).

**Fig. 2.** Thermal profiles during summer (broken line) and winter (solid line) from CTD measurements. Source: PEP database.



summer surface temperatures of the Kattegat reached 16–18°C. With depth, temperature decreased continuously towards 6–8°C due to stratification brought about by a northward flow of near-surface Baltic water, which is very cold in winter and heated considerably during the summer, and southward-flowing cool North Sea/Skagerrak water at depth. During the winter, surface temperatures fell below 2°C but increased gradually toward the bottom to about 5°C. Accordingly, within the range of DVM, krill may be exposed to shifts of temperature of up to 8°C. Seasonal variation may even exceed 12°C.

#### Trophic conditions

The trophic environments at the three locations are distinctly different. Recent results underline that *M. norvegica* is an opportunistic feeder and feeds on both phyto- and zoo-plankton (Bamstedt and Karlson 1998; Onsrud and Kaartvedt 1998). A thorough comparison of food sources in the water columns of the Clyde Sea and Kattegat and food selection by stomach contents analysis is given in Lass et al. (2000) and by lipid analysis in Virtue et al. (2000).

To concisely characterize the seasonal feeding conditions, chlorophyll *a* (Chl *a*) values are given in Table 2. Due to the complexity of food selection in krill, the energy content of the material ingested, irrespective of its source, was determined by carbon, hydrogen, and nitrogen analysis (Salonen et al. 1976) of stomach contents. These values are presented to illustrate seasonal variability in food and feeding conditions at the three sites.

To complement these “snapshot” descriptions of trophic situations encountered during the cruises, the annual course of nutritional conditions is further characterized. In the Ligurian Sea, the productive months are limited in time and only last from mid-March to the end of May. Otherwise, this area and the whole Mediterranean are typically oligotrophic (Fabiano 1984; Goffart et al. 1995). These conditions are reflected in the above values.

In the Clyde Sea, in contrast, feeding conditions are favourable, with a long period of high production: April–October (Gowen et al. 1998). During the winter cruise, both phyto- and zoo-plankton standing stocks were low, i.e., corresponded to a food-depleted winter situation.

In the Kattegat, phytoplankton levels are always low and bound to the surface layer that krill usually do not enter during DVM. However, krill rely strongly on copepods, these being advected from the Baltic and the deep Skagerrak currents (Buchholz et al. 1995). As a consequence, a long phase of enhanced production or availability of plankton lasts from April to November (Buchholz and Boysen-Ennen 1988). The winter–summer difference during the cruises was not pronounced.

#### *Meganyctiphanes norvegica* in the climatic triangle: adaptations in respiration and enzymes

##### Respiration

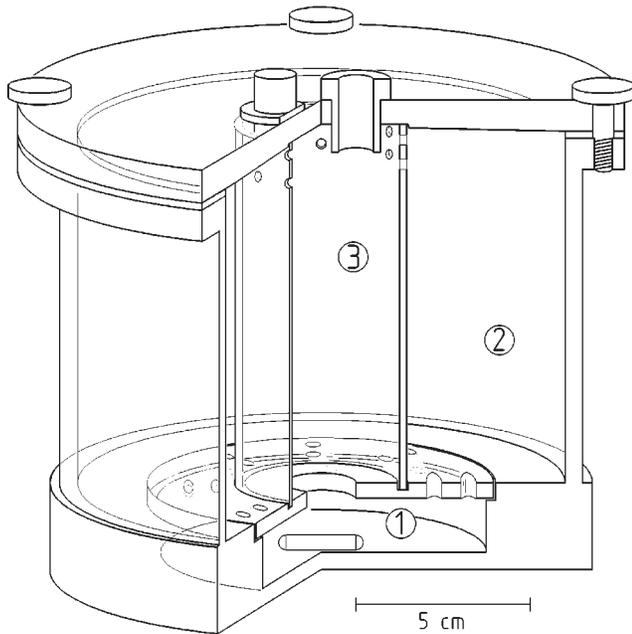
Measurement of oxygen consumption is problematic in planktonic and pelagic species. Accordingly, in order to avoid a major shortcoming, a measuring chamber was designed to prevent krill from being influenced strongly by confinement (Fig. 3). Details are given in Saborowski and Buchholz (1998).

A slow, upward-directed, and circular current was generated in the chamber. This helped the specimens to stay clear of the bottom and orient themselves to swim against the constant current. Natural swimming behaviour in krill was thus encouraged, particularly preventing krill from contacting the chamber walls too frequently.

Six chambers were immersed in a temperature-controlled water bath. One served as control. The others were equipped with two to five specimens in order to reduce individual variation. Only male krill were used to generate a reliable data set ( $n = 4\text{--}14$  per data point per temperature), and respiration measurements were normalized to a 30-mm standard krill.

In an intersite comparison (Fig. 4), the oxygen consumption rates ranged between 20 and 90  $\mu\text{mol O}_2\cdot\text{mg dry weight}^{-1}\cdot\text{h}^{-1}$  and followed exponential relationships with experimental temperatures between 4 and 16°C, corresponding to the predominant natural range that *M. norvegica* encounters. In Clyde and Kattegat krill, almost no seasonal varia-

**Fig. 3.** Respiration chamber with self-generating current system. 1, current generator; 2 and 3, outer and inner cylinder, respectively. Source: Saborowski and Buchholz (1998).



tion exists in respiration. Summer–fall values (September) in Ligurian krill overlap to a large extent with the respiration curves of krill from the other two locations. The exception is winter–spring respiration in the Ligurian krill. Here, all values are enhanced by at least 80% in relation to summer values. Winter–spring values (April) in Ligurian krill and summer values in Kattegat krill (July) were confirmed by repetition of the experiments (data not shown).

Firstly, relationships to seasonal temperature adaptation are considered. The thermal winter–summer differences in the Clyde Sea are small and could explain the lack of seasonal adjustments of overall rates. However, the high thermal variability in the Kattegat should then cause an overall adaptive shift in rates. This is apparently not the case. However, respiration rates in the Clyde Sea were consistently 20–30% lower when compared with those in the Kattegat. This may be interpreted as a thermal adaptation: a range of 30–35  $\mu\text{mol O}_2 \cdot \text{mg dry weight}^{-1} \cdot \text{h}^{-1}$  (highlighted by horizontal bars in the graphs) corresponds to the annual mean temperature of 6–9°C in the Clyde Sea. The same range of oxygen consumption corresponds to 4–7°C in the Kattegat. This range equals the annual temperature amplitude in the deeper layers in the Läsö Deep (compare with the temperature range indicated above the abscissa in Fig. 4). Accordingly, the adjustment to the low temperature ranges encountered during most times of the year may be physiologically more important at both locations than the vertical temperature differential met during summer.

At the same time, at both sites, the thermal stratification in the water column, which develops most strongly in the Kattegat with a difference of about 10°C in summer, may be of physiological advantage with respect to DVM. An enhancement of the overall metabolic rate would be advantageous as krill crossed the thermocline: the rate increase with

temperature may facilitate motility in the search for food and the ability to escape from predators. This may then be interpreted as a passive effect of metabolic enhancement because no short-term active adjustments to changing temperatures were apparent; such would have been detectable, e.g., as horizontal intersections in the respiration curves. In fact, respiration rates follow smooth exponential courses.

It is interesting to note that the range of 30–35  $\mu\text{mol O}_2 \cdot \text{mg dry weight}^{-1} \cdot \text{h}^{-1}$  equally corresponds to summer–fall respiration in the Ligurian krill at temperatures of 12–13°C prevailing below the thermocline, where krill remain for most of the time. Furthermore, when krill from all three sites are compared, the correspondence of this standard respiration rate of 30–35  $\mu\text{mol O}_2 \cdot \text{mg dry weight}^{-1} \cdot \text{h}^{-1}$  may be interpreted as an effect of metabolic temperature compensation (*sensu* Hochachka and Somero 1984). The overall respiration curves show vertical shifts and result in the same metabolic rate at the prevailing temperature at each site, i.e., at 12–13°C in the Mediterranean, 6–9°C in the Clyde Sea, and 4–7°C in the deep layers of the Kattegat.

However, winter–spring values in Ligurian krill are consistently more than 80% higher than summer–fall respiration at this site, corresponding to a vertical upward shift of the respiration curve. Most probably, seasonal trophic change plays a role in this respect.

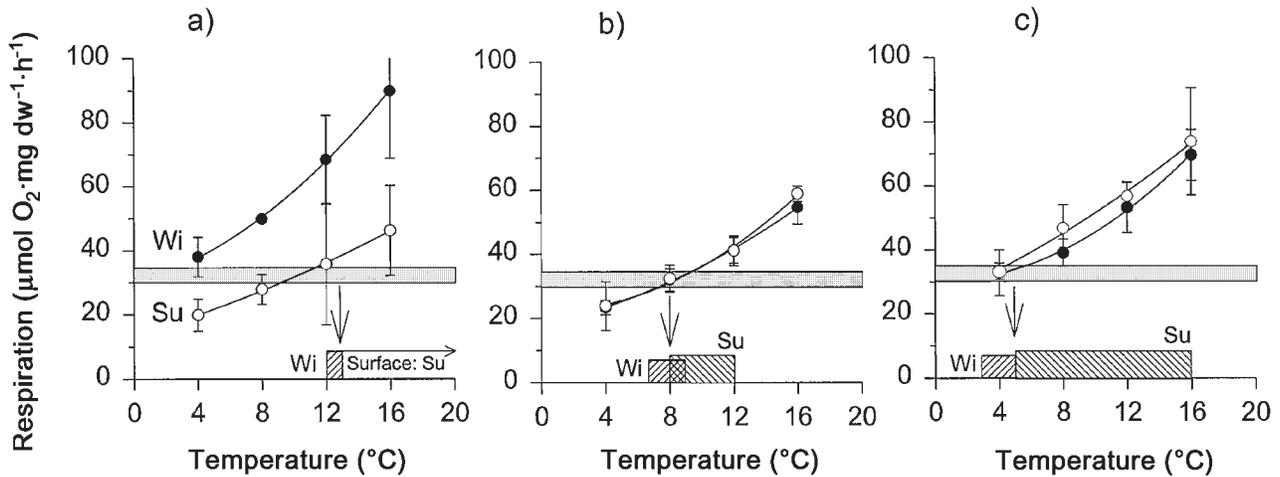
In the Ligurian Sea, the very short period of elevated primary production begins in mid-March and usually ends in May (Goffart et al. 1995), providing the basis for intensive somatic growth and reproduction in krill. In fact, krill strongly rely on phytoplankton during that time (de La Bigne 1985). Males start to produce spermatophores continuously, while females go through vitellogenesis and several consecutive spawning events (Cuzin-Roudy 1993). Compared with the Clyde Sea and the Kattegat, Ligurian krill have by far the shortest reproductive period, which, moreover, is closely interlinked with the onset of phytoplankton production. Furthermore, DVM ranges are at their maximum (Velsch 1997), and high excretion rates were measured (Roger 1978). Apparently, during the spring period, physiological and behavioural changes occur that cause an exceptional increase in overall metabolic rates in Ligurian krill.

In summary, respiration as an expression of overall metabolism shows the following features: metabolic rates in krill from different climatic regions are adjusted to maintain a comparable level tuned to the prevailing temperature regime, passive use of the temperature differential in stratified waters may enhance physiological capacity during DVM, krill from the northern locations appear to be predominantly affected by temperature, and in the Mediterranean, trophic conditions are superimposed on temperature adaptation, linked to the short productive season in an otherwise oligotrophic sea. Generally, irrespective of the sites, short-term adjustments of metabolic rates did not occur. Accordingly, systematic, long-term environmental adaptation prevails.

#### *Metabolic enzymes*

Alongside the determination of overall metabolic capacity in live krill, physiological performance and adaptive capability were also studied with regard to key metabolic enzymes. Changes in enzyme kinetics or synthesis are directly related

**Fig. 4.** Oxygen consumption in (a) the Ligurian Sea, (b) the Clyde Sea, and (c) the Kattegat normalized to a 50 mg dry weight (dw) krill (means  $\pm$  SD,  $n = 6-10$ ). Bars highlight respiration rates at ambient temperatures (see text); seasonal ranges are marked on the abscissa: Wi, winter; Su, summer. Source: R. Saborowski et al. (unpublished data) and Saborowski et al. (2000a). Method in Saborowski and Buchholz (1998).



to acclimation and adaptation to ambient temperatures, since enzyme activity is involved at all levels of metabolism (Clarke 1983). Citrate synthase (CS) is an enzyme instrumental in energy metabolism that has a control function in the citric acid cycle. Pyruvate kinase (PK), a regulatory enzyme of the glycolytic pathway, is involved in ATP production.

Work on CS and PK confirmed previous studies that showed that adjustments of the basic characteristics temperature maximum, energy of activation, and thermal stability are unimportant in temperature acclimatization in *M. norvegica* (Vetter and Buchholz 1997). Maintenance experiments showed, however, that the species adjusts specific activity of CS to different temperatures during acclimation over 11 days (Vetter 1995a).

Such a capacity to compensate thermal influences by maintaining a similar level of activity irrespective of the ambient temperature would be expected to play a role in longer term adjustments to seasonal temperature changes. The same compensatory mechanism might equally be involved as a response to short-term temperature changes that krill experience during DVM. However, determinations of specific activities in samples as compared at a standard assay temperature of 25 $^{\circ}\text{C}$  taken from the climatic triangle during both seasons did not support this hypothesis (Saborowski and Buchholz 2000). The seasonal comparison resulted in nonsignificant changes of specific activity in the Clyde Sea and the Kattegat. Equally, CS activity remained at a similar level when samples taken from the field at different ambient temperatures at different depths following a daily cycle were analysed. Accordingly, quantitative enzyme regulation by adjusting specific activity to temperature appears unimportant at both seasonal and diel time scales. The same applies to the specific activities determined for PK (Salomon et al. 2000).

With respect to season, however, a significantly diminished value of specific activity ( $-25\%$ ) was found in krill taken during summer-fall (September) in the Ligurian Sea as compared with the activity in winter (April). Temperatures are the most uniform in the Ligurian Sea (Fig. 2). Ac-

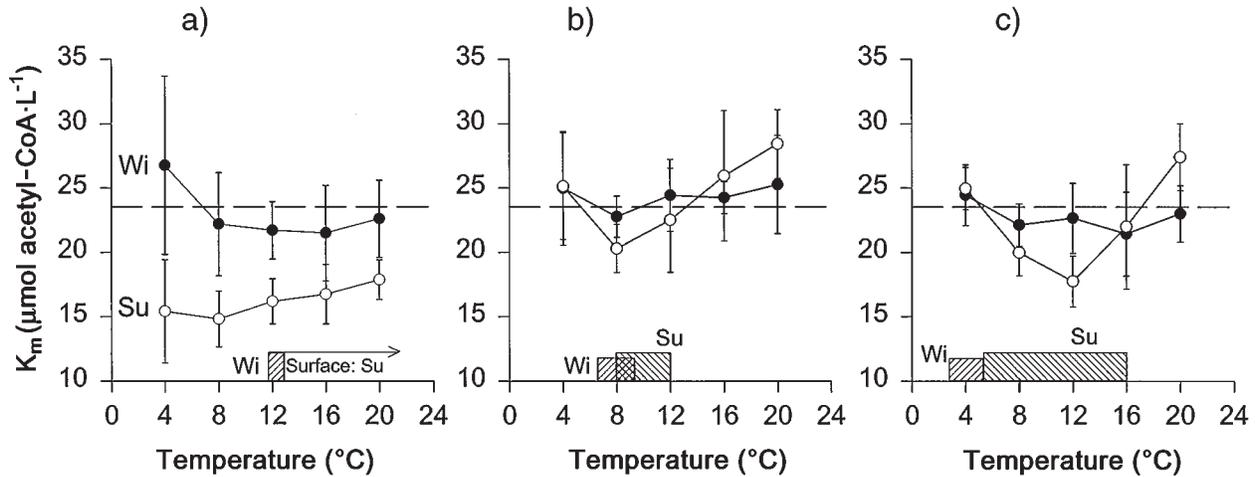
cordingly, not the thermal influence but other factors must have caused the downscaling of CS activity during September. Here again, the strong seasonal contrast of the trophic environment may have been responsible for this effect.

Variability was apparent in specific activities in CS and PK determined in the abdominal muscle tissue in the seasonal samples from all three sites. When winter and summer values were paired and the proportional activity of CS/PK calculated, these proportions fell on a straight line with a negative slope when regressed against dry weight ( $y = -0.0053 + 0.3570$ ;  $r^2 = 0.994$ ). Apparently, a fixed ratio of CS/PK exists, indicating an allometric relationship where CS correlates negatively with weight and PK positively (details are given in Saborowski and Buchholz 2000). Such an effect is known as metabolic scaling (Childress and Somero 1990; Berges and Ballantyne 1991). PK-rich cells and thus activity simply increase with weight. With respect to CS, possibly the muscle fibres of the large abdominal muscle system are multiplied to a larger extent than the most active, mitochondria-rich, but small, ventrally situated pleopod muscles. Accordingly, the relative number of mitochondria decreases and thus CS activity.

Obviously, the seasonal differences found in the specific activities of PK and CS are attributable to metabolic scaling in krill, irrespective of their geographic origin, and depend on the size of the animals. However, as indicated above, the summer-fall (September) activity in the Ligurian krill was found to be considerably depressed. As a consequence, the CS/PK ratio deviated significantly ( $<50\%$ ) from the expected value calculated from the CS/PK regression. The depressed CS activity in summer krill in the Ligurian Sea may relate to the low food availability during the long oligotrophic phase. Possibly, somatic reduction takes place to save energy during food-limiting conditions.

An adjustment of specific activities to compensate for environmental effects, particularly temperature (or nutrition, see below), can be taken as an indication of quantitative enzyme regulation (Hochachka and Somero 1984). An alternative is the qualitative strategy, i.e., to modulate substrate

**Fig. 5.** Substrate affinity ( $K_m$ ) towards acetyl-CoA of CS in relation to assay temperature from seasonal samples ( $n = 6-12$ ) in (a) the Ligurian Sea, (b) the Clyde Sea, and (c) the Kattegat. The broken line marks the mean of all measurements, irrespective of site during winter. Seasonal temperature ranges are marked on the abscissa: Wi, winter; Su, summer. Source: R. Saborowski et al. (unpublished data) and Saborowski and Buchholz (2000). Methods in Vetter (1995a).



affinity. The Michaelis–Menten constant ( $K_m$ ) is the common measure. A decrease in  $K_m$  corresponds to enhanced substrate affinity resulting in an increase in catalytic capacity and vice versa.

The determination of  $K_m$  is tedious and requires highly standardized procedures. An exceptionally large data set is needed to differentiate environmental influences. Here, it is assumed that the  $K_m$  determined at different assay temperatures in vitro reflects the changes due to structural adjustments of the enzyme in vivo. Values of  $K_m$  in CS were determined accordingly and compared under appropriate seasonal and geographic conditions.

During winter, the  $K_m$  values determined were very uniform at all three sites (Fig. 5). With little variation, all values followed nearly horizontal lines and are close to an overall average of  $23 \mu\text{mol}\cdot\text{L}^{-1}$  (broken line in the graphs). Apparently, no regulatory capacity via  $K_m$  exists during winter.

Firstly, this effect may correspond to the isothermal conditions of the water columns at the sampling locations without a need for thermal adjustment. Secondly, a fixed level of enzymatic turnover potential is maintained in CS, irrespective of the ambient temperatures, which are distinctly different at each site (Fig. 2).

Summer conditions appear to have pronounced effects on the course of  $K_m$  values. Most drastically, the complete curve is shifted to lower values in Ligurian krill. With respect to the prevailing ambient temperature of  $12-13^\circ\text{C}$ , substrate affinity is doubled at this location and results in a considerable gain of enzymatic turnover capacity.

In the Clyde Sea and the Kattegat,  $K_m$  curves were V-shaped. In the Kattegat, the  $K_m$  was significantly lowered by about 40% at a temperature of  $12^\circ\text{C}$  with respect to the corresponding winter value. In the Clyde Sea, the V-shape was generally less pronounced and showed a minimum at  $8^\circ\text{C}$ .

The winter–summer differences in  $K_m$  are partly unexpected with regard to common hypotheses of enzymatic adaptation. According to the reasoning of Baldwin (1971), Baldwin and Hochachka (1971), and Hochachka and Somero (1984),  $K_m$  adjustments of different metabolic enzymes are important in thermal adaptation. A suite of conge-

neric species, mostly fish from different latitudes, were compared as well as adaptation experiments conducted and later extended to invertebrates (e.g., Dahlhoff and Somero 1993; Vetter and Buchholz 1997). Generally,  $K_m$  values are adapted or “conserved” in different species so that  $K_m$  minima coincide with the ambient temperature range, particularly with emphasis on metabolic cold adaptation. Literature on seasonal adaptation is scarce, however. Vetter and Buchholz (1997) pointed out that a complex combination of adaptive mechanisms is needed to optimize metabolic enzymes under varying temperature regimes.

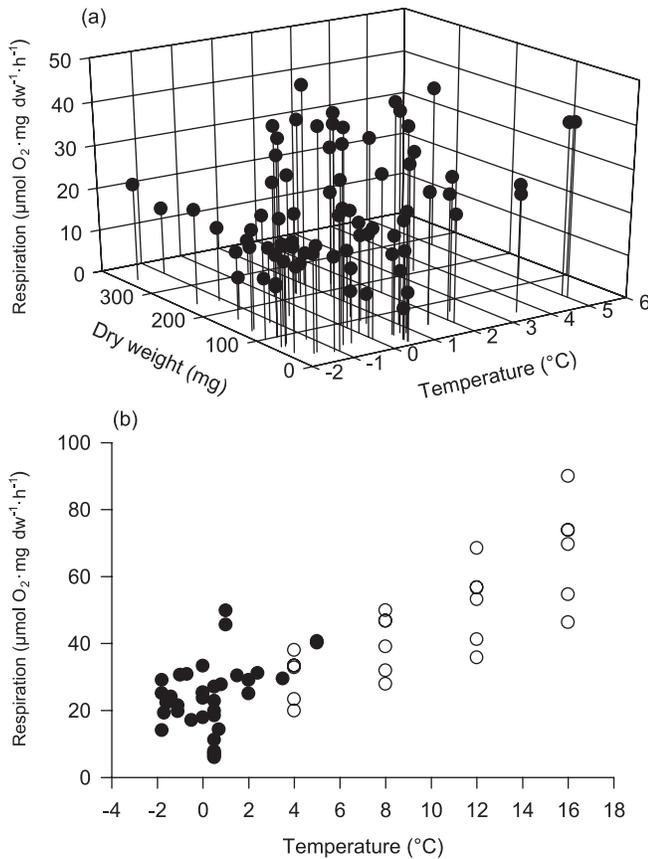
With regard to these considerations, adaptive traits of  $K_m$  variation in *M. norvegica* may be interpreted as follows. During winter, in spite of different ambient temperatures, the same level of substrate affinity is maintained at all three sites. This effect would correspond to metabolic temperature compensation. However, this is not expressed in the classically expected distinct  $K_m$  minima, which would be adjusted to the local prevailing temperatures of  $12$ ,  $7$ , and  $5^\circ\text{C}$ .

In summer in the Ligurian Sea, the qualitative adjustment of  $K_m$  presumably compensates for the quantitative reduction in terms of specific activity in muscle CS. At the same time, the strong trophic influence on metabolism such as that found in respiration in an isothermal environment appears to be confirmed. In summer in the Kattegat, thermal adaptation appears to be influential:  $K_m$  is adjusted in such a way that the same  $K_m$  as that during winter applies when krill stay in the cool deep during daytime. When krill migrate up into the warm near-surface layers during nighttime,  $K_m$  is systematically lower by about 40%, resulting in enhanced enzymatic capacity. This effect would then also help to increase motility during foraging and escape. The same effect, although less pronounced, may apply to the Clyde Sea due to the weaker temperature differential during summer in these waters.

#### Digestive enzymes

In the frame of the European Union project, a suite of digestive enzymes were analysed as indicators of qualitative and quantitative selection of planktonic food organisms in

**Fig. 6.** (a) Respiration of *E. superba* related to body weight and temperature. Source: Opalinski (1991) and Torres et al. (1994). (b) Respiration rate of *E. superba* versus temperature (solid circles; single values of krill in the weight range of 20–100 mg dry weight; source as above) and *M. norvegica* (open circles; rates normalized to a 50 mg dry weight krill; averaged data; source: Saborowski et al. 2000a).



*M. norvegica*. Results show a high potential of enzyme induction associated with sources of nutrition of both phyto- and zoo-plankton origin. Furthermore,  $K_m$  variation with temperature exists. The opportunistic behaviour in food selection (Bamstedt and Karlson 1998; Lass et al. 2000) is reflected in the repertoire and regulation of digestive enzymes. P. Mayzaud et al. (Laboratoire d'Océanographie Biochimique et d'Ecologie, Observatoire Océanologique, B.P. 28, 06230 Villefranche-sur-Mer, France, personal communication) are currently preparing a review on this subject.

The dynamics of enzymatic digestive processes, also in view of compartmentation and different ways of activation of zymogens, and a seasonal analysis of membrane-bound digestive enzymes of the midgut gland were presented by R. Saborowski at this conference (and as a personal communication). Some aspects of digestive enzyme regulation in *M. norvegica* are discussed in conjunction with more numerous results on *E. superba* in the present paper (see next section). The stomach of *M. norvegica* also contains bacteria that produce digestive enzymes. However, the potential contribution of these enzymes to krill digestion appear negligible in relation to endogenous enzyme production in the krill midgut gland and stomach (Donachie et al. 1995).

***Euphausia superba*, strictly polar: adaptations in respiration and enzymes**

*Respiration*

A multitude of data already exist on respiration rates in *E. superba*. Rates were mostly determined to establish length or weight relationships in order to assess energy requirements in krill. They were used furthermore to establish energy budgets to assess the krill's capacity to adjust to seasonal variation. Moreover, thermal adjustments are discussed.

Recently, direct winter–summer comparisons of respiration rates were also conducted with respect to seasonal adjustments, particularly in view of the unsolved riddle of how krill cope physiologically with the long winter in the Antarctic. Kawaguchi et al. (1986) were the first to report, and Quetin and Ross (1991) and Torres et al. (1994) presented data that showed metabolic reductions during winter as compared with summer values. These reductions were about 33 and 45%, respectively. Such metabolic reduction was interpreted as a way to reduce physiological activity generally in order to adjust to diminished food levels during winter. This refers to primary production in the open sea as well as that in under-ice algae, which krill are known to utilize (e.g., Marschall 1988). Since information on the feeding habits, including a possible switch to carnivory (Atkinson and Snyder 1997), is still incomplete, much debate was generated (e.g., Huntley et al. 1994).

A summary of respiratory data is published in Opalinski (1991). From available tables, a graph was constructed to give an overview of the respiratory dependence on size (weight) and temperature (Fig 6a).

From Fig. 6a, it is obvious that respiration is dependent on both body size (weight) and experimental temperatures. However, a large range of data scatter prevails. For this reason, a more thorough analysis was not attempted here, e.g., to calculate mathematical relationships. Nevertheless, a trend is visible in which both size and temperature typically follow exponential curves (Quetin et al. 1994; Torres et al. 1994). However, there is a general problem in obtaining reliable data in a pelagic (planktonic) organism under necessarily confined conditions in a measuring chamber.

In terms of temperature adaptation, comparisons within congeneric species from different latitudes have been useful (Hochachka and Somero 1984). Size apparently matters as well. Although not congeneric, *M. norvegica* is a large euphausiid and may thus be comparable to *E. superba*. Accordingly, respiration data for *E. superba* taken from the above data set were integrated into Fig. 6b in a comparable size range of 20–100 mg dry weight and were contrasted with the complete data set on *M. norvegica* from the Ligurian Sea, Clyde Sea, and Kattegat (standard size 50 mg dry weight).

The exponential curves for *M. norvegica* appear to link with the single data points for *E. superba* in the low temperature range. The spread of data in the former indicates the range of respiratory adaptation across geographic regions. An equal adaptive potential appears to relate to Antarctic krill from different areas of the polar ocean within its narrow range of temperatures (–2 to 2°C). However, metabolic cold compensation (sensu Hochachka and Somero 1984) does not seem to be pronounced in *E. superba* because a shift to

higher respiratory levels at low temperatures would have been expected in the interspecies comparison in view of the strong latitudinal thermal contrast (−2 to 16°C).

At any rate, future more thorough comparisons of the two euphausiid species appear feasible using standardized equipment. A prerequisite to following these lines would be to further optimize respiration chambers and to take utmost care in reproducing the same measuring conditions in seasonal or systematic comparisons. The same applies to intra-specific comparisons of respiration rates in relation to seasonal, particularly nutritional, adaptations.

#### *Metabolic enzymes: CS*

As in *M. norvegica*, adjustments of the basic characteristics temperature maximum and thermal stability in CS are unimportant in temperature acclimatization in *E. superba*. A substantial reduction in the energy of activation may play a role (Vetter 1995a), which was partly confirmed in our laboratory but is apparently influenced by individual and (or) geographic variation (R. Müller, Alfred Wegener Institut for Polar and Marine Research, c/o Biologische Anstalt Helgoland, Marine Station, Postfach 180, D-27483 Helgoland, Germany, personal communication). Experimental acclimation showed no capacity to adjust specific activities to varied temperatures (Vetter 1995a). All of these results underline the strictly stenothermal characteristics in the Antarctic krill.

In a north–south comparison of enzymatic regulation in different crustaceans, it appeared that one way to compensate for the rate-limiting, constantly cold environment in the Antarctic is maintaining higher enzyme concentration. Concerning CS, the polar euphausiid produces about five times the amount of CS protein as *M. norvegica* (by way of immunodetermination and ELISA; R. Müller, Alfred Wegener Institut for Polar and Marine Research, c/o Biologische Anstalt Helgoland, Marine Station, Postfach 180, D-27483 Helgoland, Germany, personal communication).

A previous comparison of northern and Antarctic krill revealed a mechanism to enhance enzyme regulation (Vetter 1995b). The compared euphausiids showed generally low levels of enzyme inhibition ( $K_i$ ), such an effect being the prerequisite for high regulatory capacity. ATP controls CS activity via feedback inhibition. At low ATP levels, the enzyme is activated. With increasing citrate production, more ATP is produced in the end, and the resulting higher levels of ATP then inhibit the turnover of the substrate acetyl-CoA. This alternating activation–inhibition mechanism is possibly unique to euphausiids and apparently is coupled to the specific strictly pelagic way of life, requiring constant swimming. A high capacity for control helps in optimizing CS activity in relation to the high and fluctuating energy demand in euphausiids.

What becomes clear is that CS activity and respiration rates do not appear to be strongly related, particularly in the case of northern krill in the Ligurian Sea. This corresponds to findings for other invertebrates (Thuesen et al. 1998). Generally, further studies on actual substrate concentrations, the control function of metabolites, and the feedback mechanisms that modulate enzyme activity appear to be a promising field in euphausiid ecophysiology.

#### *Digestive enzymes*

The structural components of chitin are amino sugars. However, chitin is one of the most resistant and hardy substances in nature. The ability to use or reuse chitin, i.e., to break it down enzymatically into the sugar components, would mean a considerable gain of energy for any organism.

Chitin-degrading enzymes are widely found in most animal taxa (Jeuniaux 1966) but have rarely been identified in marine zooplankton (Buchholz 1993). In krill, two main sources of chitin are available. Firstly, several *Thalassiosira* species contain large amounts of chitin and are very common in the Atlantic, and particularly in Antarctic waters. The species is a staple food of krill (see Saborowski and Buchholz 1999). Secondly, copepods and other small crustaceans with chitinous exoskeletons are major food items of *M. norvegica*. However, this also appears to be the case for *E. superba*, at least during the southern winter (e.g., Pakhomov et al. 1997). Accordingly, we attempted to identify in krill the enzymes necessary to cleave chitin and compare activities and the dynamics of digestive enzyme induction.

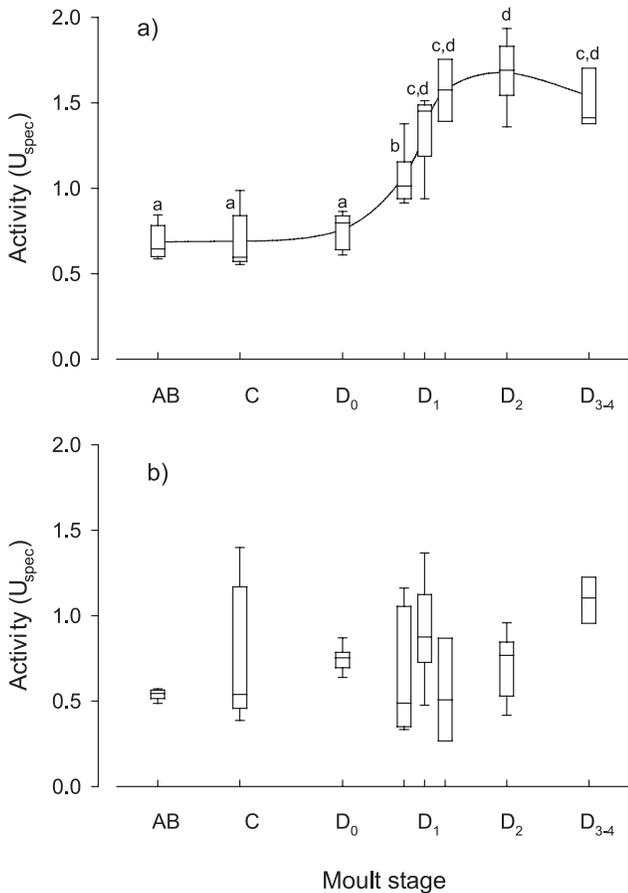
The chitin-degrading enzymes in krill consist of an endochitinase that produces oligomers and three isoforms of an exochitinase that liberate the amino sugars (Spindler and Buchholz 1988). This set of enzymes has a double function. Firstly, they show high activity associated with the moult cycle and are responsible for reabsorption of the old cuticle prior to ecdysis. It is interesting to note that the capacity for the moult-related turnover of chitin corresponds to that of tropical insects (Buchholz 1989; Buchholz and Buchholz 1989) and thus highlights the physiological performance in the Antarctic krill. Secondly, they function as true digestive enzymes and occur at high activities (Buchholz and Saborowski 1996; Saborowski and Buchholz 1999) in the midgut gland and stomach in krill. Using immunotechniques, the functions of the exochitinase (*N*-acetyl- $\beta$ -D-glucosaminidase (NAGase)) were further differentiated.

While one enzyme, NAGase B, plays a significant role in the animal's moult cycle, the other one, NAGase C, participates in the digestion of a chitin-containing diet. NAGase B followed a moult cycle related pattern of activity and was almost exclusively localized in the integument (Fig. 7a). The activity of NAGase C was independent of the moult stage of the animal and was predominantly found in the gastrointestinal tract (Fig. 7b) (Peters et al. 1998 and 1999).

The diet-induced form shows high variability in activity, reflecting most probably different nutritional states of the specimens taken from the field due to induction of NAGase synthesis by an appropriate chitinous diet. Furthermore, the lack of moult-related activity indicates that food ingestion and digestive processes are not reduced around ecdysis, such as in other crustaceans (see Buchholz 1989). The high energy demand in krill may prohibit periods of extended fasting around moult, forcing these animals to immediately resume feeding after moult. This effect may be further facilitated by the extremely rapid ecdysis, which takes no more than a few seconds (Buchholz 1991).

In a seasonal analysis, chitinolytic activity was compared with alkaline protease in the midgut gland and stomach in *E. superba* (Buchholz 1989). The digestive chitinases and the protease were similarly active during austral summer and

**Fig. 7.** Specific activities of the exochitinase (a) NAGase B (from integument extracts) and (b) NAGase C (from stomach extracts) in specimens of *E. superba* of different moult stages determined by immunotitration using a specific polyclonal antibody ( $n = 4-8$ ). Values with the same letter in (a) are nonsignificant differences (ANOVA and Student–Newman–Keuls test,  $p > 0.05$ ). Source: Peters et al. (1999).

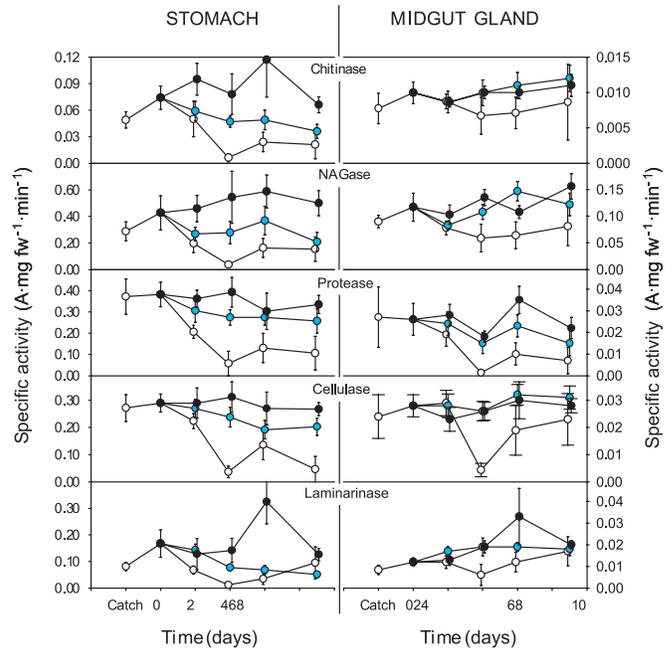


winter (August, September). This may indicate that feeding levels were maintained at a comparable level throughout the year. As phytoplankton levels were low during winter in the area of sampling (South Georgia; Priddle et al. 1990), a switch to zooplankton ingestion and associated induction of chitinases could not be excluded. A more thorough analysis in that respect is intended in the near future.

Activity levels in *M. norvegica* displayed generally considerably higher values with higher variation than in *E. superba*, probably as a consequence of the omnivorous behaviour in the northern krill species, the variability depending on the recently ingested food type. Chitinolytic activity was also analysed in the integument in *E. superba*: similar levels of activity indicate maintenance of reabsorption of cuticle chitin and thus the continuation of moulting processes during overwintering.

With the aim of testing the capacity of krill to cope with an unpredictable food supply in the Antarctic, a laboratory study of the induction of a set of digestive enzymes, including chitinases, in response to different feeding conditions was conducted (Saborowski and Buchholz 1999). Krill were either starved or fed with the diatom *Dunaliella* or alterna-

**Fig. 8.** Time courses of specific activities of digestive enzymes in the stomach and midgut gland of krill maintained in a feeding experiment for 9 days. Solid circles, fed with chitinous *Cyclotella*; grey circles, fed with nonchitinous *Dunaliella*; open circles, starved ( $n = 4-15$ ). Source: Saborowski and Buchholz (1999).



tively with *Cyclotella*, which contains chitin at 12% of its dry weight.

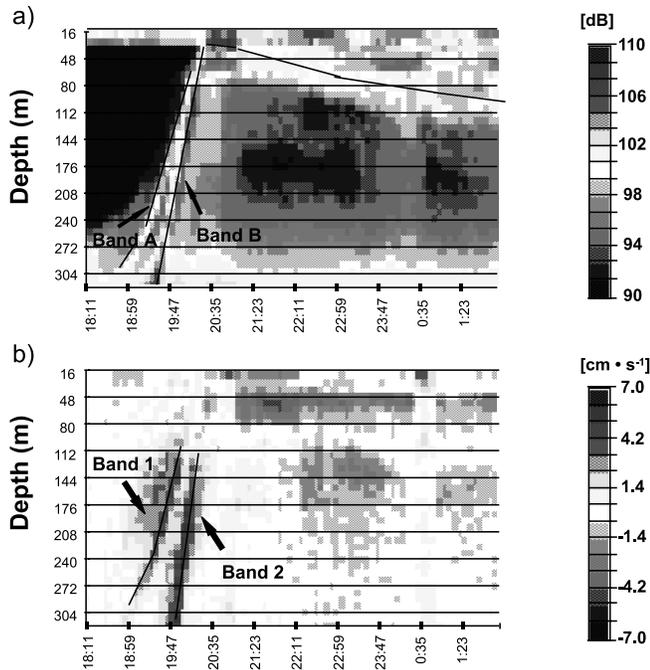
Characteristic time courses of the activities in exo- and endo-chitinase, protease, laminarinase, and cellulase were evident after 4 days. In specimens fed with *Cyclotella*, rich in both chitin and laminarin, chitinase and laminarinase were coincided above the initial value. In starved animals, in contrast, enzyme activities in the stomach decreased to a minimum after 4 days but recovered within 14 days to the values at the start of the experiment (Fig. 8; 14- and 20-day experiments not shown). Specific activities of all enzymes were affected less by feeding regime in the midgut gland than in the stomach but otherwise followed a similar pattern.

The initial decrease in activity in the stomach during starvation can be explained by diminished secretion of enzymes from the midgut gland into the stomach (Saborowski and Buchholz 1999). At the same time, persistent activities in the midgut gland provide a digestive potential that can immediately be used when a rich food patch is encountered by Antarctic krill. The ability to maintain high levels of activities in a whole set of enzymes and additionally to enhance specific enzymes with an increase in a particular substrate can be considered an efficient adaptation to the variable Antarctic trophic environment, which requires rapid and effective nutrient utilization.

### The enigma of DVM, and metabolic adaptation in *M. norvegica*

The impressive performance in DVM has long been known in *M. norvegica* (Mauchline 1960). In *E. superba*,

**Fig. 9.** Real-time ADCP recordings of DVM in zooplankton in the Ligurian Sea. (a) Backscatter at 150 kHz; (b) vertical velocities. Band B and band 2 relate to *M. norvegica*. Source: PEP database and G.A. Tarling (personal communication). Method in Tarling et al. (1999a).



analogous behaviour is much less expressed (Klusek and Godlewska 1988).

In a recent study of *M. norvegica*, behavioural investigations using sonar and multinet equipment (Tarling et al. 1998a, 1999a; Guérin-Ancey et al. 1998) were able to be combined with data on energy requirements. A short summary of DVM behaviour is given below as a background to considerations of energy metabolism in *M. norvegica*.

The largest amplitude of DVM is typical of *M. norvegica* in the Mediterranean (Fig. 9). Over depths of more than 2000 m, krill migrate in spring some 800 m daily, whereas 500–600 m appears to be the summer amplitude (Velsch 1997).

In very elegant experiments, Velsch and Champalbert (1994) and Velsch (1997) demonstrated a clear influence of an underlying endogenous control. Here, the natural light cycle of the sun is the zeitgeber. However, the moon is also influential and appears to phase “midnight sinking”, i.e., after “dipping” for a short time into the warm top layers, krill sink near midnight to depths of about 100 m before the sunrise induces the rapid morning descent (Tarling et al. 1999a).

In the Clyde Sea and Kattegat, the range of DVM is restricted by shallower depths of 150 and 130 m, respectively. Nevertheless, regular DVM prevails during summer and winter, closely following the solar light cycle, apparently setting the clock of an endogenous rhythm. However, the cyclical behaviour is modified to a large extent by external and internal cues.

Firstly, only a certain proportion of the krill seems to migrate at all (Fig. 10). This was assessed at two-thirds of the population in the Clyde Sea and at one third in the Kattegat

(Tarling et al. 1998a). Secondly, at both locations, krill not only feed during nighttime in the upper layers but also during daytime in the deep (Buchholz et al. 1995; Lass et al. 2000). Apparently, there is only a limited need for DVM in terms of trophic cues. Furthermore, a tendency for smaller krill to migrate at a larger amplitude than larger krill was confirmed (Mauchline 1960), probably dependent on the considerable increase in energetic requirements with weight (Tarling et al. 1998a; Saborowski et al. 2000b).

Energetic requirements increase drastically with temperature. The relative carbon demand was calculated from temperature-dependent respiration at the Kattegat location (Fig. 11).

If krill, for example, migrate from the deep layers at 6°C to above 50 m where the temperature increases to 12°C during summer, a 50% increase in carbon demand ensues. There seems to be a trade-off between the favourable enhancement of motility due to the temperature increase and the high energy expenditure in the upper warm layers. Krill may suspend DVM for one or more diel cycles before crossing the thermocline again (Saborowski et al. 2000b).

An intensive study of DVM in the Kattegat was conducted over 10 days during summer 1998 (Tarling et al. 1999b). It was shown that internal cues, depending on the moult and reproductive cycles, can modify DVM behaviour and the range of DVM.

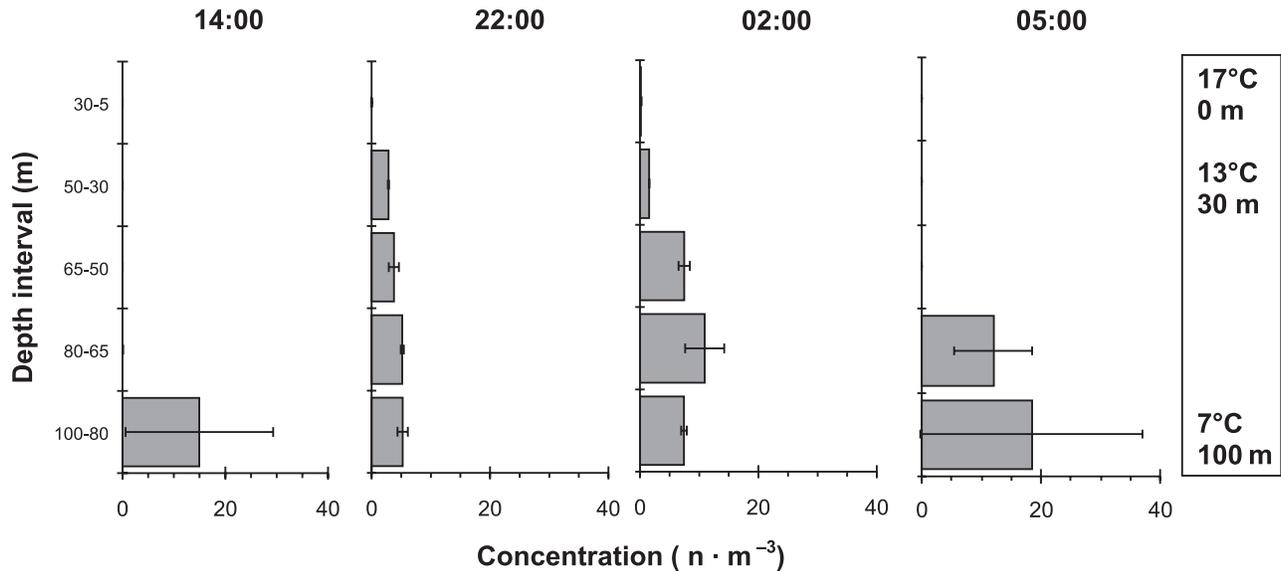
In both female krill (Fig. 12) and male krill (data not shown), an intensive phase of moulting was encountered during the summer study. Obviously, krill moulted only at depth and predominantly during nighttime. In this way, having a soft exoskeleton for about 2 days, krill can “hide” in the dark deep and thus avoid predation and (or) cannibalism during this phase of vulnerability. Moreover, due to the energy-intensive processes of shedding the old shell and hardening the new one, krill suspend DVM during this time.

In contrast, female krill that were ready to spawn (identifiable by the blue-grey tint of the ovary; Cuzin-Roudy and Buchholz 1999) were almost exclusively found in the warm upper layers of the Kattegat. By crossing the thermocline, the physiological advantage would be an acceleration of the final stages of egg maturation and of the spawning process itself. Furthermore, spawned eggs and the first larval stages would be positioned in favourable surroundings, i.e., warm and plankton rich.

In summary, krill seem to be generally better adapted to the deeper and cooler water layers at all three sites. DVM apparently is controlled endogenously, but krill are able to adapt the range of migration (and possibly the frequency of DVM) to physiological necessity to a large extent. To modify internal rhythms more or less continuously depending on a multitude of external and internal cues requires a high level of physiological plasticity, which appears to be typical for *M. norvegica*.

At any rate, energetic requirements and DVM are intimately interlinked. A practical way to differentiate and better understand the complex interdependencies would be to use a modelling approach. A first attempt to integrate physiology and behaviour in *M. norvegica* was presented by G.A. Tarling et al. (Scottish Association for Marine Science, P.O. Box 3, Oban, Argyll PA34 4AD, U.K., personal communication) during this conference in the form of an optimal-foraging model. Here, another important influencing factor

**Fig. 10.** Concentration of *M. norvegica* in the Alkor Deep, Kattegat, averaged over seven day–night cycles within the period 24 July – 3 August 1998 (with 95% confidence intervals). The pattern shows a vertical migration into the upper layers during nighttime from a daytime location in the deep.



in DVM can also be addressed and integrated: predation pressure.

**Seasonality in euphausiid physiology**

In the following, the adaptive features described above are summarized in view of seasonal adjustments of rates of respiration and metabolic and digestive enzymes in both krill species.

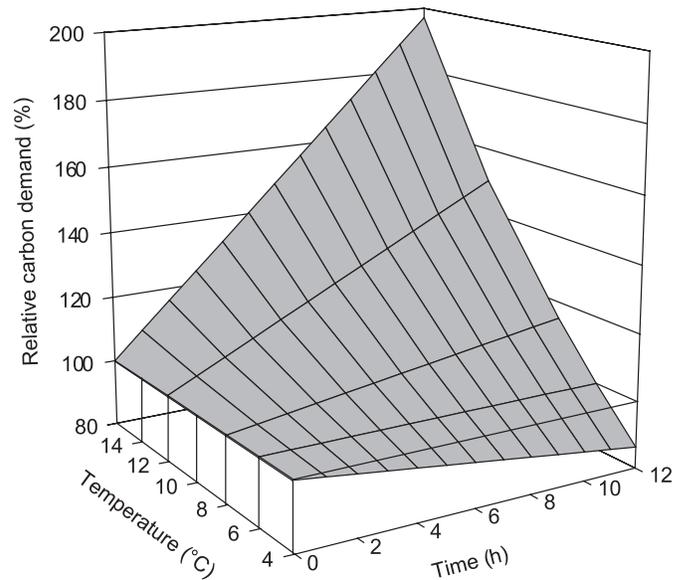
***Meganctiphanes norvegica***

Apparently, no real food shortage exists during the seasons in the Clyde Sea and Kattegat. Nevertheless, a drastic winter reduction occurs in both ambient Chl *a* levels and food ingested in the Clyde Sea krill (Table 2). In the Kattegat, in contrast, continuous advection supplies copepods, the staple food of krill at that location, during summer as well as winter. As a consequence, no seasonal respiratory adaptation is noted. However, the vertical shift of the respiration curves suggests an overall adaptation to the different thermal regimes at the two sites.

The case of the Ligurian Sea is different from both of the northern sites: *M. norvegica* has to cope with an oligotrophic situation during most of the year. Accordingly, a strong metabolic enhancement coupled in time to the short winter–spring plankton blooms occurs to maximize growth and reproduction. However, the summer–fall curve of respiration is at a similar level as in the north, although shifted parallel so that a similar level of 30–35 μmol O<sub>2</sub>·mg dry weight<sup>-1</sup>·h<sup>-1</sup> is maintained at all three sites. This respiratory value may be seen as a common basal metabolic level to maintain physiological functions.

Similarly, in CS, a basal level of *K<sub>m</sub>* values prevails in krill at all three sites during winter. At the northern locations, a summer maximization of substrate affinity is notable, apparently linked to the development of the temperature differential of the water columns. This adaptation may be seen

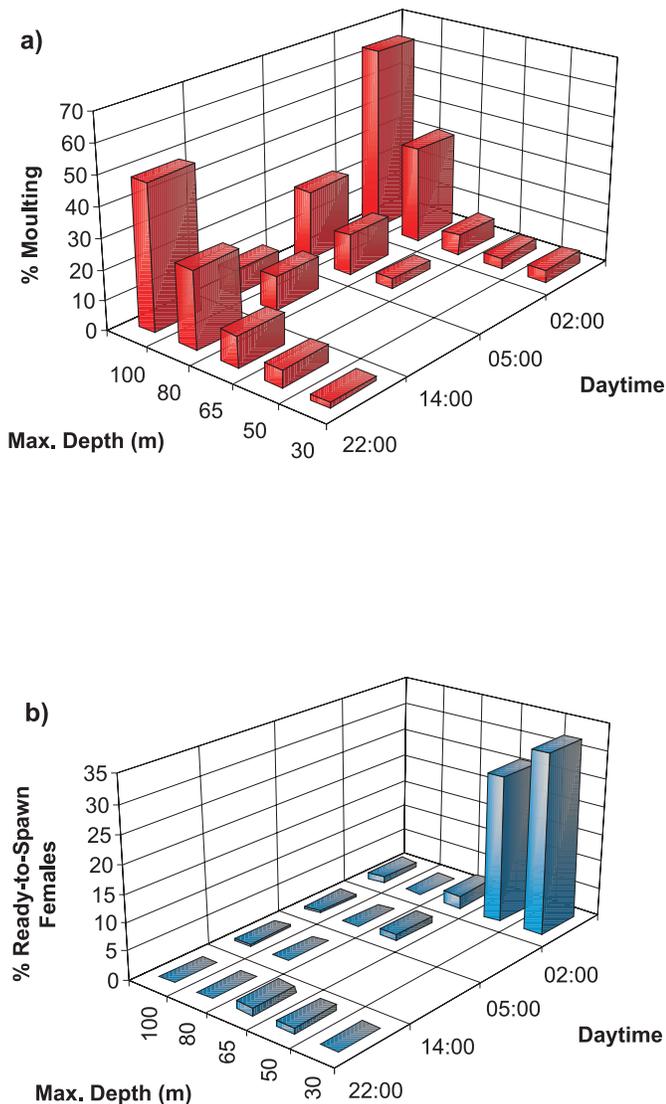
**Fig. 11.** Relative carbon demand as related to oxygen consumption versus environmental temperature and time spent at these temperatures in *M. norvegica* at the Kattegat location. Source: R. Saborowski (unpublished data) and Saborowski et al. (2000b).



as an indication that krill in fact make use of temperature-linked enhancements of physiological functions, e.g., with respect to motility and reproductive processes.

Here, the Ligurian krill is completely different again: the drastic increase in substrate affinity during the oligotrophic summer–fall phase may compensate for the somatic reduction expressed as the decrease in specific activity of muscle CS. In short, the Mediterranean krill appear to be more influenced by the seasonal trophic regime than krill at the northern sites, where temperature effects are more important. The differences found in respiratory and metabolic enzy-

**Fig. 12.** (a) Percentage of moulting *M. norvegica* versus diel depth distribution (females) and (b) percentage of ready-to-spawn female *M. norvegica* versus diel depth distribution (the ovary of ready-to-spawn krill attains a blue–grey tint and can be easily spotted in fresh samples). Averaged from data obtained from MOCNESS nets deployed over seven full day–night cycles between 24 July and 3 August 1998. Source: F. Buchholz (unpublished data). Methods in Tarling et al. (1999b).



matic adaptation may be reflected in the clear genetic separation of the Ligurian population from all Northeast Atlantic ones (Zane and Patarnello 2000; Zane et al. 2000).

*Meganyctiphanes norvegica* is a strong feeding opportunist and alternates between phyto- and zoo-plankton, making use of a wide variety of species. The inducibility of digestive enzymes including chitinases is perfectly tuned to this physiological flexibility. Furthermore, egg production and the length of the reproductive period are both controlled by the specific temperature regime and trophic conditions at the three sites (Cuzin-Roudy and Buchholz 1999; and Cuzin-Roudy 2000). Moreover, krill are able to modify DVM behaviour according to physiological necessity with respect to moult, reproduction, and overall energetics.

In conclusion, *M. norvegica* is characterized by a high level of physiological plasticity, which may be the prerequisite for the evolutionary success of the species. This is reflected in its exceptionally wide distribution.

### *Euphausia superba*

For *E. superba*, tendencies towards metabolic reduction as an overwintering strategy have been reported (Quetin et al. 1994; Torres et al. 1994). If a latitudinal comparison may be allowed, a different strategy in *M. norvegica* would appear: the northern krill maintains a comparable oxygen consumption at all three sites during winter but shows enhanced metabolism during the productive spring phase in the Mediterranean with respect to a common basal metabolic level.

However, the enzymatic repertoire of *E. superba* shows an equal potential for physiological plasticity as in *M. norvegica*. The Antarctic krill may use variable food sources wherever it finds them, i.e., phyto- and zoo-plankton as well as under-ice algae. Furthermore, the growth parameters are kept equally flexible (Buchholz 1991): krill may even shrink as a last resort to compensate for diminishing energy reserves during winter (Nicol et al. 1992).

It may be considered a less important question whether krill reduce or enhance metabolic rates. Nevertheless, krill are apparently able to make use of any improvement in environmental conditions and to transfer these immediately into maintenance of vital functions irrespective of season and as far as possible into those of growth and reproductive processes.

### Perspectives

A worldwide comparison of temperate euphausiids may be envisaged in the near future: in particular, knowledge on *M. norvegica*, *Euphausia pacifica*, and *Thysanoessa* spp. is emerging rapidly. To elucidate patterns of DVM and the underlying physiology may serve as a unifying approach. In this case, a combination of methods can be applied: at the population level, ADCP and multifrequency sonar, ground-truthed by multinet catches, may be employed. In linked physiological studies, modern energy budgets should be constructed with parameterization appropriately tuned to modelling. Progress has been achieved in combining physiological models, e.g., optimal-foraging models, and physical (advection) models. Several examples of such approaches appear to be underway, e.g., *E. pacifica* is a target species of Northeastern Pacific GLOBEC (Global Ocean Ecosystem Dynamics) and *M. norvegica* is planned to be studied in the Arctic and Northeast Atlantic fjords, which may serve as “model oceans.” A coordination of initiatives would be desirable. A transfer of methodology and results with respect to Antarctic krill appears to be sensible.

Further adaptational and life history data are needed to complement population assessments in *E. superba* in view of currently growing plans for krill utilization. To use standardized equipment, e.g., appropriate devices for respiration measurements, for consistent seasonal, latitudinal, and interspecies comparisons, appears to be a necessity. State-of-the-art methodology for studies of population genetics is available and may serve to highlight functional biodiversity,

as exemplified here for *M. norvegica* as well as large-scale gene flow. Biochemical features may be usable as spinoffs: digestive enzymes in krill may be characterized and then used in pharmaceutical technology.

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