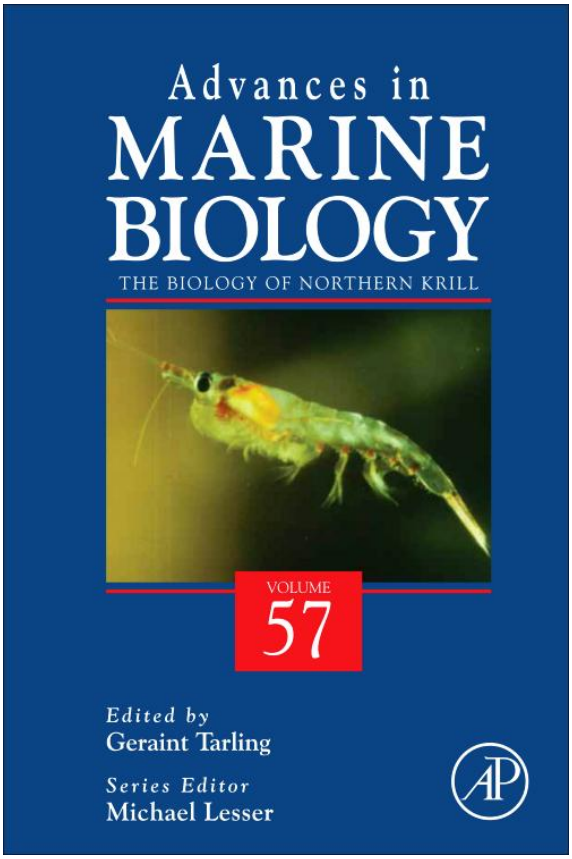

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CHAPTER FOUR

Physiology and Metabolism of Northern Krill (*Meganyctiphanes norvegica* Sars)

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Contents

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Abstract

Advances in our understanding of the physiology and metabolism of Northern krill, *Meganyctiphanes norvegica* have been sporadic but significant. Despite problems with keeping *M. norvegica* in good condition in the laboratory, those who have tried, and succeeded, have contributed to a better knowledge of krill biology and challenged our understanding of some basic biological processes. Most recent work has been concentrated in the fields of digestive physiology, lipid biochemistry, respiration and anaerobiosis, metabolic properties, and pollutants. *M. norvegica* is capable of digesting an opportunistic, omnivorous diet, showing some digestive enzyme polymorphism and high levels of enzyme activity, the latter varying with season. It also seems capable of digesting cellulose and hemicelluloses, for example, laminarin. The biochemical composition of krill is relatively well known with some recent extensive work focusing on the previously little studied lipid and fatty acid composition, particularly with reference to reproduction, overwintering energy storage and as a nutrition marker. A high aerobic metabolism (but poor anaerobic capacity) is characteristic of *M. norvegica*, and how this is affected by temperature, low O_2, and season has attracted some attention, particularly in the context of diel vertical migration (DVM) across pronounced pycnoclines. Despite determining high metabolic turnover rates and a high physiological plasticity for this species, we know little of the regulative potential of metabolites, particularly their modulative effect on enzyme activity. Certainly a modest ability to maintain aerobic metabolism when encountering hypoxia, and little or no ability to osmoregulate in hyposaline conditions, does not prevent DVM in adults of this species. The ability to maintain aerobic metabolism develops early in ontogeny at about furcilia III (i.e. concurrent with first DVM behaviour). The respiratory pigment of *M. norvegica*, haemocyanin, has a low O_2 affinity and high temperature sensitivity (although temperature has the opposite effect on O_2 binding than found for nearly every other haemocyanin). Also surprising is the apparent use of haemocyanin as an energy source/store. While recent work has focused on physiological effects, the ecophysiological effects of transuric elements and trace metals, the effects of pollution generally are widely understudied.

1. Introduction

Advances in our understanding of the physiology and metabolism of *M. norvegica* since the reviews of Mauchline [Mauchline, J., Fisher, L.R., 1969. The biology of euphausiids. *Adv. Mar. Biol.* 7, 1–454; Mauchline, J., 1980. Part II: The biology of euphausiids. *Adv. Mar. Biol.* 18, 373–623]

have been both sporadic and patchy, but there have certainly been advances. We review the literature published since the last review of krill biology, highlighting such advances but also noting areas which, despite their obvious importance, still require attention.

2. The Physiology of Digestion

The digestive systems of euphausiids are broadly similar to those of other eucarid crustaceans (Mauchline and Fisher, 1969). Since no histological and cytological studies are available for the digestive organs of *Meganyctiphanes norvegica*, a brief description based on the extensive work done on decapods (e.g. Dall and Moriarty, 1983; Loizzi, 1971) and Antarctic krill, *Euphausia superba* (e.g. Ikeda *et al.*, 1984; Ullrich *et al.*, 1991), is given as background to what follows on the digestive system and its function.

2.1. The digestive organs

The digestive system consists of an ectodermal foregut with the oesophagus and the stomach, an endodermal midgut which forms the digestive midgut gland, and the again ectodermal hindgut (Fig. 4.1). While the stomach has a primarily mechanical function in the maceration of the ingested food (see Chapter 5), the midgut gland (or hepatopancreas) is the principal organ of digestive enzyme production as well as nutrient resorption. The gland is a bunch of numerous blind ending tubules each of which are constructed from a monolayer epithelium of specialised cells. This cell layer develops from so-called embryonic cells (E-cells) at the distal tip of the tubules. The E-cells develop into at least two cell types: the R-cells which perform

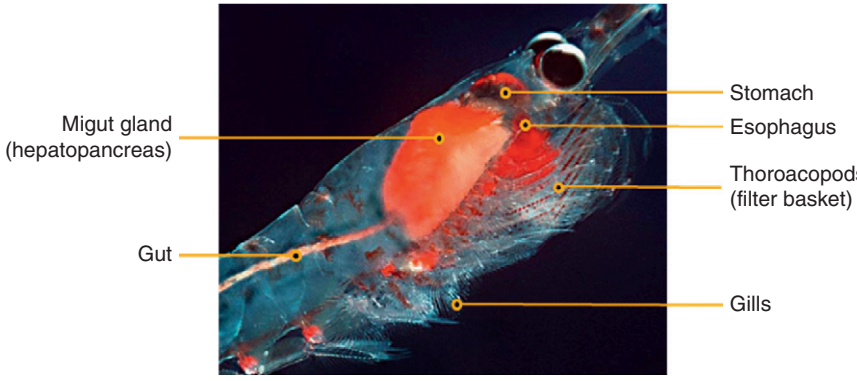

Figure 4.1 The cephalothorax of *Meganyctiphanes norvegica* showing the location of the digestive organs. Photograph provided by Uwe Kils.

nutrient resorption or the F-cells (fibrillar cells) which facilitate enzyme synthesis and secretion. The origin of a third cell type in decapods, which is characterised by the presence of a huge vacuole (blister cells or B-cells), is still under debate.

The stomach and the midgut gland form a functional unit. Enzymes synthesised in the midgut gland are released into the lumen of the tubules. The proximal ends of the tubules merge to form larger channels which, ultimately, end up as a pair of funnels into the posterior part of the stomach. The enzymes accumulate in the stomach. Food, which is ground in the stomach by the gastric mill (Ullrich *et al.*, 1991), is simultaneously mixed with the digestive enzymes initializing the extracellular digestion of the food. The chyme is then pressed through a fine filter system which allows the liquid fraction to pass into the midgut gland for nutrient resorption but retains larger solid particles such as diatom frustles, copepod mouthparts, or cuticle fragments for defecation.

2.2. The digestive enzymes

Investigations on enzyme polymorphism in *M. norvegica* included some digestive enzymes (Fevolden, 1982). Studies on the properties of digestive enzymes were primarily focused on chitinolytic and proteolytic enzymes. Spindler and Buchholz (1988) identified, in the extracts of whole animals, different forms of endo- and exochitinases with broad pH optima and high stabilities. Separate analyses of the digestive organs and the cuticle revealed the presence of specific chitinases which are involved either in moulting or in digestion (Peters *et al.*, 1998, 1999). The stomach and the midgut gland of *M. norvegica* are also rich in proteolytic enzymes (Buchholz, 1989). According to inhibition assays, the majority of endopeptidase activity results from serine-endopeptidases, including trypsin-like enzymes (Dittrich, 1992b; Kreibich *et al.*, 2010). A set of various digestive hydrolases was detected by Donachie *et al.* (1995) in the stomach and in the midgut gland (Table 4.1).

The pH of the gastric fluid of *M. norvegica* ranges between 6.5 and 6.9. Maximum *in vitro* activity of trypsin-like enzymes occurs across a broad pH-range from pH 7 to pH 10. Eighty to ninety percent of activity in trypsin-like enzymes is inhibited by soybean inhibitor (SBI) and *N*-tosyl-L-lysine chloromethyl ketone (TLCK). Salts containing trace metal ions ($HgCl_2$, $AgNO_3$, and $CuSO_4$) reduce the tryptic activity of *M. norvegica* by 40–50% (Dittrich, 1992b).

2.3. Endosymbionts

Bacteria have been detected in the stomach and in the midgut gland of *M. norvegica* from the Kattegat (Donachie *et al.*, 1995). Laboratory experiments showed that these bacteria seem to contribute to a lesser extent to the production of a range of enzymes, including chitinolytic and proteolytic

Table 4.1 Digestive enzymes detected in the stomach and in the midgut gland of *Meganyctiphanes norvegica*

Enzyme	Substrate	Reference
Proteolytic enzymes		
Total proteinase (alk.)	Azocasein	Spindler and Buchholz (1988), Buchholz (1989), Donachie *et al.* (1995), Kreibich *et al.* (2010), Kreibich (unpublished), Saborowski (unpublished)
Trypsin	BAPNA, TAME	Båmstedt (1988), Dittrich (1992a,b), Kreibich *et al.* (2010), Kreibich (unpublished), Saborowski (unpublished)
	N-Benzoyl-DL-arginine-2-naphthylamide	Donachie *et al.* (1995)
Chymotrypsin	SAAPNAA	Saborowski (unpublished)
	N-Glutaryl-phenylalanine-2-naphthylamide	Donachie *et al.* (1995)
Alanine aminopeptidase	Ala-*p*-nitrophenol	Kreibich (unpublished), Saborowski (unpublished)
Leucine arylamidase	L-Leucyl-2-naphthylamide	Fevolden (1982), Donachie *et al.* (1995)
Valine arylamidase	L-Yalyl-2-naphthylamide	Donachie *et al.* (1995)
Cysteine arylamiadse	L-Cystyl-2-naphtylamide	Donachie *et al.* (1995)
Esterases and lipases		
Esterase (C2)	1-Naphthyl acetate	Fevolden (1982)
Esterase (C4)	2-Naphthyl butyrate	Donachie *et al.* (1995)
Esterase lipase (C8)	2-Naphty caprylate	Donachie *et al.* (1995)
Lipase (C14)	2-Naphthyl myristate	Donachie *et al.* (1995)
Triacylglycerolacylhydrolase	1,2-Diglycide	Kreibich (unpublished)

(*continued*)

Table 4.1 (*continued*)

Enzyme	Substrate	Reference
Phosphatases		
Alkaline phosphatase	1-Naphthyl phosphate (pH 8.6)	Fevolden (1982)
	2-Naphthyl phosphate (pH 8.5)	Donachie *et al.* (1995)
Acid phosphatase	1-Naphthyl phosphate (pH 5.0)	Fevolden (1982)
	2-Naphthylphosphate (pH 5.4)	Donachie *et al.* (1995)
Naphthol-AS-BI-phosphohdrolase	6-Naphthol-AS-BI-phosphate	
Glycoside hydrolases		
α-Glucosidase	2-Naphthyl-α-D-glucopyranoside	Donachie *et al.* (1995)
β-Glucosidase	6-Br-Naphthyl-β-D-glucopyranoside	Donachie *et al.* (1995)
α-Galactosidase	6-Br-naphthyl-α-D-galactopyranoside	Donachie *et al.* (1995)
β-Galactosidase	2-Naphthyl-β-D-glucopyranoside	Donachie *et al.* (1995)
β-Glucuronidase	Naphthol-AS-BI-βD-glucuronide	Donachie *et al.* (1995)
α-Mannosidase	6-Br-naphthyl-βD-mannopyranoside	Donachie *et al.* (1995)
α-Fucosidase	2-Naphthyl-α-L-fucopyranoside	Donachie *et al.* (1995)
Amylase	Amylose, starch, CM-starch-RBB	Båmstedt (1988), Donachie *et al.* (1995), Saborowski (unpublished)
Cellulase	CM-cellulose-RBB	Saborowski (unpublished)
Laminarinase	CM-curdlan-RBB	Saborowski (unpublished)
Chitinase	CM-chitin-RBB, chitin (cryst.)	Spindler and Buchholz (1988), Buchholz (1989), Donachie *et al.* (1995)

enzymes. However, none of the enzymes studied in the digestive organs of krill were exclusively of bacterial origin. Moreover, rapid gut transit times of the krill and frequent moulting of the foregut, including the stomach, prevents the formation of a persistent population of symbiotic bacteria.

2.4. Ecological implications

M. norvegica is well suited to digesting food of both plant and animal origin. Moreover, it shows a certain degree of enzyme polymorphism (Fevolden, 1982) and comparatively high levels of enzyme activity (Kreibich *et al.*, 2010). Seasonally elevated levels of activity of trypsin and amylase appear during the spring (Båmstedt, 1988), probably reflecting a higher metabolic energy demand at this time of year. Northern krill is also capable of digesting cellulose and hemicelluloses such as laminarin.

3. Biochemical Composition

The biochemical composition of various euphausiid species was intensively studied in the 1970s. The results were thoroughly summarised by Mauchline and Fisher (1969) and Mauchline (1980). The data comprise the biochemical gross composition (water, ash, lipid, carbohydrate, protein, and chitin), the carbon and nitrogen amounts, the elemental composition including trace metals and pollutants, the amino acid composition, as well as the ATP and the RNA concentrations. Only a few additional studies on these parameters in *M. norvegica* have been published since. In contrast, extensive work has been carried out since on the relatively unstudied lipid and fatty acid composition. This will be reviewed in a separate section below.

3.1. Protein, lipid, ash

Falk-Petersen (1981) examined in detail some major biochemical parameters in a population of *M. norvegica* from the Norwegian Balsfjorden (70° N). Increase in weight and changes in protein and lipid contents were closely related to the seasonal cycles of primary production. A decrease in lipid content in I-group *M. norvegica* during winter was likely related to the use of energy for overwintering and the growth of gonads. The relative amount of proteins ranged from 23% to 36% DW and the amount of lipids from 30% to 47%. The ash content varied around 15% of the body dry weight. Buchholz and Prado-Fiedler (1987) studied the seasonal changes of biochemical parameters of a krill population in the Danish Kattegat. These data were also presented as percentage of the dry weight. If an average water content of 80% is assumed (Mauchline and Fisher, 1969), then the

recalculated values related to wet weight would amount to ash (2.2–5.8% WW), lipid (1.6–9.6% WW), carbohydrates (0.2–0.4% WW), protein (9.2–13.8% WW), and chitin (0.6–1.1% WW). These data for *M. norvegica* from the Kattegat are in the same range as those data from krill from various locations previously summarised by Mauchline and Fisher (1969).

3.2. Nucleic acids

The RNA concentration, or the RNA/DNA-ratio, is a widely used index to determine the physiological condition of organisms. It can serve as a proxy for the performance in terms of, for example, growth or reproduction (Chícharo and Chícharo, 2008). RNA values from *M. norvegica* sampled in the Norwegian Korsfjorden ranged from 2.5 to 20.8 $\mu g\ mg^{-1}$ DW in February and 3.8 to 21.3 $\mu g\ mg^{-1}$ DW in September (Båmstedt and Skjoldal, 1980). The authors established an allometric relationship between the RNA concentration and the dry weight of krill and presented a relationship between growth rate and RNA concentration to estimate the average growth rate. A more detailed seasonal cycle of the RNA content was determined by Båmstedt (1983) on a krill population from the Kosterfjorden, Swedish west coast. The values were highest in spring and early summer (16–18 $\mu g\ mg^{-1}$ DW), decreased to almost 4 $\mu g\ mg^{-1}$ DW in July and increased again in August to 11.5 $\mu g\ mg^{-1}$ DW. The variation was high and the seasonal changes were suggested to reflect the variation in food supply and gonad growth.

3.3. Lipids

In the past three decades, intensive research has been carried out on the lipid biochemistry of krill and other pelagic crustaceans. The functions of lipids and fatty acids in the physiology and the ecology of *M. norvegica* were studied either in relation to reproduction, as an energy store for overwintering, or as markers for nutrition. The amount of total lipids in whole body extracts of *M. norvegica* can range from as low as 7–8% DW (Kreibich *et al.*, 2010; Saether *et al.*, 1986), or 3.5% DW in krill from oligotrophic waters (Mayzaud *et al.*, 1999), to up to more than 40% DW (Sargent and Falk-Petersen, 1981). The lipid content and its composition depends strongly on season, location, sex, maturation, and, particularly, on the nutritive state.

3.3.1. Major lipid storage organs

The cephalothorax is the site of major lipid accumulation in *M. norvegica*. The thorax of krill from the Trondheimfjord contained 15.8% lipids on a dry weight basis and the abdomen just 9.1% (Saether *et al.*, 1986). Albessard and Mayzaud (2003) reported that the lipid content in the cephalothorax

from Ligurian, Kattegat, and Clyde Sea krill was 2–4 times greater than in the abdomen.

The cephalothorax contains, besides other organs, the stomach, the digestive or midgut gland, the ovaries, and the so-called fat body consisting of connective tissue (Cuzin-Roudy, 1993). The lipid concentration in the stomach can amount to 17% DW and that of the midgut gland 65% DW (Albessard *et al.*, 2001) which confirms the relevance of the midgut gland as the major lipid storage organ, for example, in Antarctic krill (*E. superba*) starvation for 19 days entailed a significant decrease in total lipids from 21% to 9% DW (Virtue *et al.*, 1993). The gonads and the fat body contain 24% DW and 20% DW, respectively.

The abdomen consists mainly of muscular tissue. It contains 8% lipids on a dry weight basis. The lipid composition reflects the predominance of phospholipids from biomembranes and shows low levels of neutral lipids like TAG (Albessard and Mayzaud, 2003). The relative amount of the body lipids in resting males from the Ligurian Sea is summarised in Table 4.2.

The lipid content in the separate organs of *M. norvegica* varied significantly with season and the reproductive cycle; details are given by Albessard *et al.* (2001) and Albessard and Mayzaud (2003).

3.3.2. Lipid classes

A basic classification of lipids can be made through separating neutral from polar lipids. The neutral lipids are generally separated further into the major classes of triacylglycerols (TAG), wax esters (WE), and sterols (ST). The polar lipid (PL) fraction comprises the phospholipids mainly represented by phosphatidylcholine (PC). Depending on the instrumentation and analytical performance, these lipid classes can be further separated and identified (Mayzaud *et al.*, 1999).

TAG: The major lipids in *M. norvegica* are triacylglycerols (TAG). Sargent and Falk-Petersen (1981) found that more than the half of total lipids in *M. norvegica* caught during winter (November/December) in Balsfjorden near Tromsø occurred as TAG and Saether *et al.* (1986) found more than 70% TAG in the total lipids. Their contents varied strongly and

Table 4.2 Lipid content of different organs in resting males from the Ligurian Sea (after Albessard *et al.*, 2001)

	Stomach	Midgut gland	Fat body	Gonads	Abdomen
% DW	14.5 ± 2.4	65.0 ± 11.2	19.7 ± 5.3	24.4 ± 10.3	8.1 ± 1.5
% of total body lipid	9.4 ± 1.7	35.1 ± 6.1	18.6 ± 4.8	9.7 ± 4.8	27.1 ± 6.1

correlated with the total lipid content (Kreibich *et al.*, 2010; Saether *et al.*, 1986) which shows that TAG serve as the major fraction of storage lipids in *M. norvegica*. Other lipid classes were present in amounts of less than 5% of the body dry weight. Their amount did not change with the total lipid content but remained constant (Fig. 4.2). This indicates that these lipids are more involved in defined physiological functions, for example, as membrane compounds.

WE: Wax esters (WE) are important and frequently occurring storage lipids in many pelagic crustaceans including euphausiids (Falk-Petersen *et al.*, 1981; Saether *et al.*, 1986). It has also been suggested that they play an important role as a 'long-term' lipid store in deep sea and high-latitude zooplankton (Lee *et al.*, 2006). However, only small amounts of WE have been detected in *M. norvegica* (Fig. 4.2). Morris (1972) determined 5% in relation to the total lipid amount in krill sampled northeast of the Azores. Krill from the Greenland Sea contained 6% WE which accounts for 1.6% DM (Kreibich *et al.*, 2010). No WE were found in krill from the Norwegian Balsfjorden (Falk-Petersen *et al.*, 1981), or the Ligurian Sea, the Scottish Clyde Sea, and the Danish Kattegat (Albessard and Mayzaud, 2003; Mayzaud *et al.*, 1999). Thus, *M. norvegica* seems not to follow a typical high-latitude lipid storage strategy where WE are preferentially accumulated. Sargent and Falk-Petersen (1981) suggested that traces of WE may be derived from copepods consumed by the krill. Mayzaud *et al.*

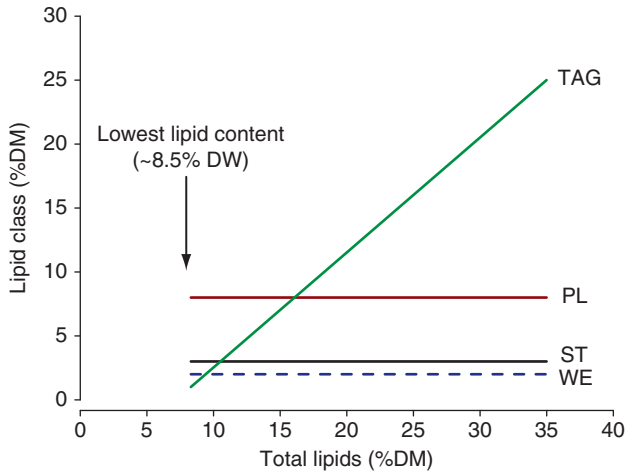

Figure 4.2 Content of the major lipid classes in relation to the total lipid content of *Meganyctiphanes norvegica*. The theoretically lowest lipid content was calculated from data given by Saether *et al.* (1986) and Kreibich *et al.* (2010) for krill from higher latitudes. It does not apply for krill from the Ligurian Sea which contained as low as 3.5% DW of lipids (Mayzaud *et al.*, 1999). PL = polar lipid fraction, ST = sterols, TAG = triacylglycerols, WE = wax esters.

(1999) found no WE in krill from the Ligurian Sea and concluded that those krill prey predominantly upon organisms that are poor in WE.

PL: The largest share of the polar lipid fraction derives from biomembranes. These polar lipids predominantly consist of phospholipids of which PC is a major component. PC is thought to play an important role as storage lipid in high-latitude euphausiids (Lee *et al.*, 2006). In krill from northern Norwegian fjords, the PL-fraction accounted for 15–20% of total lipids (Saether *et al.*, 1986) or more than 30% of total lipids, respectively (Falk-Petersen *et al.*, 1981) which is approximately the same amount as in Ligurian krill (Mayzaud *et al.*, 1999). PL in krill from the Greenland Sea made up 29% of total lipids (Kreibich *et al.*, 2010). This accounts for about 8% on a dry weight basis or 2–3% on a wet weight basis (Albessard and Mayzaud, 2003). The relative amount of PC remains constant at different total lipid amounts because, besides the formation of biomembranes, PC is involved in egg production (Albessard *et al.*, 2001; Cuzin-Roudy *et al.*, 1999).

ST: Ballantine and Roberts (1980) studied the sterol (ST) content of some pelagic marine crustaceans and found in *M. norvegica* from the Atlantic (25°N, 17°W) 3.4% ST in total lipid extracts. The major ST compound was cholesterol accounting for 98% of the ST fraction. Cholesterol was present in krill samples from various other locations usually not exceeding 1–2% of the animal's dry weight (Albessard and Mayzaud, 2003; Kreibich *et al.*, 2010; Mayzaud *et al.*, 1999).

FFA: Large amounts of free fatty acids (FFA) are probably the result of rapid lipolysis post mortem. Sargent and Falk-Petersen (1981) reported an FFA-amount of almost 20% and Falk-Petersen *et al.* (1981) reported a seasonal maximum of even about 45%. The high FFA amounts most likely result from inappropriate handling and drying of the krill samples. Saether *et al.* (1986) took special precautions to avoid *post-mortem* lipolysis. The FFA contents in his samples were low, in the range 0.6% of the dry weight of the krill. Similar values were reported by Albessard and Mayzaud (2003) and Kreibich *et al.* (2010). These latter values are probably the closest to the level of natural occurrence in krill tissues.

3.3.3. Fatty acid composition

Depending on the scientific aim of the study, the fatty acid compositions were investigated in the total lipid fraction or in separated lipid classes of either whole animals or of different body sections such as the cephalothorax or the abdomen.

The fatty acid composition of the total lipid fraction of complete individuals of *M. norvegica* was analysed by Mayzaud *et al.* (1999) and Kreibich *et al.* (2010). A selection of the most abundant fatty acids, accounting together for 80% or more of total fatty acids, is given in Table 4.3. The bulk of saturated fatty acids (SFA) were represented by myristic acid (14:0)

Table 4.3 Approximate amount of major fatty acids in *M. norvegica*

Fatty acid	% of total FA
SFA	
14:0	1.4–7.6
16:0	9.4–22.6
Σ SFA	16.2–40.3
MUFA	
16:1($n-7$)	<1.0–13.2
18:1($n-9$)	8.2–17.4
18:1($n-7$)	2.2–9.2
20:1($n-9$)	<1.0–21.9
22:1($n-11$)	<1.0–26.6
Σ MUFA	15.1–48.3
PUFA	
20:5($n-3$)	2.6–24.8
22:6($n-3$)	4.0–37.5
Σ PUFA	20.1–61.5

and, particularly, by palmitic acid (16:0). The proportion of single monounsaturated fatty acids (MUFA) varied strongly between studies indicating a strong dependence on altering intrinsic factors or nutrition. The major polyunsaturated fatty acids (PUFA) were the ώ-3 PUFA eicosapentaenoic acid [EPA, 20:5($n-3$)] and the docosahexaenoic acid [DHA, 22:3 ($n-6$)]. Pronounced differences in the amount of single fatty acids were noted between lipid fractions, sampling sites, and seasons, for example, in krill from the Ligurian Sea, the Clyde Sea, and the Kattegat, the MUFA 20:1($n-9$) was almost absent in the polar lipid fraction but amounted to 5% in the TAG-fraction (Virtue *et al.*, 2000). In the whole lipid extracts of krill from the Greenland Sea, 20:1($n-9$) accounted for 18% of fatty acids (Kreibich *et al.*, 2010).

3.4. Factors of lipid and fatty acid variation

The lipid content and the lipid composition may be influenced by several factors including food supply, sex, maturity, spawning, season, and locality (Saether *et al.*, 1986).

3.4.1. Reproduction

Falk-Petersen (1981) concluded that variation in lipid content was related to gonad maturation in winter. Båmstedt (1976) reached the same conclusion but also suggested that the loss of lipids cannot be explained solely by gonad

maturation but also by lipid catabolism. No clear relation between the lipid content and gonad maturation could be established by Buchholz and Prado-Fiedler (1987). Moreover, those authors stated, for animals from the Kattegat that lipid accumulation is not in phase with gonad maturation. By August, 60% of the females have laid their eggs and by October, virtually all the females have spawned. During that time the lipid content was still increasing.

In the Ligurian Sea, the Kattegat, and the Clyde Sea, males and ready-to-spawn females did not show significant differences in the lipid content of the cephalothorax. However, ready-to-spawn females displayed higher lipid levels than post-spawn females. The loss of lipids from the cephalothorax after spawning can amount to 55% (Albessard and Mayzaud, 2003). Consequently, there must be a significant increase of lipids between vitellogenic and ready-to-spawn females through the synthesis or allocation of lipids into the maturing eggs.

The dynamics and the amplitude of lipid store variation during the reproductive season depend on the capacity of lipid accumulation either due to food availability or due to the duration of the spawning season, for example, the changes in the lipid amount were distinct in krill from the Ligurian Sea which live in an oligotrophic environment and do not accumulate high lipid reserves. Krill from the Scottish Clyde Sea generally had higher lipid stores than Ligurian krill. Though the absolute loss of lipids due to spawning was similar in both populations, the relative change was lower in Clyde Sea krill (33%). Krill from the Kattegat did not seem to lose significant amounts of lipids compared to krill from both the other locations. Kattegat krill may perform more successive spawning cycles with a more continuous lipid uptake and less distinct change of lipid store (Albessard and Mayzaud, 2003).

3.4.2. Season, latitude, and trophic conditions

The seasonal changes in trophic conditions are major factors which determine the dynamics of lipid storage in krill populations. Different lipid levels in krill from different latitudes reflect the variation in primary production with regard to the accumulation of overwintering lipid stores. Thus, they are related to the seasonal pattern of primary and secondary production rather than directly influenced by climate.

The total lipid content of *M. norvegica* from central and northern Norwegian fjords followed a distinct seasonal cycle. It was greatest in autumn and early winter and least in spring (Saether *et al.*, 1986). A similar seasonal cycle of lipid contents was found in krill in the Danish Kattegat (Buchholz and Prado-Fiedler, 1987). The values ranged from as low as 7.8% DW in July, up to 47.8% DW in late November. The seasonal lipid variations reflect the deposition and utilisation of winter reserves.

In the Ligurian Sea, krill showed apparently a reverse pattern with maximum values in early summer (20% DW) and lowest values (~3–4% DW) in early winter (Mayzaud *et al.*, 1999). The trophic situation in the Ligurian Sea consists of a short phytoplankton bloom in late spring (April–May) and a subsequent period of zooplankton production. In summer and fall, the biomass is low in the Ligurian. Accordingly, krill accumulate lipids during the short bloom but have to utilise them again when facing the oligotrophic summer conditions (Mayzaud *et al.*, 1999). The spring-peak of lipid accumulation is also closely linked with reproduction.

A comparative study on the influence of environmental and physiological factors on the distribution of lipids was carried out on three populations of *M. norvegica* from the Kattegat, the Clyde Sea, and the Ligurian Sea (Albessard and Mayzaud, 2003; Mayzaud *et al.*, 1999, 2000). Krill at sexual rest and early in the reproductive period showed lowest lipid values in the Ligurian Sea. The lipid content increased in the Clyde Sea and was greatest in Kattegat krill. The tendency of increasing lipid amounts towards the higher latitudes was previously emphasised by Mayzaud *et al.* (1999).

3.5. Fatty acids as trophic markers

The fatty acid composition of *M. norvegica*, particularly the TAG-fraction (Virtue *et al.*, 2000), reflects to a certain extent the composition of the prey and, thus, may serve as a biochemical indicator of trophic interactions. This has been applied to Northern krill in a number of studies (Dalsgaard *et al.*, 2003; Falk-Petersen, 1981; Falk-Petersen *et al.*, 2000; Mayzaud *et al.*, 1999; Saether *et al.*, 1986; Stübing *et al.*, 2003, Virtue *et al.*, 2000).

There is no single fatty acid (FA) which is unique to any species. However, a rough distinction between primarily herbivorous or carnivorous feeding habits can readily be made. Major fatty acids in diatoms include 16:1($n-7$), C16PUFA, and 20:5($n-3$). Dinoflagellates are characterised by 18:4($n-3$), 18:5($n-3$), and 22:6($n-3$). FA 18:4($n-3$) is present in other flagellates (Virtue *et al.*, 2000). In contrast, the FAs 20:1($n-9$) and 22:1($n-11$) appear only in traces in phytoplankton. Accordingly, a low content of 16:1($n-7$) and high contents of 20:1($n-9$) and 22:1($n-11$) as found in krill from Ullsfjorden may reflect a carnivorous or omnivorous diet with copepods as the major food component (Saether *et al.* 1986). Similarly, high levels of 20:1($n-9$) and 22:1($n-11$) are indicative of heavy predation on copepods by, for example, krill from the Kattegat (Virtue *et al.*, 2000), as confirmed by stomach analysis (Lass *et al.*, 2001). Virtue *et al.* (2000) distinguished the predominant diet of krill from the Ligurian Sea, the Kattegat, and the Clyde Sea by means of their fatty acid composition. The higher latitude krill contained greater diatom and copepod signals. The Ligurian krill fed more opportunistically, probably as an adaptation to the oligotrophic conditions in Ligurian waters.

Fatty alcohols derived from WE have been found in the stomachs of krill (Lass *et al.*, 2001) but only trace amounts of WE occur in the rest of the body. Apparently, *M. norvegica* catabolizes WE and fatty alcohols from prey both quickly and efficiently. Only in krill from the Azores and from the Greenland Sea have WE been found to occur in more than trace amounts, comprising up to 6% of total lipids (Morris, 1972; Kreibich *et al.*, 2010). Both studies were carried out on total lipid extracts of whole animals so we cannot determine whether the WE were from food items or stored in the tissues.

Promising approaches to study the lipid uptake of *M. norvegica* from the food were carried out on the stomach content and faeces of krill. Virtue *et al.* (2000) analysed the FA composition of the faeces and compared it with the fatty acid composition of zooplankton catches. In the faeces, the amount of PUFAs was reduced but the amount of saturated fatty acids was higher than in the food. Apparently, dietary PUFA were selectively metabolised by krill. Lass *et al.* (2001) analysed the FA composition of the stomach contents and found high amounts of still undigested fatty alcohols from copepods and phytol from microalgae. Unfortunately, this approach is tedious due to the small amount of sample available for analysis.

4. Respiratory Gas Exchange

4.1. Effects of temperature and season on O_2 uptake

Krill may encounter both temporal (over the course of a few hours during DVM) and spatial (different locations and seasonal changes over the course of months) differences in temperature, so it is not surprising that the effect of temperature on rates of O_2 uptake (as a measure of metabolism) has been subject to much attention (Mauchline, 1980; Mauchline and Fisher, 1969). Saborowski *et al.* (2002) investigated the effect of temperature on individuals from three geographically separate populations of *M. norvegica* which experienced markedly different patterns of spatio-temporal temperature variation (Fig. 4.3) and different trophic conditions (rich to poor; Clyde > Kattegat, Ligurian Sea). Using a specially engineered device which allows the measurement of respiration in swimming krill (Saborowski and Buchholz, 1998), Saborowski *et al.* (2002) found that there was a pronounced effect of acute temperature change on O_2 uptake (over the temperature range 4–16 °C) for all individuals, of roughly the same magnitude (Fig. 4.3); Q_{10} values ≤ 2, indicating little thermal adaptation with metabolism doubling for each 10 °C increase in temperature much like the effect of temperature on chemical reactions. Such a pronounced acute temperature effect on metabolism had been noted previously for *M. norvegica* from the Alkor Deep, Kattegat (Saborowski *et al.*, 2000) and from the Gullmarsfjord (Hirche, 1984; also see Strömberg and Spicer,

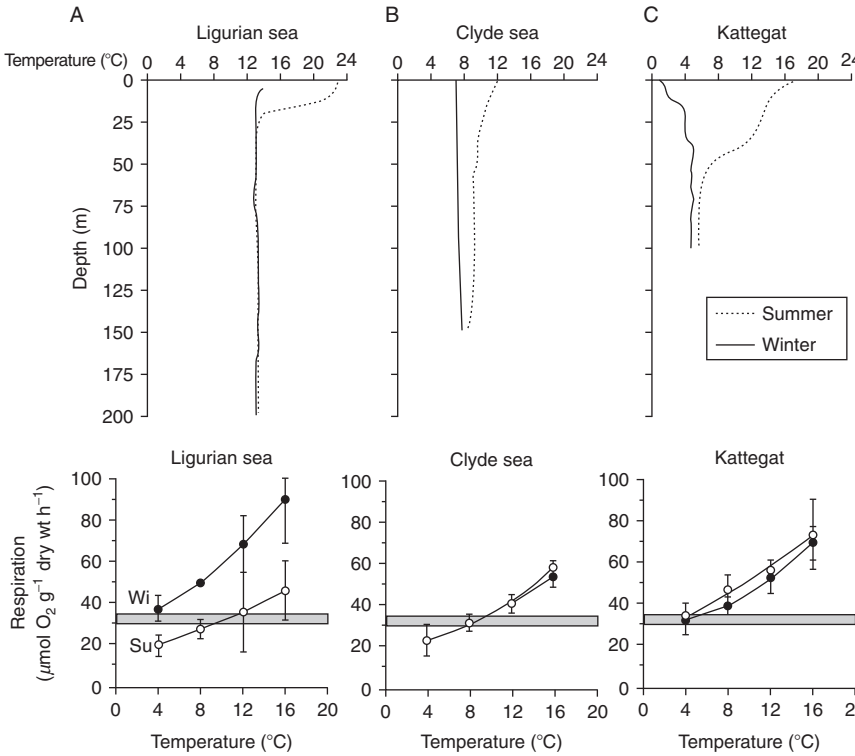

Figure 4.3 Temperature profiles of sampling locations (summer and winter) and rates of respiration (O_2 uptake: means ± SD) at different environmental temperatures of individuals of *M. norvegica* from three geographically separate populations. The shaded line represents the rate of O_2 uptake at each of the respective environmental temperatures (From Saborowski *et al.*, 2002).

2000). What is especially interesting in Saborowski *et al.* (2002) is that individuals from each of the three different populations displayed near identical rates of O_2 uptake (30–35 μmol g^{-1} DW h^{-1}) when tested at the environmental temperatures they were each experiencing (Fig. 4.3.). This indicates that the metabolism of *M. norvegica* can acclimatise to local temperature conditions over a time course greater than the period of acute temperature change used in the experiments described above, more likely in the order of days to weeks. However, there was an important exception to this phenomenon. Rates of O_2 uptake of Ligurian *M. norvegica* in the winter were twice as great as those in summer despite similar thermal regimes prevailing which Saborowski *et al.* (2002) interpreted as being in response to increased food ability. Certainly Salomon *et al.* (2000) showed that the starving of *M. norvegica* resulted in a reduction in O_2 uptake. The use of

DVM as behavioural thermoregulation of metabolism has been raised by a number of authors (e.g. Saborowski *et al.*, 2000, 2002; Strömberg and Spicer, 2000) and warrants further study. Interestingly, Mayzaud *et al.* (2005) found there was no significant difference in CO_2 excretion between *M. norvegica* kept at 12.5 °C and those kept at 17.8 °C, although there was an increase in respiratory quotient (CO_2:O_2) from 1.29 to 1.62 at the higher temperature, this increase due to rates of O_2 uptake seeming to be more sensitive to temperature change than rates of CO_2 elimination, indicating that both the physiological and ecological effects of temperature on metabolism still requires further elucidation.

In passing, comparison of rates of O_2 uptake between studies is notoriously difficult because of methodological and ecological differences and the thermal history of the animal. Not surprisingly there is a range of O_2 uptake values reported for *M. norvegica* (Table 4.4.). Certainly in few of the studies has activity, a feature that will dramatically affect rates, been quantified, though it may have been standardised. Methodological differences such as the use of closed respirometry compared with flow-through respirometry may well contribute to the large differences. Saborowski *et al.* (2002) found lower rates of O_2 uptake in closed compared with open systems. What can be said is that the metabolism of the pelagic and actively swimming *M. norvegica* is generally relatively high compared to other crustaceans.

4.2. Effects of hypoxia on O_2 uptake and anaerobic metabolism

van den Thillart *et al.* (1999) were the first to investigate the effect of exposure to acutely declining O_2 tensions, or PO_2, (hypoxia) on oxygen uptake (as a measure of metabolism) of *M. norvegica*, in individuals from the Gullmarsfjord, Sweden. Despite an earlier report which seemed to suggest that *M. norvegica* would not traverse a pycnocline into an area of low O_2 (Bergström and Strömberg, 1997), excursions into severely hypoxic bottom water (PO_2 = 3–10 kPa at a depth of 65–85 m, roughly 15–50% O_2 saturation; the waters are hypoxic because of a delay, or sometimes cessation, in annual water renewal as a result of altered currents) in the Gullmarsfjord meant that krill during the day resided at such depths (Spicer *et al.*, 1999). van den Thillart found that *M. norvegica* was able to regulate its O_2 uptake down to approx. 30% saturation (critical O_2 tension, or P_c = ~6–7 kPa, T = 10 °C). So in common with a number of other krill species *M. norvegica* displays some ability to maintain O_2 uptake in the face of declining O_2 tensions but the ability does not seem to be any more developed than that found in krill species which do not encounter hypoxia on a regular basis, if at all. Furthermore, *M. norvegica* is characterised by one of the poorest anaerobic capacities of any crustacean, surviving not more than 1 h in anoxia, and accumulating large concentrations of L-lactic acid quickly (Spicer *et al.*, 1999). So reliance on a shift from aerobic

Table 4.4 *Meganyctiphanes norvegica* (some literature data of respiration rates; from Saborowski *et al.*, 2002)

Region	Season	Temperature (°C)	Respiration rate (μmol O_2 g^{-1} dry wt h^{-1})
Ligurian Sea	Nov	5–20	23.7–43.9
Ligurian Sea	Winter	13	56.7
	Spring	13	58.0
Gulf of St. Lawrence	Feb–Aug	2–10	60.3–92.9[a]
Kosterfjorden (Sweden)	Dec–Sep	5–6	21.9–40.6
Kattegat (Alkor Deep)	Jun–Sep	5–10	50.1–73.4
Gullmarsfjorden (Sweden)	Sep	6.5	46.9
Gullmarsfjorden	Summer	10	38.7
Gullmarsfjorden	Sep	7–15	16.0–30.3[b]

[a] Rates calculated for 10-mg animals.
[b] Rates were recalculated from wet weight to dry weight assuming dry weight equals 25% of wet weight.

to anaerobic metabolism when exposed to low O_2 can barely be seen as an adaptation to migrating into hypoxic layers during DVM; indeed if krill are caged in deep hypoxic water for a greater period than they would normally reside there, they have extremely high levels of L-lactate if they survive, but survival itself is poor (Spicer *et al.*, 1999). Given this poor anaerobic capacity, it would be interesting to know the functional significance (if any) of the low levels of polymorphism of the enzyme LDH (lactate dehydrogenase—responsible for converting pyruvate to lactate) found in *M. norvegica* (Mulkiewicz *et al.*, 2001).

Strömberg and Spicer (2000) confirmed this modest regulatory ability in *M. norvegica* from the same population but also found that exposure temperature dramatically affected regulatory ability. The P_c decreased from 8–11 kPa at 15 °C to 4–6 kPa at 7 °C. Their experiments were carried out during September when 15 °C was the temperature of the upper water layers and 7 °C was the temperature below the pycnocline between 40 and 50 m deep. Thus, they suggested that a reduction in temperature dramatically improves regulatory ability (linked to a reduction in overall metabolism) and could allow excursions into cold hypoxic water. If the hypoxic deep water below the pycnocline had been characterised by the same (high) temperature as found at the surface, then krill would have been unable to regulate their metabolism, and the poor anaerobic capacity of these animals (Spicer *et al.*, 1999) would have been insufficient to permit residence in those waters for more than a few hours.

The modest regulatory ability of *M. norvegica* when challenged by acutely declining PO_2s was not present in the earliest life stages investigated (calyptopis III/early furcilia I) but began to be detectable in furcilia III (Spicer and Strömberg, 2003). Furcilia III displayed a P_c of 15.4 kPa (~75% saturation), which improved until furcilia V, $P_c = 12.6$ kPa (~63% saturation). Clearly this regulatory ability, measured at 10 °C, was not as good as the adult with a $P_c = 6$–7 kPa, from which we may surmise that regulatory ability continues to develop through the remainder of the furcilia stages, and possibly beyond. Hypoxia-related hyperventilation was achieved by an increase in pleopod (but not thoracic limb) beating frequency which appeared at or just before furcilia V. It is reasonable to suppose that the development of regulatory ability in furcilia V is intimately linked with this hyperventilation which fails at oxygen tensions lower than 11 kPa. However, the earlier appearance of regulation in furcilia III cannot be attributed to pleopod beating (even though this stage has a full complement of functional setose pleopods) as beat rate declines with declining PO_2s. The timing of the onset of regulation could not be modified, as it can in other crustaceans, by pre-exposing larvae to hypoxia, and indeed such pre-exposure resulted in significant mortality leading Spicer and Strömberg (2003) to conclude that the development of respiratory regulation in *M. norvegica* was not open to environmental influence as is the case in other crustaceans. They further speculated that, as pleopod ontogeny is intimately associated with the ontogeny of DVM behaviour and the ontogeny of respiratory regulation, this co-occurrence is fortuitous in the Gullmarsfjord as krill do not descend into hypoxic deep water until they have developed the physiology that will allow them to regulate their metabolism there.

4.3. Respiratory pigments

To our knowledge, *M. norvegica*, and krill generally, utilises just the one extracellular respiratory pigment, the copper-based haemocyanin (Hc), that is found in some crustaceans and some molluscs (Brix *et al.*, 1989; Spicer and Strömberg, 2002). Brix *et al.* (1989) was the first to characterise the O_2 binding properties of *M. norvegica* haemocyanin (Fig. 4.4). Using haemolymph pooled from 766 specimens collected from the North Sea, he investigated O_2 binding ability of the haemolymph at two different temperatures (5 and 10 °C). He found that at 5 °C, *M. norvegica* haemolymph had a very low affinity for oxygen (P_{50} or half saturation value = 50.1 mmHg [6.66 kPa], at pH = 7.9). This was very similar to P_{50} values of 6.12–6.31 kPa, pH = 7.80, $T = 7$ °C, recorded by Spicer and Strömberg (2002) for dialysed haemolymph from individuals collected from the Gullmarsfjord, Sweden. Brix *et al.* (1989) also found that the cooperativity (the 'sigmoidness' of the O_2 binding curve) was high, but not exceptional ($n_{50} = 2.5$–3.0) although the haemolymph did exhibit a marked Bohr effect

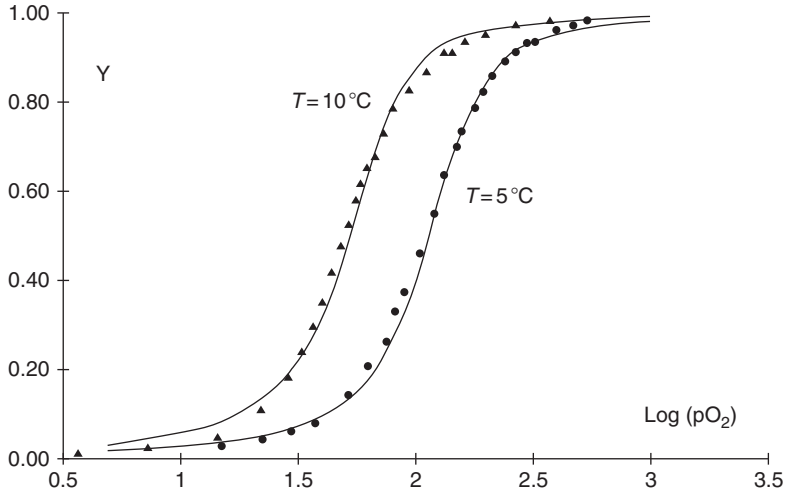

Figure 4.4 Oxygen binding curves for the haemolymph of *M. norvegica* at 5 (closed symbols) and 10 (open symbols) °C, pH = 7.7 (Brix *et al.*, 1989).

($\Delta\log PO_2/\Delta\log$ pH $= -1.99$), amongst one of the highest recorded for crustaceans. What was quite exceptional, however, was that when O_2 binding curves were constructed at a higher temperature ($T = 10$ °C), the affinity for O_2 increased markedly ($P_{50} = 18.2$ mmHg [2.42 kPa], pH = 7.8). In other words, there was a strong effect of temperature, but in the opposite direction from that recorded for every other crustacean species examined. The reaction is normally exothermic, but in this case it was endothermic as evidenced by the positive value for the heat of oxygenation ($\Delta H = 133.76$ kJ mol^{-1}, pH = 7.9). Although Brix *et al.* (1989) speculated that this increase in Hc-O_2 affinity with increasing temperature may be related to passing through thermoclines during DVM, it is difficult to be definitive about the adaptive nature (if any) of this unique feature.

Concentrations of Hc ([Hc]) in individuals of *M. norvegica* collected from the Gullmarsfjord, Sweden, were extremely variable (0.39–0.89 mmol l^{-1}), but at the upper end are some of the highest recorded in aquatic crustaceans (Spicer and Strömberg, 2002). Spicer and Strömberg (2002) found that [Hc] varied during DVM. This is one of the most exciting findings of their study, that [Hc] could and did change over a timescale of hours, rather than days, as generally believed. The [Hc] decreased with increasing depth, when measured in individuals trawled or caged at different depths. Laboratory experiments showed that this pattern could not easily be explained by differences in O_2, temperature, or salinity affecting Hc concentration. However, starvation had a dramatic effect on Hc concentration over <10 h, and this was exacerbated by an increase in temperature. Spicer

and Strömberg (2002) suggested that when *M. norvegica* migrates into deep water during the day, for whatever reason, they cannot secure enough energy to meet routine metabolic demands and so they resort to breaking down Hc and using it as an energy source. This notion, that there could be a trade-off between the respiratory function of Hc and its importance in nutrition when krill migrate into deeper, nutritionally poorer water during DVM, was further supported by Dawdry (2004) who investigated feeding and [Hc] concentrations during an actual DVM, and in the laboratory. Her work also highlighted that both sex and moult stage influences [Hc], with females having a significantly greater [Hc] than males.

5. Metabolic Properties

Due to its wide geographical distribution in the North Atlantic, and so covering different climatic zones from sub-tropical to sub-polar, *M. norvegica* is a valuable tool for comparative physiological investigations. Northern krill are found in high productive as well as in oligotrophic waters and live at temperatures from 2 to 16 °C. Thereby, krill may be exposed to persistently low temperatures at higher latitudes, constantly moderate temperatures in the Mediterranean, or seasonally variable temperatures, for example, in the Kattegat. Northern krill may even experience almost the whole range of its thermal spectrum within several hours when migrating vertically through different water strata, as happens in the Kattegat (Matthews *et al.*, 1999; Saborowski *et al.*, 2000). Accordingly, a European Union research programme (PEP) in the second half of the 1990s used this species as a model for examining adaptive metabolic responses to biotic and physical factors (Buchholz, 2003; Buchholz and Saborowski, 2000; Buchholz *et al.*, 1998), of which the results are discussed alongside others below.

5.1. Moult and digestion

Moulting: Spindler and Buchholz (1988) examined the potential adaptive properties of biocatalysts in the chitinolytic enzymes of *M. norvegica* and *E. superba*. In both species, the authors found similar temperature optima around 40–50 °C. Although enzyme activity was still high at 0 °C. In both euphausiids, activation energies were reduced at lower temperatures. The authors concluded that the enzymes showed a functional adaptation to a low temperature range. Nevertheless, the fact that the activity profiles were the same in both species, despite the fact that *M. norvegica* occurs in waters that are between 4 and 12 °C warmer than those inhabited by *E. superba*, indicates that the enzymes can operate in a relatively wide range of environments.

Digestive proteases: High apparent temperature optima appeared also in proteolytic digestive enzymes of *M. norvegica* (Dittrich, 1990). Again no shift of the optimum towards lower temperatures was evident. Michaelis–Menten constants (K_M) of trypsin-like enzymes hydrolyzing the artificial substrate BAPA were low at 0 °C and continuously increased towards 20 °C. Since low K_M-values indicate higher affinity of the enzyme towards the substrate, *M. norvegica* seems to partly compensate the rate limiting effects of low temperatures for this reaction (Dittrich, 1992a). In contrast, the activation energy of the trypsin reaction was surprisingly high and similar to tropical species (Dittrich, 1992b).

NAGase isoforms: Buchholz and Vetter (1993) isolated the different isoforms of the chitinolytic enzyme *N*-acetyl-β-D-glucosaminidase (NAGase) and studied the kinetic properties of each isoform separately. The enzymes displayed different temperature maxima and a reduced K_M-value in the range of the ambient environmental temperature. This characteristic can be interpreted as an adjustment of the species to the temperature regime (Buchholz and Vetter, 1993). The authors assumed from their study that hydrolases such as NAGase may not be regulated to a great extent but instead there is a fine tuning, with respect to temperature, of the complex molecular pathways that regulate metabolism.

5.2. Key metabolic enzymes

CS and PK: The enzymes citrate synthase (CS) and pyruvate kinase (PK) are key enzymes in metabolic pathways. The mitochondrial citrate synthase (CS), also referred to as a condensing enzyme, initiates and regulates the citric acid cycle by catalysing the condensation of acetyl-Coenzyme A (acetyl-CoA) and oxaloacetate (OA) to citric acid. The K_M-value for acetyl-CoA was up to seven times greater than that of OA. Accordingly, the catalytic rate of CS is mainly controlled by the supply of acetyl-CoA.

The cytosolic pyruvate kinase (PK) is one of the major regulatory enzymes of the glycolytic pathway; it catalyses the conversion of phosphoenolpyruvate (PEP) to pyruvate and the phosphorylation of ADP to ATP.

No distinct adaptive properties of Northern krill CS with respect to steno- or eurythermy were found in terms of specific activities, activation energy, pH/activity profiles or Michaelis–Menten constants (Vetter, 1995a). However, short-term acclimation experiments with krill from the Swedish Gullmarsfjord indicated reduced K_M-values as well as elevated specific activities at ambient maintenance temperatures (Vetter, 1995a). The author suggested that *M. norvegica* is capable of increasing CS synthesis or producing alternative CS-isoforms with a

higher specific activity to compensate enzyme activities when the animals are exposed to temperature changes.

A more detailed study on the CS and PK in *M. norvegica* from three different locations was unable to confirm adjustment of specific activities to temperature. But that study revealed that both enzymes were not equally distributed within the body and the organs of krill and that each enzyme showed a negative (CS) or positive (PK) allometric relationship (Saborowski and Buchholz, 2002). Considering this, variations in activity is most probably a result of metabolic scaling and thus only depends on the size of the animals. When the CS/PK-ratio was plotted against the sample weight, the data points of each population appeared on the same regression line. However, one exception was evident in summer krill from the Ligurian Sea which showed reduced CS/PK-ratios and stood apart from other populations. The reduced CS/PK-ratio might be due to a reduction of muscle tissue or mitochondria to cope with food-limiting conditions during the summer months in the Ligurian Sea (Saborowski and Buchholz, 2002).

CS-inhibition: Citrate synthase is controlled by cellular energy levels. Increasing ATP concentrations inhibit the CS-activity. Vetter (1995b) reported a peculiarity of the CS of *M. norvegica*: low ATP concentrations caused an increase of the maximum reaction velocity (v_{max}). The physiological consequence is that high energy demands accelerate the channelling of acetyl-CoA into the citric acid cycle. This mechanism can be understood as an adaptation to the energy demanding pelagic life style of krill.

PK-isoforms: In order to study physiological mechanisms of seasonal temperature adaptation Vetter and Buchholz (1997) isolated two isoforms of pyruvate kinase (PK) of *M. norvegica* from the Danish Kattegat. One isoform (PKI) dominated in the abdominal muscle while the other isoform, PKII, was mainly present in the cephalothorax. The K_M-values of PKI for phosphoenolpyruvate (PEP) was in summer twice as high as in winter which indicates a higher enzyme–substrate affinity to compensate for low turnover rates during the cold season. Only PKI showed features of temperature adaptation by decreasing activation energy (E_a) and K_M-values. Apparently, the requirement for temperature adaptation is more important in the energetically intensive swimming muscles of the abdomen (Vetter and Buchholz, 1997).

Dietary influence on a key metabolic enzyme: The influence of nutrition on the two PK-isoforms (PKI and PKII) of *M. norvegica* was studied by Salomon *et al.* (2000) in specimens from the Ligurian Sea. Similar to Vetter and Buchholz (1997), the authors found two PK-isoforms. A common feature of Ligurian and Kattegat krill was the inhibition of PKI and PKII by ATP and the activation of PKII by fructose-1,6-bisphosphate (FBP), a key metabolite in the glycolytic pathway. Moreover, in the

presence of FBP, the sigmoidal kinetics of PKII was shifted to hyperbolic kinetics (Vetter and Buchholz, 1997). Differences were apparent in the share of both isoforms in whole body extracts: PKI amounted to 80% in Ligurian krill, but only 44% in Kattegat krill. It is not clear yet whether this difference is intrinsic or results from differences in the chromatographic separation procedure. Neither in feeding experiments nor in field samples could the authors identify properties of PK which indicate enzyme modifications in relation to food supply.

Tissue specificity of PK-isoforms: Almost all organs contained PKI and PKII. However, PKI prevailed in the locomotive organs, that is, the abdominal muscles, the pleopods, the thoracopods, and in the thoracic muscles. PKII dominated in the eyes, the midgut glands and in the ovaries (Salomon and Saborowski, 2006). Since PKII is activated by FBP, the authors determined the adenylate energy charge and the concentrations of FBP. ATP levels did not change significantly during six days of starvation but the concentrations of FBP decreased by 30%. Moreover, FBP-values were lower in cold-acclimated than in warm-acclimated krill. The tissue-specific distribution of the two different PK-isoenzymes seems to improve the krill's physiological flexibility to successfully cope with low temperatures or limited food supply. As a consequence of food deprivation, or decreased temperature, the glycolytic energy turnover may be reduced in some organs such as the gonads and the midgut gland. Simultaneously, the locomotive organs maintain high glycolytic turnover rates due to the presence of the active PK-isoform.

6. Osmotic/Ionic Regulation and Excretion

6.1. Osmotic and ionic regulation

Osmotic, and to a lesser extent ionic, regulation by *M. norvegica* was first investigated by Forward and Fyhn (1983). They found that krill, like many other oceanic animals, were osmoconformers, at least over the salinity range 40–24 PSU ($T = 3$–7 °C). Equilibration to test salinities occurred within a few hours: while haemolymph sodium was iso-ionic within the range of experimental salinities, chloride was consistently hypo-ionic (by 50–70 mmol l^{-1}) pointing to some degree of regulation of chloride but not sodium. Amino acid regulation, probably as a means of regulating cell volume, was detected in the abdominal flexor muscle for proline (over the range 35–28 PSU) and glycine (over the range 32–24 PSU). Exposure to salinities below 24 PSU resulted in irreversible damage and death.

6.2. Fluoride accumulation and regulation

We have some knowledge of fluoride accumulation (and to some extent regulation) in the tissues of *M. norvegica*. Fluorides derive principally from the weathering of fluoride minerals and volcanic activity although a small proportion is contributed by the use of aerosols. The fluoride content of Northern krill, in agreement with what is known of other euphausiids, is quite high compared to other oceanic invertebrates with whole body burdens of 2360 $\mu g\ F^-\ g^{-1}$ dry weight (DW) (Soevik and Braekkan, 1979) and 2153 $\mu g\ F^-\ g^{-1}$ DW (Adelung *et al.*, 1987). Adelung *et al.* (1987) showed that most of the fluoride was incorporated into the exoskeleton (3343 $\mu g\ F^-\ g^{-1}$ DW), with relatively small amounts found in the tissues (e.g. muscle 5.7 $\mu g\ F^-\ g^{-1}$ DW). Tissue fluoride appears to be regulated at these low concentrations, concentrations not unlike those of vertebrates. Having found the highest concentrations of fluoride in the mouthparts of *E. superba* (12,876 $\mu g\ F^-\ g^{-1}$ DW), Sands *et al.* (1998) suggested that as the mouthparts were the hardest part of the exoskeleton, that inorganic fluoride was important in hardening krill exoskeleton. The fact that the period of maximum fluoride uptake is post-moult adds weight to this idea. Exactly how fluoride is bound into the exoskeleton of *M. norvegica* remains to be elucidated.

6.3. Excretion

Excretion occurs as the result of catabolism in animals. Rogers (1978) found that excretion rates in individuals from a Mediterranean population of *M. norvegica* were low (0.07–0.11 μg-at NH_4–N $mg^{-1}\ day^{-1}$, and 0.009–0.010 μg-at PO_4–P $mg^{-1}\ day^{-1}$) in summer, autumn, and early winter but rose sharply through the next 2 months to peak in spring (0.25 μg-at NH_4–N $mg^{-1}\ day^{-1}$ and 0.026 μg-at PO_4–P $mg^{-1}\ day^{-1}$, $T = 13\ ^\circ C$). A study of 15 zooplankton species from Kosterfjorden, west Sweden by Båmstedt (1985) included a breakdown of the different nitrogen and phosphorus excretion products for *M. norvegica*, at three different times of the year. Although not strictly comparable with Rogers (1978), the general patterns are interesting. While individuals collected in the autumn (averaged over September and October) had an ammonia excretion of 1.85 nmol mg $protein^{-1}\ h^{-1}$, individuals collected in the spring (March) showed a doubling of this rate (3.66 nmol mg $protein^{-1}\ h^{-1}$; both rates were determined in darkness, $T = 4$–$6\ ^\circ C$). However, spring rates of urea excretion were about one-third of autumn rates (1.33 compared with 5.35 nmol mg $protein^{-1}\ h^{-1}$). This pattern requires further elucidation. Both Båmstedt (1985) and Rogers (1978) record a greater value for the nitrogen:phosphorus excretion ratio in autumn (September) compared with spring (March) despite using different methods.

Saborowski *et al.* (2002) measured rates of ammonia excretion (and O:N ratios) at a number of different environmental temperatures for *M. norvegica*

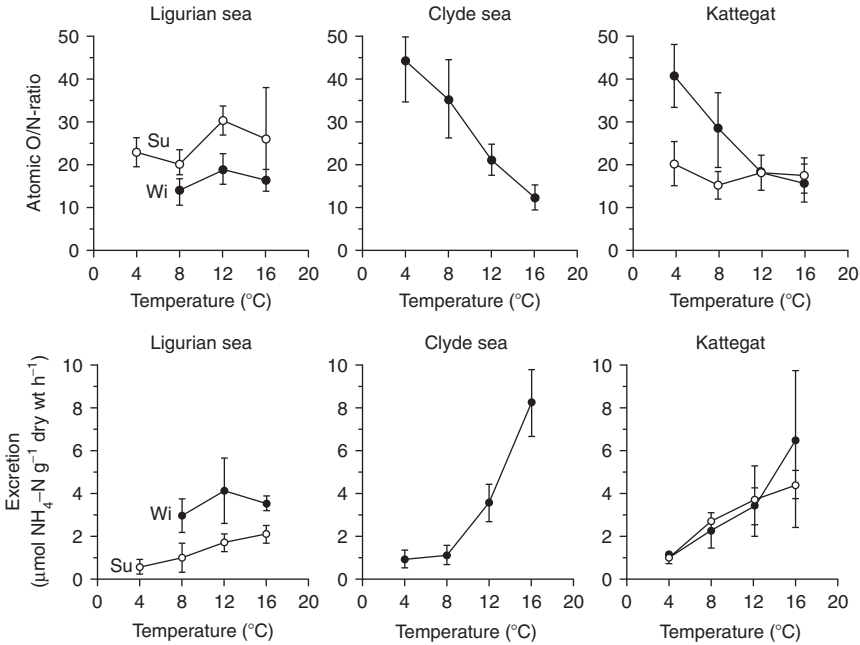

Figure 4.5 Excretion rates and O:N ratios (means ± SD) at different environmental temperatures of individuals of *M. norvegica* from three geographically separate populations (Saborowski *et al.*, 2002).

from three geographically separate populations (Fig. 4.5). There was tremendous variation in excretion rates between populations, in their response to temperature, and in seasonal modification. While the patterns obtained were quite different for different locations (Fig. 4.5), some generalisations can be made. For instance, in *M. norvegica* from the Kattegat and Clyde Sea, O:N ratios decreased with increasing temperature (winter) indicating a shift from the use of lipids (which occurs at winter environmental temperatures) to the use of protein in metabolism. O:N was equal to 35–40 in both the Kattegat and the Clyde, which Saborowski *et al.* (2002) suggest indicates equally favourable trophic conditions and similar feeding strategies of *M. norvegica* in the two locations.

7. Pollution and Trace Metals

7.1. Transuric elements

Exposure to, and accumulation of, transuric elements has commanded most attention with respect to pollution in *M. norvegica*. Fisher *et al.* (1983) demonstrated that Americium-241 (Am-241), a key radionuclide from

waste disposal, was accumulated from contaminated sea water by passive adsorption onto the exoskeleton of *M. norvegica*. The exoskeleton showed a 100-times increase in Am-241 concentration over the course of about a week. However, 96% of the total Am-241 body burden was associated with the exoskeleton, and that burden was shed during moult. There was some assimilation by internal tissues (3% after 4 days feeding on radiolabelled—2.1 kBq Am-241—diatoms *Thalassiosira pseudonana*). Thorium-234 (Th-234) is also taken up (exposure conditions = 0.03 Bq ml^{-1}) and concentrated by *M. norvegica*, reaching a steady state ($\sim$180 concentration factor) within 3 or 4 days (Baena *et al.*, 2008). As with Am-241 much of Th-234 is found in association with the exoskeleton, but not quite to the same extent. Only about 53% of the Th-234 is lost through the cast exoskeleton.

The transfer of Polonium-210 (Po-210) and Lead-210 along a food chain was examined by Stewart *et al.* (2005) who fed *M. norvegica* (NW Mediterranean) with contaminated *Artemia* adults, themselves fed on radiolabelled diatoms *T. pseudonana* and *Isochrysis galbana*. Po-210 is interesting because it is both a useful geochemical tracer and a source of high-energy alpha-emitter in marine organisms and humans: Po-210 is a naturally occurring radionuclide formed by beta decay of Pb-210. Unlike Am-241 and Th-234, it is thought that Po-210 accumulates mainly in the internal tissues *M. norvegica*, namely the hepatopancreas (midgut gland) and alimentary tract (Heyraud *et al.*, 1976).

The Po-210:Pb-210 ratio within animals increased 5–12 times with each trophic level indicating preferential bioaccumulation of Po-210 (44% assimilated by *M. norvegica*) over Pb-210 (3.5% assimilated by *M. norvegica*). The poor assimilation efficiency of Pb-210 means that the Po-210:Pb-210 ratio was 1–2 orders of magnitude smaller in krill faecal pellets than in the krill producing them. Stewart *et al.* (2005) suggest that in surface waters Po-210 has the potential to accumulate and concentrate in krill, and in the food chains of which they are a part, and be biologically recycled, whereas Pb-210 does not, making *M. norvegica* an important source of Po-210 to those predators consumed as seafood by humans. This study also appears to be the first to investigate the trophic transfer of these key radionuclides to any carnivorous planktonic organism.

7.2. 'Biomarkers'

The particle spectrum and feeding behaviour of *M. norvegica* were affected by the addition of Venezuelan crude oil ($\sim$6 $\mu l\ l^{-1}$) to sea water (Hebert and Poulet, 1980). Oil exposure had the same effect as starvation in reducing growth and survival.

Mixed function oxidase (MFO) has been proposed as a biomarker to assess the health status of krill. Fossia *et al.* (2002) measured MFO activity in Mediterranean populations by assaying benzo[a]pyrene monooxygenase

(BPMO) activities. However, there was no difference between populations with a mean value for BPMO of 1.83 (range 0.47–3.20) A.F.U. mg protein^{-1} h^{-1}. The same study also considered poly-aromatic hydrocarbons (PAH), which are byproducts of burning oil or coal. Mean PAH was 2624 (range 963.8–5038 ng g^{-1} DW) and the mean concentration of carcinogenic PAHs was 88.4 (range 61.84–141.7) ng g^{-1} DW with highest concentrations detected adjacent to the Ligurian coast. Amongst other measures, mean DDT (dichlorodiphenyltrichloroethane) levels were 72.83 (range 41.02–163.2) ng g^{-1} DW; mean PCBs (polychlorinated biphenyls), 145.4 (range 84.60–210.2) ng g^{-1} DW and mean HCB (hexachlorobenzene) = 6.08 (range 3.5–11.56) ng g^{-1} DW.

7.3. Trace metals

Rainbow (1989, 1993) provides baseline data for trace metals (essential as well as non-essential) from geographically separate populations of *M. norvegica* (Table 4.5). The first thing to notice is that there appears to be substantial spatial variability in trace metal concentrations, but also, where investigated, equally marked variability within a given population. Rainbow (1989) demonstrated a clear size dependency on whole body concentrations of copper and cadmium, but not zinc, iron, or manganese, in two geographically separate populations. This casts some doubt on mean values of copper and zinc presented in the table. However, it should be noted that Ridout *et al.* (1989) and Zauke and Schmalenbach (2006) also tested for size dependency in many of the same elements but were unable to detect any relationship. Some caution is therefore necessary when considering mean values, particularly when all size classes of krill have not been considered.

Nevertheless, there are clear population differences in cadmium and copper, particularly when we compare the North Atlantic (oceanic) and Firth of Clyde (coastal) populations (Rainbow 1989). The Atlantic population consistently had a greater concentration of both of those metals, across a range of body sizes. Furthermore, as the concentration of cadmium and copper was greater in the oceanic compared with the coastal individuals, we can discount the difference being attributable to enriched dissolved metal concentrations in coastal water, or other anthropogenic effects.

What is interesting is that the nature of the size dependency of these two metals is quite different. While copper concentration increased with increasing body mass (b is positive), cadmium concentration decreased with increasing body mass (b is negative). The negative relationship as found for cadmium might indicate that surface adsorbed metal contributes a significant proportion of total body metal burden, which is enhanced in small individuals with surface area to body mass ratios decreasing with increasing size. However, one might expect such a negative relationship for metals such as iron and manganese which show tendencies in sea water

Table 4.5 Trace metal concentrations (μg g^{-1} DW) of whole *M. norvegica* collected from different locations

Location	Cd	Cu	Pb	Zn	Fe	Mn	V	Cr	Ni	Co	As	Reference
Barents Sea	0.2	47	–	73	–	–	–	–	–	–	–	Zauke and Schmalenbach (2006)
Greenland Sea	0.4	35	<0.3	42	–	–	–	–	–	–	–	Ritterhoff and Zauke (1997)
N.E. Atlantic	0.54–6.06[1]	8.8–67.2[2]	–	43⋆	38.9⋆	5⋆	–	–	–	–	–	Rainbow (1989)
N.E. Atlantic	0.39⋆	71.9⋆	–	96.5⋆	25.6⋆	2.9⋆	0.17⋆	0.27⋆	0.80⋆	0.16⋆	59.3⋆	Ridout *et al.* (1989)
North sea/Atlantic	0.5	26	1.0	45	–	–	–	–	–	–	–	Zauke *et al.* (1996)
Firth of Clyde, Scotland	0.14–1.83[3]	30.8–72.6[4]	–	102⋆	31.8⋆	1.14⋆	–	–	–	–	–	Rainbow (1989)
Corsica, Mediterranean	0.5	25.4	4.03	59	–	–	–	–	–	–	–	Romeo and Nicolas (1986)

All values are mean values, except from those marked with a superscript (1–4) where the range of concentrations measured is presented. This is because in those cases a relationship was detected between body weight and trace metal concentration, described by the equation $_{\log}C = {}_{\log}a + b_{\log}$d.w., where C = trace metal concentration, d.w., dry weight; and a and b are constants, the values for which are as follows.

[1] $a = -1.646$, $b = -1.126$.

[2] $a = 2.591$, $b = 0.639$.

[3] $a = -0.989$, $b = -0.779$.

[4] $a = 2.031$, $b = 0.367$.

Values marked with ⋆ denotes where a relationship between dry mass and metal concentration was tested for, but none was found.

to adsorb onto resuspended particles, and no such significant relationships were detected where it was tested for in *M. norvegica*. What is clear is that the cadmium concentrations are amongst some of the lowest for planktonic crustaceans and the copper values are amongst some of the highest, the latter fact perhaps related to the comparatively high concentrations of the copper-based respiratory pigment, haemocyanin, found in the haemolymph of *M. norvegica* (Brix *et al.* 1989; Spicer and Strömberg, 2002; see Section 4.3).

The concentrations of zinc, iron, and manganese were not atypical for planktonic crustaceans in general. As there was no size dependency detected for these elements, a comparison of mean values seems valid and shows that there is considerably spatial variation in zinc (means range from 42 to 109 $\mu g\ g^{-1}$) and manganese (means range from 1.14 to 5 $\mu g\ g^{-1}$), but not so much spatial variation in iron (means range from 25.6 to 38.9 $\mu g\ g^{-1}$). Experiments on the uptake, accumulation, and excretion of trace metals by *M. norvegica* exposed to natural and enriched concentrations of trace metals remain to be performed, as does an investigation on the physiology and pathology of exposure to enriched concentrations.

8. Perspectives

With respect to our understanding of the physiology of digestion, further biochemical and cytological studies are needed to investigate the assimilation process and, in particular, the membrane transfer of nutrients within the midgut gland. Furthermore, additional information is also required about the properties of key enzymes such as those involved in lipid digestion and lipid conversion. The cellulase and laminarinase activities in Northern krill need to be investigated as a matter of urgency, and it should be determined whether they are of endogenous origin or from microbial symbionts. Unfortunately, much of this work (and a number of the studies proposed below) requires long-term maintenance of krill in captivity and Northern krill are not easy to keep either in good condition, or at all, in the laboratory. Nevertheless, more experiments are required to study the processes of lipid digestion, conversion, and synthesis in *M. norvegica*.

Our understanding of key elements of the respiratory system of krill generally, and in some cases of *M. norvegica* specifically, have advanced markedly over the past 30 years, although mainly due to a handful of papers. Krill haemocyanin has a low O_2 affinity and a high sensitivity to temperature, although in the case of *M. norvegica*, the temperature effect is the opposite to that found for the respiratory pigments of nearly every other organism, including the closely related Antarctic krill. The positive heat of oxygenation discovered for the haemocyanin of *M. norvegica*, and the fact

that the haemocyanin appears to be used as much as an energy source/store, as an O_2 transporter, deserve further, more detailed study. We are beginning to understand the effects of changing environmental factors on the physiology of *M. norvegica*, but to date no study has attempted to investigate synergistic or additive effects as a result of varying two or more environmental parameters. Furthermore as we realise that larval krill are not merely small adults in terms of their physiological capacity and tolerance, the need to consider development as central to our understanding of the ecophysiology of *M. norvegica* becomes clear.

When raising the notion of synergistic interactions, it is worth noting again that there are few studies, and none of them very detailed, on the effects of pollutants on the ecophysiology of *M. norvegica*. Certainly, we only know a little about the physiological effects and responses to transuric elements and trace metals. There is also one of the potentially most important (perceived) challenges to marine life in the twenty-first century, ocean acidification, and in particular its interaction with increasing temperature (Widdicombe and Spicer, 2008).

We have an appreciation of the high metabolic turnover rates and a high physiological plasticity of *M. norvegica*. However, very few investigators have probed the regulative potential of metabolites. Future studies should, therefore, focus more on the modulative effect of metabolites on enzyme activity levels.

M. norvegica has a physiology which is as interesting and surprising as it is beautiful to behold. Despite the problems of keeping this species in good condition in the laboratory for prolonged periods of time, those who have tried, and succeeded, have increased our knowledge of krill biology. Even more so those who have paid some attention to *Meganyctiphanes* biology have in doing so challenged our understanding of some basic biological processes, such as how respiratory pigments work and what they can be used for. There is no reason why this should not continue particularly given the key ecological role of krill in the world's oceans, and the importance of understanding their physiological responses to the pressing environmental problems of those oceans, particularly increasing CO_2 and temperature.

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