DOCTOR OF PHILOSOPHY

Latitudinal variations in the energy consumption of gammarid amphipods

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Latitudinal variations in the energy consumption of gammarid amphipods

A thesis submitted to Bangor University

by

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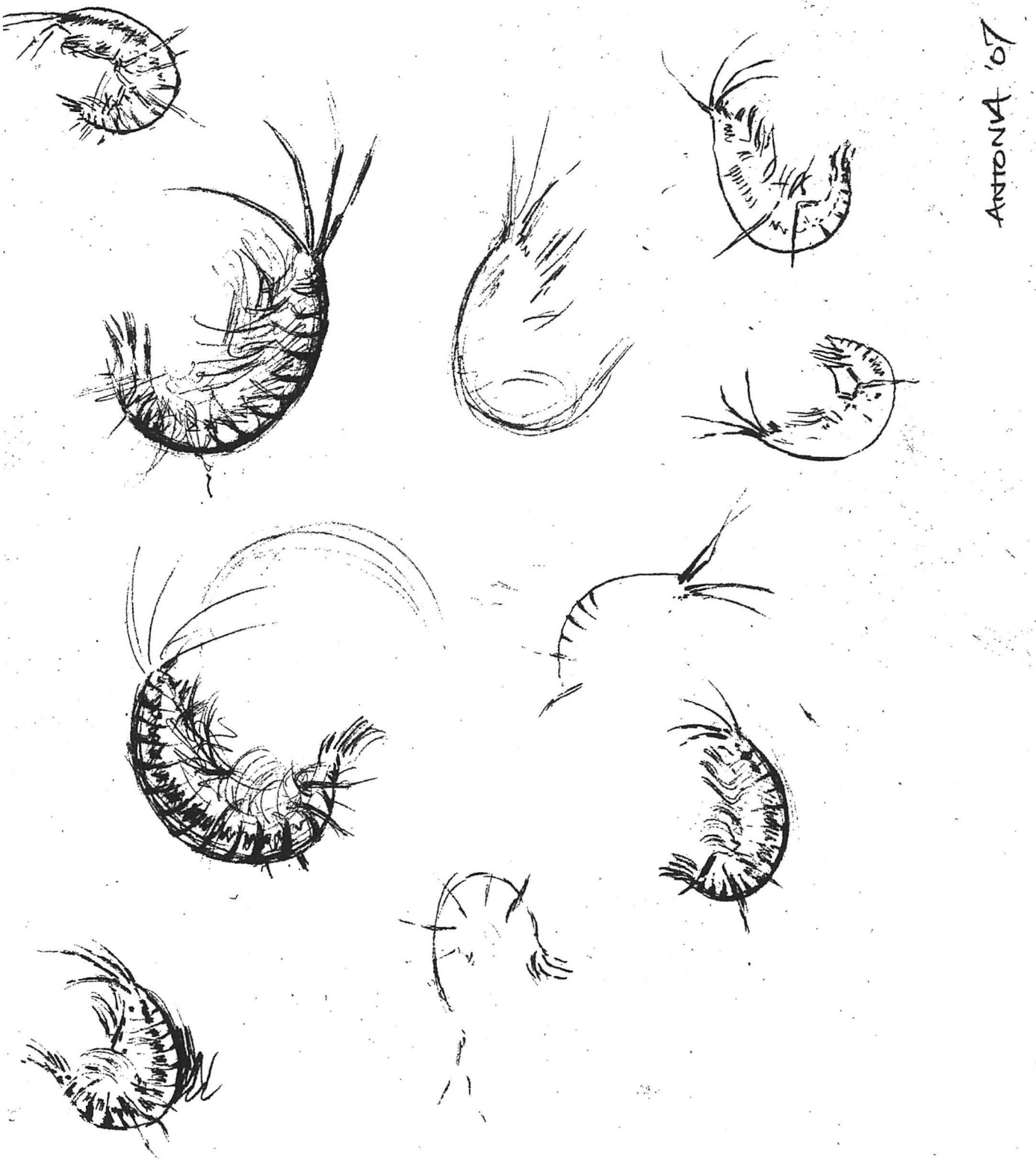
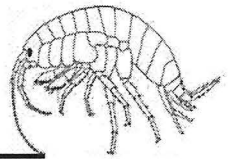
"Cogito ergo sum"

René Descartes. (1644) *Principles of Philosophy,* Part 1, article 7



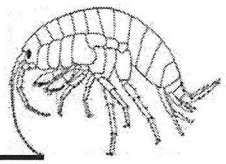
Gammarid amphipods, Sketches by wild-life artist Antonia Phillips, Ny-Ålesund, 2007.



Synopsis

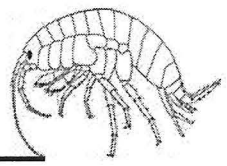
The relationship between metabolic rate and latitude in marine ectotherms has received much interest, especially for increasing our understanding of the metabolic specialisations shown by polar species. Studies have been undertaken to examine natural variations in metabolic rate (oxygen uptake) and rates of protein synthesis in an ecologically important group of marine ectothems, the gammarid amphipods, with a wide latitudinal distribution along the coasts of the NE Atlantic and Arctic Oceans.

Comparisons between populations revealed relatively low whole animal rates of oxygen uptake and protein synthesis in the subarctic populations of *Gammarus setosus* and *G. oceanicus* from Svalbard (79°N). Despite an increase in rates of both variables in *G. oceanicus* as latitude decreased, the energetic unit-costs of protein synthesis remained the same. Consequently, costs of protein synthesis, at least in the subarctic-temperate *G. oceanicus,* are fixed and independent of temperature. In sharp contrast, the temperate species, *G. locusta,* shows no variation in rates of oxygen uptake or protein synthesis across its latitudinal range, despite a variation in habitat temperature of 8°C. *G. locusta* is also characterised by decreased Arrhenius activation energies for metabolism and increased RNA efficiencies with latitude, suggesting optimisation of key enzymes to lower temperatures in the northern population (53°N). The cold-temperate upper shore species, *G. d. duebeni,* also shows no variation in rates of oxygen uptake or protein synthesis with latitude, probably to impart some independence from its highly variable environment and to compensate for the effects of shorter seasons on growth and development. Unexpectedly, *G. d. duebeni* exhibited comparatively low rates of protein synthesis, suggesting low rates of energy turnover.



This may be due to resource limitation and energy conservation in an unstable environment.

It appears that warm-temperate species compensate for temperature-related changes in metabolic rate and protein synthesis, whereas the Arctic/boreal species do not. Such differences could be related to their ancestral origins and thermal histories. Global-scale meta-analysis of rates of oxygen uptake in 48 species of amphipod indicates an overall lack of metabolic compensation with latitude between species, with low energetic costs of living observed in polar species. Low rates of energy consumption and expenditure may be advantageous in low energy polar environments; and may underlie K-selected traits such as slow rates of growth, development and reproduction reported in most polar ectotherms, including amphipods. Such traits favour stable environments and may limit adaptation to long term variations in temperature associated with climate change.



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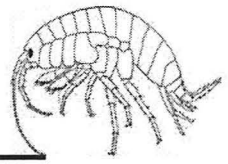

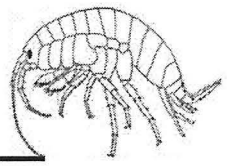

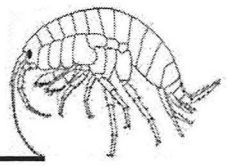

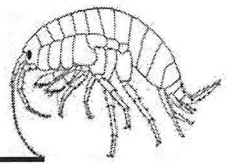

Acknowledgements

I would like to dedicate this thesis to the memory of my grandfather Raymond Fiddler (gramps) he remains an inspiration. I would also like to thank my grandmother Florence Fiddler, my mother Ann Rastrick and my farher Ken Rastrick for their love and support both emotionally and financially. I would like to thank my young brother, Joe Rastrick, for driving me to do my best and set the bar as high as possible (although there isn't much point as he always sails over it). I would also like to thank my girl friend Helen Graham for her support and putting up with having a relationship with my answer phone for the majority of my PhD.

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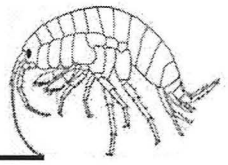

Silver for lab and filed support during my stay in Ny-Ålesund in August 2008. I also thank Ken Rastrick, Joe Rastrick, Helen Graham and Alison Bally, for assisting with amphipods collection on the Isle of Skye 2006-2008.

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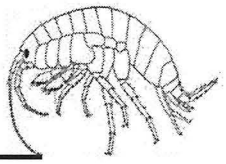


Abbreviations

ANCOVA.....Analysis of covariance

ANOVA.....Analysis of variance

A_s.....Absolute rates of protein synthesis

ATP..... Adenosine-5'-triphosphate

E_a.....Arrhenius activation energy

FW.....Fresh weight

K_{RNA}.....RNA activity

K_s.....Fractional rates of protein synthesis

LSD.....Least significant difference

MO_2.....Oxygen uptake

MCA.....Metabolic cold adaptation

n.....Sample number

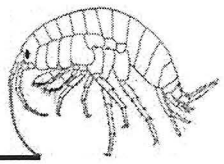
PO_2.....Oxygen partial pressure

PSRE.....Protein synthesis retention efficiency

Q_{10}.....Temperature coefficient

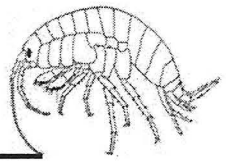
r^2..... Coefficient of determination

RNA..... Ribonucleic acid



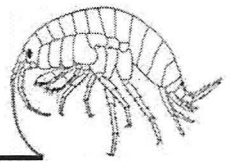
Chapter 1

General introduction



Latitude is an important environmental gradient, with large variations in temperature. thermal stability, food availability and photoperiod reported between the poles and the equator. Temperature shows an inverse relationship with latitude due to associated reductions in radiation load. Species distribution patterns and the replacement of species over this thermal gradient in the marine environment have been studied for over half a century (eg. Ekman, 1953) with many patterns in physiology and life-history traits being described (eg. Pörtner, 2006). However many of these patterns remain controversial. For example, metabolic cold adaptation (MCA) (eg. Addo-Bediako *et al.*, 2002; Lardies *et al.*, 2004) and variation in costs of protein synthesis (reviewed by Fraser & Rogers, 2007; Whiteley & Fraser, 2009). It is now known that in species adapted to a range of environmental conditions, temperature is probably the most significant environmental factor affecting organisms at all levels of biological organisation and acting as agent for selection in several species distributed over a wide latitudinal range (reviewed by Powers *et al.*,1991).

Physiological responses to short-term fluctuations in temperature involve the increase in rate functions such as metabolic rate, often determined indirectly as changes in oxygen uptake rate. However, longer term exposure can lead to acclimatisation and adaptation, and may involve modifications in metabolic rate (MCA) and rates of protein synthesis. Such variations in energy consumption and expenditure can also affect life-history traits such as growth and body size. Physiological specialisations to the thermal environment can be phenotypic or genetic and may act as a driving force of speciation. Variation in thermal tolerances between populations/species separated latitudinally are also often beyond the range of acclimation. The present thesis examines trends in energy consumption, oxygen

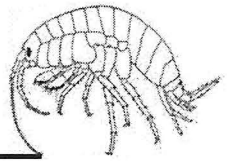


uptake and rates of protein synthesis, with latitude and discusses their relevance in terms of thermal adaptation in an ecologically important genus of amphipod crustacean, the *Gammarus*.

1.1 Variations in metabolic rate with latitude

Almost a century ago, Ege and Krogh (1914) and Krogh (1914, 1916) predicted that metabolic rates in animals adapted to cold environments should be higher at a given temperature than those adapted to warm environments. Rate functions such as jellyfish bell pulsation (Mayer, 1941) and water pumping rates in mussels (*Mytilus californianus*; Bullock, 1955) have been shown to be higher at colder latitudes when compared at the same temperature. The pumping rates of *M. californianus* are the same at *in situ* temperatures, with no significant difference in rates between mussels at 6.5°C from Friday Harbour (48°27'N) and at 12°C from Los Angeles (34°N; Bullock, 1955). Heart rates have also been shown to be similar between populations of *M. californianus* despite variations in latitude and temperature (eg. Pickens, 1965). The possible adaptive increase in metabolic rates at higher, colder latitudes is known as metabolic cold adaptation (MCA) and was first proposed by Fox (1939).

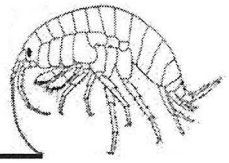
Increased metabolic rate at higher, cooler latitudes, as a result of MCA, may be advantageous in compensating for the slower catalysation rates of some enzymes at low temperatures, although many species show thermo-adaptive isoenzymes to compensate for this (Hochachka & Somero, 2002). MCA may also allow species to meet the elevated ATP costs of growth and development allowing them to complete life cycles in the colder shorter growing seasons (Somme & Block, 1991). However, elevated ATP costs of protein synthesis at lower temperatures may not be as high as



once thought (discussed below). In addition, there may be fitness costs associated with rapid growth (Gotthard *et al.*, 2000) and with the divergence of energy away from other important possesses such as reproduction. Such fitness costs make MCA unlikely to evolve in energy restricted habitats (Clarke, 1991; 1993). Instead, lower energy consumption would be expected to be advantageous in high latitude species, due to energy conservation in energy limited environments (Clarke, 1991; 1993).

To date, MCA is reported in terrestrial arthropods including beetles (eg Anuaas *et al.*, 1983; Stromme *et al.*, 1986; Schultz *et al.*, 1992), grasshoppers (Cheppell, 1983; Massion, 1983) and polar microarthropods (Block, 1977; Block & Young, 1978; Young, 1979). Addo-Bediako *et al.*, (2002) used a global-scale analysis of the standard metabolic rates of 346 species of insects and showed that once treatment temperature and body size was accounted for, higher whole-animal metabolic rates were observed in insects from low temperature environments. MCA has also been reported in aquatic arthropods, such as amphipods (Opaliński, 1979; 1982; Opaliński & Węslawski, 1989), however, such early studies have since been criticised for possibly measuring artificially high metabolic rates due to handing stress (Clarke, 1980).

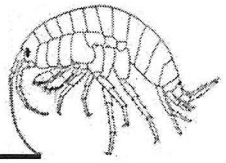
Metabolic cold adaptation has also been described between populations of the same species with latitude, for example, *Drosophila melanogaster* collected along a latitudinal gradient in Australia. When acclimated to a common temperature of 18°C, metabolic rate increased with latitude showing a 9% difference between 17°S and 41°S. However, no differences in metabolic rate occurred at higher common temperatures of 25°C (Berrigan & Partridge, 1997). In addition, *D. melanogaster* allowed to evolve in the laboratory at 18°C exhibited metabolic rates 5-7% higher



than those allowed to evolve at 25°C (Berrigan & Partridge, 1997). In the marine environment, Mangum (1963) observed intraspecific divergence in metabolic rates in two maldanid polychaete species, *Clymenella torquata* and *C. mucosa*. She showed that when metabolic rate was adjusted for body size, the northern populations from Long Island Sound, Connecticut (41°15'N) consumed more oxygen over a range of temperatures (2.5-23.5°C) than more southerly populations from Beaufort Harbor, North Carolina (34°40'N). *Clymenalla mucosa* from Bahia Parguera, Puerto Rico (18°N) showed a similar response.

The effect of latitude and temperature on rates of oxygen consumption has been widely studied in fiddler crabs (*Uca spp.*). These intertidal crustaceans show a wide latitudinal distribution throughout the USA and Brazil. Vernberg and Costlow (1966) studied three latitudinally separate populations of *Uca pugilator* from North Carolina, Massachusetts and Florida. They showed that when hatched and reared at identical temperatures, the higher latitude populations from North Carolina and Massachusetts exhibited higher rates of metabolism than the more southern, Florida population. These differences were attributed to genotypic variation and not phenotypic plasticity. In contrast, MCA is not observed in either *U. uruguayensis* as rates of oxygen uptake were higher in southerly populations, or *U. rapax* where higher metabolic rates in high latitude populations were only reported at 12°C. *U. rapax* also exhibits large intraspecific variation in metabolic rates between tropical populations exposed to near identical thermal regimes (Vernbrg & Vernberg, 1966).

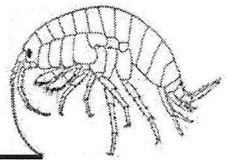
In addition, if variations in metabolism at different latitudes are dependent on the latitudinal thermal-gradient, metabolic rates of populations exposed to similar temperatures either side of the thermal equator should be the same. However, this is



not the case in populations of *U. rapax* from the USA and Brazil (Vernbrg & Vernberg, 1966). This pattern of metabolic variation across the equator is also not shown between species of mussels. For example, in the southern hemisphere mussel, *Perna* (*Mytilus*) *perna*, metabolic rates are significantly higher than in the northern hemisphere mussel *Mytilus edulis*, despite similarities in ecology and anatomy (Bayne, personal communication in Vernberg & Vernberg, 1966).

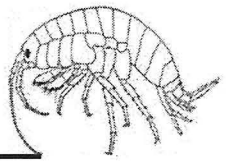
A number of studies comparing metabolic rates between temperate and Antarctic marine ectothermic species do not support the concept of MCA. For example, lower metabolic rates were reported for the Antarctic isopods, *Serolis polita* and *S. cornuta*, compared to previous data from 13 temperate and tropical species (Luxmoore, 1984). This pattern of low energy consumption in Antarctic marine ectotherms is repeated regardless of whether these comparisons are made across phyla (zooplankton; Ikeda 1985), within phylum (Crustacea; Ivleva 1980), or within a specific class (Houlihan & Allen, 1982; Peck & Conway, 2000; Clarke & Johnston, 1999; Luxmoore, 1984), including Amphipoda (Chapelle & Peck, 1995). It is now generally accepted that MCA does not occur in Antarctic marine invertebrates (Pörtner *et al.*, 2007). A recent large-scale study has also suggested that MCA does not occur in Antarctic teleost (Clarke & Johnston, 1999), although this is not consistent with some earlier studies (Wohlschlag, 1960).

Many Antarctic species are endemic and highly specialised to live in the extreme cold; therefore low metabolic rates may be a result of phylogenetic differences between Antarctic and temperate species. However, in the northern hemisphere Arctic populations can be compared to temperate populations of the same species, as the Arctic is less isolated from temperate regions than the Antarctic. In



addition, many studies (eg., Opalinski, 1982; Luxmoore, 1984) compare wide ranging species with no discussion of phylogeny; therefore it is not known whether any metabolic variations may simply be a result of genetic history. This is also true for some meta-analysis studies (e.g., Addo-Bediako, *et al.*, 2002) where it is not always clear which species are being compared (Hodkinson, 2003). Hodkinson (2003) also argued that the meta-analysis study by Addo-Bediako, *et al.* (2002) is a huge oversimplification and is based on restricted knowledge of the organisms involved. Despite the importance of meta-analyses models in summarizing and comparing disparate physiological (Chown, *et al.* 2003) and ecological (Lawtin, 1992; 1999) data, there is clearly a requirement for the direct measurement of metabolic rates and more detailed intra- and inter-specific comparisons from a range of latitudes (not just two; Garland & Adolph, 1994), in species and populations where the phylogenetic relationships have been established.

A number of studies have indicated that metabolic compensation at higher latitudes may involve variations in the Arrhenius activation energy of metabolism (E_a) and that E_a may be the best measure of MCA (Young 1979; Lee & Baust, 1982b; Aunaas *et al.*, 1983; Somme & Block, 1991; Hodkinson, 2003; Lardies *et al.*, 2004). The E_a of metabolism is calculated from the Arrhenius relationship between log metabolic rates (usually oxygen uptake) and temperature (in degrees Kelvin) and gives an estimation of the energy required for metabolic reactions, to occur. Reduction in E_a at higher latitudes indicates alterations in specific activity of the metabolic enzymes which is a typical response to cold compensation (Johnston *et al.* 1975; Sommer & Pörtner 2002; Lucassen *et al.*, 2006). Variations in E_a of metabolism

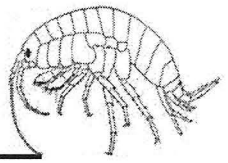


associated with reduced metabolic rate/temperature relationships will also affect thermal sensitivity.

1.2 Variations in thermal sensitivity and tolerance with latitude

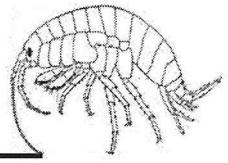
Thermal sensitivity is often described by the temperature coefficient or Q_{10}, which is the proportional increase in a rate function, such as the rate of oxygen uptake, over a 10°C change in temperature. Usually metabolic rate doubles or triples over a 10°C increase representing the kinetic change of biochemical reactions with temperature (Precht, 1958; Hoar, 1975). Temperature coefficients of more than 2 or 3 indicate increased thermal sensitivity often associated with lower temperature, for example, allowing polar species to respond rapidly to small changes in temperature (Hodkinson, 2003). However, high Q_{10}s may result from inadequate acclimation periods (eg, Ivleva, 1973) and low Q_{10}s may reflect a need to conserve energy at lower temperatures, especially if food is limited (Feder, 1985). Temperature coefficients lower than 2 suggest at least partial metabolic independence from temperature (Precht, 1958; Cossins & Bowler, 1987), as would be expected at higher latitudes if E_a of metabolism was reduced, due to MCA.

As early as the beginning of last century, studies of the thermal tolerance in the jellyfish *Aurelia aurita* and the horseshoe crab *Limulus polyphemus* showed that upper critical temperature decreased with latitude (Mayer, 1914). Hyman (1955) showed a non-reversible decrease of 20°C in upper critical temperature with latitude between laboratory cultures of the crinoid, *Antedon petasus,* from Norway and Tobago. More recent studies, such as Gaston and Spicer (1998), have also shown a non-reversible decrease in upper critical temperature with latitude between a northern



and southern population, in this case beachfleas (*Orchestia gammarellus*) from South East England and Northern Scotland. The marine gastropod, *Littorina saxatilis*, also shows lower upper critical temperatures in a sub-arctic White Sea population than in a more southern North Sea population. Cold acclimation also led to a decrease in upper critical temperature in the North Sea (Sokolova & Pörtner, 2002). However, there are exceptions to this pattern, such as thermal-tolerances in the musk turtle, *Sternotherus odouratus* (Ultsch & Cochran, 1994).

Many of these studies only examine two populations and are therefore limited in determining a latitudinal pattern, as there will always be a relationship between two points (Garland & Adolph, 1994). Studies that compare thermal tolerances along a true latitudinal thermo-gradient (more than two latitudinally separated populations) are extremely rare in the literature. A rare study comparing thermal tolerances along a true latitudinal gradient in Eastern Pacific Porcelain crabs (*Petrolisthes spp*) involved 20 species (the phylogeny of which are discussed and accounted for) from a very wide range of latitudes (43°21'- 8°50'N). The upper critical temperature was shown to be positively correlated with surface water temperature (Stillman & Somero, 2000). Within species Tanaka (1996) compared the supercooling point of the House spider, *Achaearanea tepidariorum*, from six populations in Japan ranging from 26°12' to 43°03'N. The supercooling point was lower at higher latitudes in winter but not summer animals (Tanaka, 1996). Another more recent study compared the thermal physiology of three populations of the Chilean terrestrial isopod, *Porcellio laevis* (common woodlouse) collected in Antofagasta (23°38'S), La Serena (29°55'S) and Santiago (33°23'S). *P. laevis* from low latitudes exhibited higher optimum temperatures and low cold tolerance. Although intraspecific variations in thermal

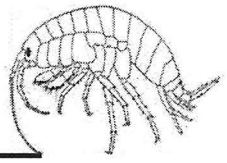


tolerance along a latitudinal thermo-gradient is a long studied biological trend there is little direct evidence from studies examining a number of latitudinally separated populations, especially comparison of Arctic and temperate populations of marine invertebrates.

1.3 Variations in protein synthesis with latitude

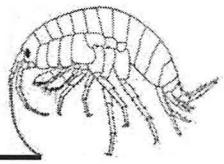
Thermal tolerance and the ability of organisms to adjust to variations in temperature is affected by general rates of protein synthesis. In the carp, *Cyprinus carpio*, a net loss of protein, in response to starvation, leads to an inability to acclimate to new temperatures (Gerlach *et al.*, 1990), while the ability to adjust thermal tolerances is lost in the European Bitterling, *Rhodeus amarus,* when treated with a protein synthesis inhibitor (Künnemann, 1973). Protein synthesis is also critical for acclimatisation and adaptation to long term variations in mean temperature (Hawkins, 1991; Reid *et al.,* 1998; Fraser & Rogers, 2007).

Limited examination of variations in rates of protein synthesis with latitude indicates that whole animal rates of protein synthesis decrease in polar marine ectotherms living at temperatures below 5°C (Whiteley *et al.*, 1996; Robertson *et al.*, 2001a; Fraser *et al.*, 2007), with the exception of the Antarctic sea urchin, *Sterechinus neumayeri* (Marsh *et al.*, 2001; Pace & Manahan, 2007). The Antarctic isopod, *Glyptonotus antarcticus,* exhibits much lower rates of protein synthesis at 0.24% day^{-1} (Whiteley *et al.*, 1996) than the temperate isopod, *Idotea rescata*, at 0.45% day^{-1} (Whiteley & Faulkner, 2005). In the Antarctic limpet, *Nacella concinna*, summer factional rates of protein synthesis were 0.8% day^{-1} (Fraser, *et al.*, 2007) and substantially lower than the mean rate of protein synthesis, approximately 2.7% day^{-1},

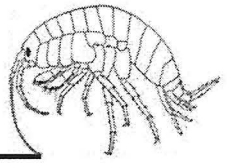


reported for temperate marine ectotherms (Fraser & Rogers, 2007). With still higher rates of 3.7% day^{-1} reported in the tropical prawn, *Macrobrachium rosenbergii,* acclimated to 30°C (Intanai *et al.*, 2009). To date protein synthesis rates, have only been measured in six species of polar marine ectotherm (Whiteley *et al.*, 1996; Robertson *et al.*, 2001a; Marsh *et al.*, 2001; Storch & Portner, 2003; Pace *et al.*, 2004; Fraser *et al.*, 2002a) and rates of protein synthesis remain to be determined between populations of the same species occupying different thermal regimes. Such an examination will help to determine whether or not rates of protein synthesis decrease with latitude.

Short-term increases in temperature, not approaching the upper thermal limit, usually result in increased rates of protein synthesis (Whiteley *et al.*, 1996; Robertson *et al.*, 2001a; b; Whiteley *et al.*, 2001). Rates of protein synthesis also decrease with temperature in species acclimated to a range of different temperatures as shown in the wolfish, *Anarhichas lupus* (eg. McCarthy *et al.*, 1999). Temperature has a direct effect on the kinetics of protein synthesis due to variations in RNA activity (K_{RNA}) with temperature (Goolish *et al.*, 1984; Houlihan, 1991; Foster *et al.*, 1992; 1993a; b; McCarthy *et al.*, 1999; Robertson *et al.*, 2001b; Fraser *et al.*, 2002a; Storch *et al.*, 2003; Treberg *et al.*, 2005; Whiteley & Faulkner, 2005; Intanai *et al.,* 2009). For example, in the temperate isopod, *I. rescata*, K_{RNA} decreased from 1.10ugRNA^{-1}.day^{-1} in animals acclimated to 14°C to 0.21ugRNA^{-1}.day^{-1} in animals acclimated to 4°C. However, temperature depressed overall metabolic rates and ATP synthesis may also affect the level of energy available for protein synthesis. Temperature also has an indirect affect on rates of protein synthesis via its positive effect on the rate of food consumption, as increased amino acid uptake increases the rate of protein synthesis at



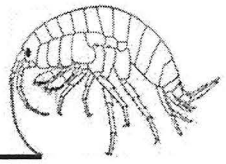
a common temperature (McCarthy *et al.*, 1993; 1994). For example, the polar isopod, *Saduria entomon,* acclimated to 4°C, exhibited rates of protein synthesis of 1.5%day^{-1}. However in starved isopods acclimated to the same conditions, rates of protein synthesis fell to 0.6% day^{-1} (Robertson *et al.*, 2001a). Seasonal variations in rates of protein synthesis are also like to be affected by variations in both temperature and food availability (Hawkins, 1985; Kreeger *et al.*, 1995; Fraser *et al.*, 2002a; b; Whiteley & Faulkner, 2005). In the temperate isopod, *Ligia oceanica,* summer rates of protein synthesis at 20°C were almost 8-fold higher than the rates in winter animals acclimatised to 5°C (Whiteley & Faulkner, 2005). Summer rates of protein synthesis are also 3.5-fold higher than winter rates, in the temperate mussel, *Mytilus edulis*, (Hawkins, 1985; Kreeger *et al.*, 1995). The Antarctic limpet, *Nacella concinna*, also exhibits summer rates of protein synthesis as high as twice that in winter (Fraser *et al.*, 2002a; b). This decrease in summer rates is most likely due to a tenfold decrease in nutrient consumption (determined from faecal egestion), and not the 2°C difference in temperature between summer and winter. In this case rates of protein synthesis appear to be affected by food availability more than temperature, possibly because of high seasonal fluctuations in both benthic and pelagic primary productivity reported for Antarctic marine ecosystems (Clarke, 1988; Clarke *et al.*, 1988; Gilbert, 1991; Fraser *et al.*, 2004; Grange *et al.*, 2004). However, despite primary productivity being highly seasonal, it would be misleading to suggest that at high-latitudes primary productivity is necessarily low particularly in summer (eg., Eilersen, *et al.*, 1989), when most measurements of protein synthesis are taken.



1.4 Variations in growth rate with latitude

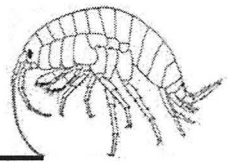
Protein synthesis is an important determinant of growth with maximum growth occurring at temperatures conducive to maximum rates of protein synthesis (Loughna & Goldspink, 1985; Pannevis & Houlihan, 1992; Carter & Houlihan, 2001). Growth is therefore likely to be affected by temperature and food variation with latitude and season. Lower growth rates at high latitudes were shown in the clam, *Siliqua patula*, over 70 years ago (Weymouth *et al.*, 1931). This pattern has since been described in a number of other studies (eg. Steele & Steele, 1970; 1972; Peck *et al.*, 1997; Clarke *et al.*, 2004). Lower growth rates reported in most polar ectotherms is in part likely to be due to low rates of protein synthesis (Peck, 2002). Although, nutrition (Cowey, 1992; Hewitt, 1992; Paul *et al.*, 1994), feeding regime (Mente *et al.*, 2001; Bolliet *et al.*, 2000; Schofield, 2004), temperature (Laurence, 1975; Jobling, 1995) and social interactions (Carter *et al.*, 1992; McCarthy *et al.*, 1992) have been shown to affect growth, most if not all of these factors affect rates of metabolism and protein synthesis.

The trend of decreased growth rate with latitude, however, is not supported by all studies. For instance, Jensen *et al.*, (2000) measured annual growth rates in 22 Norwegian brown trout (*Salmo trutta*) populations from a range of latitudes (61-70°N), they also analysed previously collected data from one Spanish, fifteen British and four Danish populations collected between 44-58°N. Overall, it was shown that in high latitude rivers with mean annual temperatures of less than 5.1°C, growth rates were higher than expected. Therefore, growth rates may be conserved across different latitudes, and species/populations from high latitudes may exhibit higher growth rates than those from lower latitudes at a given temperature (Levinton, 1983). A similar



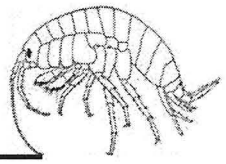
response was observed in *Drosophila melanogaster* because at higher latitudes flies mature at a larger size and in a shorter time than warm-adapted individuals at the same temperature (James *et al.*, 1995). Levinton and Monahan (1983) showed that at low temperatures, laboratory-reared copepods derived from higher latitudes exhibited higher growth rates that those derived from lower latitude conspecifics. This suggests that growth compensation is genetically based (Levinton, 1983). Intraspecific variation in growth compensation has also been shown in the copepod, *Scottolana candensis*, from a range of latitudes (27°-43°N) and temperatures (15°, 20°, 25°, and 30°C; Lonsdale & Levinton, 1985). When reared for several generations in the laboratory under their natural physiological conditions, it was shown that at low, but not higher, temperature individuals from higher latitude populations grew faster (Lonsdale & Levinton, 1985). Such adaptations may act to maximize available energy for growth at a particular physiological temperature.

Summer seasons can decline in length by a factor of approximately 2.5 with increasing latitude, which considerably affects the time available for growth at higher latitudes. Therefore, a higher capacity for growth in high-latitude genotypes may allow high latitude populations to exhibit increased growth rates over shorter summer growing seasons, possibly as a response to size-related winter mortality (Yamahira & Conover, 2002). This strategy is supported in a number of teleost species including: Atlantic salmon, *Salmo salar* (Nicieza et al., 1994); Atlantic Silversides, *menidia menidia*, (Conover & Present, 1990); Mummichog, *Fundulus heteroclitus* (Schultz *et al.*, 1996; DiMichele & Westerman, 1997); Striped bass, *Morone saxatilis* (Conover, *et al.,* 1997) and Atlantic halibut, *Hippoglossus hippoglossus* (Jonassen, *et al.*, 2000). More recently, Yamahira and Takeshi (2008) have demonstrated higher capacity for



growth in higher latitudinal populations (12 populations were surveyed) of the medaka, *Oryzias latipes*, acclimated to a common temperature of 28°C.

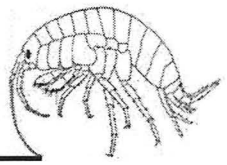
In contrast the Local Temperature Adaptation Model suggests that higher latitude populations exhibit low temperature adaptation, optimising growth rates to the lower environmental temperatures experienced (Levinton, 1983; Levinton & Monahan, 1983; Lonsdale & Levinton, 1985). This does not involve an up-regulation of maximum growth rates (growth capacity) at higher-latitudes but a downward shift in the growth temperature curve and optimum temperature for growth (Yamahira & Conover, 2002). The Local Temperature Adaptation Model is demonstrated between populations of the temperate copepod, *S. candensis,* ranging from 27-43°N. After rearing several generations under natural temperatures (15-30°C) growth rates at low temperatures were higher in northern populations (Lonsdale & Levinton, 1985). However, at higher temperatures growth rates were higher in southern populations. This is due to the growth temperature curves of northern populations crossing those of the southern populations, as growth rates decrease above optimal temperatures in the northern populations and growth increases with temperature in the southern populations (Lonsdale & Levinton, 1985). Therefore the adaptation of growth to local environmental / latitudinal temperatures entails a performance trade-off, with higher-latitude ectotherms growing faster than low-latitude ectotherms at low temperatures but more slowly at higher temperatures (Yamahira & Conover, 2002). Both these models for growth compensation can also interact and are probably not mutually exclusive within and between species of gammarids as both temperature and seasonality vary with latitude (Conover & Present, 1990). Studies of *Menidia menidia* and *M. peninsulae* show that both counter-gradient variation and temperature



adaptation contribute to interspecific growth compensation. In contrast, only counter-gradient variation contributes to growth compensation within species (Yamahira & Conover, 2002). However, elevated growth in high latitude low energy environments are likely to be associated with increased fitness costs due to the divergence of energy away from reproduction. Growth compensation is also difficult to explain in the absence of elevated energy consumption associated with MCA.

1.5 Variations in protein retention and growth efficiency with latitude

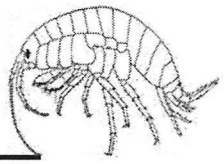
It is possible that variation in growth and body size with latitude are due to variations in growth efficiency, as described by The Assimilation Efficiency Hypothesis (Lonsdale & Levinton, 1985). It has previously been speculated that low energetic costs of living due to selection for lower energy turnover in more stenothermal polar environments leads to more energy being available for growth and high growth efficiencies (Hawkins *et al.*, 1989; Wieser, 1994; Hawkins & Day, 1996; Heilmayer *et al.*, 2004, Pörtner *et al.*, 2005). Indeed, growth experiments in scallops and eel pout indicate that growth efficiencies are higher at lower temperatures (reviewed by Pörtner *et al.*, 2005). However, recent measurements of protein synthesis retention efficiency (PSRE) suggest that growth efficiency decreases with temperature at higher latitudes (reviewed by Fraser & Rogers, 2007; Whiteley & Fraser, 2009). PSRE describes the percentage of synthesised protein retained as protein growth (Houlihan *et al.,* 1995). A high proportion of protein synthesised is degraded through a variety of pathways, such as ubiquitisation, and are never retained as growth (Herschko & Ciechanover, 1982; 1998). The rate of protein degradation, indicated by level of ubiquitin conjugates of protein, is also reported to be elevated in high latitude



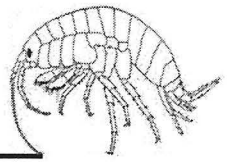
ectotherms compared to temperate species (Place *et al.*, 2004; Place & Hofmann, 2005). Protein degradation is also elevated in individuals approaching their upper and lower critical temperatures, as shown in fish (McCarthy *et al.*, 1999; Katersky & Carter, 2007). Rates of protein synthesis in the Wolffish, *Anarhichas lupus*, increased with temperature from 5 to 14°C, however, growth decreased from 11°C due to the effect of increased protein degradation at higher temperatures on PSRE (McCarthy *et al.*, 1999). This increase in PSRE at higher temperatures may explain decreases in growth often reported at temperatures just prior to the upper thermal limit (Brett, 1979; McCarthy & Houlihan, 1996; McCarthy *et al.*, 1998; 1999). Recent measurements of PSRE in the Antarctic limpet, *Nacella concinna,* revealed extremely low PSRE in summer (15.7% at -0.5°C) and winter limpets (20.6% at -1.6°C) compared to the majority of non-polar ectotherms where values can be as high as 95% (Houlihan *et al.*, 1995; Fraser *et al.*, 2007). Low PSRE in polar ectotherms is also indicated by recent meta-analysis of 15 species from a range of temperatures and taxa (teleosts, molluscs and crustaceans) (Fraser *et al.*, 2007). Consequently, low rates of growth usually reported in polar ectotherms (Peck *et al.*, 1997; Clarke *et al.,* 2004; Barnes *et al.*, 2006; Bowden *et al.*, 2006) may be due to low protein retention as well as low synthesis rates at low temperatures (Fraser & Rogers, 2007; Whiteley & Fraser, 2009).

1.6 Variations in RNA concentrations and RNA activity with latitude

Compensation of growth may be possible due to the thermal compensation of rates of protein synthesis with latitude. Thermal independence of protein synthesis rates have been reported between (Fraser & Rogers, 2007; Pace & Manahan, 2007)

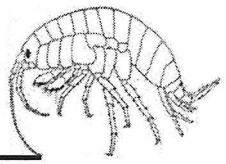


and within (Whiteley & Faulkner, 2005) species of marine ectotherms. Modification of RNA concentration is the most common mechanism to counter temperature dependent decreases in K_{RNA} (Goolish *et al.*, 1984; Houlihan, 1991; Foster *et al.*, 1992; 1993a; b; McCarthy *et al.*, 1999; Robertson *et al.*, 2001b; Fraser *et al.*, 2002a; Storch *et al.*, 2003; Treberg *et al.*, 2005; Whiteley & Faulkner, 2005; Intanai *et al.*, 2009). In the wolfish, *Anarhichas lupus*, whole animal and white muscle RNA concentrations increase as K_{RNA} decreases at lower temperatures (McCarthy *et al.*, 1999). However, due to energetic costs associated with maintaining elevated RNA concentrations at lower temperatures (Fraser *et al.*, 2002a) direct compensation of K_{RNA} may be more advantageous at low temperatures. For example, the Antarctic scallop, *Adamussium colbecki*, living at 0°C showed a nine-fold increase in K_{RNA} compared to the temperate scallop, *Aequipecten opercularis* living at 25°C, while RNA concentrations remained the same (Storch *et al.*, 2003). In the temperate eelpout, *Zoarces viviparous, in vitro* E_a of protein synthesis was lower at lower temperatures (Storch *et al.*, 2005). A similar response was demonstrated in eel hepatocytes. Collectively these studies suggest cold-acclimatisation in RNA efficiency, via the optimisation of enzymes and pathways involved in protein synthesis to lower temperatures (Jankowsky *et al.*, 1981). The Antarctic scallop, *Adamussium colbecki*, does not show this variation in RNA efficiency at different temperatures, perhaps suggesting a more restricted response in polar stenotherms (Storch *et al.*, 2003).



1.7 Variations in costs of protein synthesis with latitude

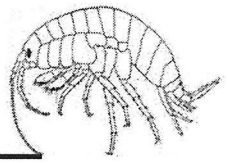
Compensation of protein synthesis rates in polar environments may involve a reduction in the energetic costs of protein synthesis in response to a low energy environment, as reported for the sea urchin, *Sterechinus neumayeri* (Place & Manahan, 2007). Protein synthesis costs of 2.2 μmol O_2 mg^{-1} reported for *S. neumayeri* during development are lower than the theoretical minimum of 8.3 μmol O_2 mg^{-1} (Reeds *et al.*, 1985). However, such low costs may allow rates of protein synthesis to remain high despite an energy limited environment (-1°C), ranging from 0.5 to 7.1% day^{-1} (Place & Manahan, 2007) compared to rates of protein synthesis in warmer temperate sea urchins; *Lyechinus pictus,* at 0.6 % h^{-1} at 15°C (Place & Manahan, 2006); *Strongylocentrotus purpuratus*, 1.1 % h^{-1} at 16°C (Goustin & Wilt, 1981); *Arbacia punctulata*, 1.9 % h^{-1} at 25°C (Fry & Gross, 1970; reviewed by Place & Manahan, 2007). Low costs of 1.9 μmol O_2 mg^{-1} are also reported in the Antarctic starfish, *Odontaster vadlidus* (Pace *et al.*, 2004). However, costs of protein synthesis vary considerably over two orders of magnitude from 0.92 μmol O_2 mg^{-1} in sea urchin larvae, *S. neumayeri* (Marsh *et al.*, 2001), to 147.5 μmol O_2 mg^{-1} in the isopod, *G. antarcticus* (Whiteley *et al.*, 1996). This unlikely variation is possibly due to methodological differences between studies (Bowgen *et al.,* 2007), as well as the limited number of studies cared out to date that include only 5 polar species. Of these, two species show no variation in costs of protein synthesis with temperature (Storch & Portner, 2003; Bowgen, *et al.*, 2007), suggesting that costs are fixed, as there is no known mechanism to change the stoichiometry of peptide bond formation (Bowgen, *et al.*, 2007). However, additional costs such as RNA synthesis, peptide transport and the translation initiation and termination of proteins or post-translational modification



of newly synthesised polypeptide chains may vary between species (Waterlow *et al.*, 1978a; b; Reeds *et al.*, 1985; Storch & Portner, 2003). In a study measuring protein synthesis and oxygen uptake simultaneously in isolated rainbow trout (*Oncorhynchus* mykiss) hepatocytes at a range of temperatures, Pannevis and Houlihan (1992) showed that energetic costs decreased at higher rates of protein synthesis associated with higher temperatures. They suggested two cost components of protein synthesis: a fixed component involving peptide bond formation that is independent of synthesis rate; and a variable component of additional costs that is dependent on synthesis rate. Comparisons of whole animal rates and costs of protein synthesis between the Antarctic isopod, *Glyptonotus antarcticus*, at 0°C and the temperate, *Idotea rescata*, at 4°C support this hypothesis, as energetic costs of protein synthesis were 4-times higher in the Antarctic compared with the temperate isopod. High costs of protein synthesis in high latitude animals may lead to slower growth rates as reported in the majority of high latitude ectotherms (Peck *et al.*, 1997; Clarke *et al.*, 2004; Barnes *et al.*, 2006; Bowden *et al.*, 2006). Despite low rates of growth, associated with low rates of protein synthesis, low protein retention rates and possibly higher synthesis costs, larger body sizes are often observed in polar ectotherms.

1.8 Variations in body size with latitude

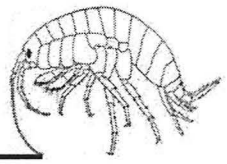
Bergmann (1847) proposed that within a species of endothermic vertebrates larger bodied populations inhabit lower temperature environments, such as high latitudes, and that the mechanism driving this trend is heat conservation. However, the heat conservation mechanism requires endothermy and so can not explain body size variation in ectotherms (Ashton, 2002). Other factors that can be closely linked to



temperature or latitude, such as predation, food availability or seasonality will interact differently in different species and can also affect body size. Some authors support Bergmann's rule in ectotherms (eg., Ray, 1960; Lindsey, 1966; Van Voorhies, 1996; Atkinson & Sibly, 1997). However, others suggest that ectotherms actually reverse the trend (eg., Cowles, 1945; Mousseau, 1997). Most studies have focussed on terrestrial ectotherms, with Bergmann's rule supported in squamates (Schuster, 1950) and amphibians (Ashton, 2002). Amphibians have also been shown to increase in size at lower temperatures (Schuster, 1950).However, the relationship between size and environmental temperature is often less clear than the relationship between size and latitude (Ashton, 2002).

In insects Bergmann's rule is supported, for example, in *Drosophila kikkawai* (Karan *et al.*, 1998), *Leptothorax acervorum* (Heinze, 2003) and *Myrmeleon immaculatus* (Arnett & Gotelli, 1999a;b; 2003), however, the converse of Bergmann's rule is also reported within species of orthopteran (Mousseau, 1997) and geometridae (Brehm & Fiedler, 2004). With photoperiod (Arnett & Gotelli, 1999a), food availability (Arnett & Gotelli, 1999b), fasting endurance (Arnett & Gotelli 2003), seasonal temperature variation (Marcondes *et al.*, 1999) and an interaction between environmental stability, growing season length and generation time (Chown & Gaston, 1999) being suggested to have a greater affect on body size with latitude than temperature.

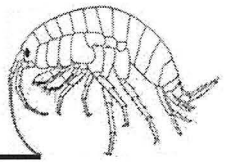
In aquatic ectotherms Belk and Houston (2002) demonstrated that out of 18 species of freshwater fish none exhibit a significant pattern of increasing body size with increasing latitude and many follow the converse of Bergmann's rule. This pattern is consistent with other studies in the sturgeon, *Acipenser fulvescens* (Power &



McKinley, 1997), and the European minnow, *Phoxinus phoxinus* (Mill, 1988). However, Bergmann's rule in supported in chelonians (turtles) with 19 of 23 species increasing size with latitude and 40 of 56 species increasing in size with temperature (Ashton & Feldman, 2003). The spotted turtle, *Clemmys guttate*, shows an increase in size with latitude (Litzgus *et al.*, 2004) although the authers conceded that when a population of large turtles at the northern extreme of the species range (45°N) is removed from analysis, Bergmann's rule is no longer supported, suggesting polar gigantism rather than a cline in body size with latitude. Litzgus *et al.* (2004) concluded that factors such as female size at maturity and reproductive cycles are responsible for the observed patterns in body size.

Predation risk can also affect body-size in marine ectotherms and can vary with latitude. The intertidal snail, *Littorina obtusata,* increases shell size and thickness with decreasing latitude, contrary to Bergmann's rule, due to a larger predator (*Carcinus maenas*) population at higher temperatures. Although the soft body mass, as an index of size, tends to increase with latitude in support of Bergmann's rule, this is constrained by the shell morphology (Trussell, 2000). Bergmann's principle can also be applied to deep-water marine crustaceans where body size increases with latitude but also along a depth-related thermal gradient. The low temperatures with depth lead to increased cell size and longevity that may lead to increased body size in deep-water marine ectothems (Timofeev, 2001).

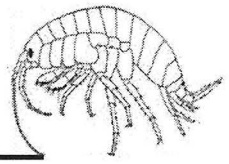
Cell size, like body size, has been shown to increase in higher latitude marine ectotherms. For example adult notothenioids (Antarctic teleosts) have large myotomes with fast muscle fibres over 500μm in diameter and slow muscle up to 100μm (Johnston, 2003; Johnston *et* al., 2003; Fernández *et al.*, 2005). In temperate and



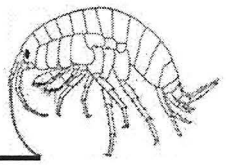
tropical teleost muscle fibre diameters are much less. For example the Pacific Blue Marlin, *Makaira nigricans*, has maximum fibre diameters of 50μm in slow muscle and only 120μm in fast muscle, despite growing to over 100kg (Johnston *et al.*, 2003). In these larger cells oxygen may become limiting due to an increase in diffusion distances. This would particularly affect the contractile activity of slow muscle fibres which are dependent on aerobic metabolism. However, low metabolic demand in polar ectotherms may release constraints on diffusion distance and cell size (Fernández *et al.*, 2000; Johnston, 2003; Johnston *et* al., 2003). It is also suggested that at low temperatures increased mitochondrial density and a decrease of the mean spacing between mitochondria may reduce diffusion distances (Eggington & Sidell, 1989; Jonston *et al.*, 1998). Despite larger cell size, growth rates are slow and maximum body sizes are modest in notothenioids (reviewed by Johnston, 1993). Increased cell size has an effect on aspects of life history including longevity and fecundity; this may result from the possible inverse effect of cell size on metabolism (Cavalier-Smith, 1978). The depression of metabolic rate in higher latitude species with larger cell sizes is shown in a number of ectotherms (Gregory, 2001).

1.9 Implications of variation in energy consumption with latitude

Lower metabolic rates reported in the majority of polar ectothems may relax physiological constants on body/cell size, as if energetic costs are lower and less oxygen is required, it is possible for diffusion distances to increase and for surface area to volume ratios to decrease. Such a response may be supported by higher oxygen saturation levels in polar environments due to the effect of temperature on oxygen solubility coefficients relaxing physiological constraints on body size

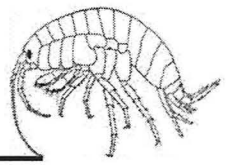


(Chappelle & Peck, 1999a;b). This hypothesis, originally demonstrated in amphipods (Chapelle & Peck, 1999a), remains controversial (Spicer & Gaston, 1999; Chapelle & Peck, 1999b McClan & Rex, 2001; Chapelle & Peck, 2004; Woods & Moran, 2008). However recent measurements of locomotory performance in Antarctic pycnogonid crustaceans of different sizes under hypoxic conditions has cast doubt on this explanation of polar gigantism (Woods *et al.*, 2009). Body size also does not appear to be limited by hypoxia in intertidal amphipods. For example, the mid- to high- shore species *G. marinus* is more tolerant of hypoxia than the smaller lower shore species *G. locusta* (S.P.S. Rastrick personal observations). This is because marine ectotherms show a number of adaptations, other than body size, to compensate for low oxygen environments such as increased gill areas, high ventilation volumes and adjustments to respiratory pigments to increase affinity for oxygen (Childress & Seibel, 1998; Seibel *et al.*, 1999). Adaptations in gill structure are also reported in crustaceans exposed to low oxygen saturations, for example, in the intertidal crab, *Carcinus maenas* (eg., Taylor & Butler, 1978). In a response to Chapelle and Peck's (1999a) original paper, Spicer and Gaston (1999) also point out that gas exchange over respiratory surfaces is driven by partial pressure of oxygen and not oxygen concentration. Although oxygen concentration is likely to be higher at higher latitudes due to the effect of low temperature on oxygen solubility, partial pressure of oxygen is unlikely to very with latitude (Spicer & Gaston, 1999). However, higher energy consumption may result in a maximum body size beyond which maintenance costs can not be supported by nutrient uptake. This is known as the Metabolic Constraint Hypothesis (Lonsdale & Levinton, 1985).



Smaller body sizes may be an advantage in eurythermal temperate environments where it is speculated that increased surface area to volume ratios may facilitate oxygen uptake in organisms with high metabolic demands (Pörtner *et al.*, 2007). At more eurythermal latitudes, increased selection for faster rates of development and reproduction may also lead to smaller body sizes at maturity, known as the Development Rate Hypothesis (Lonsdale & Levinton, 1985). Selection for faster development at temperate latitudes may result in the reallocation of energy towards reproduction soon after maturity, depleting resources for future growth and survival, perhaps leading to smaller adult body sizes and increasing adult mortality, thus, reinforcing selection for fast development and early reproduction. However, size at maturity could be constrained at high latitudes due to short growing seasons (Garvey & Marschall 2003).

Selection for faster development during short growing seasons, shown in some higher latitude ectotherms (discussed above), could therefore also lead to the converse of Bergmann's rule. Selection for energy conservation and slower rates of growth and development exhibited by most polar ectotherms may lead to larger body sizes due to an increase in longevity. Variation in body size due to latitudinal variation in life span is known as the Latitude-dependent Age-specific Mortality hypothesis (Lonsdale & Levinton, 1985). For almost 80 years it has been suggested that larger body sizes in polar populations are a result of low rates of growth sustained over a longer life span (Weymouth *et al.*, 1931). Pearl (1928), and Alpatov and Pearl (1929) suggest that life span is inversely related to metabolic rate. Voorhies (2002) suggests that extended longevity in some nematode mutants, *Caenorhabditis elegans*, may be due to lower metabolic rates. The use of oxygen by cells during normal aerobic metabolism leads

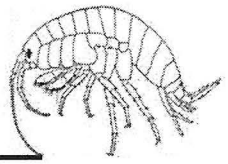


to the generation of deleterious reactive oxygen metabolites. These accumulate as an organism ages and cause oxidative damage thought to be a significant factor in the onset of senescence (Sohal & Weindruch, 1996). A recent paleontological study has revealed extreme longevity of over 100 years in the fossil bivalve *Cucullaea* that inhabited the relatively warm (14°C) shallow seas of the Antarctic Eocene (Buick & Ivany, 2004). This suggests perhaps low light and food availability are more significant as factors than temperature (Buick & Ivany, 2004), although these factors could still affect energy consumption.

Variations in energy consumption with latitude have an affect on rates of growth/development, longevity and body size. However, studies that have separated the effect of latitude and temperature demonstrate that temperature is not the only latitudinal variable having an affect. Many other latitudinal variables such as: seasonality; photoperiod; length of growing season; primary productivity; food quality; starvation resistance; predation risk; and reproductive cycles have also been shown to be important due to their effect on levels of energy consumption.

1.10 Rational

The relationships between rates of metabolism, rates of protein synthesis, growth and body size within a species distributed along a latitudinal thermal gradient are still far from clear. It is clear from the literature that marine ectotherms exhibit variations in upper and lower critical temperatures with latitude (Mayer, 1914; Hyman, 1955; Ansell *et* al., 1986; Gaston & Spicer, 1998; Sokolova & Pörtner, 2002) indicating that adjustments to latitude are taking place. Acclimatisation to different temperatures may involve variations in body size, metabolic rates and rates/costs of protein synthesis as

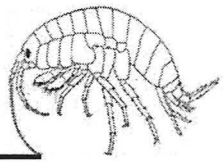


a determinant of growth. Some adjustments in life-history traits to changes in latitude are reported to be phenotypic responses (Skadsheim, 1989), other variables, such as metabolic rate may have an underlying genetic basis to latitudinal variation, suggested for insects (Addo-Bediako *et al.*, 2002) and copepods (Lonsdale & Levington, 1989). Latitudinal studies can therefore provide an insight into physiological variation along natural environmental clines in local conditions including temperature. However, most latitudinal studies to date focus on just two populations/species and the majority of polar species studied are from the isolated waters of the Southern Ocean with little consideration of genetic diversity or thermal history. Therefore the present thesis aims to:

1) Determine whole animal rates of oxygen uptake (Chapter 2) and protein synthesis (Chapter 3) in the same natural populations of marine ectotherms adapted/acclimatised to different latitudinal conditions; by comparing populations and species with known phylogenies and overlapping latitudinal distributions, including subarctic (79°N and 5°C) to warm-temperate (38°N and 21°C) environments. The present thesis is the first to examine intraspecific variation in whole animal rates of protein synthesis across a latitudinal gradient.

2) Determine if whole animal costs of protein synthesis are fixed between populations of the same species acclimatised to sub-arctic and temperate latitudinal conditions (Chapter 4).

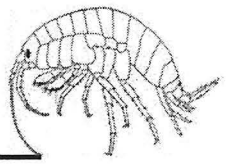
3) Discuss how variations in energy consumption associated with different latitudinal conditions, including variations in mean temperature and temperature stability, may affect and be affected by species distributions and life-history traits (Chapter 5). Understanding the effect of latitudinal thermal gradients on energy



consumption as a determinant of growth/development, may help to explain macroecological patterns in body size and r/K selection in natural populations. Such patterns will become increasingly important to the emerging fields of Macrophysiology (Gaston *et al.*, 2009) and Physiological Conservation (Franklin, 2009). As understanding the underlying physiological mechanisms, for example how temperature affects distributions and reproductive output of endangered species, may help in their conservation. This more integrated understanding of how ectotherms respond to natural thermal gradients may also help us to understand how organisms may respond to future climate change.

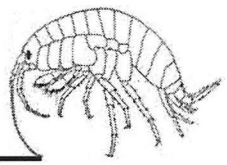
1.11 Gammarid amphipods

The genera *Gammarus* comprise of 204 marine, freshwater and in some cases terrestrial species, currently described (Väinölä *et al.*, 2008). This genera play a key role in ecosystem functioning both as an important food source for other aquatic and terrestrial predators (eg., Costa & Costa, 2000) and as detritivores, shredders, grazers and predators (eg., Kelly *et al.*, 2002; Christie & Kraufvelin, 2003) within the food chain. As parasites some species can also have an ecological and economic impact on fisheries (Lefever *et al.*, 2008). Gammarids are frequently used in the study of ecotoxicology (eg., Clason & Zauke, 2000; Costa *et al.*, 2005; Fialkowski & Rainbow, 2006; Prato *et al.*, 2006) and parasitology (eg., Ironside *et al.*, 2003; MacNeil *et al.*, 2004; Kostadinova & Mavrodieva, 2005; Rolbiecki & Normant, 2005). Gammarids have also been used to study the relationship between range size and environmental tolerance (Gaston & Spicer, 2001).



The present thesis focuses on four northern hemisphere species of intertidal *gammarids*; the circumpolar species, *Gammarus setosus*; the sub-arctic/temperate species, *G. oceanicus*; the warm-temperate species, *G. locusta* and the more eurythermal boreal/temperate species, *G. duebeni duebeni*. All species are abundant and are found under rocks and fucoids were they graze, feeding mainly on micro-algae. Of the four species *G. locusta* shows the most southerly latitudinal distribution extending from southern Spain to the northern the coast of Norway (Bulnheim, 1979; Costa *et al.*, 2004). However, in the northern UK it tends to become sub-littoral and is replaced in the intertidal environment by *G. oceanicus* (Wim Vader, personal communication; S.P.S. Rastrick, personal observation). *G. locusta* is normally found at salinities above 15psu (Fenchel & Kolding, 1979) and is the least tolerant of the four species studied to environmental change, possibly due to its low intertidal to sub-littoral distribution (Gaston & Spicer, 2001; Rock *et al.*, 2009). Recent phylogenetic analysis using the mitochondrial cytochrome c oxidase I (COI) gene has shown that *G. locusta* is more closely related to Mediterranean and Black Sea gammarids than other Northern European species, whereas, both *G. oceanicus* and *G. setosus* share a putative phylogroup with other northern European and Arctic species (Costa *et al.*, 2009). *G. oceanicus* and *G. setosus* are also adapted for life at lower temperatures and show variations in life history traits with latitude (Steele & Steele 1970;1972)

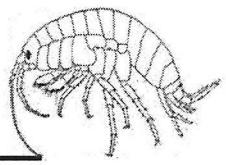
Gammarus oceanicus shows a similar intertidal/ sub-littoral distribution to *G. locusta*, but, has a more northerly distribution that extends up the coastal fringes of western Europe from Scotland in the British Isles to Iceland, Svalbard and Greenland. It is also found in the Baltic and White Seas and on the Eastern Seaboard of North America northwards from the Gulf of Saint Lawrence (Steele & Steele, 1972;



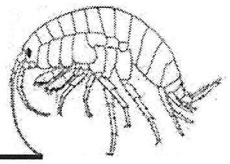
Opalinski & Weslawski, 1989). *G. oceanicus* is usually found at salinities between 20-33psu (Fenchel & Kolding, 1979). However it can be found at lower salinities of 5psu in the Gulf of Finland (Segerstrale, 1949). The circumpolar species *G. setosus* is also tolerant of brackish waters and estuaries (Steele & Steele, 1970; Wim Vader, personal communication). This species extends northwards from Nova Scotia and Northern Norway to the most northerly extending land masses (Lincoln, 1979). Although *G. oceanicus* and *G. setosus* occupy the same intertidal sites in Svalbard, *G. setosus* is found slightly higher on the shore.

G. d. duebeni is the marine haplotype of the species (Rock, *et al.*, 2007) and is a interesting addition to the study due to its high-intertidal distribution and its high tolerance of environmental change (Gaston & Spicer, 2001; Rock *et al.*, 2009). *G. d. duebeni* is tolerant of both hypo- and hyper-saline water and is often associated with small steams, ditches, storm drains and other freshwater runoffs in to the intertidal (Fenchel & Kolding, 1979). *G. d. duebeni* are found on both sides of the Atlantic and range in Europe from the English Channel to Northern Norway (Lincoln, 1979).

These intertidal gammarids are excellent models for the study of physiological variations with latitude as they inhabit a wide range of thermal habitats and latitudes with some species such a *Gammarus oceanicus* showing a continuous distribution from subarctic to temperate environments, allowing for intra and inter-specific comparisons. Gammarid*s* also inhabit a range of tidal heights allowing congeners from the lower shore such as *G. locusta* to be compared with extremely thermal-tolerant upper shore species such as *G. duebeni* at the same latitude. Many intertidal gammarids are also easily collected from the shore and maintainable in the laboratory. The phylogeny of various species and populations of gammarids has also recently



been investigated (Costa *et al.*, 2009), allowing the effects of phylogeny on physiological traits to be discussed. Such amphipods also comply with Bergmann's rule exhibiting large intra- and inter-specific variations in body size with latitude (De Broyer 1977) and exhibit a trend from r- to K-selection with increasing latitude (Weslawski & Legezynska, 2002), allowing the present thesis to explore the implications of variations in energy consumption with latitude on life-histories.

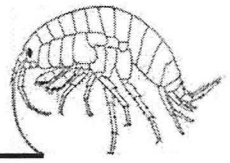


Chapter 2

The ability to conserve metabolic rate between natural populations of gammarid amphipods varies with latitude

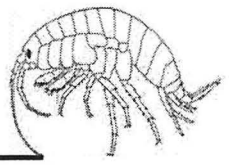
Submitted to: Marine Ecology Progress Series

S.P.S. Rastrick and N.M. Whiteley



2.1 Abstract

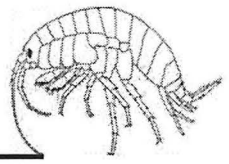
Whole-animal rates of oxygen uptake ($\dot{M}O_2$) were determined in acclimatized gammarid amphipods with overlapping latitudinal distribution patterns in the NE Atlantic and Arctic Oceans to examine metabolic diversity between latitudinal populations under natural environmental conditions. Such an approach revealed two different responses depending on the latitudinal distribution and environmental tolerances of the species involved. In the subarctic-temperate species, *Gammarus oceanicus*, $\dot{M}O_2$ declined as habitat temperature decreased with latitude so that rates of energy consumption were lower in subarctic (79°N) relative to temperate (58°N) populations, and similar to the values in the Arctic-boreal species, *G. setosus*, at the same latitude. Consequently, there was no evidence for metabolic rate compensation in the cold-water, high latitude populations. Further examination of the specific effects of temperature on $\dot{M}O_2$ in *G. oceanicus* revealed similarities in $\dot{M}O_2$ between populations at the same acclimation temperature, and similarities in thermal sensitivity (Q_{10}) and activation energies (E_a) on exposure to acute temperature change, indicating a lack of metabolic diversity between populations. In sharp contrast, the boreal-temperate (*G. d. duebeni*) and the temperate species (*G. locusta*) showed no variation in $\dot{M}O_2$ with latitude. Instead, species conserved relatively high metabolic rates either to maintain a warm-water life-histories and activity levels or to confer some independence from highly variable environments. Acclimation to 10°C and acute temperature exposure in *G. locusta* revealed metabolic diversity between populations which may in part be due to adaptation to local conditions. The differing abilities of closely related congeneric amphipods to conserve metabolic rate across natural latitudinal gradients is related to latitude via associated changes in environmental temperature and resource availability.



2.2 Introduction

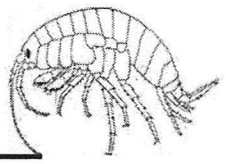
Metabolic rate is an important whole-animal response which provides empirical evidence for costs of living and the rate at which available environmental resources are used. The relationship between metabolic rate and temperature in aquatic ectotherms, and any predictable patterns that may emerge along natural thermal gradients, has been under scrutiny for nearly a century (Krogh, 1916). Early species-related comparisons between Arctic and tropical aquatic ectotherms concluded that there was considerable, although incomplete, metabolic cold adaptation in Arctic species because their metabolic rates were elevated relative to tropical species (Scholander *et al.*, 1953) More extensive comparisons between Antarctic and distantly related temperate marine species, have largely rejected the concept (Houlihan & Allen, 1982; Luxmoore, 1984; Clarke, 1991; 1993; Chapelle & Peck, 1995; Whiteley *et al.,* 1996; Clarke & Johnston, 1999; Peck & Conway, 2000). Instead, resting metabolic rates in Antarctic aquatic ectotherms are lower than those of temperate and tropical species extrapolated back to the same temperatures indicating a selective advantage for low energy consumption and turnover in the extreme cold (Clarke, 2003; Pörtner *et al.* 2007). Whether it is the stability and harshness of the environment, limited resource availability or the low temperatures that are selecting for low costs of living is unclear and frequently debated (Clarke 1993; 2003; Pörtner *et al.,* 2005).

Although the concept of metabolic cold adaptation has largely been dismissed in polar, mainly Antarctic, marine ectotherms, metabolic compensation (elevation in metabolic rate to avoid the slowing effects of lower temperatures) has been documented between populations of the same species living at lower latitudes. All of these studies have been conducted in the northern hemisphere on individuals



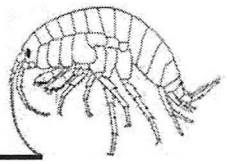
acclimated to comparable temperatures for several weeks. The results show that individuals from colder, more northerly populations have higher metabolic rates than their counterparts from warmer, more southerly populations when measured at the same temperature (Vernberg, 1962; Mangum, 1963; Lonsdale & Levinton, 1989; Sommer & Pörtner, 2002), although there are exceptions (Sokolova & Pörtner, 2003). Such intraspecific variation in metabolism is attributed to shifts in the relationship between metabolic rate and temperature, termed R/T curves by Prosser (1950), where a change in R/T gradient signifies alterations in Q_{10} and activation energies for metabolism (E_a), and parallel shifts demonstrate changes in enzyme concentrations. The overall effect is the conservation of metabolic rate at the normal habitat temperatures of each population imparting some independence of rate function from the direct effects of habitat temperature (Bullock, 1955; Prosser, 1950).

By raising individuals in a common environment for several generations, it has been shown that intraspecific variations in rate functions are genetically based indicating adaptation for local temperature regimes (Lonsdale & Levinton, 1989; Dittman, 1997). Latitudinal studies are therefore extremely valuable because they can allow for an examination of evolutionary adaptation to temperature in ecologically similar and closely related taxa. Unfortunately, latitudinal comparisons in marine invertebrates are limited and frequently confounded by variations in factors such as season and body size (eg., Vernberg, 1962). In addition, most intraspecific studies to date have concentrated on comparisons between tropical and temperate populations, or temperate populations over limited latitudinal ranges. Surprisingly little is known of the differences in metabolic rate between Arctic species and populations of the same species living at lower latitudes, apart from two studies comparing subpolar and boreal populations, where opposing responses are observed (Sommer & Pörtner,



2002; Sokolova & Pörtner, 2003). Consequently, the key question of whether or not Arctic and subarctic marine invertebrates are capable of compensating their metabolism at polar latitudes remains unclear. It may be that shorter histories at low temperatures (0.7 my as opposed to 16 my in the Antarctic), less isolation than the Antarctic and wider geographical ranges may increase the capacity of Arctic/boreal species to compensate rate functions in the cold. This question is of key importance to marine ectotherms living in the rapidly changing Arctic, as it is not known whether these species are specialised to function at low temperatures and whether they are metabolically limited with respect to further environmental change. In addition, it is unclear whether polar marine invertebrates have the metabolic capacity to compete with warmer-water invasive species/populations with higher energetic requirements and rates of resource use.

The present study investigated the relationship between metabolic rate and latitude in a widely distributed genus of amphipod crustacean, *Gammarus*, where sequences from the mitochondrial cytochrome c oxidase I (COI) gene show much higher genetic diversity between species than within species (Costa *et al.* 2009). The study concentrated on four gammarid species with different but overlapping latitudinal distributions along the coastal fringes of the NE Atlantic and Arctic Oceans to include both Arctic, subarctic, boreal and temperate representatives. All species are abundant, especially at high latitudes, and are found under rocks and fucoids where they graze, feeding mainly on micro-algae (Wim Vader, personal communication). Their ability to tolerate environmental change, however, differs as does their vertical distribution in the intertidal (Gaston & Spicer, 2001) In all species, metabolic rates were determined in animals within 48h of capture at their normal environmental conditions (acclimatised animals) in order to assess whether metabolic compensation

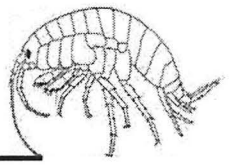


occurs between natural populations. The specific effects of temperature on metabolic rate were subsequently investigated between latitudinal populations of the two intertidal species which occupy the same ecological niche but have different latitudinal ranges. Using this approach it was possible to examine intraspecific variations in metabolic rate in species with similar ecologies and known phylogeny, but different latitudinal distribution patterns and environmental tolerances.

2.3 Materials and methods

2.3.1 Animal Collection

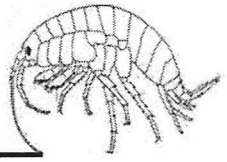
Four species of marine gammarid amphipod were investigated including: the circumpolar Arctic-boreal species, *Gammarus setosus*; the subarctic-boreal species, *G. oceanicus*; the temperate species, *G. locusta;* and the more eurythermal boreal-temperate species and marine haplotype, *G. duebeni duebeni* (Rock *et al.,* 2007). All species inhabit the low intertidal apart from *G. d. duebeni* which inhabits areas under freshwater influence in the mid to high intertidal (Rock *et al.*, 2009). Environmental tolerances vary between the species with *G. d. duebeni* showing the widest range of tolerances and physiological performances under environmental change and *G. locusta* the least (Bulnheim, 1979). Collection sites (Fig. 1) ranged from the most southerly population in Portugal (38.48˚N) up the coast of Western Europe to include mid-latitude sites in Brittany/France (47.85˚N), Anglesey/Wales (53.20˚N), and Isle of Skye/Scotland (57.66˚N), as well as two high latitude sites in Norway: Tromsø (69.61˚N) and Ny-Ålesund on Kongsfjorden, Svalbard (78.92˚N). Collectively the various gammarid populations represented a range of climate regimes: warm-temperate (Portugal and France), cold–temperate (Wales and Scotland), boreal (Tromsø) and subarctic in Svalbard because of the warming influence of the Atlantic



Ocean in the summer (Opaliński & Węslawski, 1989; Willis *et al.,* 2006). The sea surface temperatures at each collection site were: Portugal (15-22°C); France (9-18°C); Wales (6-16°C); Scotland (6-14 °C); Tromsø (4-11 °C); and Svalbard (-1.89-6°C). All collections were made at low tide between June and Sept (2006 - 2007), avoiding brooding females and moulting animals. Fucoids and microalgae were abundant at all sampling sites, including Svalbard, indicating that food supply was not limiting during the summer.

2.3.2 Resting MO2 in acclimatised animals

In order to determine resting rate of oxygen uptake (MO_2) in acclimatised animals, amphipods were removed from the shore and held at their respective environmental conditions at the time of capture (temperature, PO_2, salinity and photoperiod), and allowed to recover from handling and feeding effects which can artificially elevate MO_2. All determinations were made within 48h. Three latitudinal populations were sampled for each species apart from *G. setosus* which was only collected in Svalbard (79°N) (Fig.1). After collection, individual amphipods were held at their respective capture temperatures in fully aerated seawater, apart from *G. d. duebeni* which was held in 50% seawater. To determine MO_2 measurements within 48h of capture, animals were returned immediately to one of three sites: Bangor University, Wales, for the amphipod populations in Scotland, Wales and France; NERC's Arctic Research Station in Ny-Ålesund for populations collected in Tromsø and Ny-Ålesund; and Universidade Nova de Lisboa, Lisbon, for the population collected in Portugal. For the transport of amphipods between sites, individuals were wrapped in paper soaked in seawater and maintained at a constant temperature. This is reported to be the most effective means of transporting amphipods ensuring maximum survival rates



(J. Ironside, personal communication). Preliminary studies also showed no variation in $\dot{M}O_2$ before and after transportation. On arrival they were returned to aerated seawater at the appropriate capture temperature as soon as possible and left for 24 h to recover before $\dot{M}O_2$ measurements commenced.

2.3.3 Thermal sensitivity of $\dot{M}O_2$ in acclimatised animals

A second experiment characterised the specific effects of temperature by testing the temperature sensitivity of resting $\dot{M}O_2$ in acclimatised animals and by estimating maximal $\dot{M}O_2$ values when exposed to an acute increase in temperature. To determine the effects of acute temperature change on resting $\dot{M}O_2$ in acclimatised animals, two latitudinal populations of *G. oceanicus* (58 and 79°N) and *G. locusta* (38 and 53°N) were investigated. In each case animals were held in fully aerated seawater at their respective environmental conditions at the time of capture for the maximum of 5 days. Animals were fed throughout this period on algal fish food (Tetra*Veg*®, Tetra GmbH, Germany) but were starved for 24h before $\dot{M}O_2$ measurements commenced.

2.3.4 Resting $\dot{M}O_2$ in acclimated animals

Amphipods from two latitudinal populations of *G. oceanicus* (58 and 70°N) and *G. locusta* (38 and 53°N) were returned to Bangor University and acclimated to a common temperature of 10°C in fully aerated, full strength seawater in a 12L:12D regime for 4 weeks. Individual *G. oceanicus* from Svalbard were not included in the acclimation studies because they were unable to survive longer then 2 weeks at 8°C. In contrast, *G. locusta* from Portugal continued to feed and remained active for 4 weeks at 10°C and no mortalities were reported. A third of the water was replaced each week before feeding with algal fish food (Tetra*Veg*®, Tetra GmbH, Germany).

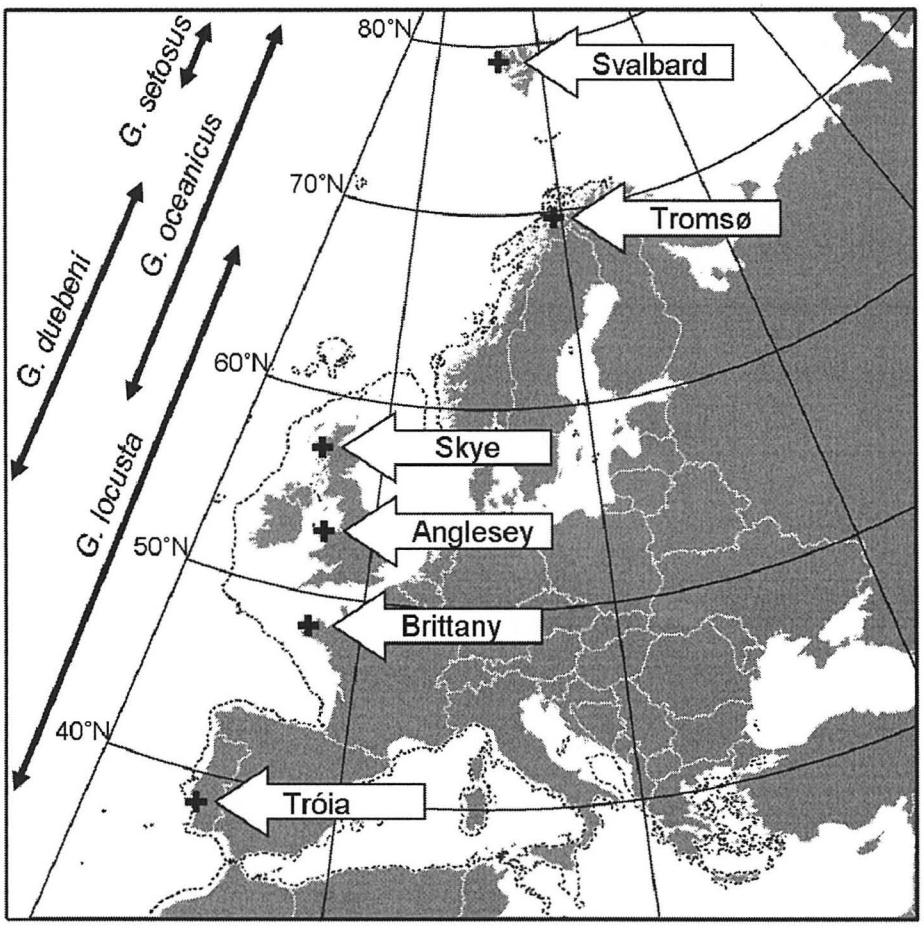
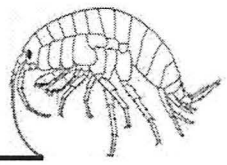


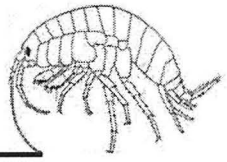
Figure 1.1. Map of Europe to show the various collection sites for gammarid amphipods. These include: Ny-Ålesund, Svalbard (79°N); Tromsø, Norway (70°N); Isle of Skye, Scotland (58°N); Anglesey, Wales (53°N); Brittany, France (48°N); and Tróia, Portugal (38°N). The latitudinal distribution patterns of the four gammarid species examined in this study are shown by the diagonal arrows on the left of the map. More northerly populations of *G. locusta* from Scotland and Norway (56-70°N) are subtidal and were not included in this study. Populations of *G. oceanicus* were collected from populations in Ny-Ålesund, Tromsø and Isle of Skye. *G. setosus* was only collected in Ny-Ålesund. *G. d. duebeni* was collected from populations in Tromsø, Isle of Skye and Anglesey, and *G. locusta* was collected from Anglesey, Brittany and Tróia. Temperatures at the time of collection were; 5°C at 79°N, 15°C at 70°N, 13°C at 58 and 53°N, 18°C at 48°N, and 21°C at 38°N. The latitudinal distribution of the species is based on Opaliński & Węslawski (1989), Klekowski & Węslawski (1990) and Costa *et al.* (2004).



2.3.5 Determination of $\dot{M}O_2$

$\dot{M}O_2$ was measured in each individual using stop flow respirometers (vol=14 ml) held at a constant temperature by the presence of a water jacket. Each respirometer was supplied with fully aerated seawater at the desired temperature and salinity and stirred by means of a magnetic stirrer, separated by a perforated platform, to prevent the formation of oxygen partial pressure (PO_2) gradients. Each respirometer contained a number of beads to provide shelter for the animals and to reduce activity levels. In each case, animals were placed into the respirometers and allowed to settle for 4h, which was found to be the minimum time necessary for resting $\dot{M}O_2$ (SPS Rastrick, unpublished data). After the animals had settled the water flow was stopped for 30 min and the decline in PO_2 in the seawater measured at regular intervals. In the study on acute temperature change, the flow of fully aerated seawater through the respirometers was resumed once resting $\dot{M}O_2$ had been determined and warmed by 4°C. After 30 min, each respirometer was resealed and PO_2 measurements taken for a further 30 min. This procedure was repeated until the animal succumbed to heat exhaustion and $\dot{M}O_2$ levels fell precipitously. Consequently, each amphipod was exposed to an increase in water temperature at a rate of 4°C h^{-1}. In all cases, control respirometers were run without any animals.

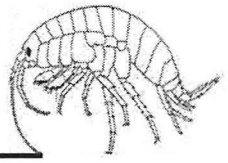
Oxygen partial pressures (PO_2) were determined using an OxySense®101 Non-invasive Oxygen Analyzer System (OxySense® Inc., Dallas, Texas, USA). The OxySense® system utilises an optical method to determine water PO_2 levels by measuring the fluorescent energy released from an oxygen-sensitive dot placed onto the inside surface of the respirometer. Changes in fluorescence occur in proportion to changes in water PO_2 and are detected by an external reader pen assembly (fluorimeter) which was held firmly in position by a Perspex holder projecting



horizontally from the side of the respirometer to align the pen assembly parallel to the oxygen-sensitive dot. The pen assembly was connected via a fibre optic cable to a converter and a PC for control and data acquisition using OxySense®101 software (OxySense® Inc., Dallas, Texas, USA). Calibration of the OxySense® system was carried out for each new batch of oxygen-sensitive dots according to the manufacturer's instructions. Each batch of oxygen-sensitive dots are supplied pre-calibrated from the manufacturer, however, minimum and maximum PO_2 was checked using nitrogen or air saturated sea water, respectively. Fluorescent measurements were logged every 5 seconds for the length of the stop-flow period. In each case, the decline in PO_2 was linear over the measurement period and kept above 17 kPa to avoid hypoxia. All measurements were taken in low light conditions: first, as ambient light causes high noise to the live fluorescence signal; and second, to minimise any disturbance to the animal.

2.3.6 Data Analysis

OxySense®101 software (OxySense Inc., Dallas, Texas, USA) converted fluorescent readings into changes in seawater PO_2 against time. Rates of oxygen uptake were calculated as the change in PO_2 per hour multiplied by the solubility coefficient for oxygen, which was adjusted for salinity and temperature (Harvey 1955), and the volume of water within each respirometer. Whole animal values for $\dot{M}O_2$ in $\mu l\ O_2.h^{-1}$ were standardised to S.T.D.P. and expressed as nmol O_2. $animal^{-1}$. h^{-1}. As body mass increased with latitude, all $\dot{M}O_2$ data was standardised to a wet body mass of 1mg using a mass exponent of 0.62. This exponent was calculated empirically from the relationships between log whole-animal $\dot{M}O_2$ and log wet body mass and did not vary

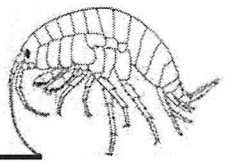


significantly between the 4 gammarid species investigated in the present study (ANCOVA, P=0.89) (S.P.S. Rastrick and T. Potter, unpublished data).

For the temperature sensitivity experiments, maximal aerobic threshold temperature was taken as the temperature at which $\dot{M}O_2$ reached its maximum value before falling precipitously. In preliminary studies it was shown that this temperature corresponded to the upper thermal tolerance of ventilatory activity (determined as changes in the beating frequency of the pleopods), and circulatory capacity (determined as changes in heart rate) (SPS Rastrick, unpublished data). The specific relationship between acute temperature change and acclimatised $\dot{M}O_2$ was investigated by calculating the mean temperature coefficient (Q_{10}) and the mean Arrhenius activation energy for metabolism (E_a) for each population. Q_{10} values were calculated using the Van't Hoff equation:

$$Q_{10} = R_2 / R_1 \,^{(10 / T2 - T1)}$$

Where T_2 is the temperature at the maximal aerobic threshold and T_1 is the temperature after the first temperature increase (capture temperature plus 4 °C). R_1 and R_2 represent whole-animal $\dot{M}O_2$ values (nmol O_2. animal^{-1}. h^{-1}) recorded at T_1 and T_2, respectively. E_a values (kJ. mol^{-1}) were calculated, over the same range as Q_{10}, from the gradient of the natural log of $\dot{M}O_2$ plotted against the reciprocal of temperature (1/°K), multiplied by the negative molar gas constant (-8.3144 J. K^{-1}. mol^{-1}) (Lee & Baust 1982). All Arrhenius relationships were linear and negative over the temperature ranges studied. Mean aerobic scopes were taken as the differences between $\dot{M}O_2$ at rest and at the maximal aerobic threshold.



2.3.6 Statistical Analysis

Values are given as means ± SEM with the number of observations in parenthesis. Data were tested for normality using a Kolmogorov-Smirnov test and for homogeneity of variances using Levene's test. As all data were parametric, one-way analysis of variance (ANOVA) and the least significant difference (LSD) post-hoc test was used to test for any significant differences in either $\dot{M}O_2$, body size, Q_{10} and E_a between populations. Comparisons between two species at the same latitude, and two populations after temperature acclimation were made using an independent sample *t*-test. Within populations, any significant variation in $\dot{M}O_2$ with acute change in temperature was tested using repeated measurers ANOVA. The linear relationship between aerobic scope and latitude was analysed using least-squares regression analysis. Results were considered to be significant at the 5% confidence interval ($P<0.05$). Statistical analyses were performed using SPSS software (SPSS version 12; SPSS INC., Chicago, IL, USA).

2.4 Results

2.4.1 Body mass and latitude

Between species, mean body mass of the individuals used to determine resting $\dot{M}O_2$ in acclimatised amphipods varied significantly with latitude (ANOVA, $P<0.001$) (Fig. 2). Within species, latitude had a significant effect on body size in *G. oceanicus* and in *G. locusta* (ANOVA, $P<0.001$). In *G. oceanicus,* individuals from Svalbard were over twice the size of individuals from Scotland (58°N), and in *G. locusta,* individuals from Wales (53°N) were over 3 times larger than individuals from Portugal (38°N). In contrast, latitude had no effect on body mass in *G. d. duebeni*. In Tromsø (70°N), *G. d. duebeni* were significantly smaller than *G. oceanicus* (*t*-test, $P<0.0001$).

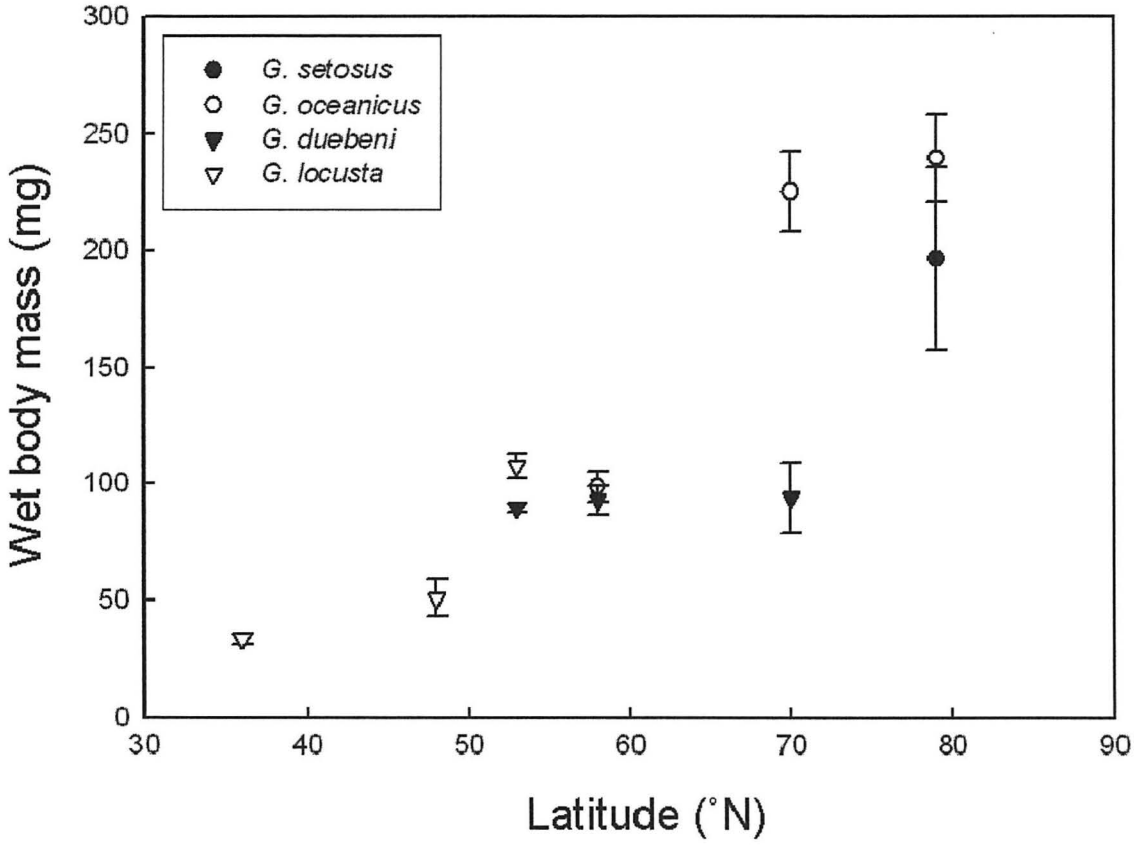
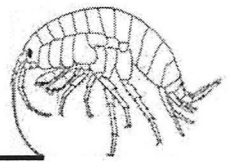


Figure 2.2. Mean body mass (mg) of the animals used to determine resting MO_2 in acclimatised animals plotted against the latitudes at which they were caught. Mean values given ±SEM. (In *G. setosus*: n=6 at 79°N and 5°C. In *G. oceanicus*: n= 15 at 79°N and 5°C; n=12 at 70°N and 15°C; n=17 at 58°N and 13°C. In *G. d .duebeni*: n=16 at 70°N and 15°C; n=19 at 58°N and 13°C; and n=15 at 53°N and 13°C. In *G. locusta*: n=18 at 53°N and 13°C; and n=10 at 48°N and 18°C; and n=9 at 38°N and 21°C).



2.4.3 Resting MO_2 in acclimatised animals

Mean resting whole-animal MO_2 for acclimatised animals is plotted against latitude in Fig. 2.3a and against capture temperature in Fig. 2.3b. In *G. oceanicus*, there was a significant variation in mean MO_2 between latitudinal populations (ANOVA, $P<0.05$) (Fig. 3a), marking a 50% drop in MO_2 from 28.2±4.8(12) nmol O_2. animal^{-1}. h^{-1} in the population from Scotland to 14.8±1.7(15) nmol O_2. animal^{-1}. h^{-1} in the population from Svalbard (LSD test, $P<0.001$). This decline in MO_2 occurred over a 8°C drop in capture temperature at a Q_{10} of 2.24. In contrast, whole animal MO_2 remained unchanged between latitudinal populations of *G. locusta* and latitudinal populations of *G. d. duebeni,* despite changes in capture temperature (Figs. 2.3a and 2.3b).

2.4.4 Resting MO_2 in acclimated animals

When acclimated to 10°C for four weeks there was no difference in mean MO_2 between *G. oceanicus* from Tromsø and *G. oceanicus* from Scotland as mean values were 10.4±1.5(6) and 9.3±1.2(6) nmol O_2. animal^{-1}. h^{-1}, respectively (*t*-test, $P=0.56$) (Fig. 2.4). In *G. locusta*, however, mean MO_2 was two-fold higher in the population from Wales at 35.4±5.5(5) nmol O_2. animal^{-1}. h^{-1} compared with the population from Portugal where MO_2 was 19.0±2.4(6) nmolO_2. animal^{-1}. h^{-1} (*t*-test, $P<0.05$).

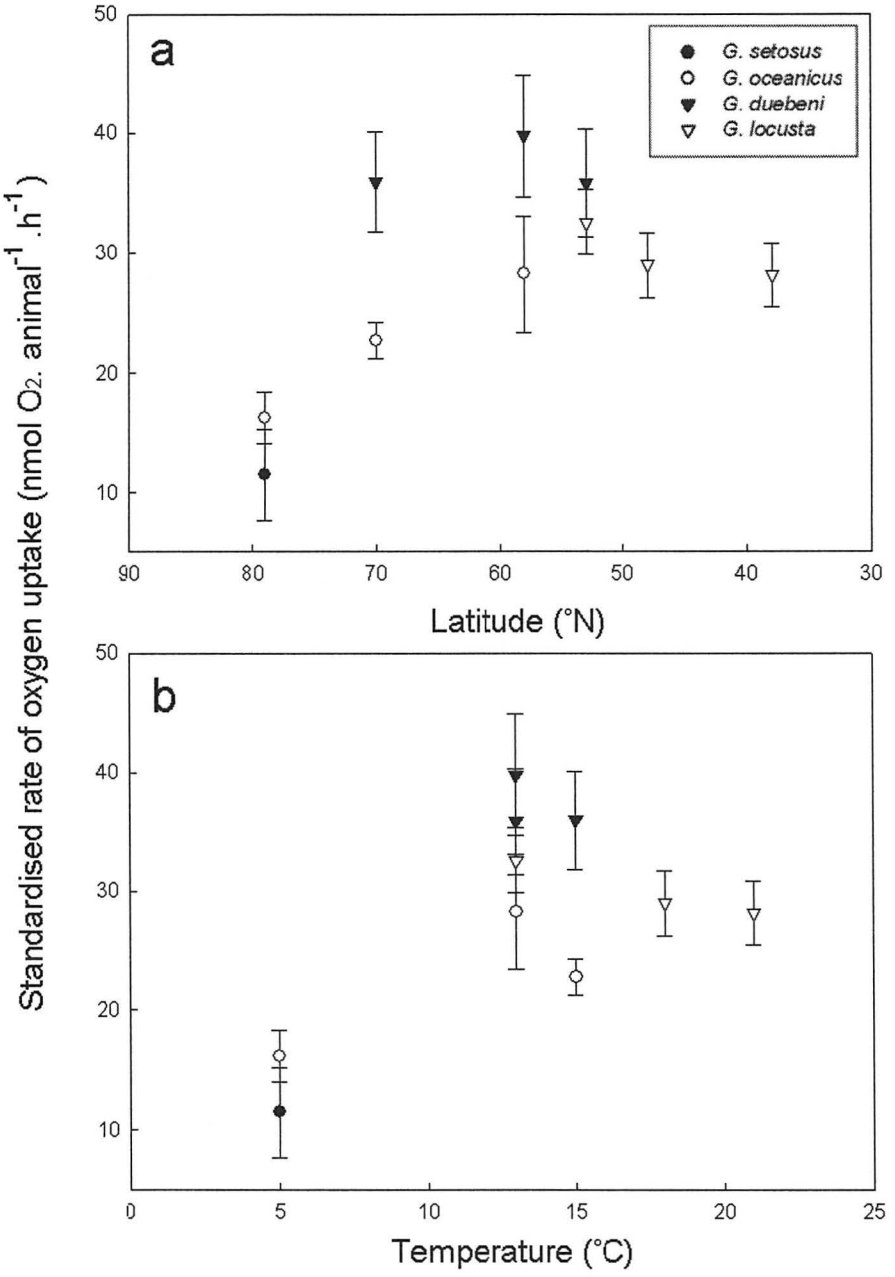


Figure 2.3. Whole-animal MO_2 values standardised for a body mass of 1mg plotted against (a) latitude, or (b) capture temperature. Mean values given ±SEM. (In *G. setosus*: n=6 at 79°N and 5°C. In *G. oceanicus*: n= 15 at 79°N and 5°C; n=12 at 70°N and 15°C; n=17 at 58°N and 13°C. In *G. d. duebeni*: n=16 at 70°N and 15°C; n=19 at 58°N and 13°C; and n=15 at 53°N and 13°C. In *G. locusta*: n=18 at 53°N and 13°C; and n=10 at 48°N and 18°C; and n=9 at 38°N and 21°C).

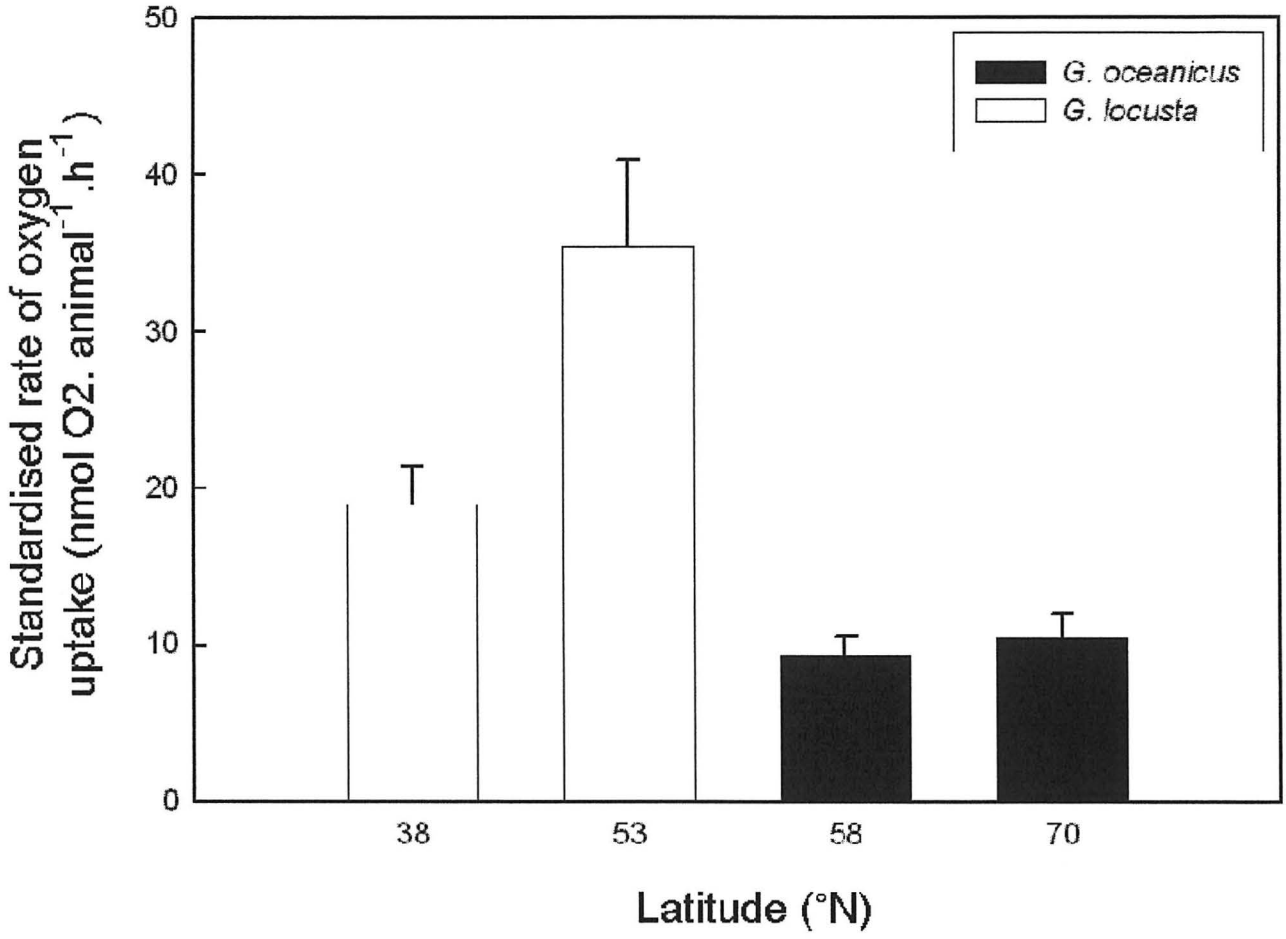
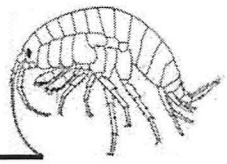


Figure 2.4. Resting whole-animal MO_2 values standardised for a body mass of 1mg for animals acclimated to 10°C for 4 weeks. Mean values given ±SEM to include *G. locusta* collected from the populations in Portugal (38°N) and Wales (53°N), and *G. oceanicus* collected from the populations in Scotland (58°N) and Tromsø (70°N). In *G. oceanicus* at 58 and 70°N and *G. locusta* at 53°N: n=6. In *G. locusta* at 38°N: n=8.



2.4.5 Thermal sensitivity of MO_2 in acclimatised animals

In both populations of *G. locusta* and *G. oceanicus*, exposure to an acute increase in temperature resulted in an exponential and significant increase in $\dot{M}O_2$ until the maximal aerobic threshold temperature was reached (ANOVA, $P<0.01$) (Table 2.1 & Fig. 2.5). The maximal aerobic threshold varied with latitude in both species, as did the maximum $\dot{M}O_2$ reached at this temperature. The highest maximal aerobic threshold of 33°C was observed in the southern population of *G. locusta* from Portugal at a maximum $\dot{M}O_2$ of 44.8±7.4(6) nmol O_2. $animal^{-1}$. h^{-1} (Fig. 2.5). This value was reduced to 29°C in the northern population of *G. locusta* even though maximum $\dot{M}O_2$ remained unchanged at 52.3±7.1(10) nmol O_2. $animal^{-1}$. h^{-1}. In contrast, *G. oceanicus* from Scotland (58°N) was characterised by an maximal aerobic threshold of 29°C, and a maximum $\dot{M}O_2$ of 37.7±4.0(7) nmol O_2. $animal^{-1}$. h^{-1}. The population of *G. oceanicus* from Svalbard (79°N) exhibited the lowest maximal aerobic threshold at 25°C and had a significantly lower maximum $\dot{M}O_2$ of 23.6±2.1(6) nmol O_2. $animal^{-1}$. h^{-1} compared with the population from Scotland (*t*-test, $P<0.01$) (Table 2.1 & Fig 2.5).

The rate of change in $\dot{M}O_2$ with temperature (Q_{10}) in *G. locusta* from Portugal was twice the value in *G. locusta* from Wales which was a significant difference (LSD test, $P<0.01$) (Table 2.1). Activation energies for metabolism (E_a) followed a similar trend with *G. locusta* from Portugal exhibiting significantly higher E_a values than *G. locusta* from Wales (LSD test, $P<0.05$). In contrast, there was no significant variation in either mean Q_{10} or E_a between the two populations of *G. oceanicus* (Table 2.1). Aerobic scope exhibited a significant negative relationship with latitude (ANOVA, $P<0.05$) resulting in a 3-fold reduction in aerobic scope between *G. locusta* from Portugal at 38°N and *G. oceanicus* from Svalbard at 79°N (Table 2.1).

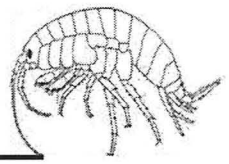


Table 2.1. Temperature sensitivity of MO_2 in two latitudinal populations of acclimatized *G. oceanicus* and *G. locusta* exposed to an acute change in temperature at a rate of 4°C h^{-1}. Values are given as means±SEM to include maximal aerobic threshold temperatures, aerobic scopes, temperature coefficients (Q_{10}), activation energies of metabolism (E_a), and the body mass of the individuals sampled along with the number of observations in parenthesis. *P*-values generated using repeated measures ANOVA show the significance of any variation in MO_2 with acute temperature change.

Species	Latitude (°N)	Maximal aerobic threshold (°C)	Aerobic scope (nmol O_2. animal $^{-1}$ h^{-1})	Q_{10}	E_a (kJ.mol^{-1})	Body mass (mg)	*P*
G. oceanicus	79	25	8.5±0.2	1.52±0.16	40.6±9.4	166±10(6)	0.019
G. oceanicus	58	29	14.6±3.7	1.68±0.12	37.2±4.8	81±8(7)	0.019
G. locusta	53	29	20.9±7.8	1.78±0.16	46.0±5.1	92±6(10)	0.011
G. locusta	38	33	27.3±7.7	3.51±0.97	81.8±19.9	33±3(6)	0.046

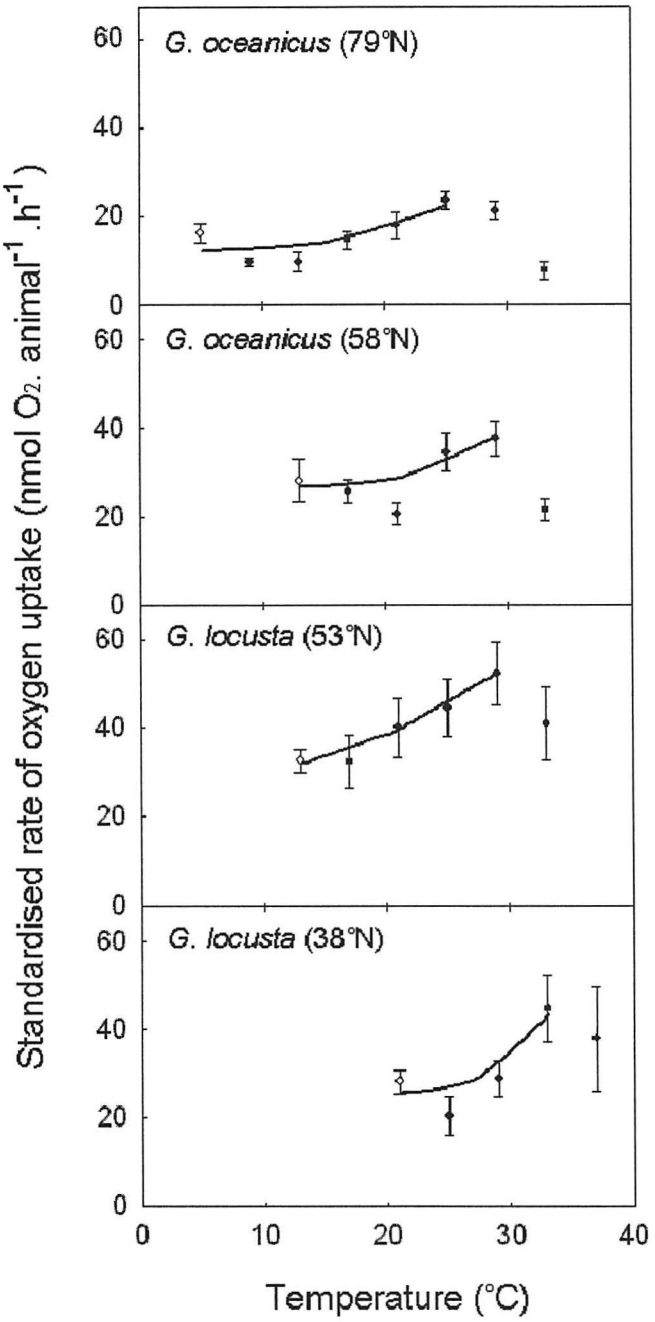
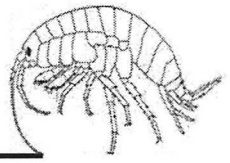
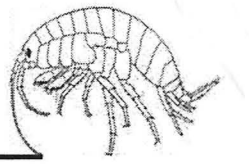


Figure 2.5. The effect of an acute change in temperature (4°C h^{-1}) on mean whole-animal $\dot{M}O_2$ values standardised for a body mass of 1mg for *G. oceanicus* collected from Scotland (58°N) and Svalbard (79°N), and in *G. locusta* collected from Portugal (38°N) and Wales (53°N). Open circles represent resting $\dot{M}O_2$ values at the respective capture temperature of each population. Curves were fitted using locally weighted polynomial regression (LOESS) with a polynomial degree and a smoothing parameter of 1. Values are given as means±SEM (In *G. oceanicus*: n= 6 at 79°N and n=7 at 58°N. In *G. locusta*: n=10 at 53°N and n=6 at 38°N).



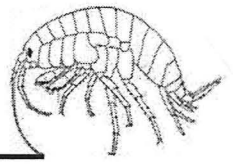
2.5 Discussion

The relatively low metabolic rates observed in subarctic populations of *G. oceanicus* and *G. setosus,* and the decline in MO_2 with latitude in *G. oceanicus,* demonstrate that high latitude gammarid populations do not up regulate their metabolic rates in the cold i.e. they do not show metabolic cold adaptation. Subarctic populations of *G. oceanicus* and *G. setosus* are therefore low energy consumers with low rates of energy turnover in keeping with many Antarctic benthic marine invertebrates. The latter are specialized to live in the extreme cold in an isolated environment characterized by marked seasonal changes in environmental conditions and resource availability (Clarke, 2003; Pörtner *et al.,* 2007). The subarctic population of *G. oceanicus,* however, appears to be less specialised, at least in terms of metabolism, as there was no evidence for metabolic divergence between the various latitudinal populations. Instead, metabolic rate in acclimatised *G. oceanicus* declined with habitat temperature at a Q_{10} of 2.24 which is typical for temperature-dependent changes in rate function. In addition, there were no differences in metabolic rate between populations at a common acclimation temperature, and no differences in the relationship between MO_2 and acute temperature change, described by Q_{10} and E_a, indicating similarities in enzyme kinetics and metabolic pathways. Low levels of energy consumption in Antarctic marine invertebrates are associated with relatively high longevity, and an increase in body size despite slower growth rates compared with warmer, low latitude species (Pörtner *et al.*, 2007), including gammarid amphipods (De Broyer, 1977). Similar characteristics are observed in *G. oceanicus* as life-span and body size increase, and growth rates decline in subarctic relative to temperate populations (Steele & Steele, 1972; Skadsheim, 1984; Węslawski & Legeżyńska, 2002). Certain life-history traits also vary with number of broods in *G. oceanicus* declining from



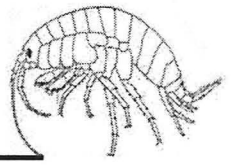
three to one between populations living at 59 and 79°N (Skadsheim 1984). In the Southern Ocean, low energy life-history traits are thought to be evolutionary adaptations to low energy environments where costs of living may also be resource limited, especially in those species reliant on seasonal primary production (Clarke, 2003). The wide latitudinal distribution of *G. oceanicus* combined with the observation that latitudinal differences in egg size and number of broods are phenotypic responses (Skadsheim, 1989), suggests that the low energy life-history traits of *G. oceanicus* from Svalbard are less likely to be an adaptive response and more likely to be a direct result of living in a cold and restricted thermal environment.

The dependence of metabolic rate on temperature and the lack of metabolic specialization between populations resulted in higher rates of metabolism in the temperate population of *G. oceanicus*. This population was characterised by higher aerobic scopes and maximal threshold temperatures compared with the population at higher latitudes. Such observations demonstrate that *G. oceanicus* is capable of increasing metabolic rates and changing thermal tolerances at temperate latitudes where the environment is less restrictive and energy budgets higher (Clarke, 2003). Interestingly, *G. oceanicus* does not show metabolic compensation with season (Einarson, 1993), suggesting a general inability to compensate for change, which is further supported by at the molecular level by a recent study on sequence variation in a functionally significant region of the myosin heavy chain gene (Rock *et al.*, 2009). The lack of myosin sequence diversity in *G. oceanicus* with latitude demonstrates a general toleration to change rather than an adaptive response. Moreover, recent phylogenetic analysis of the genus *Gammarus* based on nucleotide COI sequences revealed that within Europe, there is little genetic diversity between populations of *G. oceanicus*. Collectively these observations indicate that *G. oceanicus* is more of a



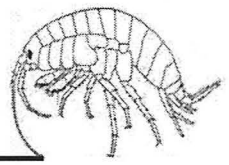
generalist than a typical Antarctic marine invertebrate species, as it survives environments that are not permanently cold and limited by resource availability, probably due to phenotypic adjustments to environmental change.

In sharp contrast to *G. oceanicus*, metabolic rate in the temperate species, *G. locusta*, and the boreal-temperate species, *G. d. duebeni*, remained unchanged with latitude, suggesting compensation for changes in environmental conditions to conserve metabolic rate. Compensatory responses are normally described during temperature acclimation when an increase in rate function occurs in cold acclimated animals to counteract the slowing effect of reduced temperatures. A similar response is reported between latitudinal populations after temperature acclimation in several tropical/temperate marine invertebrates such as the fiddler crab, *Uca rupax* (Vernberg, 1962), and the polychaete, *Clymenella torquata* (Mangum, 1963). It also occurs in acclimatised animals as demonstrated in the present study and in a classic study on water pumping rates in *Mytilus californianus* (Bullock, 1955), in which mussels from Friday Harbor (48°N) at 6.5°C pumped water at the same rate as mussels from Los Angeles (34°N) at 12°C. The resulting conservation of metabolic rate at the normal habitat temperature of each latitudinal population indicates alterations in metabolism can be attributed to shifts in the relationship between rate function and temperature (R/T curves described by Prosser, 1950). Shifts in R/T curves are likely to exist between latitudinal populations of *G. locusta* because individuals from Wales (53°N) had higher $\dot{M}O_2$ values than individuals from Portugal (38°N) when measured at a common acclimation temperature. In addition, the reduction of E_a in the northern population signifies alterations in the specific activity of metabolic enzymes resulting in a lowering of the energy barriers for catalysis, a typical compensatory response between polar or subpolar ectotherms and their temperate conspecifics or confamilials

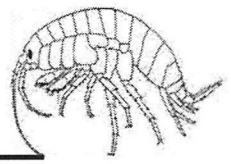


(Johnston *et al.*, 1975; Sommer & Pörtner, 2002; Lucassen *et al.*, 2006). In addition, the drop in Q_{10} in the northern population of *G. locusta* reflected a drop in thermal sensitivity of metabolism at higher latitudes which was accompanied by a reduction in maximal aerobic threshold temperature and aerobic scope. Such alterations are characteristic of cold adapted eurytherms at subpolar latitudes in the northern hemisphere where increased mitochondrial capacities lead to higher maintenance costs and an increase in whole-organism standard metabolic rates (Sommer & Pörtner, 2002; 2004; Pörtner *et al.*, 2005). It is possible that *G. locusta* responds in a similar fashion, albeit at a different range of latitudes, as initial studies show a 7-fold increase in mitochondrial densities in the abdominal muscles of *G. locusta* with latitude (C. Field & N. M. Whiteley, unpublished data). Regardless of the mechanisms involved, the conservation of metabolic rates in *G. locusta* and *G. d. duebeni* indicates metabolic diversity between populations.

The conservation of relatively high rates of metabolism in *G. locusta* and *G. d. duebeni* indicate high rates of energy consumption and high energy life-styles. Preliminary growth studies, for instance, show that juvenile growth rates are 3 times higher in *G. locusta* from Wales at 1.02±0.06(10) mm $week^{-1}$ compared with *G. oceanicus* from Scotland at 0.30±0.02(10) mm $week^{-1}$ (A. M. Posacka & S.P.S. Rastrick, unpublished data). There could be a number of reasons for conserving high metabolic rates across a range of latitudes, all of which depend on the continued availability of food to fuel elevated rates of energy consumption (Lonsdale & Levinton, 1989). In the low intertidal *G. locusta*, high energy turnover may be related to its warm-water ancestry. Phylogenetic analysis revealed that *G. locusta* shares a putative phylogroup with other Mediterranean species (Costa *et al.*, 2009). Consequently, metabolic compensation may occur between latitudinal populations of



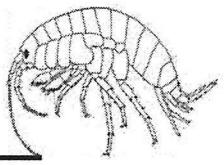
G. locusta to maintain higher energy traits such as high rates of growth and activity. Moreover, deep intraspecific diversity occurs between populations of *G. locusta* from Portugal and Wales of ~4% suggesting that the two populations were separated before the last glaciation event (Costa *et al.*, 2009). Indeed, *G. locusta* has the longest history of reproductive isolation of the four species included in the present study. Genetic divergence between populations suggests that metabolic conservation at respective habitat temperatures may result from adaptation to local conditions. Unlike *G. locusta*, the conservation of a relatively high energy budget in *G. d. duebeni* could be less to do with its ancestry and more to do with to its ability to tolerate environmental change. *G. d. duebeni* groups in a different phylogroup to *G. locusta* and is more closely related to the high Arctic species, *G. wilkitzkii*, and to the cold-water species, *G. oceancicus* and *G. setosus* (Costa *et al.*, 2009). Despite its cold-water ancestry, *G. d. duebeni* inhabits the high intertidal where it experiences wide and rapidly changing environmental conditions. As a result a number of biochemical and physiological adjustments are needed to impart some independence of rate function from environmental variability (Bulnheim, 1979; Gaston & Spicer 2001). The ability of *G. d. duebeni* to show metabolic compensation at high latitudes could be related to its high degree of eurythermy as described in another intertidal species, *Arenicola marina* (Pörtner *et al.*, 2005; 2007). Metabolic compensation may only occur in boreal–temperate species in more eurythermal habitats such as the upper shore, as along as winter conditions as are not too extreme and food is not limiting, because some species experience metabolic depression in the winter (Sommer & Pörtner, 2002; Sokolova & Pörtner, 2003). The conservation of metabolism in temperate and more eurythermal boreal-temperate gammarids supports the proposition that metabolic conservation is advantageous in a less stable thermal environment because



the alternative: high thermal sensitivities within the environmental range, is associated with increased long-term metabolic costs and a lower tolerance to extreme temperatures (Hawkins, 1995). As with *G. locusta*, the observed metabolic diversity in *G. d. duebeni* may in part be explained by adaptation to local conditions as grater genetic diversity was also reported between populations of *G. d. duebeni* than between populations of *G. oceanicus* (Costa *et al.*, 2009).

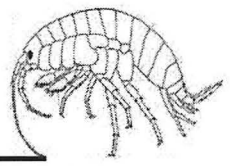
2.5.1 Summary

In summary, metabolic rates in acclimatised gammarid amphipods are conserved across populations in the temperate and boreal-temperate species, *G. locusta* and *G. d. duebeni*, but not in the subarctic-boreal species, *G. oceanicus*. In the latter, there was no evidence of metabolic compensation and therefore metabolic diversity between populations, suggesting that the reduction in energy consumption observed in the subarctic population was a direct result of restricted environmental conditions, especially temperature. The general lack of metabolic diversity and population structure in *G. oceanicus* suggest that associated changes in thermal tolerances between populations may be phenotypic. Metabolic conservation across populations is a feature of high energy turnover either to maintain a warm water life-style or to confer some independence from highly variable environmental conditions. Metabolic divergence between populations may in part be due to adaptive responses as some population structure is observed in both *G. locusta* and *G. d. duebeni*.



Chapter 3

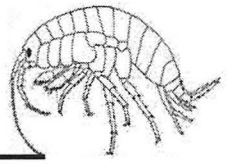
Conservation of protein synthesis rates between natural populations of gammarid amphipods is dependent on latitude but not environmental tolerance



3.1 Abstract

To examine the rates of energy expenditure in marine ectothems along a latitudinal thermal gradient, whole-animal rates of protein synthesis were determined in gammarid amphipods acclimatised to natural environmental conditions. Protein synthesis rates represent a significant metabolic expense and are a major determinant of growth. This study included congeneric species of known phylogeny and similar ecologies but different latitudinal and vertical distribution patterns on the shore.

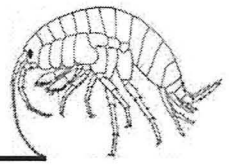
Relatively low whole-animal fractional rates of protein synthesis, associated with low energy consumption and slow rates of growth, were recorded in *Gammarus setosus* and sub-arctic populations of *G. oceanicus* from Svalbard (79°N). In contrast, the warm-temperate species *G. locusta* showed no variation in rates of protein synthesis across its latitudinal range, despite a variation in habitat temperature of 8°C. *G. locusta* also showed direct compensation of RNA activities (K_{RNA}) with latitude, indicating elevated RNA efficiencies and suggesting the optimisation of enzymes to lower temperatures in the northern population (53°N). *G. d. duebeni* also showed no variation in rates of protein synthesis with latitude, perhaps allowing the compensation of growth/development across its range and to impart independence from temperature in its eurythermal environment. Conservation of protein synthesis rates in *G. d. duebeni* is facilitated by an elevation in RNA concentrations to counteract temperature depressed K_{RNA} in northern populations (70°N). Unexpectedly, this high shore eurythermal species exhibited low whole-animal rates of protein synthesis, suggesting low rates of energy turn over. This may be due to resource limitation and energy conservation in an unstable environment.



3.2 Introduction

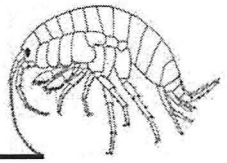
Protein synthesis rates are an important determinant of growth and cellular metabolism, and are of fundamental importance to the turnover and repair of proteins. It is well documented that whole body protein synthesis rates are influenced by both temperature and food availability (reviewed by Fraser and Rogers, 2007; Whiteley & Fraser, 2008). Both of these factors vary over natural spatial gradients, such as latitude, yet little is known about the variation in protein synthesis rates in ecologically important marine ectotherms with wide latitudinal distribution patterns.

In general, rates of protein synthesis decrease with temperature both within species acclimated to different temperatures (eg. McCarthy *et al.*, 1999) and between species acclimatised to natural seasonal changes in temperature and food availability (Whiteley & Faulkner, 2005). For example, in the temperate isopod, *Ligia oceanica,* summer rates of protein synthesis in animals acclimatised to 20°C at 2.3% day^{-1} were almost 8-fold higher than the rates in winter animals acclimatised to 5°C (Whiteley & Faulkner, 2005). Temperature has a direct effect on rates of protein synthesis, via changes in kinetic energy, but it also has an indirect affect on rates of protein synthesis, via its positive effect on the rate of food consumption, as increased amino acid uptake increases the rate of protein synthesis at a common temperature (McCarthy *et al.*, 1993, 1994). Greater food consumption at higher temperatures also makes the independent roles of temperature and nutrition unclear, even during acclimation studies. Therefore, ectotherms are acclimatised to temperature when feed *ad libitum* and can never be truly acclimated to just temperature (McCarthy & Houlihan,1996; Fraser & Rogers, 2008; Whiteley & Fraser, 2008). Limited examination of protein synthesis rates between polar and temperate marine ectothermic species has revealed that, with the exception of the Antarctic sea urchin,

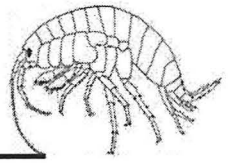


Sterechinus neumayeri (Marsh *et al.*, 2001; Pace & Manahan, 2007), whole animal rates of protein synthesis decrease in polar marine ectothems living at temperatures below 5°C (Whiteley *et al.*, 1996; Robertson *et al.*, 2001a; Fraser *et al.*, 2007). In the polar stenothermic isopod, *Saduria entomon,* rates of protein synthesis range from 1.5% day^{-1} in fed animals to 0.6% day^{-1} in starved animals at 4°C (Robertson *et al.*, 2001a). Both values are substantially lower than the mean rate of protein synthesis, approximately 2.7% day^{-1}, reported for temperate marine ectothems (Fraser & Rogers, 2007). Rates of protein synthesis, however, have only been examined in 5 species of polar marine ectotherm (Whiteley *et al.*, 1996; Robertson *et al.*, 2001a; Marsh *et al.*, 2001; Storch & Pörtner, 2003; Pace *et al.*, 2004; Fraser *et al.*, 2007). These include species from different taxa and represent different stages of development. To date there has been no investigation of *in situ* rates of protein synthesis in closely related (congeneric) species or populations acclimatised to a wide range of latitudinal and local conditions.

Protein synthesis is critical to cellular metabolism due to the catalytic effect of enzymes and the importance of membrane proteins in ion regulation. The ability to synthesise isozymes optimised to different temperatures is also crucial to the ability of organisms to enable acclimatisation to long term variations in temperature (reviewed by Hochachka & Somero, 2002). The ability of cells to protect metabolic pathways from short-term increases in temperature also depends on rates of protein synthesis both in the replacement of denatured enzymes and in the production of molecular chaperones, such as heat-shock proteins to repair and protect existing proteins (Morimoto *et al.*, 1990; Hartl, 1996; Nover & Scarf, 1997; Bukau & Horwich, 1998; Somero, 2002; Ali *et al.*, 2003, Hofmann, 2005). Protein synthesis is also an important determinent of growth and development in individual organisms with

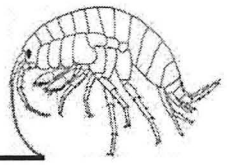


optimum growth occurring at temperatures conducive to maximum rates of protein synthesis (Loughna & Goldspink, 1985; Pannevis & Houlihan, 1992; Carter & Houlihan, 2001). However, not all synthesised protein is retained as growth, as a high percentage is degraded through a variety of pathways such as ubiquitisation (Herschko & Ciechanover, 1982; 1998). Studies in fish have shown that at upper and lower critical temperatures, protein degradation increases leading to less protein being retained (McCarthy *et al.*, 1999: Katersky & Carter, 2007). The efficiency of growth can be expressed as ether protein growth efficiency (PPV; protein growth / protein consumption) or protein synthesis retention efficiency (PSRE; protein growth / fractional rate of protein synthesis). The latter is more typically used to express protein deposition and describes the percentage of synthesised protein retained as protein growth (Houlihan *et al.,* 1995). Acclimation studies in juvenile barramundi, *Lates calcacarifer,* acclimated to a range of temperatures from 21-39°C also revealed that feeding, growth rate, and protein growth efficiency (PPV) increased with temperature reaching maximum values at similar temperatures before decreasing precipitously (Katersky & Carter, 2007). In the Wolffish, *Anarhichas lupus,* rates of protein synthesis increased between 5 and 14°C, but growth decreased from 11°C due to the effect of increased protein degradation on PSRE at higher temperatures (McCarthy *et al.*, 1999). Subsequently, thermal differences between protein synthesis and degradation may explain why growth decreases at temperatures just prior to the upper thermal limit (Brett, 1979; McCarthy & Houlihan, 1996; McCarthy *et al.*, 1998; 1999). Interspecific comparisons between 15 species of teleosts, molluscs and crustaceans showed that PSRE generally decreases with temperature (Whiteley & Fraser, 2009). Low PSRE values are also observed in polar ectotherms such as the Antarctic limpet *Nacella concinna* (Fraser *et al.*, 2007). Levels of ubiquitin conjugates



of protein, used as an indicator of protein degradation, are also high in polar marine ectotherms (Place *et al.*, 2004; Place & Hofmann, 2005). This suggests that low rates of growth reported in polar ectotherms (Peck *et al.*, 1997; Clarke *et al.,* 2004; Barnes *et al.*, 2006; Bowden *et al.*, 2006) may be due to low protein retention and synthesis rates at low temperatures (Fraser & Rogers, 2007; Whiteley & Fraser, 2009). Conversely, life-history traits such as higher rates of growth and development, associated with southern populations and species of gammarids (Steele & Steele, 1972; 1973) may necessitate underlying selection for higher rates of protein synthesis and turnover in higher temperature, more eurythermal temperate environments (Pörtner, 2005).

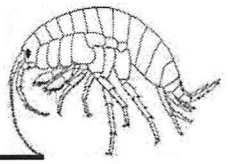
Contary to recent measurements of growth efficiencies discussed above, it was previously proposed that selection for lower protein turnover and maintenances costs in more stable higher latitude environments may lead to more energy being available to growth and theoretically higher growth efficiencies (Pörtner *et* al., 2005). Indeed, growth does not always decrease in higher latitude species (Dayton *et al.,* 1974; Dayton, 1989; Rauschert, 1991; Barnes, 1995) or populations (Lonsdale & Levinton, 1985; Conover & Present, 1990; Conover, 1990; Jensen *et al.*, 2000) and thermal independence of protein synthesis has been reported between (Fraser & Rogers, 2007; Pace & Manahan, 2007) and within (Whiteley & Faulkner, 2005) species of marine ectotherms. Rates of protein synthesis and as a consequence growth may be maintained due to modifications of RNA concentrations, compensating for temperature dependent decreases in RNA activities (K_{RNA}; Goolish *et al.*, 1984; Houlihan, 1991; Foster *et al.*, 1992: 1993a; b; McCarthy *et al.*, 1999; Robertson *et al.*, 2001b; Fraser *et al.*, 2002a; Storch *et al.*, 2003; Treberg *et al.*, 2005; Whiteley &



Faulkner, 2005; Intanai *et al.*, 2009); or via direct compensation of K_{RNA} (Storch *et al.*, 2003; 2005).

Higher rates of growth and development can increase individual fitness (Yamahira & Conorer, 2002) by limiting the time spent as non-reproductive, smaller juveniles that are at greater risk from predation (Laurence, 1975; Peterson & Wroblewski, 1984). Desiccation-tolerance has also been positively correlated with body size in ectotherms (Hobday, 1995; Gilchrist *et al*, 2008), and thermal-tolerance with developmental stage in gammarids (Steele & Steele, 1969); both may affect the ability of marine ectothermic species to survive climate change. Lower rates of growth, development, and reproduction due to lower rates of protein synthesis and retention may also affect rates of evolution and adaption in the face of ecosystem change (Hawkins, 1991; Reid *et al.,* 1998; Fraser & Rogers, 2007). Rates of growth and development also affect, and are limited by, resource availability and species density, as an environment can support fewer individuals if those individuals consume resourses at a greater rate in order to maintain high rates of protein synthesis. Variation in the size of individuals and density of prey species may also affect the flow of energy though ecosystems.

The ability of marine ectotherms to adapt or acclimatise to long- or short-term variations in temperature may depend on variations in rates of protein synthesis between species, with lower rates indicating a reduced ability to adapt to change (Hawkins, 1991; Reid *et al.*, 1998; Fraser & Rogers, 2007). Polar marine ectotherms with low rates of protein synthesis are therefore likely to be more vulnerable to climate change. To examine whether low rates of protein synthesis are a feature of high latitude populations of the same species, comparisons were made between naturally acclimatised populations of congeneric species, the gammarid amphipods,

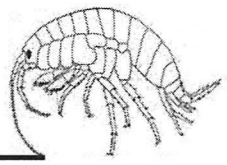


over a latitudinal gradient of 40°N. This will allow examination of some of the underlying mechanisms determining rates of growth and development between populations and species along a latitudinal thermal gradient. Such studies are ecologically relevant and may increases our understanding of varying susceptibilities to climate change.

3.3 Materials and Methods

3.3.1 Animal collection and husbandry

To examine whole-animal fractional rates of protein synthesis at different latitudes, four species of gammarid amphipod were collected from various latitudinally separated populations between June and September 2007/2008. The overlapping distribution of each species meant that gammarid amphipods were collected from sites across a latitudinal range of 79 to 38°N representing a gradient of 5 to 21°C in habitat temperature. Populations of each species were collected from the same sites as those described in Chapter 2. The subarctic/boreal low shore species, *Gammarus oceanicus,* was collected from Ny-Ålesund in Svalbard, Norway (78.92°N-11.92°E); Tromsø, Norway (69.61°N-18.9°E), and Isle of Skye, Scotland (57.66°N-5.33°W). The temperate low shore species, *G. locusta,* was collected from Isle of Anglesey, Wales (53.2°N-4.5°W), and from Troia, Portugal (38.48°N-3.88°W). The temperate high shore species, *G. d. duebeni,* was collected from populations in Tromsø, Isle of Skye and Anglesey. The Arctic species, *G. setosus,* was only collected from Ny-Ålesund, Svalbard (see Fig 2.1, Chapter 2). Northern populations were sampled first due to the shorter summer season. Rates of protein synthesis were not measured in brooding females or moulting animals. Microhabitat temperature was recorded at each site at

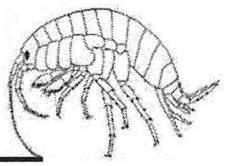


the time of collection, by use of a thermocouple placed under the particular rocks and sediment were animals were found, these capture temperatures are shown in Table 3.1.

Table 3.1. The temperatures recorded at the time of collection and the natural temperature range that each population usually experiences. The collection site and latitude of each species is also shown.

Species	Site	Latitude (°N)	Capture Temperature (°C)
G. setosus	Svalbard	79	5
G. oceanicus	Svalbard	79	5
G. oceanicus	Tromsø	70	10
G. oceanicus	Scotland	58	13
G. locusta	Wales	53	13
G. locusta	Portugal	36	21
G. d. duebeni	Tromsø	70	15
G. d. duebeni	Scotland	58	13
G. d. duebeni	Wales	53	13

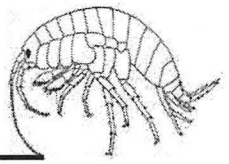
After capture, amphipods were held in seawater from the same site as they were collected, this was fully aerated and maintained at the respective capture temperature of each population. Animals were allowed access to natural food items to maintain the animals as close to the conditions on the shore as possible. Within 48h of capture individuals from each population had been returned to Bangor University, UK, for the determination of whole-animal protein synthesis rates. During transit animals were maintained between sheets of damp filter paper at their respective capture temperatures. On arrival animals were allowed to recover over night in aerated sea water collected from each site and maintained at their respective capture temperatures. The combination of transit and recovery time resulted in approximately 12h of starvation prior to experimentation.



To further investigate the effect of temperature on protein synthesis *G. d. duebeni* from the north (Tromsø; 70°N) and south (Scotland; 58°N) of their range, as well as a boreal population of *G. oceanicus* form Tromsø were acclimated to a common temperature. In each case, amphipods were held in fully aerated seawater at a constant temperature of 10°C and a 12L:12D regime for 4 weeks. A third of the water was replaced each week before the amphipods were fed a diet of algal fish food (Tetra*Veg*®, Tetra GmbH, Germany).

3.3.2. Determination of protein synthesis rate: injection regime

Fractional rates of protein synthesis were determined using the flooding dose method (Garlick *et al.*, 1980) modified for use in crustaceans (Houlihan *et al.*, 1990; Whiteley *et al.*, 1996). Each animal was injected with 2µl $50mg^{-1}$ wet body mass of crab saline (Pantin, 1934) containing 150 mmol l^{-1} of unlabelled L-phenylalanine and 3.7 MBq ml^{-1} of L- [2,3,4,5,6-^{3}H] phenylalanine (G. E. Healthcare. Specific Activity 4.37 TBq $mmol^{-1}$). A micro-droplet manipulation system modified from Tomos *et al.* (1994) was used to deliver a small volume of labelling cocktail into the haemolymph, via the bulbus arteriosus of the heart. To this end, a microcapillary was orientated obliquely between the 1st and 2nd mesenteric plates using a micromanipulator under a dissecting microscope (Wild, M32, Heerbrugy, Switzerland). Amphipods were held in place with the pereopods submerged in water but the dorsal surface emersed and accessible for injection. A bright light shone from the side ensured that the bulbus arteriosus could be observed. The microcapillary was then left in place for 10s as preliminary injections of 0.01% Toluene blue in crab saline revealed that this was sufficient time to ensure complete circulation of the labelling cocktail. Each injection took no longer than 2 min to minimalise disturbance to the animals. This system allowed animals as

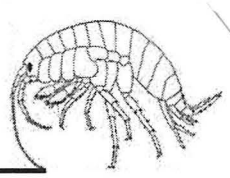


small as 25mg to be injected. After injection animals were incubated at their respective capture temperatures in fully aerated seawater in 100%, with the exception of *G. d. duebeni* which was incubated in 50% seawater. *G. d. duebeni* and *G. locusta* were incubated for 1h and *G. oceanicus* and *G. setosus,* were incubated for 1 and 2 h. Incubation time was determined in the validation experiments described below. After incubation, amphipods were sacrificed, snap frozen in liquid nitrogen and stored at -80°C for later analysis.

3.3.3. Validation of the flooding dose methodology

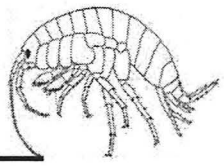
In order to validate the flooding dose technique for use in amphipod crustaceans the low-shore species *G. oceanicus* (58°N) and the high-shore species *G. d. duebeni* (53°N) were injected with the labelling cocktail and incubated at 13°C for 30, 60 or 120 min. This determined the optimum time-course for the maximum incorporation of the labelled phenylalanine into the protein fraction. The effect of temperature on the time-course of incorporation was also considered, as *G. oceanicus* from the high latitude population (79°N) were incubated at 5°C for 60, 120 or 180 min.

In order to test the assumption that the intracellular free-pools were completely flooded by phenylalanine during the incubation period, 5 additional *G .oceanicus* (69°N) and *G. locusta* (53°N) were injected with crab saline containing no phenylalanine and incubated for 60min. The resulting samples were analysed as described below and comparisons of phenylalanine levels made between animals injected with saline and those from the same population injected with the flooding dose. All validation experiments in each population were carried out at their respective capture temperatures.



3.3.4 Determination of protein synthesis rate: analysis

Each individual was treated as a separate sample unless animals weighed less than 50 mg in which case up to four individuals from the same population were pooled together. Samples were analysed as previously described by Garlick *et al.* (1980) and McCleary and Whiteley (in preparation). Samples were ground under liquid nitrogen and vortexed with 3 ml of 2% PCA before being centrifuged (ALK, Multispeed refrigerated centrifuge, PK 121R; 15000rpm for 20min at 4˚C) to separate the intracellular free-pools from the precipitated protein and RNA pellet. The pellet was washed twice more in 3 ml of 2% PCA and then incubated in 4 ml of 0.3N NaOH for 1 hour at 37˚C, to redissolve the protein. The alkali-soluble protein was determined from a 20µl sub-sample using a modified Lowry method (Peterson, 1977). The rest of the sample was vortexed with 2.5 ml of 12% PCA and incubated for 10 min at 4˚C to precipitate the protein. After centrifuging, the pellet was washed in 2% PCA and supernatants decanted for the determination of RNA levels. The protein pellet was hydrolysed in 3 ml of 6N HCl for 18-24 hours at 110˚C. The acid was evaporated from the hydrolysed protein before the enzymatic conversion of the protein-bound and the intracellular free-pool phenylalanine to β-phenylethylamine (PEA) using tyrosine decarboxylase (Worthingtons, 44C335A). Before enzyme incubation all samples were sonicated in 5 second bursts for a total of 20 seconds to increase enzyme efficiency (McCleary & Whiteley, in preparation). The efficiency of tyrosine decarboxylase in converting phenylalanine into β-phenylethylamine was determined for each batch of enzyme by converting and analysing known standards of phenylalanine (150, 100 and 50 nm ml^{-1}). Phenylethlamine was extracted and determined flourometrically using a multipliable counter (Wallac, Victor $^{2}{}_{TM}$, 1420). RNA levels were measured by ultraviolet absorption at 232 and 260nm (Bio-Tek Kontron, Uvikon 943; after



Ashfrod & Pain, 1986) and verified using known standards (RNA, Sigma R 8508). The specific radioactivities of the protein-bound (S_b) and the intracellular free-pool (S_a) faction were determined by scintillation counting (Wallac, WinSpectral™, 1414 Liquid scintillation counter) and expressed as disintegrations per min (dpm) per nmole of phenylethylamine. Counting efficiency was 37% (Whiteley & Faulkner, 2005).

3.3.5 Calculations

Whole-animal fractional rates of protein synthesis (k_s; % day^{-1}) were calculated using the equation (Garlick *et al.*, 1980):

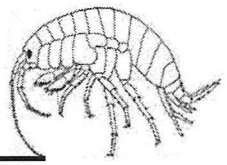
$$K_s = \frac{S_a}{S_b} \times \frac{24}{t} \times 100$$

Where k_s= % protein mass synthesised per day (% day^{-1}); S_b= protein-bound specific radioactivity of phenylalanine (dpm/nmol phe); Sa= free-pool specific radioactivity of phenylalanine (dpm/nmol phe); t = incubation time in hours; 24= number of hours in a day. Absolute rates of protein synthesis (A_s; mg protein.day^{-1}) were calculated from the equation:

$$A_s = \frac{K_s}{100} \times (\text{protein mass})$$

RNA to protein ratios (µg RNA. mg protein^{-1}) were used to express RNA concentrations, and the capacity for protein synthesis. RNA activity (K_{RNA}; mg protein. mg^{-1} RNA day^{-1}) was calculated using the equation (after Preedy *et al.*, 1988):

$$K_{RNA} = \frac{10\,K_s}{\text{RNA : protein}}$$



As body size varied significantly between species and populations of the same species all data was standardised to 1g wet body mass so that comparisons could be made between the measured variables. Data were standardised using the regression coefficients 0.7 for whole animal values (A_s) and -0.2 for weight-specific values (k_s, K_{RNA}, RNA:protein) (Houlihan *et al.*, 1990).

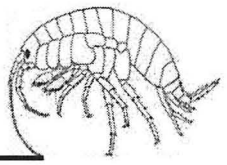
3.3.6 Statistical analysis

All data were tested for normality using Kolmgorov-Smirnov tests and Levene's tests for homogeneity of variances. As fractional rates of protein synthesis represent proportional data, all k_s values were arcsine square root transformed before further analysis. Parametric data were subjected to t-tests or one-way analysis of variance (ANOVA) with LSD post hoc tests. The linear incorporation of the labelled phenylalanine into the protein fraction over time was tested using least-squares liner regression. All statistical analyses were performed using SPSS software (SPSS INC., Chicago. IL, USA). All values are means±SEM with the number of observations in parentheses.

3.4 Results

3.4.1. Validation of flooding dose methodology

The time course for phenylalanine radioactivities in the intracellular free-pools and protein-bound fractions of both *G. oceanicus* and *G. d. duebeni* are displayed in Fig 3.2. In *G. oceanicus*, no significant change in intracellular free-pool specific radioactivity was observed between 30 and 120 min in individuals collected from Skye at 13°C (ANOVA, f=0.167, P=0.848); and between 60 and 180 min in individuals collected from Svalbard at 5°C (ANOVA f=0.828, P=0.459). In contrast,



specific radioactivities of phenylalanine in the free-pools of *G. d. duebeni* were stable between 30 and 60 min, but levels fell significantly between 60 and 120 min from 1291±251(8) to 656±144(8) dpm.nmol^{-1} phenylalanine (ANOVA, f=3.75, $P<0.05$; LSD, $P=0.028$).

Incorporation of labelled phenylalanine into protein was significant (Table 3.2) and linear between 30 and 120 min at 13°C in *G. oceanicus* and *G. d. duebeni* from the UK (Fig. 3.1). In *G. d. duebeni* protein-bound phenylalanine radioactivities increased from 0.3±0.08(8) dpm.nmol^{-1} at 30 min to 1.8±0.9(8) dpm.nmol^{-1} at 120 min (Table 3.2 and Fig 3.2d). In *G. oceanicus* a similar increase was observed from 0.35±0.07(5) dpm.nmol^{-1} to 1.49±0.69(6) dpm.nmol^{-1} over the 1.5h incubation period (Table 2 and Fig 2c). The subarctic population of *G. oceanicus* also exhibited a significant linear increase in protein-bound phenylalanine radioactivities from 0.29±0.08(8) dpm.nmol^{-1} at 60 min to 0.59±0.14(5) dpm.nmol^{-1} at 180 min (Table 3.2 and Fig 3.2c). However, at the lower temperature of 5°C this population showed rates of incorporation over 5 times lower than the that of their southern conspecifics at 13°C (Table 3.2). In each case the intercept of the regression model for each population/species was not significantly different from the origin (Table 3.2). Base-line phenylalanine levels in saline injected amphipods were 3.9±1.1 (5) nm.ml^{-1} in *G. d. duebeni* (53°N) and 1.9±1.0(5) nm.ml^{-1} in *G. locusta* (53°N). Therefore, the flooding-dose increased baseline phenylalanine levels 3-6 fold.

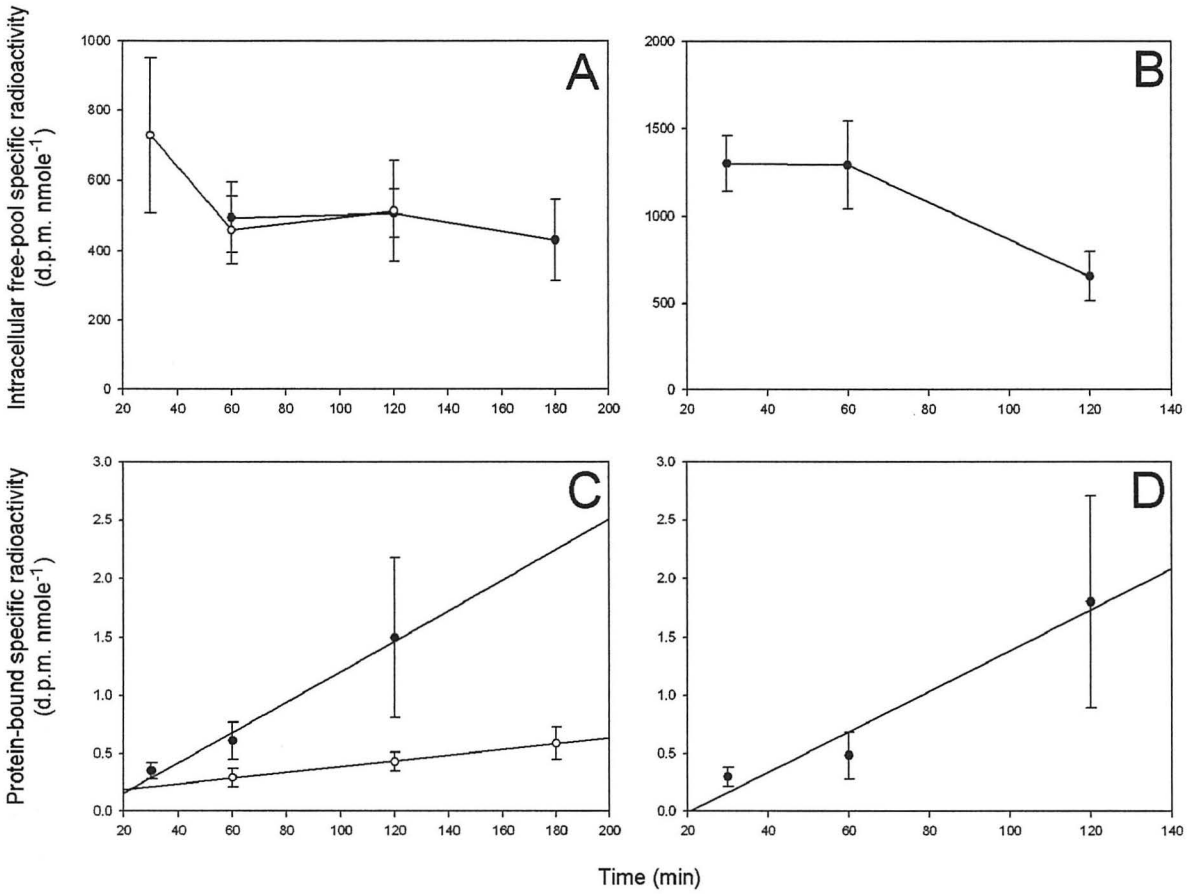


Fig. 3.1. The specific radioactivity of phenylalanine (dpm nmol^{-1}) in the intracellular free-pools and in the protein-bound fraction at different incubation times (min). (A) Intracellular free-pool specific radioactivities for phenylalanine in *G. oceanicus* from 79°N held at 5°C (open circles; n=6) and *G. oceanicus* from 58°N held at 13°C (closed circles; n=5); (B) Intracellular free-pool specific radioactivities for phenylalanine in *G. d. duebeni* from 58°N held at 13°C (n=8); (C) The significant positive linear relationship between protein-bound specific radioactivities of phenylalanine and time in *G. oceanicus* from 79°N held at 5°C (open circles; n=6) and *G. oceanicus* from 58°N held at 13°C (closed circles; n=5); (D) The significant positive linear relationship between protein-bound specific radioactivities of phenylalanine and time in *d. duebeni* from 58°N held at 13°C (n=8). The equations for the linear regression lines in C and D are given in Table 2. Mean values given ±SEM.

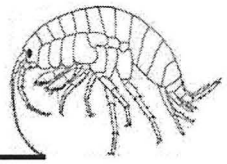


Table 3.2. Results of the least-squares regression analysis for the relationship between protein-bound phenylalanine radioactivity and incubation time illustrated in Fig 3.1. The regression coefficient (b) or the gradient of the relationship characterises the rate of labelled phenylalanine incorporation into proteins (dpm $nmole^{-1}$. min^{-1}). The *p*-value (p_b) showing the significance of least-squares regression model is shown. As is the *p*-value (p_a) of the significance of the variation between the intercept (a) and the origin is also shown. All values are means± SEM.

Species	Latitude(°N)	Temperature(°C)	N	b	p_b	a	p_a
G. d. duebeni	58	13	24	0.017±0.008	0.044*	-0.36±0.65	0.58
G. oceanicus	58	13	15	0.013±0.006	0.045*	-0.1±0.46	0.83
G. oceanicus	79	5	18	0.002±0.001	0.044*	0.14±0.14	0.33

ANOVA, * = *P<0.05*

3.4.2 Body size

An 11-fold increase in wet body mass was observed with latitude between *G. locusta* from Portugal and *G. setosus* from Svalbard (Fig. 3.2). Within species body mass increased with latitude. In the lower shore species, *G. locusta,* a significant increase in body mass of 60% was recorded with latitude (t-test, t=6.85, *P*<0.001). Values ranged from 25.3±2.1(18) mg at 38°N to 64±6.6(10) mg at 53°N. A similar increase of 65%, from 97.4±7.3(10) mg at 58°N to 274.4±27.6(11) mg at 79°N, was observed in *G. oceanicus* (ANOVA, F=26.1, *P*<0.001). Body mass also varied significantly in the high-shore species, *G. d. duebeni*, increasing with latitude from 71.5±3.9(8) mg at 58°N to 128.1±5(8) mg at 70°N (ANOVA, F=22.2, *P*<0.001). The only significant difference in body size between species was at 70°N were *G. d. duebeni* on the high shore were less than half the size of *G. oceanicus* on the lower shore (t-test, t=6.8, *P*<0.001).

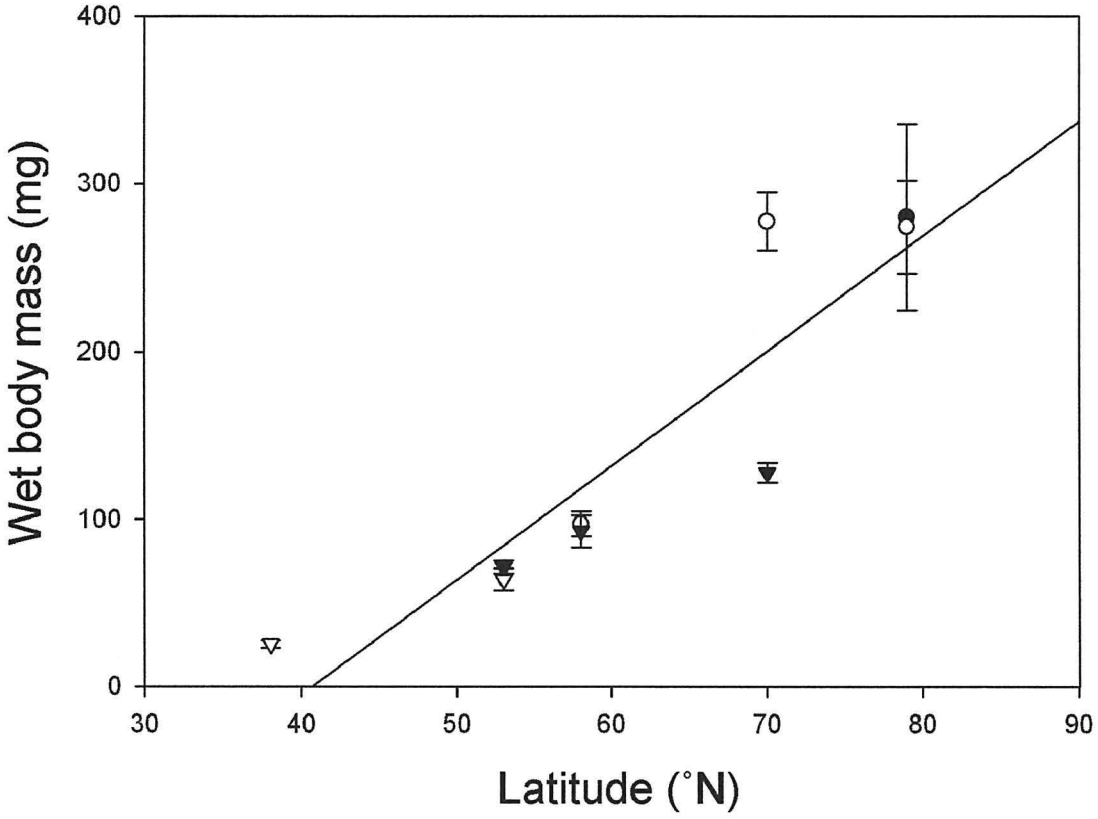
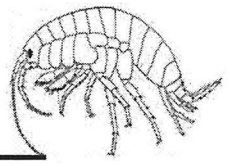


Fig. 3.2 The positive relationship between wet body mass (mg) and latitude (˚N) for *Gammarus setosus* (closed circles); *G. oceanicus* (open circles); *G. d. duebeni* (closed triangles) and *G. locusta* (open triangles). Line fitted using least-squares regression Y= 6.8X -277.9 (r^2= 0.82). Mean values given ±SEM. (In *G. setosus*: n=12 at 79°N. In *G. oceanicus*: n= 11 at 79°N; n=12 at 70°N; n=10 at 58°N. In *G. d. duebeni*: n=8 at 70°N; n=6 at 58°N; and n=8 at 53°N. In *G. locusta*: n=10 at 53°N; n=18 at 38°N). All values are means± SEM.

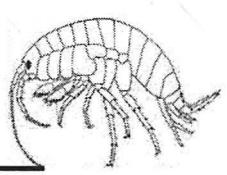


3.4.3 Fractional and absolute rates of protein synthesis

Whole-animal fractional and absolute rates of protein synthesis in the various species and their populations are plotted against latitude and against habitat temperature in Fig. 3.3. In general, whole-animal k_s and A_s values decreased with latitude and temperature. In *G. oceanicus* collected at 5°C (79°N) k_s and A_s values were 12- and 25-times lower than measured in *G. locusta* collected at 21°C (36°N).

Interspecifically, the mid-shore species *G. setosus* exhibited whole-animal k_s values (1.14±0.16(12) % day^{-1}) that were 1.5 fold higher than in *G. oceanicus* (t-test, t=2.3 P=<0.05) at the same latitude (79°N) and temperature (5°C). A_s (0.78±0.24 (12) values were also significantly higher in *G. setosus* (t-test, t=6.7 P=<0.001). In contrast, the upper-shore species *G. d. duebeni* exhibited k_s values that were 4-fold lower (t-test, t=2.2 P=<0.05) and A_s values that were 8.5-fold lower (t-test, t=-3.7 P=<0.05) than the lower-shore species *G. locusta* at the same latitude (53°N) and temperature (13°C). The high-shore species *G. oceanicus* at 69°N also exhibited significantly higher k_s (t-test, t=-6.7 P=<0.001) and A_s values (t-test, t=-5.7 P=<0.001) than *G. d. duebeni* captured at the same latitude.

Intraspecifically, a decrease in k_s and A_s with temperature is reported between latitudinal populations of the subarctic-boreal species *G. oceanicus*. Rates of protein synthesis decreased significantly from 1.96±0.8(10)% day^{-1} at 13°C in the population from Scotland (58°N) to 0.25±0.1(11) % day^{-1} at 5°C in the population from Svalbard (79°N; ANOVA, F=3.7, P>0.05). This marked an 87% decrease in k_s over an 8°C fall in habitat temperature. A_s values also decreased significantly with temperature from 1.6±0.77 mg day^{-1} at 13°C to 0.18±0.07(10)mg day^{-1} at 5°C, marking a 9-fold reduction in synthesis rates (ANOVA, F = 4.41, P<0.05). In contrast, the temperate species, *G. locusta,* showed no significant variation in either whole-animal k_s (t-test,



t= -0.65, P=0.37) or A_s (t-test, t = -0.47, P=8.75) with latitude despite an 8°C difference in capture temperature between the populations from Wales (53°N) and Portugal (38°N). Likewise there was no significant variation in whole-animal k_s in the boreal-temperate species, *G. d. duebeni,* between the 3 latitudinal populations (ANOVA, F =3.1, P=0.069).

When acclimated to a common temperature of 10°C for 4 weeks there was no significant difference in k_s between *G. oceanicus* and *G. d. duebeni* from Tromsø. After acclimation *G. d. duebeni* also showed no significant difference in k_s between populations collected form Tromsø (1.7±0.8(7) % day^{-1}) and Wales (0.9±0.4(6) mg day^{-1}).

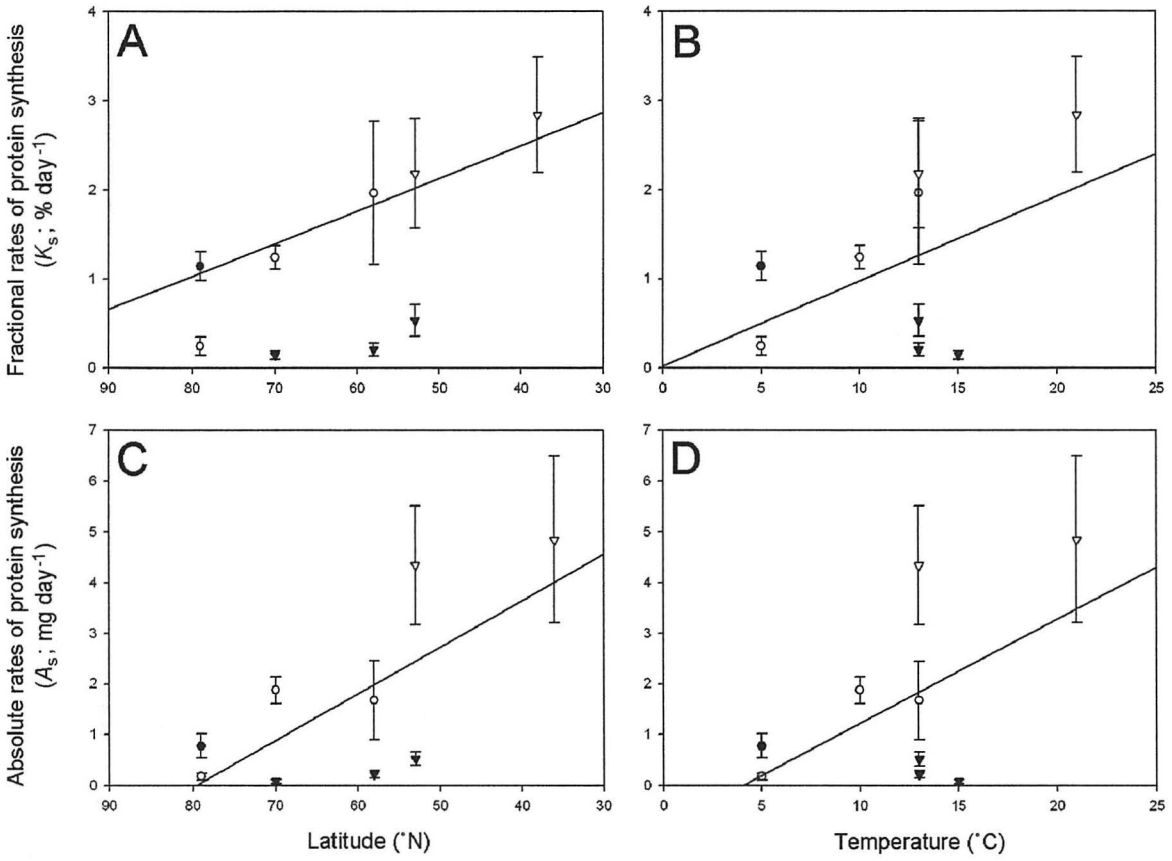
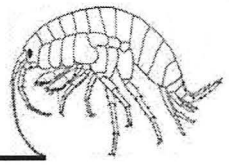


Fig 3.3. Fractional (k_s; % day^{-1}) and absolute (A_s; mg day^{-1}) rates of protein synthesis in populations captured at different latitudes (°N) and temperatures (°C). Species include: *Gammarus setosus* (closed circles); *G. oceanicus* (open circles); *G. d. duebeni* (closed triangles) and *G. locusta* (open triangles). (A) Relationship between fractional rates of protein synthesis and latitude (Y = -0.05X + 3.96; r^2=0.39). (B) Relationship between fractional rates of protein synthesis and habitat temperature (Y = 0.1X + 0.02; r^2=0.23). (C) Relationship between absolute rates of protein synthesis and latitude (Y = -0.09X + 7.32; r^2=0.48). (D) Relationship between absolute rates of protein synthesis and temperature (Y = 0.21X + -0.84; r^2=0.32). Lines fitted using least-squares regression. Mean values given ±SEM. (In *G. setosus*: n=12 at 79°N. In *G. oceanicus*: n= 13 at 79°N; n=11 at 70°N; n=11 at 58°N. In *G. d. duebeni*: n=8 at 70°N; n=6 at 58°N; and n=8 at 53°N. In *G. locusta*: n=8 at 53°N; n=7 at 38°N).



3.4.4 RNA concentrations and activities

In general, RNA activities (K_{RNA}) decreased with temperature between 0.13±0.04(11) mg protein mg RNA^{-1} day^{-1} in *G. oceanicus* captured at 79°N and 5°C, and 2.49±1.02(18) mg protein mg RNA^{-1} day^{-1} measured in *G. locusta* from 38°N and 21°C (Fig.3.4a). Comparisons between species at the same latitude revealed that K_{RNA} was lower in the high-shore species *G. d. duebeni* compared with *G. oceanicus* or *G. locusta* collected from the lower shore at the same latitude, in keeping with low k_s.

Intraspecifically, K_{RNA} values followed a similar pattern to A_S with latitude and temperature. *G. oceanicus* exhibited a 96% decrease in mean K_{RNA} over a 8°C drop in temperature from 1.36±0.58(11) mg protein mg RNA^{-1} day^{-1} at 58°N to 0.1±0.04(11) mg protein mg RNA^{-1} day^{-1} at 79°N (ANOVA, F=4.8, P=0.05). In *G. d.duebeni*, there was a 79% decrease in K_{RNA} from 0.63±0.15(8) to 0.13±0.08(8) mg protein mg RNA^{-1} day^{-1} between populations from 53°N and 69°N (ANOVA, f=4.8, P<0.01). In contrast, there was no change in K_{RNA} between populations of *G .locusta* from Portugal at 38°N (2.43±1.02 (6)mg protein mg RNA^{-1} day^{-1}) and Wales at 53°N (2.29±0.74 (6)mg protein mgRNA^{-1} day^{-1}) despite the 8°C drop in temperature (t-test, t=-0.16, P=0.88).

Overall, RNA concentrations, expressed as RNA:protein ratios, increased in gammarid amphipods with latitude (Fig 3.4b). Intraspecific comparisons showed that *G. d. duebeni* was the only species to show a significant increase in RNA:protein ratios as latitude increased with an 5.2-fold increase in values between 6.37±1.61(8) µg RNA.mg^{-1} protein at 53°N and 33.17±10.91(8) µg mg^{-1} at 69°N. RNA:protein ratios remained unchanged between latitudinal populations of *G. oceanicus* at 79°N (15.5±0.039 (11)µg mg^{-1}), 69°N (7.72±0.15 (11)µg mg^{-1}) and 58°N (14.46±0.58 (10)µg mg^{-1}; ANOVA, F=2.09 P=0.14) and populations of *G. locusta* at 53°N (5.7±1.38 (6)µg mg^{-1};) and 38°N (9.31±1.74 (6)µg mg^{-1}; t-test, t=-1.62, P=0.14).

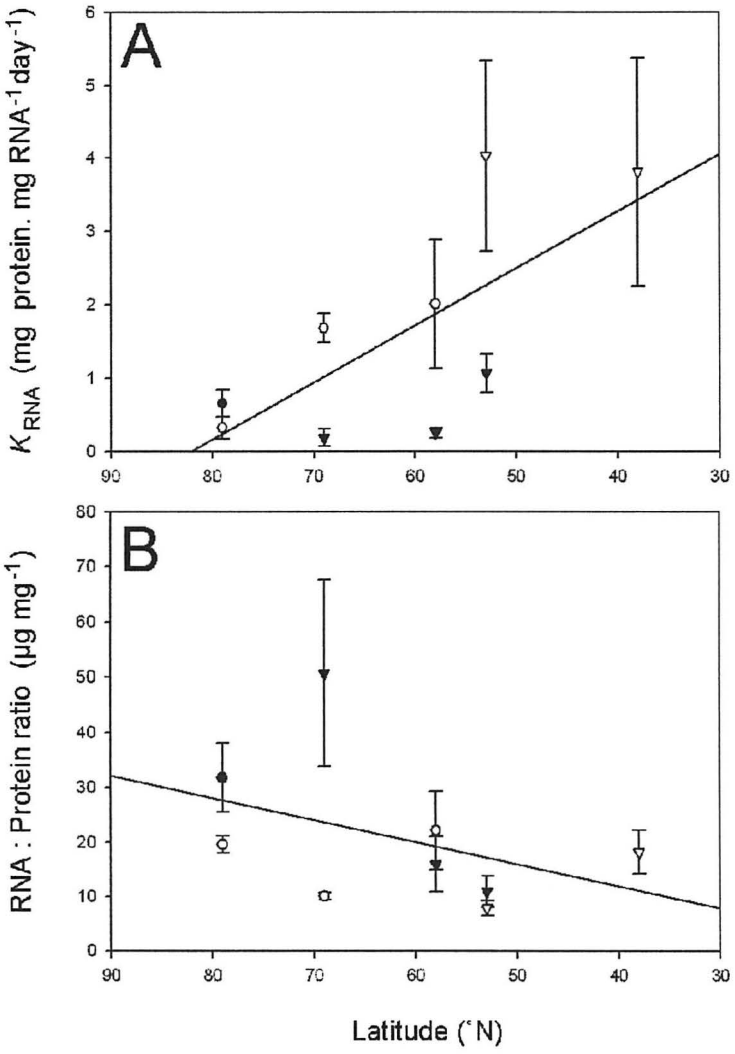
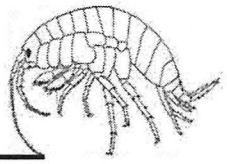


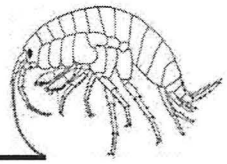
Fig. 3.4. The effect of latitude (°N) on RNA activities (K_{RNA}; mg protein mg^{-1} RNA day^{-1}) and concentrations (RNA: protein; µg mg^{-1}) in: *Gammarus setosus* (closed circles); *G. oceanicus* (open circles); *G. d. duebeni* (closed triangles) and *G. locusta* (open triangles). (A) Relationship between K_{RNA} and latitude (Y = -0.078X + 6.38; r^2= 0.52). (B) Relationship between RNA:protein and latitude (Y = -0.35X + 0.41; r^2= 0.17). Lines fitted using least-squares regression Mean values given ±SEM. (In *G. setosus*: n=12 at 79°N. In *G. oceanicus*: n= 13 at 79°N; n=11 at 70°N; n=11 at 58°N. In *G. d. duebeni*: n=8 at 70°N; n=6 at 58°N; and n=8 at 53°N. In *G. locusta*: n=8 at 53°N; n=7 at 38°N).



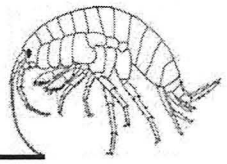
3.5. Discussion

In general, whole body fractional rates of protein synthesis are lower in marine ectotherms from polar latitudes (Fraser & Rogers, 2007; Whiteley & Fraser, 2009). This general pattern is also observed in other marine crustaceans. For instance, the Antarctic isopod, *Glyptonotus antarcticus,* living permanently at 0°C has much lower standardised whole animal fractional rates of protein synthesis at 0.24±0.04% day^{-1} (Whiteley *et al.*, 1996) than the temperate isopod, *Idotea rescata*, at 0.45±0.58 % day^{-1} (Faulkner & Whiteley, 2005) or the tropical prawn, *Macrobrachium rosenbergii,* at 3.7±1.54% day^{-1} acclimated to 30°C (Intanai *et al.*, 2009). In the present study, rates of protein synthesis were higher in populations of the temperate species, *G. locusta,* at 13°C and 21°C then the Arctic species, *G. setosus,* at 5°C. In addition, rates of protein synthesis were also low in the subarctic population of *G. oceanicus* living in Kongsfjorden, Svalbard, compared with the temperate population from Scotland. Not all polar ectotherms exhibit low rates of protein synthesis, fractional rates of protein synthesis during development of the Antarctic sea urchin, *Sterechinus neumayeri*, range from 12 -170% day^{-1} (Place & Manahan, 2007). These values are higher or in the same range as those reported in temperate sea urchins: *Lyechinus pictus,* 14 % day^{-1} at 15°C (Place & Manahan, 2006); *Strongylocentrotus purpuratus*, 26 % day^{-1} at 16°C (Goustin & Wilt, 1981); *Arbacia punctulata*, 46 % day^{-1} at 25°C (Fry & Gross, 1970; reviewed by Place & Manahan, 2007). However, *S. neumayeri* is unusual among polar ectotherms and rates of protein synthesis substantially decrease in most polar species living below 5°C (Houlihan, 1991; Houlihan *et al.*, 1995 ; Carter & Houlihan, 2001; Fraser & Rogers, 2007; Whiteley & Fraser, 2009).

In the laboratory, short-term increases in temperature, not approaching the upper thermal limit, usually result in increased whole animal rates of protein synthesis

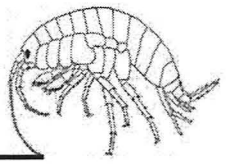


(Whiteley *et al.*, 1996; Robertson *et al.*, 2001a; b; Whiteley *et al.*, 2001). In natural populations, variations in protein synthesis across natural thermal gradients, such as latitude and season, are associated with fluctuations in both temperature and food availability. For example, the temperate mussel *Mytilus edulis* has summer rates that are 3.5-fold higher than winter rates (Hawkins, 1985; Kreeger *et al.*, 1995). The effect of seasonal variations in protein synthesis rates on growth is less clear as protein degradation and protein synthesis retention efficiency (PSRE) also vary noticeably with season. In *M. edulis* rates of protein synthesis were only greater than rates of degradation in June and October, and then only in fed animals (Hawkins, 1985). In a more recent study, the Antarctic limpet, *Nacella concinna*, exhibited winter rates of protein synthesis as low as half that of summer values (Fraser *et al.*, 2002a; b). This was attributed to a tenfold decrease in nutrient consumption (determined from faecal egestion), and not the 2°C difference in temperature between summer and winter. In *N. concinna*, it appears that food availability is the primary factor effecting protein synthesis over temperature, which is likely to be associated with high seasonal variability of primary productivity in both benthic and pelagic Antarctic marine ecosystems (Clarke, 1988; Clarke *et al.*, 1988; Gilbert, 1991; Fraser *et al.*, 2004; Grange *et al.*, 2004). Despite natural variations in nutrient availability and temperature, absolute protein synthesis rates, PSRE and therefore growth does not vary with season in *N. concinna* (Fraser *et al.*, 2007). Although nutrient availability can affect whole animal protein synthesis, microalgae on which gammarids mainly feed were abundant at all of the lower shore sites surveyed in the present study, at least in summer when the measurements were taken. Kongsfjorden, where subarctic *G. oceanicus* and *G. setosus* were collected, also has high summer marine primary productivity (Eilersen *et al.*, 1989). Therefore, at least in the lower-shore species,



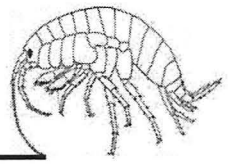
temperature is likely to be the primary factor affecting rates of protein synthesis in the high latitude populations of gammarid amphipods.

Low rates of protein synthesis indicate low energy expenditure which matches the latitudinal patterns in metabolic rate, or energy consumption, outlined for the same gammarid species and populations in Chapter 2. Thus high latitude species, such as *G. setosus* and high latitude populations of *G. oceanicus* are characterised by low rates of energy consumption and expenditure which has been reported in other polar ectotherms (Clarke, 2003; Pörtner *et al.*, 2007) including the amphipod, *Waideckia obesa* (Chapelle & Peck, 1995). A similar response has been reported in other polar invertebrates, such as *N. concinna*, where both rates of oxygen consumption and protein synthesis were considerably lower than temperate species (Bowgen *et al.*, 2007). Low energy consumption and expenditure has been associated with reduced metabolic sensitivities, and high growth efficiencies (Hawkins *et al.*, 1989; Wieser, 1994; Hawkins & Day, 1996; Pörtner *et al.*, 2005). In cold-water, high latitude species, it has been postulated that low rates of energy expenditure lead to lower maintenance costs meaning that a larger proportion of available energy is allocated to growth (Heilmayer *et al.*, 2004; Pörtner *et al.*, 2005). Growth experiments on scallops and eel pout suggest that growth efficiencies are higher in the cold (reviewed by Pörtner *et al.*, 2005). Growth rates depend not only on energy made available for protein synthesis but also on the proportion of synthesised protein degraded or retained. It is possible that in stable polar environments protein degradation and turnover rates are lower than in temperate environments where selection for high protein turnover is associated with climate variability (Pörtner, 2004), allowing, theoretically, more protein to be retained as growth. Although rates of protein degradation and turnover in the current study are not known, rates of protein degradation in polar ectotherms are reported to be high



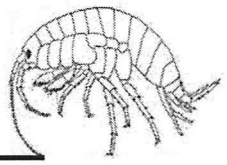
compared to temperate species (Place *et al.*, 2004; Place & Hofmann, 2005). At lower temperatures polar ectotherms are also reported to degrade about twice as much protein as tropical ectotherms, suggesting that protein growth is actually less efficient at higher latitudes (reviewed by Fraser *et al.*, 2007; Whiteley & Fraser, 2009). Indeed, meta-analysis of 12 species of teleost, 2 species of molluscs, and one species of crustacean showed that PSRE generally decreases with temperature (Fraser *et al.*, 2007). Recent measurement of PSRE in the Antarctic limpet, *N. concinna,* revealed extremely low PSRE in summer (15.7% at -0.5°C) and winter (20.6% at -1.6°C) compared to the majority of values reported in non-Antarctic ectotherms were values can be as high as 95% (Houlihan *et al.*, 1995; Fraser *et al.*, 2007). This observation contradicts the previous hypothesis indicating that growth efficiency is limited by temperature in high latitude species/populations. Acclimation studies in the wolfish, *Anarhichas lupus,* acclimated to a range of temperatures between 5 and 14°C, reported increases in growth efficiency with temperature to an optimum whole animal protein growth efficiency (PPV) of 34% and PSRE of 51% between 9 and 10°C, before decreasing as temperatures approached the upper thermal limit. Low growth rates reported in polar marine ectotherms (Peck *et al.*, 1997; Clarke *et al.*, 2004) could therefore be a result of low PSREs as well as low rates of protein synthesis. Low growth rates have also been reported in *G. setosus* and Arctic populations of *G. oceanicus* (Steele & Steele, 1970; 1971).

The low rates of growth and protein synthesis observed in high latitude populations may also be constrained by reduction in energy consumption with temperature depressed metabolic rates. High growth rates, particularly with high rates of protein degradation at lower temperatures, would lead to greater demand on protein synthesis and increases in associated maintenance costs. It has been suggested that

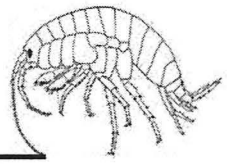


increased metabolic rates within the limited energy budgets of polar environments could lead to fitness costs associated with the divergence of energy away from reproduction (Clarke, 2003). Thus, low rates of growth and development may be beneficial in low energy Arctic environments. Low rates of metabolism and ATP synthesis may also reflect lower demands of protein synthesis in more stenothermal polar environments. For example, the Antarctic gammarid, *Paraceradocus gibber,* has lost the ability to synthesise heat-shock proteins due to its stenothermal environment (Clark, 2008), whereas in more eurythermal temperate ectotherms the synthesis of heat-shock proteins is thought to constitute a substantial metabolic expense (Feder & Hofmann, 1999). Therefore, in temperate less stenothermal environments higher rates of whole animal protein synthesis in populations of *G. oceanicus* may be a result of selection for greater protein turnover enforced by greater climate variability (Pörtner, 2004), as well as a direct effect of higher mean temperatures on rate processes. Higher protein synthesis rates could be a necessity for living in higher temperature more variable temperate environments where higher rates of growth, development and reproduction dominate.

Despite the predictions given above, the most eurythermal species, *G. d. duebeni,* in the present study displayed relatively low rates of protein synthesis. This is unexpected as higher rates of protein synthesis and turnover are associated with an ability to compensate for environmental change, at least in molluscs (Hawkins *et al.*, 1987). *G. d. duebeni* can tolerate the greatest range of temperatures and salinities and is the most robust of the four species included in the present study (Bulnheim, 1979; Gaston & Spicer, 2001) allowing it to survive on the upper shore. Low rates of protein synthesis reported for *G. d. duebeni* are associated with lower juvenile growth rates compared to the lower shore species, *G. locusta,* from the same latitude measured at a

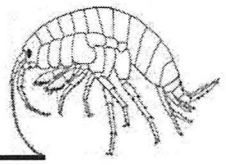


common temperature of 15°C (A. M. Posacka & S.P.S. Rastrick, unpublished data). Slower rates of development are also reported in *G. d. duebeni* compared to other gammarid species over a range of temperatures from -1.5 to 15°C (Steele & Steele, 1973). At 10°C, eggs of *G. duebeni* took 30 days to develop. This time was reduced to 17 days in *G. lawrencianus*, 26 days in *G. obtusatus*, and 25 days in *G. oceanicus*. The only species with longer times for egg development than *G. duebeni*, at any common temperature, was the Arctic species, *G. setosus, at* 35 days at 10°C (Steele & Steele, 1973). These lower rates are partly a result of tradeoffs between energy for growth and increased base line turnover in eurythermal environments (Pörtner *et al.*, 2005), as despite low rates of protein synthesis, selection for a eurythermal environment may still enforce proportionally higher rate of protein turnover. However, excess resource availability may be a precondition for the selection of higher protein synthesis and turnover in eurythermal environments (Mueller & Diamond, 2001). In high-intertidal species such as *G. duebeni* increased risk from desiccation particularly during the summer (when measurements were taken) may limit foraging activity during increased periods of emersion, as shown in other high-shore invertebrates (eg, Little, 1989). This could lead to low rates of protein synthesis due to amino acid limitation (McCarthy et al., 1993; 1994). It is possible that the maintenance of protein synthesis rates could be an important component of the overall energy budget, and divert energy away from the costs associated with ion regulation in this euryhaline environment. Indeed, another high-shore crustacean, *Ligia oceanica,* found at similar latitudes to *G. d. duebeni* also exhibits low rates of protein synthesis of 0.5±0.3 % day^{-1} (Whiteley & Faulkner, 2005), indicating that low rates of synthesis could be characteristic of high intertidal, highly tolerant species. Demonstrating that, high rates of growth and development associated with high rates of protein synthesis are not a

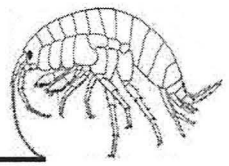


universal feature of eurythermal species. However, further investigation of protein synthesis rates and turnover in other taxa of intertidal invertebrates is required.

As well as having relatively low rates of protein synthesis both *G. d. duebeni* and *L. oceanica* also show physiological compensation for changes in temperature with latitude (Whiteley & Faulkner, 2005). Consequently, rates of protein synthesis are conserved between the various latitudinal populations of *G. d. duebeni* as observed for metabolic rate (Chapter 2). The conservation of protein synthesis rates, and therefore energy expenditure, imparts some partial energetic independence from temperature variation in order to survive a rapidly changing physical environment, as previously described in *Arenicola marina* sampled over a similar latitudinal range (Sommer & Pörtner, 2002). At temperate and tropical latitudes conservation of protein synthesis rate is also reported between different species. Recent comparisons using meta-analysis of seven species of teleost, four species of crustacean and two species of mollusc from polar, temperate and tropical environments demonstrated no significant difference in rates of protein synthesis (approximately 2.7% day^{-1}) between 10 and 30°C, with lower rates observed in polar stenotherms (Fraser & Rogers, 2007). Rates of protein synthesis were also conserved across latitudinal populations in *G. locusta* which inhabits a less variable environment than *G. d duebeni*. In this species, the northerly population of *G. locusta* might conserve higher protein synthesis rates despite the fall in habitat temperature to preserve relatively high rates of growth in order to minimise the time spent as a non-reproductive juvenile exposed to increased risk of predation (Bervan *et al.*, 1979; Lonsdale & Levinton, 1985). The conservation of protein synthesis rates despite the latitudinal drop in temperature may also be related to the increase in protein degradation predicted at lower temperatures (Place *et al.*, 2004; Place & Hofmann, 2005).

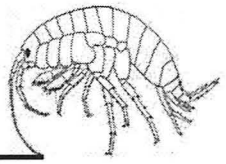


Growth compensation at higher latitudes is supported in a number of marine ectotherms, for example: Atlantic salmon, *Salmo salar* (Nicieza et al., 1994); Atlantic Silversides, *menidia menidia*, (Conover & Present, 1990); Mummichog, *Fundulus heteroclitus* (Schultz *et al.*, 1996; DiMichele & Westerman, 1997); Striped bass, *Morone saxatilis* (Conover *et al.,* 1997); Atlantic halibut, *Hippoglossus hippoglossus* (Jonassen *et al.*, 2000). This normally involves an increased capacity for growth in high latitude populations resulting in higher growth rates than lower latitude populations/species when acclimated to a common temperature. However, when *G. d. duebeni* from Wales (53°N) and Scotland (58°N) were acclimated to a common temperature of 15°C, no variation in growth rate was observed (A. M. Posacka & S.P.S. Rastrick, unpublished data). Steele and Steele (1969) also reported that *G. d. duebeni* would be expected to conserve life-history traits such as development rate and embryo size across its range. This is in keeping with similar rates of protein synthesis reported in the present study between northern (69°N) and southern (53°N) populations of *G. d. duebeni* even when acclimated to a common temperature of 10°C. Suggesting that possibly higher latitude populations exhibit low temperature adaptation, optimising growth rates to the lower environmental temperatures experienced (Levinton, 1983; Levinton & Monahan, 1983; Lonsdale & Levinton, 1985), not by evolving an up-regulation of maximum growth rates at higher-latitudes but by a downward shift in the growth temperature curve and optimum temperature for growth (Yamahira & Conover, 2002). As described by the Local Temperature Adaptation Model for growth reviewed in Chapter 1. A similar shift in R/T curves for metabolism in *G. locusta* and *G. d. duebeni* with latitude is also suggested in Chapter 2. Such a shift in the growth temperature curve without an elevation of growth rates in higher latitude populations is unusual within species and normally only accounts for

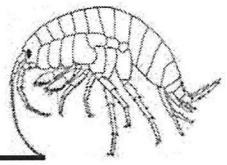


growth compensation between species (Yamahira & Conover, 2002) This may allow *G. d. duebeni* to survive in a highly variably environment, although whether this is adaptive or due to phenotypic plasticity involves further investigation.

Whatever the environmental factors and life-history trade-offs associated with compensation of protein synthesis across a latitudinal thermal gradient, the primary mechanism of compensation appears to involve an upregulation of RNA concentrations in an attempt to compensate for a reduction in RNA activities (K_{RNA}) at lower temperatures (Goolish *et al.*, 1984; Houlihan, 1991; Foster *et al.*, 1992, 1993a; b; McCarthy *et al.*, 1999; Robertson *et al.*, 2001b; Fraser *et al.*, 2002a; Storch *et al.*, 2003; Treberg *et al.*, 2005; Whiteley & Faulkner, 2005; Intanai *et al.,* 2009). Meta-analysis of thirteen latitudinally distinct species of marine teleost, crustaceans and molluscs also show a significant decrease in K_{RNA} and an associated increase in RNA concentration with temperature (McCarthy & Houlihan, 1996; Fraser *et al.*, 2002a). A similar relationship with temperature between whole animal and white muscle RNA concentrations and activities is also reported in the Wolffish, *Anarhichas lupus* (McCarthy *et al.*, 1999). In the present study, *G. d. duebeni* also shows an increase in RNA concentration and decrease in K_{RNA} at higher latitudes. However, not all studies report variations in RNA concentrations with temperature (eg. Storch *et al.*, 2005; Whiteley & Faulkner, 2005). Maintaining high concentrations of RNA at higher latitudes may be energetically expensive (Fraser *et al.*, 2002a) unless elevated RNA concentrations are a result of low rates of RNA turnover and enhanced RNA stability (Storch *et al.*, 2005). The higher energetic costs associated with elevated RNA concentrations may explain the lack of RNA upregulation, to compensate for low K_{RNA}, in energy-limited subarctic populations of *G. oceanicus*. The benefits of not maintaining elevated and costly RNA concentrations may also explain why *G. locusta*

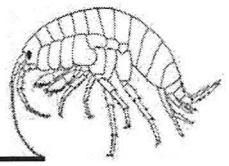


exhibits no variation in RNA between Wales (31°C) and Portugal (21°C). Suggesting that rather than maintaining high levels of the RNA machinery for protein synthesis, *G. locusta* optimises the efficiency of that machinery at lower temperatures, thus conserving rates of protein synthesis though conservation of K_{RNA}. Although never previously reported at the whole animal level, cold compensation of K_{RNA} has been reported at the cellular level in a teleost and a molluscan species (Storch *et al.*, 2003; 2005). The Antarctic scallop, *Adamussium colbecki*, living at 0°C showed similar *in vitro* gill tissue RNA concentrations to the temperate scallop, *Aequipecten opercularis* (25°C), but a nine-fold increase in K_{RNA} indicating cold-adaptation in RNA efficiency (Storch, *et al.*, 2003). In addition, an *in vitro* acclimation study of two eelpout species (Zoarcidae) from Antarctic (*Pachycara brachycephalum*) and temperate (*Zoarces viviparous*) environments, revealed lower activation energies (E_a) of protein synthesis at lower temperatures in temperate but not polar species (Storch *et al.*, 2005). Lower E_a of protein synthesis in response to cold-acclimation has also been demonstrated in eel hepatocytes suggesting the optimisation of enzymes and pathways involved in protein synthesis to lower temperatures (Jankowsky *et al.*, 1981). This is the first time that compensation for K_{RNA} has been reported between natural temperate populations of a marine ectotherm species. Therefore, optimising rates of protein synthesis and K_{RNA} to lower temperatures via reduced E_a and the optimisation of enzymes may be a feature of less stenothermal temperate species such as *G. locusta*. Although the E_a of protein synthesis in *G. locusta* and *G. oceanicus* is unknown, *G. locusta* does exhibit lower E_a of metabolism in the northerly population, whereas *G. oceanicus* does not (Chapter 2). The reduction of E_a via expression of cold-adapted isozymes that counteract restricted enzyme activities at lower temperatures, is well cited in a number of marine ectotherms (reviewed by Hochachka & Somero, 2002). Further study of the



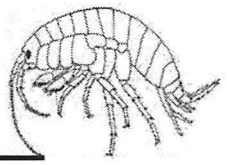
kinetics of key enzymes involved in metabolism and protein synthesis across latitudinal populations of Arctic/boreal and less stenothermal temperate species is required. However, the ability to express functional proteins with differing enzyme activities may depend on rapid temporal variations in temperature rather than progressive changes in temperature over large spatial scales (Rock *et al.,* 2009). It appears that partial independence from environmental temperature can occur in temperate species/populations given sufficient time. Indeed populations of *G. oceanicus* examined in the present study shows less genetic divergence than between the populations of *G. locusta* (Costa *et al.*, 2009).

Ectotherms have the ability to acclimatise or adapt rates of protein synthesis to varying temperature assuming the organism has the phenotypic or genotypic capacity (Houlihan *et al.*, 1993; McCarthy & Houlihan, 1996), however, this may depend on the thermal history of the species. Recent phylogenic study of gammarid species/populations using CO1 revealed that *G. oceanicus* shares a putative phylogroup with other more stenothermal North European species including the Arctic species, *G. wilkizkii*, and the circumpolar species, *G. setosus* (Costa *et al.*, 2009). Conversely, *G. locusta* is more closely related to other warmer water, less stenothermal Mediterranean and Black Sea gammarids (Costa *et al.*, 2009). It is also suggested that variations in the ability to show metabolic compensation, between *G. oceanicus* and *G. locusta* over the same latitudinal range as the present study, may in part be due to variations in ancestral thermal experience (Chapter 2). Perhaps the varying ability of natural populations to conserve rates of protein synthesis across their range is at least in part due to variations in ancestral thermal history and possible associated variations in genotypic capacity.

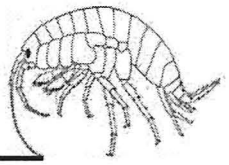


3.5.1 Summary

In summary, there is a fundamental difference in the ability of the subarctic/boreal species, *G. oceanicus,* and the temperate species, *G. locusta,* to conserve rates of protein synthesis across latitudinal thermally distinct populations. *G. oceanicus* shows no compensation of protein synthesis rates across its thermal range, with low rates of protein synthesis in subarctic, low temperature stenothermal populations, leading to low growth and development (Steele & Steele, 1972). Rates of protein synthesis may be constrained by low metabolic rates (Chapter 2), low RNA capacities, and possibly high rates of protein degradation and low PSRE as observed in another polar ectothermic species (Place *et al.*, 2004; Place & Hofmann, 2005; Fraser *et al.*, 2007); as well as the direct effect of temperature on kinetics of protein synthesis. On the contrary, *G. locusta* conserves rates of protein synthesis between populations from Wales (53˚N) and Portugal (38˚N) despite an 8°C difference in habitat temperature. This may allow partial compensation of growth across its range and compensate for possibly higher rates of protein degradation and lower PSRE at lower temperatures (Place *et al.*, 2004; Place & Hofmann, 2005; Fraser *et al.*, 2007). Higher rates of protein synthesis may also reflect a higher rate of protein turnover imposed by increased environmental variability at temperate latitudes (Pörtner, 2004; Pörtner *et al.*, 2005). The conservation of protein synthesis rates in *G. d. duebeni* may occur for different reasons. In this species the level of eurythermy may be important, and this may account for the relatively low rates of-protein synthesis observed in all 3 latitudinal populations. As low rates of protein synthesis may be imposed by restricted resource availability, leading to smaller body sizes and low rates of growth and development (Steele & Steele, 1973; Chapter 2; A. M. Posacka & S.P.S. Rastrick, unpublished data). Protein synthesis is usually conserved by compensating for low

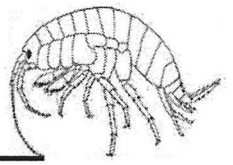


K_{RNA} at lower temperatures by upregulating RNA content (reviewed, Fraser and Rogers, 2007; Whiteley & Fraser, 2008). This response was observed in *G. d. duebeni*, but not in *G. locusta* where K_{RNA} increased instead of RNA:protein in the northern relative to the southern population. Such changes indicate alterations in enzyme activities in the more northerly population. In addition to the variations in thermal range and temperature stability with latitude, the level of genetic divergence between populations and ancestral thermal experience may also be important. Restricted growth and development, and increasing time to reproductive maturity, associated with low rates of protein synthesis, may affect the ability of polar ectotherms to adapt and evolve the long-term variations in temperature predated by climate models. It has also been suggested that low rates of protein synthesis may affect the ability of these polar ectotherms to survive short-term variations in temperature due to a restricted protein turnover. However low rates of protein synthesis measured in the high-shore eurytherm *G. d. dubeni* suggests that restricted protein turnover dues not necessarily restrict thermal-tolerance, although further direct measurement of rates of protein turnover in *G. d. dubeni* and other eurythermal marine ectotherms is required.



Chapter 4

The energetic costs of protein synthesis in, *Gammarus oceanicus*, is not dependent on latitude.



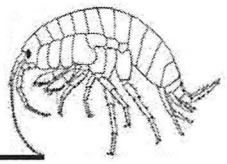
4.1 Abstract

Protein synthesis can account for a substantial portion of basal metabolic rate. The present study is the first to compare costs of whole-animal protein synthesis between latitudinally separated populations of the same species.

Estimates of the energetic costs of protein synthesis vary greatly, possibly due to the species studied, the methodology used, and the lack of detailed validation of inhibitor concentration and incubation time in some previous studies.

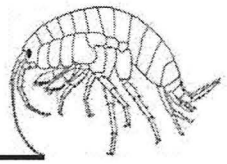
Costs of protein synthesis were determined using an inhibitor method in two populations of *Gammarus oceanicus*; a sub-arctic population from Svalbard (79°N) at 5°C and a temperate population from Scotland (58°N) at 13°C. The inhibitor (cycloheximide) concentration and incubation time was validated using a locally abundant species, *G. marinus*, acclimated to either 10 and 5°C.

Despite variability in whole-animal rates of protein synthesis between populations of *G. oceanicus,* no variations in the energetic costs of protein synthesis were observed, with both populations exhibiting costs of 7 μmol O_2 mg^{-1} close to the theoretical minimum. Therefore, costs of protein synthesis appear to be fixed and independent of temperature, at least within this species. Reported costs of protein synthesis in *G. oceanicus* at both latitudes were low and close to the theoretical minimum, perhaps indicating energy conservation in this Northern European species. Similarly, low costs of protein synthesis were also observed using the correlative method, utilising rates of oxygen uptake and protein synthesis from populations of *G. setosus*, *G. oceanicus*, *G. locusta* determined in Chapters 2 and 3.



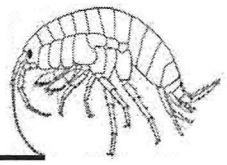
4.2 Introduction

The growth and development of organisms in order to reach reproductive maturity and pass genetic information on to the next generation is one of the major driving forces of evolution (Jobling, 2002). However, little is known about the energetic costs of growth and development and how these may change with environmental temperature. The dominant cost of growth is that of protein synthesis with costs typically within the range of 70-100 mmol ATP g^{-1} compared to lipids at 15-25 mmol ATP g^{-1} and glycogen at 10-12 mmol ATP g^{-1} (Jobling,1985). In reality the costs of protein synthesis associated with growth and development are even higher due to the greater degradation rate and turnover of proteins compared to lipids or carbohydrates (Reeds *et al.*, 1985; Houlihan *et al.*, 1995). Minimum costs of protein synthesis, in a range of endo- and ectotherms, have been estimated to account for 11-42% of total oxygen uptake ($\dot{M}O_2$), which constitutes a significant energetic expense (Houlihan *et al.*, 1995). Despite multiple attempts to estimate costs of protein synthesis, the data cited in the literature are conflicting and vary over an unlikely range (reviewed by Fraser & Rogers, 2007; Bowgen *et al.,* 2007). This is particularly true in polar ectotherms where values vary over two orders of magnitude from 0.92 μmol O_2 mg^{-1} in sea urchin larvae, *Sterechinus neumayeri* (Marsh *et al.*, 2001), to 147.5 μmol O_2 mg^{-1} in the isopod, *Glyptonotus antarcticus* (Whiteley *et al.*, 1996). To date, costs of protein synthesis have been measured in only five polar species, all from Antarctica (Whiteley *et al.*, 1996; Marsh *et al.*, 2001; Storch & Portner, 2003; Pace *et al.*, 2004; Bowgen, *et al.,* 2007) where marine invertebrates have a number of cold-water specialisations that may be unique to the isolated waters of the Southern Ocean



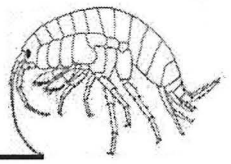
(Pörtner *et al.*, 2007. The present study is the first to determine costs of protein synthesis in a polar population from the northern hemisphere.

It has been suggested that low energetic costs of protein synthesis may be an advantage in low energy polar environments. Pace *et al.* (2004) reported comparatively low costs of 1.9 μmol O_2 mg^{-1} in the Antarctic starfish, *Odontaster vadlidus*. In the sea urchin, *S. neumayeri*, low mean costs of protein synthesis during development may allow rates of protein synthesis to remain high, ranging from 0.5 to 7.1% day^{-1} at -1°C (Place & Manahan, 2007), compared to rates of protein synthesis in warmer temperate sea urchins (Fry & Gross, 1970; Goustin & Wilt, 1981; Place & Manahan, 2007). Conserved rates of protein synthesis at higher latitudes facilitated by low costs, as shown in *S. neumayeri*, may compensate for higher rates of protein degradation (Place *et al.*, 2004; Place & Hofmann, 2005) and slow rates of growth and development normally associated with low temperatures (Peck *et al.*, 1997; Clarke *et al.,* 2004; Barnes *et al.*, 2006; reviewed in Chapter 3). Low energetic costs of protein synthesis, close to the theoretical minimum, are also associated with elevated rates of protein synthesis and growth during the yolk-sac development of the tropical African catfish, *Clarias gariepinus* (Conceicao *et al.*, 1997). Low energetic costs may, therefore, facilitate higher rates of protein synthesis. Conversely, it is possible that high rates of protein synthesis may also lead to lower energetic costs. It has been postulated that costs of protein synthesis may consist of a fixed component independent of synthesis rate and a variable component dependent on synthesis rate (Pannevis & Houlihan, 1992). At low rates the fixed cost dominates and protein is proportionately more energetically expensive to synthesise (Pannevis & Houlihan, 1992; Smith & Houlihan, 1995; Whiteley *et al.* 1996; Pedersen, 1997). Therefore,



lower rates of protein synthesis reported in most polar ectoderms (Whiteley *et al.*, 1996; Robertson *et al.*, 2001a; Fraser *et al.*, 2007; reviewed in Chapter 2) could lead to elevated costs of protein synthesis and in turn growth. Indeed, costs of protein synthesis have been shown to be higher in the Antarctic isopod, *Glyptonotus antarcticus*, at 0°C, compared with the temperate isopod, *Idotea rescata,* at 4°C (Whiteley *et al.,* 1996). However the literature is confusing and more recently, Storch and Pörtner, (2003) have shown that costs of protein synthesis do not vary between the Antarctic and the temperate scallop *Adamussium colbecki* and *Aequipecten opercularis.*

To further investigate whether polar marine ectotherms exhibit higher costs of protein synthesis as a result of lower fractional rates of protein synthesis (k_s) (Whiteley *et al.,* 1996), costs of protein synthesis were determined for a subarctic (79°N) and temperate (58°N) population of *G. oceanicus*, using the inhibitor method. *G. oceanicus* was selected as northern populations acclimatised to 5°C exhibit lower whole animal k_s than southern populations acclimatised to 13°C, due to temperature dependent decreases in RNA activities (discussed in Chapter 3). The present study is the first time that two natural populations of the same species, from different latitudes and acclimatised to different thermal regimes, have been compared.

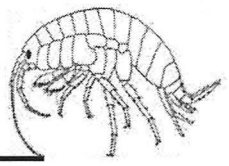


4.3 Materials and methods

4.3.1 Validation of the inhibitor methodology

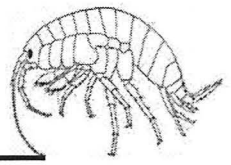
In order to validate the use of cycloheximide for determining costs of protein synthesis in amphipod crustaceans, preliminary experiments were carried out on a locally available gammarid species, *Gammarus marinus*. Two criteria were established: the minimum dose of inhibitor required to significantly inhibit fractional rates of protein synthesis; and the optimal time required for the inhibitor to have an effect at 5 and at 10°C. It was important to carry out a dose response trial as high concentrations of cycloheximide are likely to have secondary effects (Bowgen *et al.*, 2007) while low cycloheximide concentrations can be dissolved directly into saline without the use of ethanol which can have adverse affects. A time course trial was necessary as some authors have reported cycloheximide inhibition to be reversible (Frankel, 1970). For both the dose response and time course trials, rates of oxygen uptake were used as a proxy for rates of protein synthesis. This decision was made as measuring oxygen uptake is non-invasive allowing multiple measurements to be taken from the same individual amphipod. In addition, the measurement of oxygen uptake rates after placebo injections (i.e. saline) can also be used to determine the effects of the injection procedure itself.

G. marinus was collected from the Menai Straits (53.22°N, 4.13°W) and returned to Bangor University where they were acclimated at 5 or 10°C for 4 weeks in fully aerated sea water at 33‰ in a 12L:12D regime. One-third of the sea water was changed every week. After each water change, animals were feed *ad libitum* on *Tetra min* algal flakes (Tetra*Veg*®, Tetra GmbH, Germany). Salinity, temperature, and water quality were monitored at regular intervals.



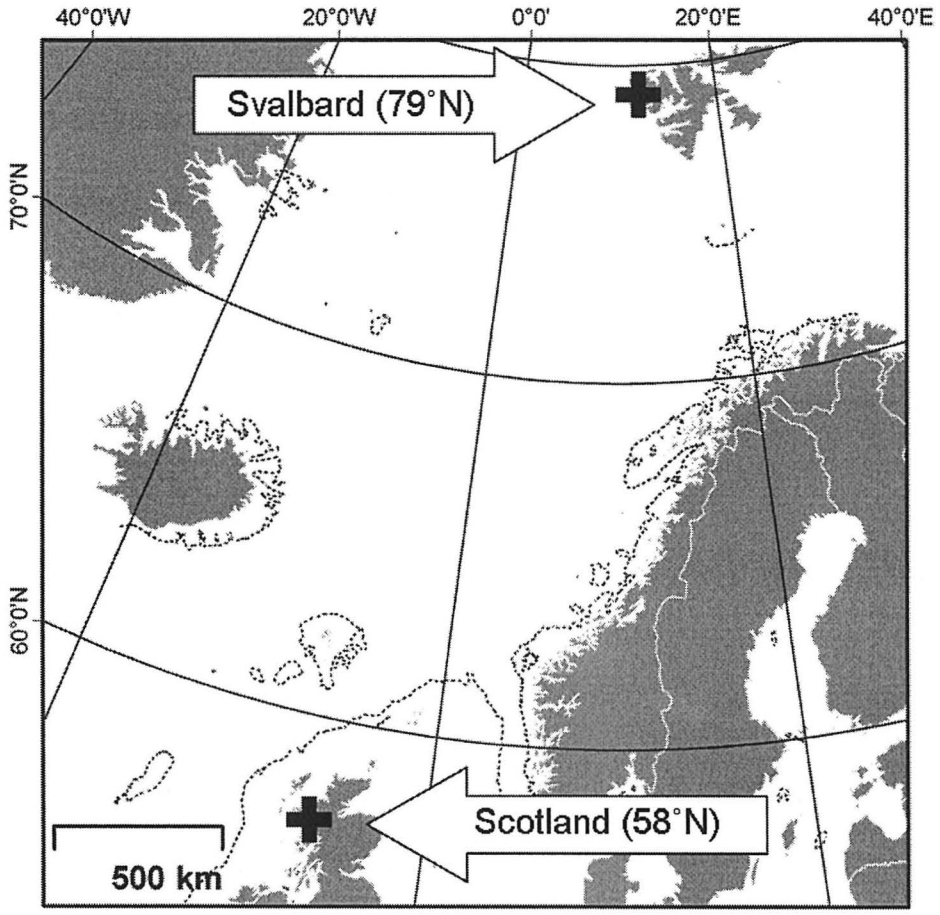
To determine the minimum dose of cycloheximide needed to significantly reduce whole animal rates of oxygen uptake, *G. marinus* acclimated to 10°C were divided into 5 groups. Amphipods in the first group of 13 animals (control animals) were injected with crab saline (Pantin, 1934). Amphipods in the remaining 4 groups of 6-8 animals were either injected with 1, 2, 10 or 20 mmoles cycloheximide in crab saline resulting in tissue concentrations after injection of 0.005, 0.01, 0.05 or 0.1mg cycloheximide g^{-1} fresh mass. Before injection, amphipods were placed into individual stop-flow respirometers maintained at 10°C and allowed to settle in order to determine resting rates of oxygen uptake. Each individual amphipod was then carefully removed from its respirometer and either injected with 2µl of saline or 2 µl diluted cycloheximide per 50 mg fresh mass. Injections were made directly into the bulbus arteriosus of the heart using a micro-droplet manipulation system as described previously (Chapter 3). After injection each animal was returned to its respective respirometer and rates of oxygen uptake measured after 30, 60 and 120 min. For animals injected with saline, 0.005 mg cycloheximide g^{-1} and 0.05 mg cycloheximide g^{-1} further oxygen readings were taken at 180, 240, 300 and 360 min. Rates of oxygen uptake were determined as described in Chapter 2 using stop-flow respirometry and the OxySense®101 Non-invasive Oxygen Analyzer System (OxySense® Inc., Dallas, Texas, USA).

To assess the possible affect of temperature on incubation time, the experiment was repeated with *G. marinus* acclimated to 5°C. In this experiment 6 amphipods were injected with cycloheximide at 0.05 mg g^{-1} and 6 amphipods with crab saline. Rates of oxygen uptake were determined in resting individuals and at 30, 60, 120, 180, 240, 300 and 360 min after injection.

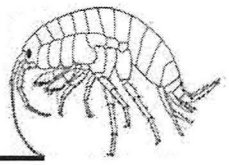


4.3.2 Determination of metabolic costs of protein synthesis by the inhibitor method

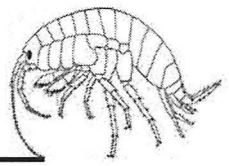
For the determination of metabolic costs of protein synthesis, *G. oceanicus* were collected from a subarctic and a temperate population inhabiting the low intertidal. The subarctic population of *G. oceanicus* was collected from Ny-Ålesund, Svalbard (78.92°N-11.92°E) at a habitat temperature of 5°C. The southern population was collected from Duntum on the Isle of Skye, Scotland (57.66°N-5.33°W; Fig. 4.1) at a habitat temperature of 13°C. All collections were made between July and August 2007. Animals were returned to Bangor University, North Wales, within 24h of collection. During transit animals were maintained between sheets of damp filter paper at the temperature of capture. After transit individuals from each population were maintained in fully aerated sea water at 33‰, in a 12L:12D regime and at their respective capture temperatures of either 5 or 13°C. All animals were feed *ad libitum* on *Tetra min* algal flakes (Tetra*Veg*®, Tetra GmbH, Germany). Costs of protein synthesis were determined within 7 days of capture.



Fig, 4.1. Position of collection sites at the Isle of Skye in Scotland (58°N) at 13°C and Ny-Ålesund in Svalbard (79°N) at 5°C.



To determine costs of protein synthesis in the subarctic population of *G. oceanicus* acclimatised to 5°C and the temperate population acclimatised to 13°C, 7-8 animals from each population were placed into individual stop-flow respirometers maintained at the appropriate temperature and allowed to settle in the apparatus for 4h. After this period, the respirometers were closed for 30 min to determine resting rates of oxygen uptake. Animals were then removed from the respirometers and injected with 10 mmoles cycloheximide at 2µl 50 mg^{-1} fresh mass to give a tissue concentration of 0.05 mg cycloheximide g^{-1} fresh mass as this was the concentration shown to have the most significant effect on $\dot{M}O_2$. In addition, 7-8 individuals from each population were injected with saline to act as controls. After injection, individuals were returned to their respective respirometers and left in flow-through recirculated sea water at the appropriate temperature for 2h (optimal time for cycloheximide inhibition from the validation time course trial). Rates of oxygen uptake were determined 30 min and one h after injection. At the end of the 2h post-injection incubation period, individual amphipods were carefully removed from the respirometers and injected with 2µl 50 mg^{-1} fresh mass of crab saline containing 150 mmol l^{-1} of unlabelled L- phenylalanine and 3.7 MBq ml^{-1} of L- [2,3,4,5,6-^{3}H] phenylalanine (G. E. Healthcare, Specific Activity 4.37 TBq $mmol^{-1}$), as described in Chapter 3. Amphipods were then returned to their respirometers for a further 2 h (4 h after cycloheximide injection) to allow for the incorporation of [^{3}H] phenylanine into proteins (validated in Chapter 3). During this time $\dot{M}O_2$ was determined at 30 min to assess handling stress at 2 h to provide data for the calculation of costs of protein synthesis. After the 4h post-injection period, all animals were sacrificed, frozen in liquid nitrogen and stored at -80°C for the analysis of protein synthesis rates.

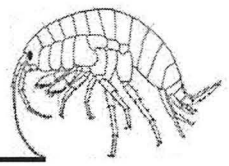


4.3.3 Analysis and calculations

Whole animal fractional rates of protein synthesis were analysed following the procedure described by Garlick *et al.* (1980), as detailed in Chapter 3. Absolute rates of protein synthesis (A_s; mg protein. animal^{-1}. day^{-1}) were determined from fractional rates as outlined in Chapter 3. Whole animal $\dot{M}O_2$ data (µmol O_2. animal^{-1}. h^{-1}) was as described in Chapter 2. As wet mass was significantly higher in the northern population of *G. oceanicus* (t-test, t=8.5, $P< 0.001$) , all MO_2 data was standardised for a fresh mass of 1g using a weight exponent of 0.62 specifically determined for gammarid amphipods (S.P.S.Rastrick and T. Potter, Chapter 2). Absolutes rates of protein synthesis were scaled using a weight exponent of 0.7 (Houlihan *et al.*, 1990). Whole animal energetic costs of protein synthesis (µmol O_2 mg^{-1}protein) were calculated for the subarctic and temperate populations of *G. oceanicus* using a modification of the equation of Bowgen et al (2007):

$$\text{Cost of Protein synthesis} = \frac{\dot{M}O_2^{\text{Saline}} - \dot{M}O_2^{\text{CHX}}}{A_s^{\text{Saline}} - A_s^{\text{CHX}}}$$

Where $\dot{M}O_2^{\text{Saline}}$ is the rate of oxygen uptake in the saline injected animals and $\dot{M}O_2^{\text{CHX}}$ is the rate of oxygen uptake in the animals injected with cycloheximide .The difference between the two represents cycloheximide-sensitive rates of oxygen uptake. A_s^{Saline} is the mean absolute rate of protein synthesis of the saline injected animals and A_s^{CHX} is the absolute rate of synthesis in animals injected with cycloheximide, recoded after 2 h incubation with the labelling cocktail. Costs of protein synthesis in cycloheximide injected individuals were then expressed as µmol ATP mg protein^{-1}, assuming that one mole of oxygen is equivalent to 6 moles of ATP.

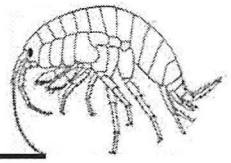


4.3.4 Correlative costs of protein synthesis

Correlative costs were estimated for a range of gammarid species using whole animal standardised (1g fresh mass) resting $\dot{M}O_2$ and absolute rates of protein synthesis (A_s) for *G. setosus* collected at 79°N, *G. oceanicus* from populations living at 79 and 58°N, and *G. locusta* from populations living at 53 and 38°N. $\dot{M}O_2$ data from Chapter 2 was converted to μmol O_2. animal^{-1}. day^{-1} and scaled to 1g fresh mass using the mass exponent of 0.62 and plotted against A_s (mg protein. animal^{-1}. day^{-1}) obtained from the same populations in Chapter 3.

4.3.5 Statistical analysis

All data were tested for normality using Kolmgorov-Smirnov tests and the Levene's tests for homogeneity of variances. As $\dot{M}O_2$ data from *G. marinus* was non-parametric, within treatment comparisons between resting and all post-injection $\dot{M}O_2$ values were made using a Wilcoxon test for related samples. Comparisons between saline and cycloheximide injected *G. marinus* were tested at common incubation times using the Mann-Whitney U test for two independent samples. A_s and MO_2 data for determining costs of protein synthesis in *G. oceanicus* were parametric, and therefore means were compared using an independent sample t-test. The relationship between A_s and $\dot{M}O_2$ used to determine the correlative costs of protein synthesis was analysed using least-squares liner regression. The slope coefficient equalled the estimated costs of protein synthesis expressed as μmol O_2 mg^{-1} protein. All statistical analyses were performed using SPSS software (SPSS INC., Chicago. IL, USA). All values are means±SEM with the number of animals in parentheses.

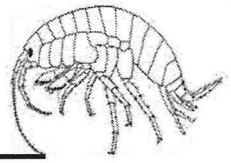


4.4 Results

4.4.1 Validation of inhibitor methodology

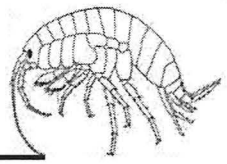
Resting, pre-treatment $\dot{M}O_2$ values in *G. marinus* acclimated to 10°C did not differ significantly between the various groups including the control (Fig. 4.2). Mean resting values ranged from 18.9±3.3(6) μmol O_2. $animal^{-1}$. h^{-1} in animals before injection with cycloheximide at 0.005 mg g^{-1} fresh mass to 32.1±3.3(8) μmol O_2. $animal^{-1}$. h^{-1} in animals before injection with 0.1 mg cycloheximide g^{-1} FW. There was a significant increase in $\dot{M}O_2$ from resting values after 30 min in controls and at each concentration of cycloheximide (Wilcoxon $P<0.05$) with the exception of animals injected with 0.05 mg cycloheximide g^{-1}. In control animals $\dot{M}O_2$ increased from 27.3±2.2(6) μmol O_2. $animal^{-1}$. h^{-1} in resting animals to 43.6±6.7(6) μmol O_2. $animal^{-1}$. h^{-1} at 30 min post saline-injection. $\dot{M}O_2$ returned to resting values after 60 min in all groups with the exception of the group injected with the strongest concentration of cycloheximide at 0.1 mg g^{-1} which remained elevated. $\dot{M}O_2$ in controls remained stable for the remainder of the incubation period being 26.9±2.7(6) μmol O_2. $animal^{-1}$. h^{-1} at 60 min and 19.5±3.0(6) μmol O_2. $animal^{-1}$. h^{-1} at 300 min. In animals injected with 0.05 mg cycloheximide g^{-1}, $\dot{M}O_2$ values decreased below the resting , pre-treatment values at 120 min post-injection and remained lower for the duration of the time cures (Fig. 4.2).

Comparisons between treatment groups at specific time intervals showed that $\dot{M}O_2$ levels were significantly higher in amphipods injected with cycloheximide at 0.1 mg g^{-1} compared to the control group, 60 min after injection (Mann-Whitney U, $P<0.05$; Fig. 4.2). At the lowest tissue concentration of 0.005 mg cycloheximide g^{-1}, $\dot{M}O_2$ did not differ from the control group until 180 min post-injection (Mann-



Whitney U; P <0.05). The effect of cycloheximide on $\dot{M}O_2$ at this concentration was also transitory and by 240 min there was no significant difference between treated and control animals (Fig. 4.2). The cylcoheximide injection of 0.01 mg g^{-1} had no significant effect on $\dot{M}O_2$ when compared to the control group at any time point. At the higher dose of 0.05 mg g^{-1}, $\dot{M}O_2$ values were significantly lower than controls at 60 min post-injection (Mann-Whitney U, P<0.05) and continued to remain significantly lower after 120 min (Mann-Whitney U, P<0.0001) and 180 min (Mann-Whitney U, P<0.05). Consequently, a cycloheximide dose of 0.05 mg g^{-1}fresh mass was chosen for the determination of metabolic costs because this was the minimum dose necessary to significantly reduce $\dot{M}O_2$ for the period necessary for radiolabelled amino acid incorporation.

In *G. marinus* acclimated to 5°C there was no significant difference in resting $\dot{M}O_2$ between the treatment group at 20.5±2.4(6) µmol O_2. $animal^{-1}$. h^{-1} and control group at 22.6±2.4(6) µmol O_2. $animal^{-1}$. h^{-1} (Mann-Whitney U, P=0.26). At 5°C, $\dot{M}O_2$ increased significantly above resting values to 22.6±3.1(6) µmol O_2. $animal^{-1}$. h^{-1} in animals injected with 0.05 mg cycloheximide g^{-1} fresh mass (Wilcoxon, P<0.05) and 34.1±2.5(6) µmol O_2. $animal^{-1}$. h^{-1} (Wilcoxon, P<0.05) in controls, 30min after injection. When compared to controls, a dose of 0.05 mg cycloheximide g^{-1}fresh mass significantly reduced $\dot{M}O_2$ to 14.2±0.9(6) µmol O_2. $animal^{-1}$. h^{-1} after 60 min at 5°C. This effect was persistent and at 360 min $\dot{M}O_2$ was still significantly lower at 17.3±6.6(6) µmol O_2. $animal^{-1}$. h^{-1} than mean $\dot{M}O_2$ in the control group at 31.1±2.0(6) µmol O_2. $animal^{-1}$. h^{-1} (Mann-Whitney U, P<0.001. Fig. 4.3). Overall, acclimation temperature had no effect on the time required for cycloheximide to take affect. Therefore, an incubation time of 120 min was deemed to be an appropriate



time for the inhibition of protein synthesis by cycloheximide to take fall affect, at both 5 and 10°C. After this incubation period of 2h animals were injected with the radiolabel amino acid and incubated for a further 2h before being sacrificed, resulting in a total incubation time of 4h. This time interval had the added advantage of allowing amphipods to recover from handling and injection stress before the second injection to determine rates of protein synthesis.

4.4.2 Energetic costs of protein synthesis - correlative approach

The relationship between whole-animal $\dot{M}O_2$ and A_s in a range of gammarid species is given in Fig. 4.4. There was a significant linear relationship between the two variables ($r^2 = 0.65$, ANOVA, $P<0.05$). The slope of the relationship gives an estimated correlated cost of 5.9 μmol O_2 mg $^{-1}$ protein synthesised or 35.4μmolATP mg protein^{-1}.

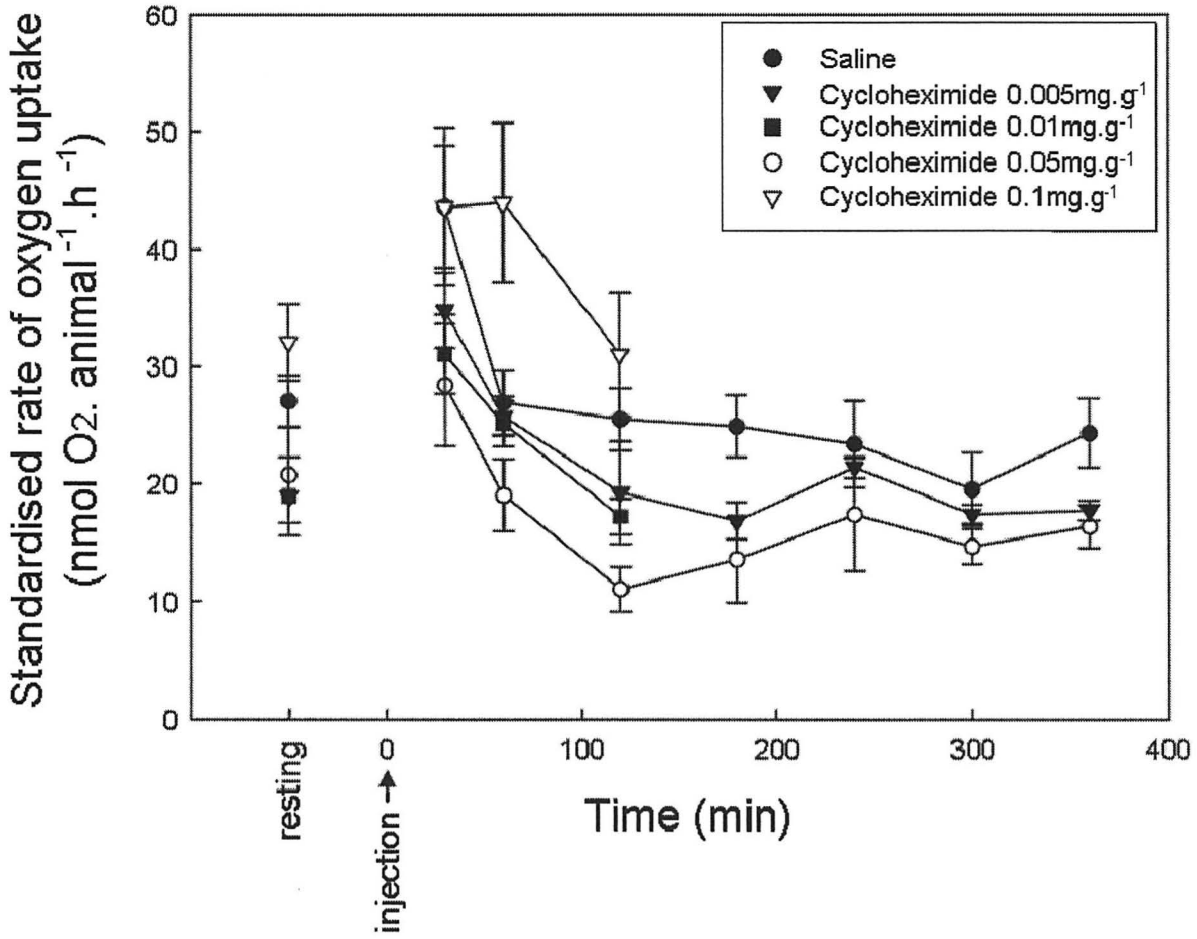


Figure 4.2. The effects of various concentrations of cycloheximide on whole animal rates of oxygen uptake ($\dot{M}O_2$), standardised for a body mass of 1mg, in *G. marinus* over an incubation period of 360 min. Control animals were injected with saline. Resting values represent $\dot{M}O_2$ values from animals allowed to settle in the respirometers for 4h before injection with either cycloheximide or saline. All values are means ± SEM. (In saline controls n= 13; at 0.01 mg cycloheximide g^{-1} fresh mass, n=8; at 0.05 mg cycloheximide g^{-1} fresh mass, n=6; at 0.005 mg cycloheximide g^{-1} fresh mass, n=6; at 0.05 mg cycloheximide g^{-1} fresh mass, n=6).

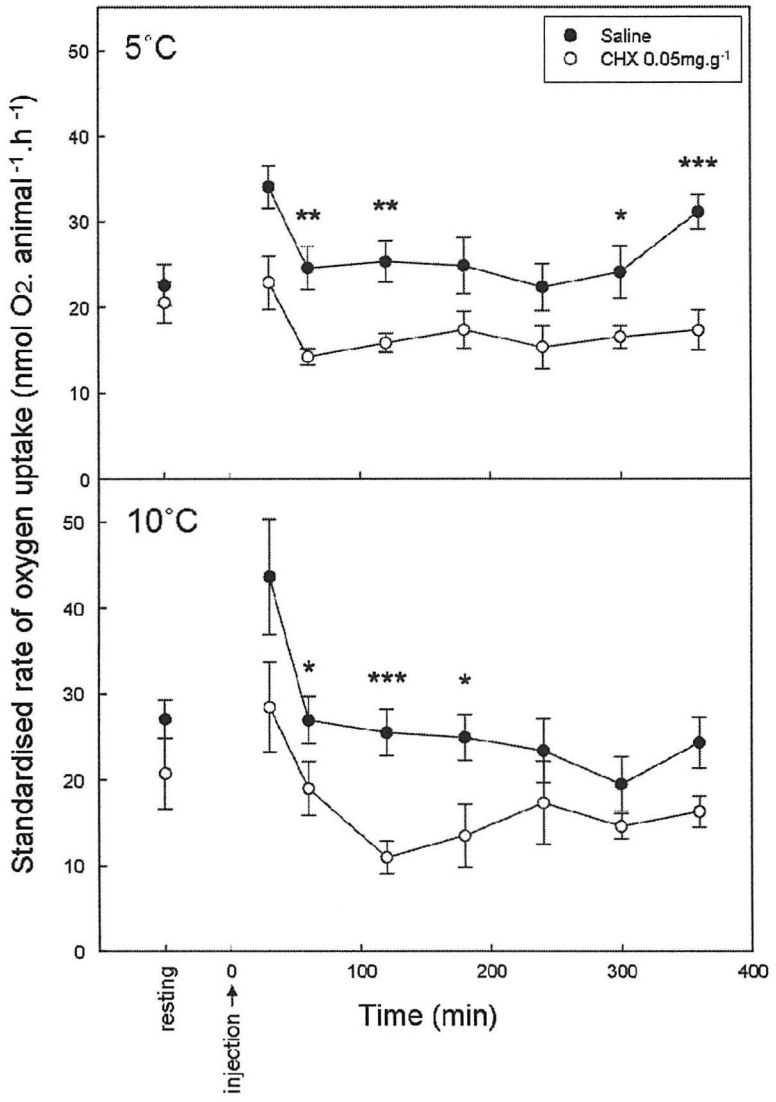


Figure 4.3. Whole animal rates of oxygen uptake, standardised for a body mass of 1mg, in *G. marinus* injected with either saline (controls) or cycloheximide at a dose of 0.05 mg g^{-1} fresh mass in animals acclimated to 5 or 10°C. Resting values refer to $\dot{M}O_2$ measured pre-injection. All values are means ± SEM (at 10°C, saline n=13, 0.05 mg cycloheximide g^{-1} fresh mass n=6; at 5°C, saline n=6, 0.05 mg cycloheximide g^{-1} fresh mass n=6). Any statistical differences between groups are represented by: *= $P<0.05$, **= $P<0.01$, ***= $P<0.001$ (Mann-Whitney U).

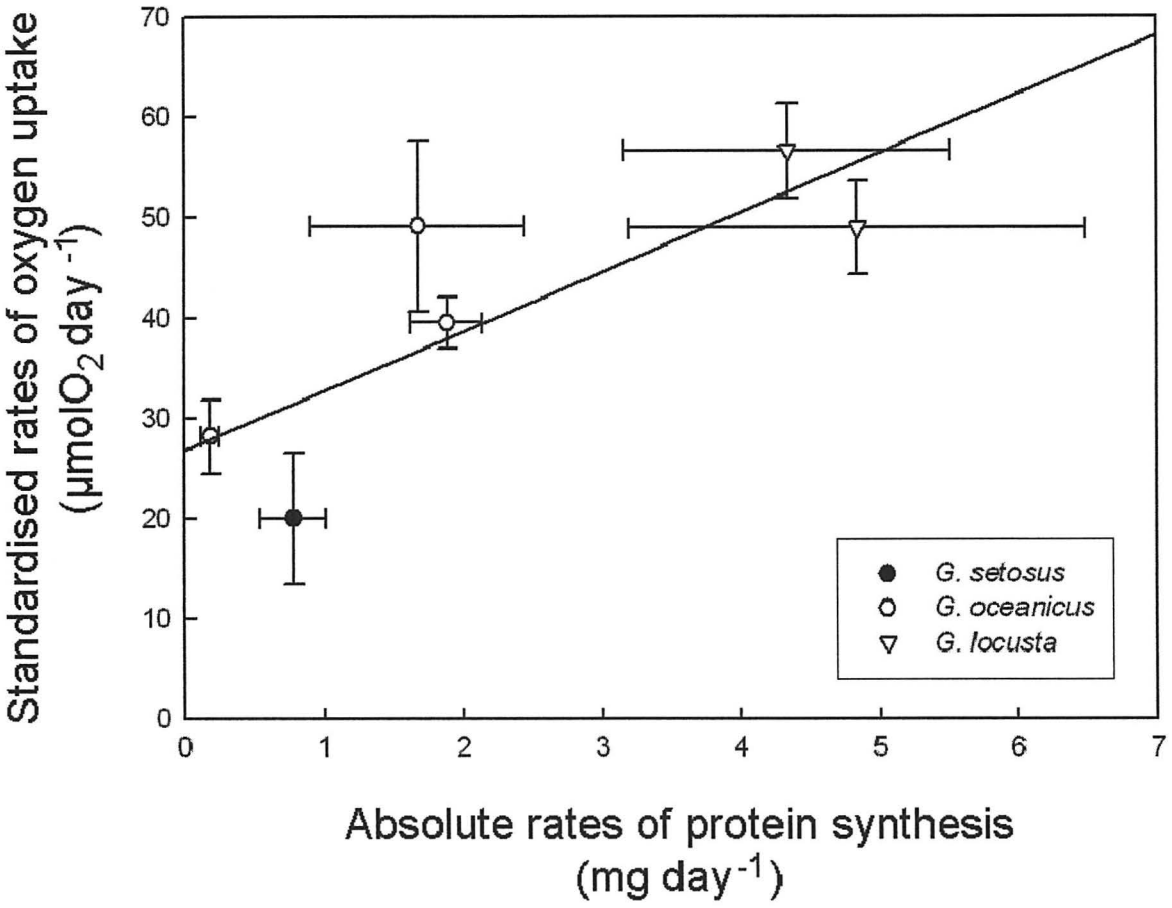
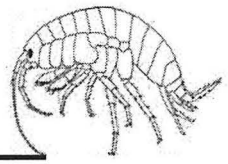


Figure 4.4. The correlation between whole animal rates of oxygen uptake standardised for a body mass of 1mg and expressed as µmol O_2 per animal per day and whole animal rates of absolute protein synthesis expressed as mg protein synthesised per day. All data standardised to a wet body mass of 1g. Mean values given ±SEM (In *G. setosus*: $\dot{M}O_2$ n=6, A_s n=12. In *G. oceanicus*: $\dot{M}O_2$ n=15, A_s n=11 at 79°N; $\dot{M}O_2$ n=12, A_s n=11 at 70°N; and $\dot{M}O_2$ n=17, A_s n=10 at 58°N. In *G. locusta* $\dot{M}O_2$ n=18, A_s n=6 at 53°N; and $\dot{M}O_2$ n=9, A_s n=6 at 38°N). Line fitted using lest-squares liner regression, Y= 5.9X + 26.9 (r^2=0.64).

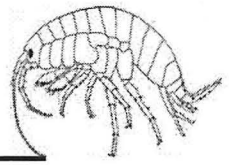


4.4.3 Energetic costs of protein synthesis- direct approach

After 4h incubation at a cycloheximide dose of 0.05 mg g^{-1} fresh mass and 2h with the radiolabel, mean $\dot{M}O_2$ and A_s were significantly lower than controls in *G. oceanicus* acclimatised to both 5°C (t-test, $P<0.05$) and 13°C (t-test, $P<0.001$) (Table 1). In the subarctic population $\dot{M}O_2$ was 25% lower in the treatment group versus the controls, and in the temperate population $\dot{M}O_2$ was 26% lower. Cycloheximide inhibited As means by 91% in the subarctic population and by 89% in the temperate population (t-test, $P<0.05$). Energetic costs of protein synthesis were relatively low in both populations of *G. oceanicus* at around 7 µmol O_2 mg $protein^{-1}$ (Table 4.1), and not significantly different (t-test, $P=0.89$). Costs of protein synthesis expressed as ATP equivalents were 40.4±5.7(7) µmol ATP mg $protein^{-1}$ in the subarctic population at 5°C, and 42.1±12.4(8) µmol ATP mg $protein^{-1}$ in the temperate population at 13°C.

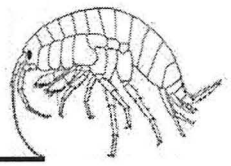
Table 4.1. Effect of saline and cycloheximide on whole-animal rates of oxygen uptake and absolute rates of protein synthesis in two latitudinal populations of *G. oceanicus*. Costs of protein synthesis were calculated as described in the text. All values are means±SEM with the number of animals in parentheses.

Latitude	Temperature	Variable	Saline	[Cycloheximide] (0.05 mg g^{-1} FW)	Costs (µmol O_2 mg $protein^{-1}$)
79°N	5°C	$\dot{M}O_2$ (µmol O_2. $animal^{-1}$.day^{-1})	36.89±1.87(7)	27.79±1.28(7)*	6.74±0.94
79°N	5°C	A_s (mg protein. $animal^{-1}$. day^{-1})	1.48±0.45(7)	0.13±0.02(7)*	
58°N	13°C	$\dot{M}O_2$ (µmol O_2. $animal^{-1}$.day^{-1})	33.93±1.07(8)	24.99±1.80(8)**	7.01±1.87
58°N	13°C	A_s (mg protein. $animal^{-1}$. day^{-1})	1.50±0.59(8)	0.17±0.06(8)*	

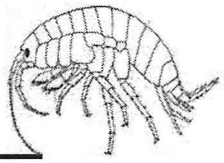


4.5 Discussion

The whole animal costs of protein synthesis obtained in both the subarctic (6.74±0.94μmol O_2 mg^{-1}) and temperate populations (7.01±1.87μmol O_2 mg^{-1}) were close to the theoretical minimum of 8.3 μmol O_2 mg^{-1} (Reeds *et al.*, 1985). Similar low costs of 7 μmol O_2 mg^{-1} are also reported in the Antarctic scallop, *Adamussium colbecki* (Storch & Portner, 2003) and in the Antarctic sea urchin, *Sterechinus neumayeri* (Pace *et al.*, 2004; Place & Manahan, 2007). Low costs of protein synthesis (i.e. costs close to or below the theoretical minimum) may be an advantage in low energy polar environments; perhaps facilitating compensation of protein synthesis rates and so growth/development in low temperature environments, as suggested in *S. neumayeri* (Place & Manahan, 2007). Energetic costs determined using the inhibitor method, however, vary over 3 orders of magnitude in Antarctic species from 0.92 μmol O_2 mg^{-1} in *S. neumayeri* (Marsh *et al.*, 2001), to 147.5 μmol O_2 mg^{-1} in the Antarctic isopod, *Glyptonotus antarcticus* (Whiteley *et al.*, 1996). Costs of protein synthesis in the Antarctic limpet, *Nacella concinna*, are 2- and 3-fold higher than costs shown in the present study, using the inhibitor method (13.95±0.77 μmol O_2 mg^{-1}) and the correlative approach (19.58 μmol O_2 mg^{-1}), respectively (Bowgen *et al.*, 2007). Some variation in the cited values may be due to differences between the correlative or inhibitor approach. Correlative costs of protein synthesis in gammarids estimated in the present study (5.9 μmol O_2 mg^{-1}) were similar to those reported for *G. oceanicus* using the inhibitor method. In theory, correlative costs should be higher than costs measured using the inhibitor method, as correlative costs include RNA synthesis and peptide transport whereas cycloheximide only inhibits peptide bonding (Bowgen *et al.*, 2007). In the limpet, *N. concinna*, correlative costs of

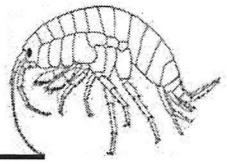


protein synthesis were almost 50% higher than those determined using the inhibitor method (Bowgen *et al.,* 2007). However, this is not always the case as in the sea urchin, *Lytechinus pictus,* correlative costs estimated in starved (13 $\mu molO_2\ mg^{-1}$) and feed (12 $\mu mol\ O_2\ mg^{-1}$) animals were lower than measured costs (20 $\mu molO_2\ mg^{-1}$; Pace & Manahan, 2001). It is not known whether RNA synthesis and/or peptide mobilisation is affected by the inhibition of polypeptide formation. If this is the case then inhibition by cycloheximide could affect the whole process of protein synthesis and account for similarities in estimated costs by both the direct and correlative methods. In the present study the correlative costs were also slightly lower than those estimated by the direct inhibitor method. Although this may be because the A_s and $\dot{M}O_2$ values used in the present correlation were population means from 3 different species and not simultaneous measurements from the same individuals of a common species. Values used in the present study are also from acclimatised animals. Further controlled experiments are needed to confirm whether costs of protein synthesis are likely to differ in animals held under controlled conditions in the laboratory. Some of the variation in energetic costs reported in the literature may indeed be due to differences between the correlative and inhibitor methods, however, values reported using the inhibitor method also appear to vary unrealistically (Bowgen *et al.*, 2007). It is possible that variations in methodology between studies, such as inhibitor concentration (Bowgen *et al.*, 2007), type of inhibitor, or the temperature and time of the incubation period results in the range of observed costs. Validation and standardisation of inhibitor methodology is important for further comparisons between costs of protein synthesis estimated using the inhibitor method.



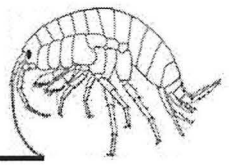
4.5.1 Validation of cycloheximide dose and incubation time

A range of inhibitors have been used to inhibit protein synthesis for a number of reasons including the assessment of energetic costs. For instance: cycloheximide, emetine and puromycin, which inhibit the transfer of RNA during protein synthesis (Yarmolinsky & Haba, 1959; Grollman, 1966); or actiomycin D which inhibits RNA polymerase directed transcription (Kersten *et al.*, 1960; Hurwitz *et al.,* 1962; Hamilton *et al.*, 1963). In each case it is important to validate the inhibitor for the subject species. For example, in the common oat plant, *Avena sativa*, cycloheximide is a more effective protein synthesis inhibitor than pactamycin (Bates & Cleland, 1979), but in the sea slug, *Aplysia*, pactamycin was reported to be more effective than cycloheximide (Schwartz *et al.*, 1971). Anisomycin (Burton, 1992), puromycin (Coleman *et al.*, 1994; Horst, 1989; Kwast & Hand, 1996; Horst, 1990) and cycloheximide (Burton, 1991; Whiteley *et al*, 1996; Pedreira *et al*, 1995; 2003) have all been use to successfully inhibit protein synthesis in crustaceans. Other inhibitors are less effective. For example, actiomycin D has even been shown to stimulate protein synthesis in the crustacean, *Upogebia littoralis* (Pataryas *et al.*, 1972). At lower concentrations actiomycin D does inhibit protein synthesis, but at higher doses it can stimulate the production of some enzymes (Garren *et al.*, 1964; Thompson *et al.,* 1966). Therefore, the inhibition of portion synthesis may vary between different inhibitors and different concentrations. Cycloheximide, as used in the present study, is the most common inhibitor used to investigate costs of protein synthesis (reviewed by Bowgen *et al.,* 2007). However, emetine and anisomycin have also been used and it is possible that variations in the non-specific affects of these inhibitors may explain some of the variation in the reported energetic costs for protein synthesis. In the

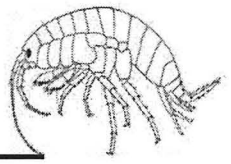


Antarctic sea urchin, *S. neumayeri,* the costs of protein synthesis determined using emetine and Anisomycin (Place & Manahan, 2007) were much lower than the theoretical minimum of 8.3 μmol O_2 mg^{-1} (Reeds *et al.*, 1985). Costs of protein synthesis in *S. neumayeri* were also 6-fold lower than costs of 13.95 μmol O_2 mg^{-1} reported in another Antarctic marine invertebrate, *N. concinna,* using cycloheximide (Bowgen *et al.,* 2007). It is possible that differences in reported costs are partly due to the inhibitor used. Rates of protein synthesis in *S. neumayerii* were also determined using a different label and amino acid, [^{14}C] alanine, instead of [^{3}H] phenylalanine as used in *N. concinna* and in the present study (Bowgen *et al.,* 2007; Place & Manahan, 2007). Therefore, methodologies should be scrutinised and care taken before comparisons of costs of protein synthesis are made between studies.

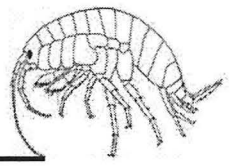
Examination of the concentration of inhibitors injected is also important. The lack of consideration of the optimal concentration needed to inhibit protein synthesis may explain some of the variation in costs of protein synthesis estiamted using the inhibitor method (Bowgen *et al.,* 2007). In crustaceans, previous estimations of protein synthesis costs by means of inhibition with cycloheximide used concentrations as high as 5 mg g^{-1} (Whiteley *et al.*, 1996), which is 100 times greater than the concentration used in the present study. Even though such high concentrations of cycloheximide significantly inhibited both rates of oxygen uptake and protein synthesis in an Antarctic and temperate isopod species it is possible that the high concentration affected other important ATP demanding processes as cycloheximide is known to inhibit a wide range of metabolic processes not necessarily associated with protein synthesis (Ellis & MacDonald, 1970; McMahon, 1975). Cycloheximide may also become unstable in slightly alkaline seawater which is of concern during



determination of costs in marine environments (Schwartz *et al.*, 1971). These secondary effects could cause an over estimation of protein synthesis costs. Indeed, the energetic costs of synthesising proteins in *Glyptonotus antarcticus* is one of the highest recorded to date. Clearly the methodology needs standardisation and validation is needed to confirm concentrations and time courses. In the present study several preliminary trials were carried out in preparation for the determination of metabolic costs in *G. oceanicus*. Due to their abundance, ease of availability and similarities in size, *G. marinus* was collected locally and used for these preliminary experiments. The highest cycloheximide dose of 0.1 mg g^{-1} used in the present study caused an elevation in $\dot{M}O_2$ which remained higher than resting, pre-treatment values for the first 60 min after injection. It appears that the higher cycloheximide tissue concentration of 0.1 mg g^{-1} elevated $\dot{M}O_2$ at a time when control animals showed a decrease from injection disturbance, indicating that the higher concentration had a direct effect on metabolic rate. A similar response was found in the high shore temperate isopod, *Ligia oceanica*, after 3h incubation at a high cycloheximide concentration of 5mg g^{-1} body mass. Extensive experiments were carried out on isopods acclimated to five different temperatures in both winter and summer. In the majority of cases, $\dot{M}O_2$ values increased after the 3h incubation period (L. S. Faulkner & N. M. Whiteley, unpublished observations). In addition, Bowgen *et al.* (2007) reported a weak (r^2=0.2) but significant positive relationship between the concentration of cycloheximide injected and the estimated energetic costs of protein synthesis. *In vitro* studies on cell cultures have also revealed that high concentrations of cycloheximide interfere with cellular metabolism and may lead to spurious results (Wieser & Krumschnabel, 2001). Therefore, as in the present study, it is important to



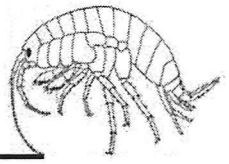
use the lowest concentration possible in order to minimise any nonspecific effects of cycloheximide. In addition, it is only necessary to inhibit enough protein metabolism in order to induce a significant reduction in rates of oxygen uptake and protein synthesis throughout the incubation period (Bowgen *et al.,* 2007). In fact, even studies utilising high concentrations of cycloheximide, such as 5 mg g^{-1}, reduce protein synthesis by 65% (Whiteley *et al.*, 1996), less than the 89-91% inhibition reported in the present study. Cycloheximide concentration may also affect the incubation time needed for the inhibitor to take effect. In the present study, lower concentrations of 0.005 mg cycloheximide g^{-1} had a limited effect and then only after 180 min. Timing of the inhibition effect is therefore crucial. Incubation times between the first cycloheximide injection and the second injection with the radiolabel also vary tremendously, ranging from 7h in *N. concinna*, giving a total incubation time of 9h, (Bowgen *et al.,* 2007) to 2 min in the isopod, *Glyptonotus antarcticus*, resulting in a total incubation time of 2h (Whiteley *et al.*, 1996). In the present study, 0.05 mg cycloheximide g^{-1} had a significant effect after 60 min at both 5 and 10°C, with the initial increase in $\dot{M}O_2$ above resting values being due to the effects of handling stress and the injection procedure. At 5°C the handling stress was less marked, possibly due to temperature-related reductions in biological rate processes including activity rates, and therefore oxygen demand. Significant variations in $\dot{M}O_2$ with time in saline controls and most of the treatment groups as a result of handling stress and the injection procedure makes comparisons of $\dot{M}O_2$ before and after injection unreliable. This also highlights one of the main problems with the inhibitor method as measurements of protein synthesis rates with the flooding dose technique are terminal and therefore repeated measurements of synthesis rates from the same individual are



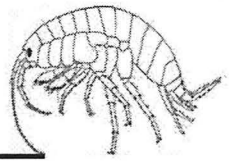
impossible. As a result the inclusion of a saline injected control group is essential. By comparing $\dot{M}O_2$ values between treatment and control groups, it was possible to show that the inhibitory effects of cycloheximide at 0.05 mg g^{-1} fresh mass diminished after 240 min suggesting an ability of *G. oceanicus* to reverse the effects of the inhibitor. Recovery of metabolic rates after cycloheximide administration has been reported in the ciliate, *Terahymena thermophila*, which shows recovery of protein synthesis in 1h and can even become insensitive to a second dose (Frankel, 1970). In contrast, in *G. oceanicus* at 5°C, metabolism of cycloheximide did not occur, at least within 360 min, suggesting that this process takes longer at the lower temperature. Other low temperature experiments at 0 and 3°C in *N. concinna* have also shown no recovery of protein synthesis rates after cycloheximide injection, at least for 24 h (Bowgen, *et al.*, 2007). In the present study a dose of 0.05 mg cycloheximide g^{-1} fresh mass was found to be the minimum concentration required to reduce $\dot{M}O_2$, and therefore protein synthesis, for the 240 min duration of the experiment at both 5 and 10°C.

4.5.2 Energetic costs of protein synthesis

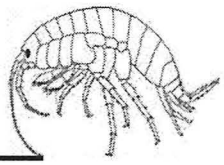
Energetic costs of protein synthesis were observed to be the same in *G. oceanicus* from Ny-Ålesund (79°N) and Scotland (58°N) despite differences in habitat temperature and whole animal acclimatised rates of protein synthesis between the populations (Chapter 3). Previous studies on isolated hepatocytes in rainbow trout, *Oncorhynchus mykiss*, have shown elevated costs of protein synthesis associated with lower fractional rates of protein synthesis (k_s) (Pannevis & Houlihan, 1992; Smith & Houlihan, 1995). In contrast, the similarity in the energetic costs of protein synthesis in *G. oceanicus* suggests that costs of protein synthesis are fixed as described in other



temperate and Antarctic marine invertebrate species (Bowgen *et al.*, 2007; Pace & Manahan, 2001; 2006; 2007). For instance, the Antarctic limpet, *N. concinna*, showed no variation in costs of protein synthesis despite rates varying significantly between 0.91 mg protein day^{-1} at 0°C and 1.16 mg protein day^{-1} at 3°C (Bowgen *et al.*, 2007). In *N. concinna* the proportion of estimated energy ($\%\dot{M}O_2$) allocated for protein synthesis did not vary with season despite a 10-fold decrease in food consumption and associated decrease in k_s during the winter. The temperate sea urchin, *Lytechinus pictus*, also showed no variation in costs of protein synthesis between starved and fed individuals despite associated variations in k_s (Place & Manahan, 2001; 2006). In addition, energetic costs of protein synthesis remain constant despite k_s varying over 2 orders of magnitude between different developmental stages (Place & Manahan, 2006). Developmental stage also has no effect on the energetic costs estimated in the Antarctic sea urchin, *S. neumayeri*, despite a 2-fold difference in rates of protein synthesis (Pace & Manahan, 2007). Bowgen *et al.* (2007) suggest that costs of protein synthesis are fixed as there is no known mechanism for altering the stoichimetry of ATP per peptide bond synthesised, although the authors concede that other costs, such as amino acid transport may vary. Indeed, on average, measured costs of protein synthesis are 5-fold higher than those estimated from the stoichiometery of boned formation, as a result of additional costs associated with RNA synthesis and amino acid transport (Waterlow & Millward, 1989). Although theoretical costs of peptide bond formation would not be expected to vary between populations/species, this may not be the case for additional costs (Waterlow *et al.*, 1978a; b; Reeds *et al.*, 1985). Muller *et al.* (1986) showed that in tumour cells, RNA synthesis accounted for up to 14% of MO2, a significant energetic cost. However, as stated above, more research is



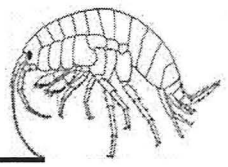
needed to investigate any additional costs that may be inhibited by cycloheximide and whether these additional costs are affected by dose. Translation initiation and termination could also lead to additional costs as might post-translational modification of newly synthesised polypeptide chains, and these costs may very between species (Storch & Pörtner, 2003). Costs of protein synthesis may be more likely to vary between species than between populations due to greater genetic diversity, particularly in highly adapted Antarctic species. Comparisons of anoxia-sensitive and anoxia–tolerant teleost species have shown that physiological adaptation to an energy limited environment can affect the percentage of ATP made available for protein synthesis and therefore possibly costs (Wiser & Krumschnabel, 2001). Indeed, Whiteley *et al.* (1996) demonstrated that energetic costs of protein synthesis were 4-times higher in the Antarctic isopod, *Glyptonotus antarcticus*, at 0°C, compared with the temperate isopod, *Idotea rescata,* at 4°C. It is possible that energetic costs were influenced by the stability of the environment with the Antarctic isopod characterised by low rates of protein synthesis and turnover (Whiteley *et al.*, 1996). More recently, Storch and Pörtner (2003) have shown that costs of protein synthesis did not vary between the Antarctic scallop, *Adamussium colbecki*, at 7 μmol O_2 mg^{-1} and the temperate scallop, *Aequipecten opercularis,* at 9 μmol O_2 mg^{-1}. Although this study was carried out *in vitro* in a cell-free system and therefore excludes additional costs such as amino acid transport that may vary between species. Recent meta-analysis has also reported no relationship between costs of protein synthesis and temperature, suggesting that energetic costs are fixed despite variations in k_s with temperature between polar and temperate species, although the authors concede that some of the



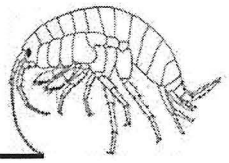
data should be interpreted with caution due to methodological differences between studies (Bowgen *et al.*, 2007).

4.5.3 Summary

The present study is the first to compare costs of whole animal protein synthesis between subarctic and temperate populations of the same species. Despite variations in whole animal acclimatised k_s between populations of *G. oceanicus,* no variations in the energetic costs of protein synthesis were observed. This supports a recent intraspecific study of Antarctic marine ectotherms acclimated to summer and winter temperatures (Bowgen *et al.,* 2007). The finding, however contradicts the hypothesis that costs of protein synthesis consist of a fixed cost of 4 ATP (2ATP and 2 GTP) equivalents per peptide bond (Reeds *et* al., 1985) which is independent of k_s and a variable cost dependent on k_s (Pannevis & Houlihan, 1992). The latter could involve the costs associated with amino acid transport, RNA synthesis, translation initiation and termination, and the post-translational modification of newly synthesised polypeptide chains. The present study suggests these additional costs of protein synthesis are fixed between subarctic and temperate populations of *G. oceanicus*. Previous metabolic observations demonstrated a lack of metabolic diversity between the same populations suggesting there is little evolutionary adaptation to local conditions (Chapter 2). This may explain why the costs of protein synthesis are the same between the two populations. To the contrary, previous whole animal inter-specific comparisons of Antarctic and temperate isopods do suggest that high costs are associated with lower rates of protein synthesis (Whiteley *et al.*, 1996). Such differences may be due to the greater evolutionary distances between species



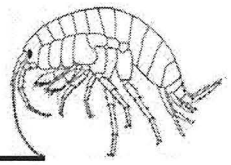
compared to populations, particularly Antarctic species that are often highly adapted and evolutionary isolated from temperate confamilials. However, the data are limited and other studies show no variation in costs between related Antarctic and temperate species (Storch & Pörtner, 2003). Comparisons between studies should also be made with caution due to variations in methodology. For the direct method it is important to appreciate that variations in energetic costs can be brought about by differences in the type of inhibitor used, the dose injected, the temperature and the time the inhibitor is allowed to take affect. A failure of some previous studies to carryout sufficient validation trials may explain some of the variation in costs of protein synthesis in the current literature.



Chapter 5

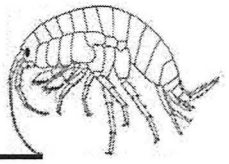
Summarising the physiological evidence:

Ecological relevance



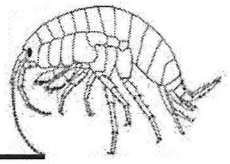
The individual studies on oxygen uptake (Chapter 2) and rates of protein synthesis (Chapter 3) that make up this thesis are intended to increase understanding of the rates of energy consumption and expenditure in an ecologically important group of marine ectothems, the gammarid amphipods. This final Chapter summarises the physiological data presented so far and discusses it in terms of the effects of latitudinal variations in energy consumption on life-history trends in r- and K-selection with latitude and the possible implications of climate change.

The present thesis shows no evidence of MCA in polar species/populations of gammarid amphipods and suggests that mean temperature, as well as other factors such as food availability, environmental stability and selection for life history-traits (e.g. faster growth) may affect metabolic rates with latitude. This is demonstrated by lower metabolic rates in the circumpolar species, *Gammarus setosus*, and the subarctic population of *G. oceanicus*. Low rates of energy consumption have been reported in other polar ectotherms (Clarke, 2003; Pörtner *et al.*, 2007) including the amphipod, *Waideckia obesa* (Chapelle & Peck, 1995). It has previously been suggested that low rates of oxygen uptake and protein synthesis (Chapter 2 and 3) may explain low growth rates reported in the majority of polar ectotherms (Peck, 2002). Particularly when combined with low protein synthesis retention efficiency (PSRE) and elevated protein degradation, associated with higher latitude ectotherms (reviewed by Fraser and Rogers, 2007; Whiteley & Fraser, 2009). Growth and development of organisms in order to reach reproductive maturity and pass genetic information to the next generation is one of the major driving forces of evolution (Jobling, 2002). Protein synthesis is an important determinant as well as the dominant cost of growth (Jobling, 1985), with optimum growth occurring at temperatures

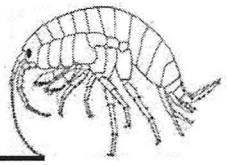


conducive to maximum rates of protein synthesis (Loughna & Goldspink, 1985; Pannevis & Houlihan, 1992; Carter & Houlihan, 2001).

Minimum costs of protein synthesis are estimated to account for 11-42% of total energy consumption, which is a significant energetic expense (Houlihan *et al.*, 1995). It is also possible that lower rates of protein synthesis result in higher costs (Pannevis & Houlihan, 1992), further limiting growth in polar ectotherms. However, the present studies suggest that within species costs may be fixed between polar and temperate ectotherms and independent of temperature, as previously shown in the Antarctic limpet, *Nacella concinna* (Bowgen, *et al.,* 2007), and the temperate sea urchin, *Lytechinus pictus* (Place & Manahan, 2001; 2006). Additional costs of protein synthesis; such as RNA synthesis, peptide transport, the translation initiation and termination of proteins or post-translational modification of newly synthesised polypeptide chains (Waterlow *et al.*, 1978a; b; Reeds *et al.*, 1985; Storch & Portner, 2003); may be more likely to vary between species than populations. Therefore, previous variations in whole animal costs of protein synthesis reported between polar and temperate species such as the Antarctic isopod, *Glyptonotus antarcticus* and the temperate isopod, *Idotea rescata*, may be reliable. Indeed, a previous study of the Antarctic scallop, *Adamussium colbecki*, and the temperate scallop, *Aequipecten opercularis,* suggested fixed costs between species, but in this case the study carried out *in vitro* in a cell-free system and therefore excluded additional costs such as amino acid transport (Storch & Pörtner, 2003). Although variation in costs of protein synthesis may be more likely between species than populations, comparisons drawn from the literature are confused by a lack of consistency in experimental methods (Bowgen *et al.,* 2007).

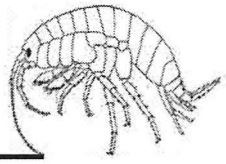


In addition to low growth rates, reduced development rates, delayed maturity, reduced number of larger eggs and reduced reproductive output are also life-history traits associated with high latitude marine invertebrates (Bosch *et al.*, 1987; Clarke, 1987; Arntz *et al.,* 1994) including Antarctic and Arctic amphipods (Steele & Steele, 1970; 1972; 1973; Bregazzi, 1972; Bluhm *et al.*, 2001). These traits show that polar ectotherms are predominantly K-selected compared to less stenothermal temperate species/populations. K-selection is often associated with stable environments, favouring slower rates of development and a greater investment in fewer offspring. Weslawski and Legezynska (2002) concluded that 17 of the 18 Arctic amphipod species they studied were K-strategists. These high-latitude K-selected traits can also be associated with restricted protein synthesis and less efficient protein retention both in general maintenance and growth (examined in Chapter 3) and more specifically in protein synthesis related to gametogenesis and reproduction that is assumed to be equally affected by temperature. Arctic populations (79°N) of *G. oceanicus* also exhibit K-selected traits, such as low reproductive rates, and are limited to only one brood per year (Sainte Marie, 1991; Weslawski & Legezynska, 2002) compared to 3 broods per year at lower latitudes (59°N; Skadsheim, 1984). Longer life-spans of 3-4 years are also reported in Arctic populations compared to only 15 months at more temperate latitudes (Weslawski & Legezynska, 2002; Skadsheim, 1984). Increased longevity in polar ectotherms my be directly related to lower energy consumption and expenditure. Eighty years ago Pearl (1928), and Alpatov and Pearl (1929) first suggested that life span is inversely related to metabolic rate and for almost as long, larger body sizes in polar ectotherms have been associated with low rates of growth sustained over a longer life span (Weymouth *et al.*, 1931). Shorter life spans and



smaller body sizes reported in lower latitude ectotherms may therefore be associated with higher energy consumption and expenditure, and with faster growth and development. Selection for fast development and early reproduction may also result in the divergence of energy towards reproduction soon after maturity, depleting recourses for future growth and survival. Perhaps leading to smaller adult body sizes and increasing adult mortality reinforcing selection for fast development and early reproduction (see Lonsdale & Levinton, 1985). At lower latitudes, selection for higher energy life-histories may lead to body size being constrained by higher energy demands, as above a maximum body size maintenance costs can not be supported by nutrient or oxygen uptake (Lonsdale & Levinton, 1985). Especially as oxygen saturations decrease at lower latitudes due to the effect of temperature on oxygen solubility (Chappelle & Peck, 1999a;b). However, the hypothesis that lower oxygen saturations limit body size remains controversial (eg. Spicer & Gaston, 1999; Woods *et al.*, 2009)

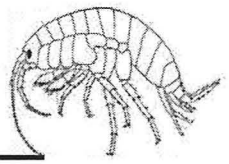
Lower overall energy budgets at higher latitudes may also explain fundamental differences in the ability of sub-arctic/boral species (i.e. *G. oceanicus*) and warm temperate species (i.e. *G. locusta*) to exhibit compensation of energy consumption and expenditure across a latitudinal thermal gradient. Clarke (1991; 1993) suggests that MCA would be unlikely to evolve in energy-limited environments, as a trade-off between metabolism and other energetically demanding processes, such as reproduction, would lead to inherent fitness costs. However, at warmer temperate latitudes where food supply may not be as seasonal, higher energy budgets may allow MCA to evolve. Low energy consumption and energy conservation may also be adaptive in low energy environments, especially in those species/populations reliant



on seasonal primary production, perhaps leading to K-selection for low energy life-history traits (Clarke, 1991; 1993; 2003), reported in polar amphipods (Steele & Steele, 1972; 1973; Weslawski & Legezynska, 2002).

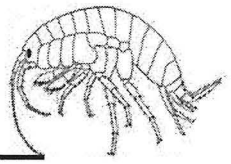
Conversely, r-selected traits, such as higher rates of growth, development and reproduction, associated with southern species/populations of amphipods including *G. oceanicus* (Steele & Steele, 1972; 1973; Weslawski & Legezynska, 2002) may lead to selection for higher rates of energy turnover in higher temperature more eurythermal temperate environments (Pörtner, 2005). As well as, the direct effect of higher mean temperatures on the kinetics of ATP sensitive processes such as protein synthesis. However, the high intertidal eurytherm *G. d. duebeni* only exhibited higher metabolic rates than its low-intertidal congeneric *G. locusta* or *G. oceanicus* at 69°N. As well as showing little variation in energy consumption, *G. d. duebeni* shows no evidence of high energy turnover or expenditure. In fact, it exhibits comparatively low rates of protein synthesis despite its unstable environment. Low rates of protein synthesis are in keeping with low rates of growth and development reported in *G. d. duebeni* (Steele & Steele, 1973; A. M. Posacka & S.P.S. Rastrick, unpublished data). Such K-selected traits are unusual in a eurythermal species inhabiting an unstable environment and may be a result of resource limitation.

Pörtner *et al.* (2005; 2007) suggests that the level of eurythermy at the low temperature extreme of a species range, known as cold-eurythermy, determines the level of MCA. This is supported by MCA reported between terrestrial species of insects (Addo-Bediako, *et al.,* 2002) but not between teleost species from more stable marine environments (Clarke & Johnston, 1999). By determining the level of MCA in high latitude species/population, the degree of cold-eurythermy, determines the level



of ATP available for protein synthesis, (Pörtner *et al.* 2005; 2007). Facilitating intraspecific compensation of protein synthesis and possibly growth in the eurytherm *G. d. duebeni,* despite inhabiting latitudes of 70°N which is well inside the Arctic Circle (Chapter 2).

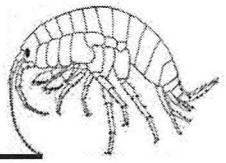
As well as elevated oxygen uptake and ATP availability for protein synthesis, the RNA machinery of protein synthesis is also up regulated in lower temperature populations of *G. d. duebeni* (Chapter 3). This compensatory response in response to temperature dependent decreases in RNA activities (K_{RNA}) is well supported in the literature (Goolish *et al.*, 1984; Houlihan, 1991; Foster *et al.*, 1992; 1993a; b; McCarthy *et al.*, 1999; Robertson *et al.*, 2001b; Fraser *et al.* 2002a; Storch *et al.*, 2003; Treberg *et al.*, 2005; Whiteley & Faulkner, 2005; Intanai *et al.*, 2009). However, compensation of protein synthesis rates reported between latitudinal populations of *G. locusta* is not reliant on variations in RNA concentration but via direct compensation of K_{RNA} (Chapter 3). Direct optimisation of RNA efficiency to varying temperature has only previously been reported *in vitro* in the Antarctic scallop, *Adamussium colbecki* and the temperate eelpout, *Zoarces viviparous* (Storch *et al.*, 2003; 2005). Lower activation energies (E_a) of protein synthesis at lower temperatures in *Z. viviparous* (Storch *et al.*, 2005) indicates the optimisation of enzymes and pathways involved in protein synthesis to lower temperatures (Jankowsky *et al* ., 1981). The optimisation of enzymes to lower temperatures in northern populations of *G. locusta* is also indicated by a reduction in the E_a of metabolism with latitude (Chapter 2). Possible variations in isozyme expression in latitudinally distinct populations of *G. locusta* warrants future investigation. This intraspecific variation may possibly be supported by greater genetic diversity reported between populations of *G. locusta*



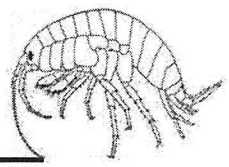
compared to *G .oceanicus* (Costa *et al.*, 2009). Recent phylogenic study of gammarid species/populations using CO1 revealed that *G. oceanicus* and *G. setosus* share a putative phylogroup with other more stenothermal Arctic species, such as *G. wilkizkii*. Whereas, *G. locusta* is more closely related to other warmer water, less stenothermal Mediterranean and Black Sea gammarids (Costa *et al.*, 2009), perhaps suggesting that ancestral thermal history and phylogeny is important in determining energy consumption and expenditure. This may suggest a genetic basis for intraspecific variation in energy consumption, a hypothesis supported in insects (Addo-Bediako, *et al.*, 2002).

The ability of marine ectotherms to adapt or acclimatise to long or short term variations in temperature may depend on variations in rates of energy consumption, perhaps putting polar ectotherms at greater risk from climate change. In the face of future climate change the outlook for ecologically important Arctic-boreal crustaceans, such as *G. setosus* and northern populations of *G. oceanicus,* may be bleak due to reduced aerobic scopes and the inability to tolerate increases in temperature (Chapter 2). Lower rates of protein synthesis may also affect the ability of polar ectotherms to survive climate change (Hawkins, 1991; Reid *et al.*, 1998; Fraser & Rogers, 2007). The ability of polar ectotherms to survive short-term fluctuations in temperature may be affected by restricted protein turnover. However, the present study has demonstrated that species such as *G. d. dubeni* that are extremely tolerant of temperature change may exhibit low rates of protein synthesis and possibly protein turnover.

K-selected traits such as slow growth and development, increasing time to reproductive maturity and lower reproductive output associated with low energy



consumption and expenditure may also affect the ability of marine ectotherms to adapt and evolve to long-term variations in mean temperature. It is also possible that temperate marine ectotherms, such as *G. locusta*, may migrate north as temperatures increase and out-compete Arctic species and populations; due to high rates of activity, growth and development associated elevated rates of ATP and protein synthesis. In addition to a greater capacity for adaptation due to shorter generation times and greater reproductive output supported by MCA. Therefore, not only are polar ectotherms restricted geographically as temperatures increase, but are also restricted physiologically.



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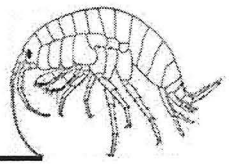

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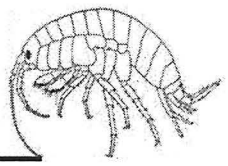

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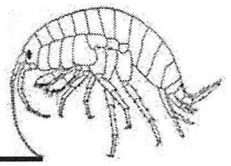

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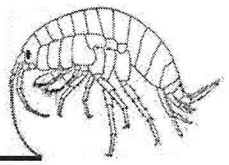

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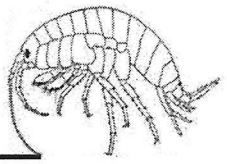

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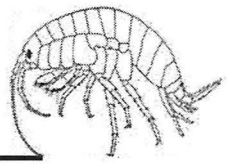

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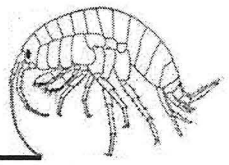

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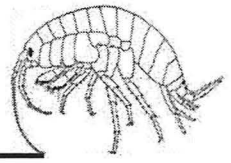

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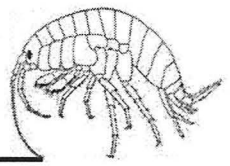

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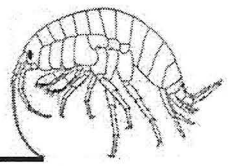

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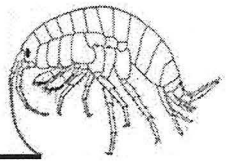

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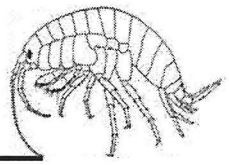

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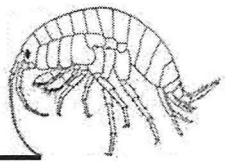

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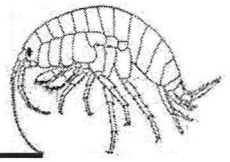

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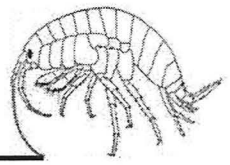

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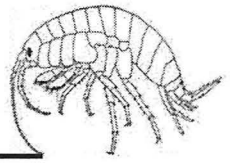

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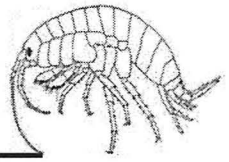

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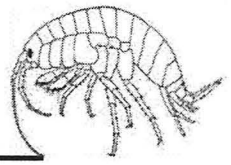

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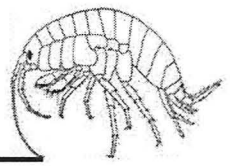

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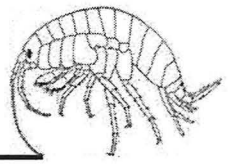

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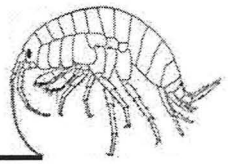

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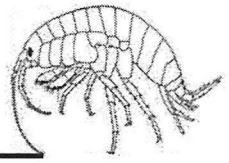

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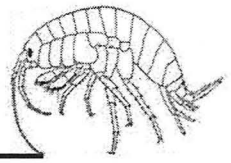

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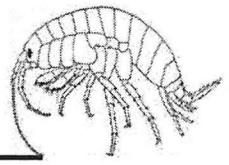

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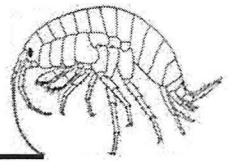

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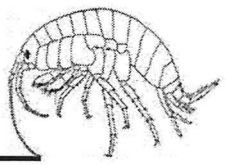

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