Environmental Effects on the Growth, Maturation and Physiology in Antarctic Krill (*Euphausia superba*) Over an Annual Cycle: An Experimental Approach

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-- Declaration --

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-- Abstract --

Antarctic krill, *Euphausia superba*, is a keystone species in the Antarctic ecosystem, being a major food source for most predators and the target of a substantial fishery. Despite being a critical component in the Southern Ocean, limited information exists on krill growth, maturation and physiology under various light, diet and temperature regimes throughout a full year. Without a comprehensive understanding of these factors, forecasting adaptations in a changing environment is hampered. This study examines the effects of the key environmental parameters (light, food availability and temperature) on growth, maturation and physiology in krill.

Krill were incubated for an annual cycle under natural light (emulating the field environment), and constant food supply and temperature. Krill showed a clear seasonal cycle of growth and maturity in all three temperature treatments (-1°C, 1°C, 3°C). Sex significantly affected the relationship with growth over a year. Overall, females showed higher growth rates than males, and growth rapidly decreased after the peak growth period towards the end of spring. Males peaked in growth and matured earlier than females and decreased growth at a considerably slower rate. Negative growth occurred towards the end of January for both sexes, coinciding with the regression of external sexual characteristics. There was a significant decline in intermoult period (IMP) with increasing temperature and some evidence to suggest that 1°C was optimum for krill growth. The IMP was significantly lower at 1°C than at -1°C, but the difference in growth increment (GI) between the two temperatures was not significantly different, with all growth variables significantly lower at 3°C. For the first time, this study has confirmed that compensation mechanisms do exist between IMP and instantaneous growth rate (IGR) for krill, resulting in short IMP/small IGR to long IMP/large IGR.

Based on external sexual characteristics (female – thelycum; male - petasma), krill exposed to a natural Antarctic light cycle or a fixed light/dark regime, progress under a natural maturation cycle of regression and re-maturation. However, when krill were maintained in complete darkness during sexual regression, the rate of regression accelerated and re-maturation occurred three months earlier in the following season. This flexible maturation cycle in response to conditions of total darkness at the time of regression means that krill can flexibly adjust their seasonal physiological cycle. Overall, light (in this case darkness) appears to be one of the most important factors influencing the krill maturation cycle.

There was a strong significant increasing trend of respiration rates in krill with month in all experimental conditions; natural light cycle versus complete darkness, fed versus starved and different temperature regimes (-1°C, 1°C and 3°C). The interaction of treatment with month, as well as generally the main effect of each treatment, was non-significant. Overall, from this study, it appears that light, food availability and temperature may not be the dominant environmental variables influencing the observed seasonal changes in metabolic rates.

There was no significant difference throughout the year (except February) in total lipid and fatty acid content and composition of immature krill, and also between mature males and females in summer. The lipid and fatty acid concentrations were near depletion in February for all krill, indicating these reserves were possibly used for reproductive purposes rather than as an overwintering source. Mated females were only observed at -1°C in November. Lipid and fatty acid levels were lower in mated compared to un-mated females, indicating utilisation of lipids during the mating process. There was no clear temperature effect on lipid and fatty acid content and composition at the various time points sampled; however, krill at the lower temperature, -1°C, generally contained higher lipid and fatty acid content.

This study has provided a solid basis for understanding the life history of krill over an annual cycle, which will enable more robust modelling for accurate assessments and management for the krill fishery. This research has further helped elucidate the effects of key environmental parameters on the growth, maturation and physiology in krill. It is crucial to expand on this knowledge so as to comprehend seasonal adaptation and survival of krill in a changing habitat, in light of predicted climatic change.

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-- Co-Authorship --

Manuscripts representing the majority of this thesis include:

i. **Brown, M**., Kawaguchi, S., Candy, S. and Virtue, P. (2010). Temperature effects on the growth and maturation of Antarctic krill (*Euphausia superba*). *Deep-Sea Research II*. **57**, 672-682. ⟶ **Chapter 2**

ii. **Brown, M**., Kawaguchi, S., King, R., Virtue, P. and. Nicol, S. Flexible adaptation of the seasonal krill maturation cycle in the laboratory. *Submitted to Journal of Plankton Research*. ⟶ **Chapter 3**

iii. **Brown, M**., Kawaguchi, S., Candy, S., Yoshida, T., Virtue, P. and Nicol, S. The long-term effect of photoperiod, temperature and feeding regimes on the respiration rates of Antarctic krill (*Euphausia superba*) in the laboratory. *Submitted to Marine Ecology Progress Series*. ⟶ **Chapter 4**

iv. **Brown, M**., Virtue, P., Nichols, P., Kawaguchi, S. and Nicol, S. Effects of temperature and constant food supply on immature Antarctic krill (*Euphausia superba*) over a full year: Lipid and fatty acid content and composition. *Submitted to Comparative Biochemistry and Physiology, Part B*.
⟶ **Chapter 5**

v. **Brown, M.,** Virtue, P., Nichols, P. and Kawaguchi, S. and Nicol, S. Effects of temperature and constant food supply on lipid and fatty acid content and composition with respect to sex and body tissue of Antarctic krill (*Euphausia superba*) in summer. *Submitted to Comparative Biochemistry and Physiology, Part B*. ⟶ **Chapter 6**

The following specifies contributions of all authors and supervisors to the above listed chapters/manuscripts:

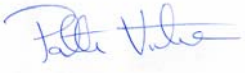
- The concept and design of this thesis was developed by M. Brown and S. Kawaguchi.

- S. Kawaguchi, P. Virtue, P. Nichols and S. Nicol assisted with the general supervision of this thesis. This included experimental design, general advice, interpretation of data, and proof reading and contributing to the above listed chapters/manuscripts.

- All laboratory experiments and measurements in this thesis were conducted by M. Brown. In Chapter 4, some of the laboratory measurements were performed by T. Yoshida. Laboratory assistance was provided by P. Virtue and P. Nichols with lipid and fatty acid analyses in Chapters 5 and 6.

- Statistical analysis in Chapter 2 and 4 was conducted by S. Candy. Statistics in the remainder of the thesis was performed by M. Brown, with advice from S. Candy.

We the undersigned agree with the above stated “proportion of work undertaken” for each of the above submitted peer-reviewed chapters/manuscripts contributing to this thesis.


Supervisor:	Supervisor:	Supervisor:	Supervisor:
Dr. So Kawaguchi	Dr. Patti Virtue	Dr. Peter Nichols	Dr. Steve Nicol

-- Acronyms and Abbreviations --

Definitions of the main acronyms used throughout the thesis:

Acronym	Definition
°C	Degrees Celcius
AA	Arachidonic acid (20:4ω6)
AAD	Australian Antarctic Division
ANOVA	Analysis of variance
BAV	Between-animal variance
CCAMLR	Commission for (and Convention on) the Conservation of Antarctic Marine Living Resources
Chl *a*	Chlorophyll *a*
DAG	Diacylglycerol
DHA	Docosahexaenoic acid (22:6ω3)
DGR	Daily growth rate (mm day^{-1})
DW	Dry weight (mg)
EPA	Eicosapentaenoic acid (20:5ω3)
F	Female
FA	Fatty acid
FAME	Fatty acid methyl esters
g	Gram
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometer
GI	Growth increment in total length (mm)
H1	Holding tank 1
H2	Holding tank 2
HC	Hydrocarbon
hr	Hour
IGR	Instantaneous growth rate (%)
ind	Individual
IMP	Intermoult period (days)
L	Litre
LA	Linoleic acid (18:2ω6)
LMM	Linear mixed model
M	Male
m	Metre
mL	Milli-Litre
month.f	Month as a factor, with a set of integer values ranging between 4-17 (Apr 06 – May 07)
MUFA	Monounsaturated fatty acid
MS	Maturity score
NVE	Night vision equipment
O_2	Oxygen
PL	Polar lipid
PUFA	Polyunsaturated fatty acid
RMT	Rectangular mid-water trawl (net)
RSV	Research and scientific vessel

Acronym	Definition
SD	Standard deviation
SE	Standard error
SFA	Saturated fatty acid
sp.	Species
TAG	Triacylglycerol
TLC-FID	Thin-layer chromatography-flame ionization detector
TL	Total length of the krill (Standard length 1)
TL.f	Total length as a factor, with classes of <31,31-33,33-35, 35-37, 37-39, >39mm
TSE	Total solvent extract
UL	Uropod length (mm)
µL	Micro-Litre
WAV	Within-animal variance
WE	Wax ester

-- Chapter one --

General Introduction

1.1. Overview of Antarctic krill

Euphausiids are small pelagic marine shrimp-like crustaceans that are distributed throughout global oceans. There are 86 known species of euphausiids (Baker *et al*. 1990), seven of which occur in Antarctic waters (Everson 2000). Euphausiids are commonly known as "krill," Norwegian for whale food, but more frequently the name krill is confined to the dominant and most ecologically significant Antarctic species, *Euphausia superba* Dana (Figure 1.1). For this thesis Antarctic krill (*Euphausia superba*) will be referred to as krill.

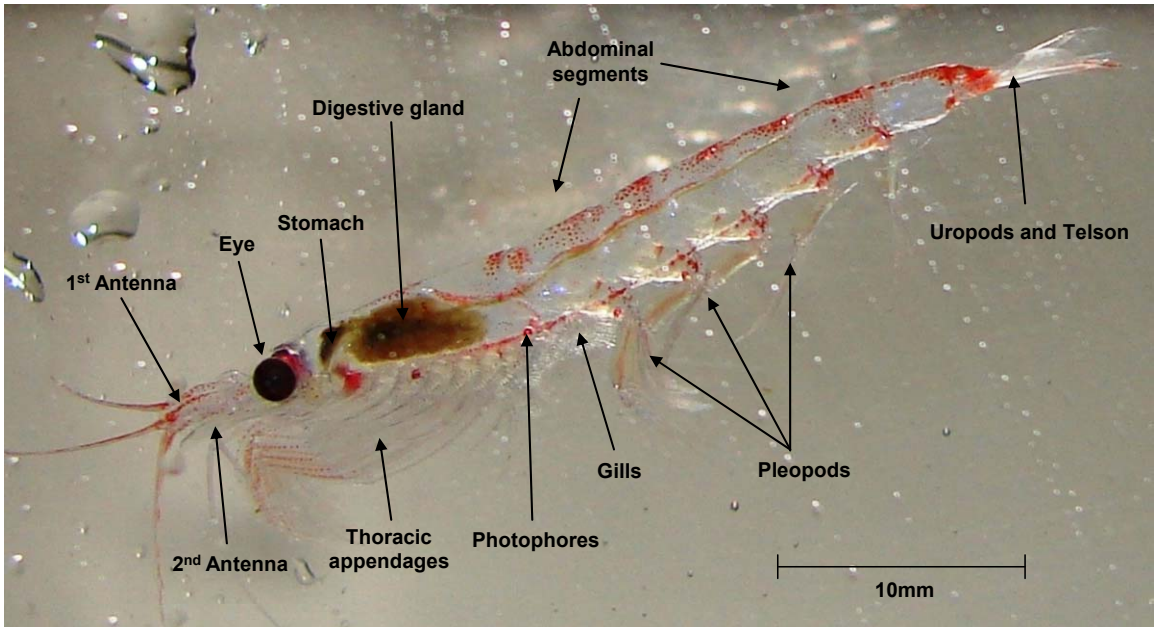


Figure 1.1. The general morphology of Antarctic krill.

Krill is one of the largest species of euphausiids, growing to a maximum of 65mm in length (Nicol and Endo 1999) with a life span of four to seven years (Siegel 2000*a*). Although biomass estimates vary greatly, krill occur in vast numbers and has by far the greatest biomass of all euphausiids. The most recent biomass estimate ranges between 117-379 million tonnes (Atkinson *et al*. 2009), but this calculation still holds uncertainty and possibly underestimates the actual total biomass. Estimating an absolute krill biomass from net samples and acoustics is extremely difficult due

mainly to the seasonal changes in sea-ice concentration, mobility and versatile behaviour of krill, sampling problems, and the overall large area of the Southern Ocean that krill inhabit (Watkins *et al*. 2000; Atkinson *et al*. 2009).

The geographical distribution of krill in the Southern Ocean is circumpolar and is restricted to the extreme north by the Antarctic Polar Front. The distributional range of krill has recently been suggested to span 19 x 10^6 km^2 in the Southern Ocean (Atkinson *et al*. 2009), which is considerably higher than previous estimates; 8 x 10^6 km^2 (Nicol *et al*. 2000) and 12 x 10^6 km^2 (Siegel 2005). Krill distribution is influenced by the annual advance and retreat of sea-ice (Marr 1962; Mackintosh 1972, 1973) and also by large-scale circulatory patterns, such as currents and gyres (Miller and Hampton 1989; Quetin and Ross 1991). Furthermore, the distribution of krill is patchy and is subject to temporal, spatial and inter-annual variability (Everson and Miller 1994; Siegel *et al*. 1997; Murphy *et al*. 1998), with some areas perennially exhibiting large abundances of krill and others much less (Daly and Macaulay 1991). The largest known concentrations of krill are located in the Atlantic sector of the Southern Ocean (Laws 1985; Brierley *et al*. 1997; Murphy *et al*. 1998; Siegel 2000*b;* Atkinson *et al*. 2004; Atkinson *et al*. 2009), with a predicted 70% of total krill stocks concentrated between longitudes 0° and 90°W (Atkinson *et al*. 2008).

Krill is behaviourally complex and for much of their life cycle they swim constantly in the upper 200m of the water column, forming densely packed social aggregations, known as schools (Marr 1962; Mauchline 1980; Hamner *et al*. 1983). These highly polarised schools can consist of densities as high as between 20,000 and 30,000 individuals per cubic metre (Nicol 2006), with school lengths ranging from several metres to several kilometres (Quetin and Ross 1991; Hamner and Hamner 2000).

1.1.1. Food chain

Krill is a keystone species in the Antarctic ecosystem, playing a pivotal and central role in the Southern Ocean food chain (Miller and Hampton 1989; Mangel and Nicol 2000; Alonzo *et al.* 2003*a*) (Figure 1.2). Krill primarily feed on phytoplankton and act as a major link in the transfer of energy between primary producers and higher organisms such as fish, squid, seabirds, penguins, seals and baleen whales (Ikeda and Dixon 1984; Laws 1985; Quetin and Ross 1991; Loeb *et al*. 1997). Most vertebrates in the Antarctic ecosystem are either direct predators of krill, or just one trophic level away from them (Mangel and Nicol 2000). However, this proposed short, simple and linear food chain is an over-simplification, as it fails to take in account the complex interaction of other invertebrates, such as salps and copepods, particularly

in areas of low krill abundance (Clarke 1985). Furthermore, the microbial loop is also considered to be a vital component in the Antarctic food web (Weber and El-Sayed 1987). None the less, much of the energy to upper trophic levels flows through this single species making much of the Southern Ocean a "wasp-waist" ecosystem (Bakun 2006).

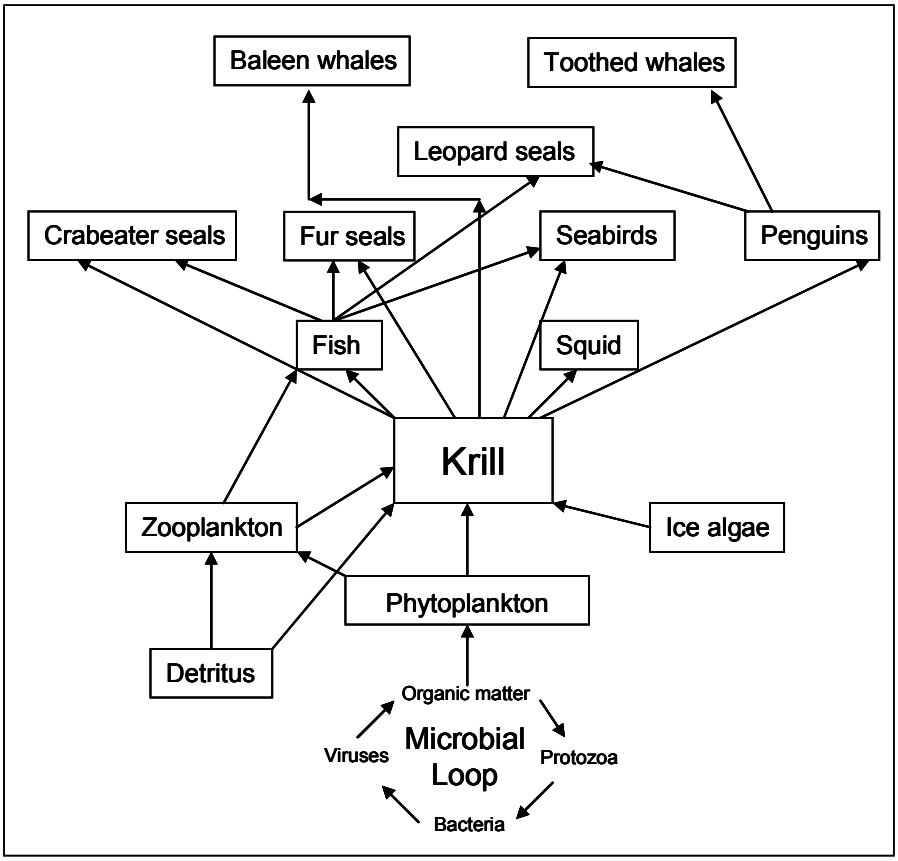


Figure 1.2. A simplified representation of the Southern Ocean food chain showing the central and important role of krill and the major groups of vertebrate predators.

Primary productivity throughout the Southern Ocean has large temporal and spatial variability, and thus, the quality and quantity of phytoplankton are unevenly distributed (Price *et al*. 1988). Even though krill is essentially a herbivore and predominantly filter feeds on phytoplankton (Miller and Hampton 1989), they appear to be able to utilise whatever food is available and have developed a variety of feeding strategies to do so (Quetin and Ross 1991). Krill exploit whatever particulate matter is in the water column including elements of the microbial loop and marine snow (Clarke and Tyler 2008). It is now well established that krill is capable of scraping ice-algae from under the sea-ice (Price *et al*. 1988, Stretch *et al*. 1988), exploit and consume other zooplankton, such as copepods (Price *et al*. 1988; Daly 1990; Huntley *et al*. 1994; Atkinson and Snÿder 1997) and undertake general

omnivorous feeding such as on sea-floor detritus (Kawaguchi *et al*. 1986). There is evidence of krill occupying a epibenthic habitat (Gutt and Siegel 1994) and they have been observed nose-diving into sediment and feeding on sea-floor detritus at depths of 3,500m in Marguerite Bay, west of Antarctic Peninsula (Clarke and Tyler 2008).

1.1.2. Krill fishery and management

Krill is important ecologically in the Antarctic ecosystem, and they are also becoming increasingly important economically with the developing krill fishery (Everson and Goss 1991; Nicol and Endo 1999; Nicol *et al*. 2000*a*). Commercial fishing of krill began in the early 1970s and peaked in 1982 with reports of over 528,000 tonnes harvested, exceeding that of the Antarctic finfish catch (Nicol and Endo 1999). Krill is harvested mainly for human consumption, as feed for aquaculture, sport fishing bait and more recently for pharmaceutical benefits (Nicol *et al*. 2000*a*). As these and other new commercially marketed krill products increase so will the fishing pressure (Nicol and Foster 2003).

With a potentially very large annual catch there was a major concern that harvesting krill in the Southern Ocean might have indirect effects on krill predators (Laws 1985; Miller and Hampton 1989). In response to this pressure, the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) was negotiated in 1980 and came into force in 1982 (Hewitt and Linen low 2000; Kock *et al*. 2007). The aim of CCAMLR is to conserve and regulate harvesting of marine living resources in the Southern Ocean and to protect the diversity and stability of target species, as well as those species that are dependent upon krill (Constable *et al*. 2000; Kock *et al*. 2007). It is fundamental to this process to ensure that harvesting of krill is conducted at a level which can be sustained over a long period of time (Everson and de la Mare 1996).

With high operating costs, lack of demand, and marketing and processing problems, such as rapid spoilage and high fluoride content in the exoskeleton (Nicol and Foster 2003), the development of the krill fishery has been slower than originally anticipated. The annual catch of krill for the last decade has stayed below 120,000 tonnes, all taken from the Atlantic Ocean sector (CCAMLR 2008). This catch level is a small fraction of even the most conservative estimates of the total krill annual production (Hewitt and Linen Low 2000; Nicol and Foster 2003; Kock *et al*. 2007), and well below the current total precautionary catch limit of 6.56 million tonnes in all CCAMLR statistical areas (CCAMLR 2008).

Over-exploitation of krill could have major effects on the whole Antarctic ecosystem and it is therefore imperative to have sound management procedures in

place before the krill fishery expands (Hewitt and Linen low 2000). A key to adequately managing this important and expanding fishery is to understand the life history traits of krill, particularly growth and reproduction (Ross and Quetin 2000), which will be reviewed in sections 1.3 and 1.4, respectively. Despite the recognised importance and extensive research on krill, current knowledge of the ecology and biology is far from complete (Nicol 2003).

1.2. Methodology of past research

In the past, it has been difficult to make direct observations and sample live krill, as they are an open ocean species with an extensive distribution, which is coupled with the Southern Ocean being extremely vast, hostile and complex to work in (Nicol 2003). Furthermore, it is complicated by the inter-annual variability in krill abundance (Brierley *et al*. 1997), and the fact that krill are mobile and are capable of swimming large distances (Kanda *et al*. 1982). Because research ship-time is extremely expensive and logistically complicated it becomes problematic to search and locate krill and to conduct long-term studies. Additionally, the ocean immediately surrounding the Antarctic continent is covered in ice in winter, making it inaccessible and difficult to actually collect and study krill, especially by net, as much of the krill population is probably located under the sea-ice (Ikeda 1987; Siegel *et al.* 1990). Sampling and data collection has therefore been mostly restricted to the summer months and to laboratory studies (Nicol 2000). The advent of newer and much improved ice breaking research vessels ships has allowed greater access to more regions in the Southern Ocean and during different seasons, as well as providing facilities to allow on-board experimentation to improve knowledge of krill biology and behaviour.

1.2.1. Field studies

Much of our previous knowledge on krill has come from demographic studies on preserved specimens obtained from net sampling in the field (Nicol 2000). Even though net collection is the most established field method to sample krill, nets are thought to be selective on various maturity stages and sizes of krill. The degree to which net surveys accurately represent the population remains questionable and are not yet fully resolved (Nicol 2000). Research on krill has also been undertaken from diet samples of predators, but this also has biases that are largely unquantified (Hill *et al*. 1996; Reid 2001). Previous data from net catches has also focussed on actual estimations of distribution and abundance of krill (Nicol 2003). More recently, however, there have been technological advances, such as hydro-acoustic echo-

sounders, which enable the density and vertical extent of krill aggregations to be estimated, and thus, a more accurate biomass prediction over large scales of the Southern Ocean (Nicol 2006). The deployment of nets is still utilised so that species compositions, size, sex, and maturity stage of the specimens can be determined during these surveys.

From preserved krill collected in net surveys, growth rates and subsequent predictions of longevity and population dynamics have been most commonly derived from field based sequential length-frequency analysis on cohorts taken throughout a season (Quetin *et al*. 1994). This is based on estimates of the change in total mean length of the population over time. A length-frequency histogram illustrates the range of size classes within the sampled population graphed against how often this size class appears in the population as a percentage of the entire sample. Peaks in the modal size classes are interpreted as successive age classes. There is a considerable variation when using this technique, as estimating age from modal size groups can easily lead to misinterpretations because of the strongly overlapping size classes of different age groups. Ettershank (1984) also discussed uncertainties with this method and the validity of krill increasing in size with age (age is not clearly related to size) after Ikeda and Dixon (1982) discovered evidence of krill shrinking in captivity. Even though this method has been used consistently and provided substantial knowledge in the past, this approach relies on assumptions that the same population is repeatedly sampled and that mortality is not size dependent, which is difficult to verify in the field (Nicol 2000; Arnold *et al*. 2004). Recovering measurements on exactly the same krill population from one or more occasions is unlikely, due to their high degree of patchiness, and their strong ability to swim and travel large distances (Kanda *et al*. 1982). Also, length-frequency data may not be consistently collected between locations in the Southern Ocean and it is difficult to account for recruitment and mortality. Although length-frequency analysis is still used, and a number of statistical techniques have been developed to identify year classes (i.e. Reid *et al*. 2002), there remain problems with the underlying assumptions.

1.2.2. Laboratory studies

Laboratory studies on krill biology have been intensively undertaken since the 1970s, particularly during the past 30 years at the Australian Antarctic Division, using live krill transported from Antarctica. Krill have been successfully maintained, experimentally examined, and even grown from eggs and larvae, with the data contributing to many remarkable new insights and important outcomes. Ikeda and

Dixon (1982) showed the first evidence that krill undergo shrinkage or negative growth during food deprivation, which was later confirmed by Nicol *et al*. (1992) from the field. Additionally, under laboratory conditions, Ikeda and Thomas (1987) maintained krill for over three years, concluding that krill can potentially live for at least five years in the field and even showed krill living for 11 years in captivity. At the time, this result was highly significant considering the fact that previous length-frequency analysis from the field suggested that krill only lived between two (Ruud 1932; Bargmann 1945; Marr 1962; Mackintosh 1972) and four (Ivanov 1970) years and possibly only spawned once and died. Fecundity estimates have therefore increased substantially now with the hypothesis that spawning can occur more than once with the longer life spans observed in captivity (Quetin *et al*. 1994). Thomas and Ikeda (1987) further demonstrated that female krill underwent a regression of external sexual characteristics after spawning, which was accompanied by negative growth and an increase in intermoult period (a process described further in the next section), in both starved and fed krill.

While it is acknowledged that the natural behaviour of krill is affected by maintenance conditions and can cause some stress, the methods used to maintain live krill in laboratories are now improving significantly with the advent of sophisticated climate-controlled aquaria facilities. Under these conditions, krill can be observed behaving in a reasonably natural fashion exhibiting schooling and successful breeding (Kawaguchi *et al*. 2010). As a consequence, recent laboratory studies are providing valuable information on krill to broaden our knowledge on life history over a full life cycle and to improve our understanding on the fundamental ecological parameters such as longevity, fecundity, recruitment, mortality, maturation, growth and physiology. Overall, it is still difficult to simulate natural conditions and to mimic exact food concentrations and quality available in the field, but important information is still obtained from such captive studies and can be used to validate and test hypotheses derived from field studies.

1.3. Krill growth

Krill possess a rigid and chitinous exoskeleton that provides protection for their soft interior tissues and facilitates basic functions of movement (Horst and Freeman 1993). Like all crustaceans, krill are required to shed this toughened outer layer in order to change size, which is a step-wise process. This periodic shedding of the exoskeleton is termed moulting or ecdysis, a process that has been observed to last between 10 and 20 seconds (Buchholz 1991). Unlike other crustaceans, krill do not have a terminal moult (Rosenberg *et al*. 1986) and can continue to moult

throughout their lifetime. Therefore, adults have the ability to adapt their body size to the changing environmental conditions. The period between successful moults is known as the intermoult period (IMP), which can range from approximately 10 to 40 days (i.e. Buchholz 1991). Temperature is the major influence on the rate of moulting, but food availability, light regime, body length, sex and maturity stage and parasite infection also play a role (Clarke 1976; Murano *et al*. 1979; Poleck and Denys 1982; Morris and Keck 1984; Ikeda *et al*. 1985; Thomas and Ikeda 1987; Buchholz 1991; Quetin *et al*. 1994; Ross *et al*. 2000; Kawaguchi *et al*. 2006; Kawaguchi *et al*. 2007*a*).

Growth rates have been most commonly derived from traditional field based length-frequency distributions (Quetin *et al*. 1994). Growth rates have also been estimated from repeated acoustic surveys (Miller and Hampton 1989; El-Sayed 1994), quantifying DNA:RNA ratios (Shin *et al*. 2003), monitoring changes in size of krill in the diet of predators (Reid 2001) and rearing populations in captive studies (Murano *et al*. 1979; Poleck and Denys 1982; Ikeda and Dixon 1982; Buchholz 1985; Ikeda *et al*. 1985; Ikeda and Thomas 1987; Buchholz 1991). To examine growth in the laboratory, krill need to be maintained for sufficient time to measure both IMP and growth increment (GI) at moult. However, it is difficult to relate laboratory derived growth rates to those derived from field studies. This led to the instantaneous growth rate method (IGR) being developed, which is an alternative approach to overcome problems associated with long-term laboratory studies and the underlying assumptions involved in length-frequency analysis (Quetin and Ross 1991; Nicol *et al*. 1992).

IGR is a laboratory based technique to monitor the change in size of freshly collected animals, by directly measuring growth using the size difference between the post-moult animal and its moulted exoskeleton on short time scales (Quetin and Ross 1991). Krill retain no structural records of growth, and the only indication of previous size is lost with the moulted exoskeleton (Clarke and Morris 1983). The IGR method is now considered as the most reliable representation of *in situ* growth. The IGR technique was later modified by Nicol *et al*. (1992) so as to provide a large amount of data on various sizes of krill from a single population. Although the IGR method was developed for use on research vessels, it can also be used on individual krill in laboratory populations (i.e. Ikeda *et al*. 1985; Buchholz 1991; Buchholz *et al*. 1996). Another advantage of this method is that it can be modelled on an individual level, and thus, growth rates can easily be transferred to other locations and times. This allows assessment of seasonal and regional variations in growth, when growth is directly related to ambient environmental conditions such as temperature, food

availability and size (Ross *et al*. 2000). Recently, statistical models have also been developed using large amounts of IGR data to predict average population growth from various regions of the Southern Ocean (Candy and Kawaguchi 2006; Atkinson *et al*. 2006; Kawaguchi *et al*. 2006; Tarling *et al*. 2006).

Krill growth rates can be determined by the GI at moult and the length of the IMP (Ross *et al*. 2000). Growth rate has been reported to be governed by the nutritional and physiological condition of the krill during the previous moult cycle (Shin 2000). Initially, under IGR experimental conditions, only those krill that moulted within the first five days of capture were used to represent the natural growth rates (Nicol *et al*. 1992). Beyond this time period, krill are affected by the confinement and maintenance conditions causing a decline in growth (Nicol *et al*. 1992; Kawaguchi *et al*. 2006). However, Tarling *et al*. (2006) found that the GI of krill declined immediately and rapidly after capture, particularly when growth was initially high. Tarling *et al*. (2006) therefore concluded that the conditions at time of moulting also influence the GI. Such apparent differences can be investigated by modifying the IGR technique within the laboratory. For example, in order to better understand daily growth rates and IMP, krill can be incubated under IGR conditions in longer term experiments, which is not possible to duplicate in the field.

Daily growth rates (DGR) of krill have recently been estimated by using extensive datasets of field IGR experiments employing two different approaches. One study estimated DGR from IGR data and IMP (Kawaguchi *et al*. 2006). In this study, IMP was predicted from temperature using published data on direct observations of IMP, and IGR was modelled as a function of sex, length, season and region (Kawaguchi *et al*. 2006). This approach allowed the generation of lifetime growth trajectories using seasonal temperature trends to determine IMP and monthly trends in average IGR by length class (Candy and Kawaguchi 2006), but was not able to quantify the direct effect of some seasonal variables that determine growth such as food availability and quality. The other approach estimated IMP from proportions of observed moulting events during IGR experiments using data from individual moults that were classified by sex, maturity status, length and temperature variables (Tarling *et al*. 2006). Combining IGR with predicted IMP, DGR was modelled as a function of length, temperature and food concentration (Atkinson *et al*. 2006). This approach focussed on detailed evaluation of environmental effects on growth including chlorophyll and temperature as well as maturity stages (Atkinson *et al*. 2006), but was not used to generate growth trajectories.

Many experimental studies have related krill growth to environmental factors such as food availability and temperature, as well as examining factors that influence

growth, including IMP and GI. Temperature is the major influence on the rate of moulting and a rise in temperature has resulted in a reduction of the IMP (Hartnoll 2001). Temperature has been used as the common predictor variable of IMP to model seasonal trends in IGR of krill (Kawaguchi *et al*. 2006). However, this approach has been limited by a lack of studies examining growth over the entire year, as assumptions have had to be made concerning the winter period. For example, Candy and Kawaguchi (2006) used growth increments measured from December through to April and Atkinson *et al*. (2006) modelled measurements from January to February. Predicting growth during winter is problematic and data is limited. As a result, the major focus of this thesis was the investigation of the factors that affect krill growth during a full seasonal cycle under controlled experimental conditions.

IMP can also be affected by many other factors other than temperature such as size, sex, maturity and food supply (Kawaguchi *et al*. 2006). Therefore, this study also examined interactions between IGR and IMP with the effects of size, sex and maturity stage on growth, and incorporated these interactions into existing temperature driven models. This undertaking identified key factors that affect moulting and growth. This obtained information made it possible to quantify the degree of compensation at both the between- and within-animal level. Compensation effects of faster moult times corresponding to slower growth increments have been observed for crustaceans in general (Hartnoll 2001), but has never been examined in krill. By understanding the compensation effects of krill, this study will facilitate modelling of growth trajectories in the wild to quantify the distribution of length given age, and overall, enable more accurate, improved and robust model predictions for temperature-dependent growth of krill.

1.4. Maturation and reproduction of krill

Krill have separate sexes which can easily be distinguished in adults by their secondary sexual organs (Everson 2000). In males, the endopodites on the first pair of pleopods are modified to form a copulatory organ, known as the petasma (Appendix A). The petasma is a complex and swollen structure that is used to transfer spermatophores to the female. The external reproductive organ of females is known as the thelycum, which is an external opening to the reproductive tract. The thelycum is a tri-lobed structure that is situated on the ventral surface between the last two pairs of thoracic appendages. The thelycum consists of projections known as holdfasts, which are thought to anchor the spermatophores in the thelycum (Makarov and Denys 1980; Baker *et al*. 1990; Cuzin-Roudy and Amsler 1991; Gibbons *et al*.

1999; Everson 2000). Other external differences between mature males and females include males having smaller cephalothoraxes, and bigger and more robust appendages compared to similarly sized females (Figure 1.3) (Makarov and Denys 1980).

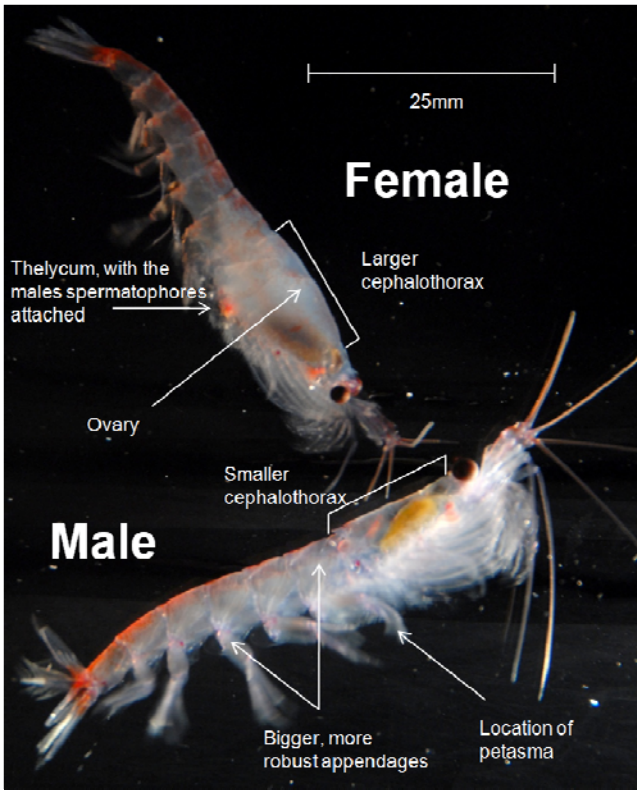


Figure 1.3. External morphology of mature female and male krill. Source of photo: R. King.

Reproduction in krill is an energetically demanding process that is limited to a short period during the Antarctic summer when conditions are favourable. The ovary of female krill generally begins to mature in spring, September to October, which is fuelled by the phytoplankton bloom. During the reproductive summer, krill undergo successive cycles of vitellogenesis, maturation and spawning (Ross and Quetin 2000). The ovaries undergo a cyclic egg production (Cuzin-Roudy 2000), which enables krill to lay up to 3,500 eggs in a single batch and are capable of recycling their ovary and repeating this up to nine times in a season (Ross and Quetin 2000). Gravid females release their batch of eggs into the open ocean, and the negatively buoyant eggs sink and hatch at approximate depths of 850 to 1,000m after four to six days (Quetin and Ross 1984; Hofmann *et al*. 1992). However, since most net tows occur in the upper 200m of the water column, there is minimal information on larval krill below this depth. The depth of hatching will depend on water column structure

such as density. Upon hatching, the larvae develop to juvenile through 12 stages, including Nauplius I, II and Matanauplius (non-feeding stages), and then the following feeding stages of Calyptopis I-III and Furcilia I-VI (Frazer 1936). The nauplius larvae ascend rapidly to the surface and metamorphose into calyptopis where phytoplankton levels are abundant (Ikeda 1984*a*). This generally begins to occur in January, but the first feeding stages of larvae have been observed until about April (Quetin *et al*. 1996). Larvae have low lipid levels (Hagen *et al*. 2001) and thus need adequate food levels within 10 to 14 days to survive, grow and develop through winter (Yoshida *et al*. 2009). They primarily feed on phytoplankton in the water column when available, but during the winter period furcilia also rely on ice-algae (Quetin *et al*. 2003). Not only is sea-ice a concentrated food source for overwintering larvae, but also provides an important nursery area and refuge from predators during a vital period of its life cycle (Daly 1990). Following winter, larval krill develop into juveniles to make the most of the phytoplankton blooms in spring/summer, about 200 days after hatching (Quetin and Ross 1991), and in turn, krill will begin spawning in their third summer of existence (Miller and Hampton 1989; Cuzin-Roudy 2000).

Previous field and laboratory studies have described the seasonal maturation cycle of krill (Denys and McWhinnie 1982; Poleck and Denys 1982; Ikeda 1985; Thomas and Ikeda 1987; Siegel *et al*. 2004; Kawaguchi *et al*. 2007*a*). Following summer spawning, females undergo a long gonadal resting period and re-organisation of the ovary throughout winter. During the same period, the female external reproductive organ, thelycum, regresses to an immature stage, before a progression and re-maturation of sexual characteristics again during spring, which is in time for the more favourable reproductive season (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*). Much less is known about the maturation of males, but it is thought that it occurs before the females and happens at a much faster rate (Ross and Quetin 2000; Kawaguchi *et al*. 2007*b*).

1.4.1. Environmental effects on reproductive processes

With such a large biomass in the Southern Ocean and the predicted high fecundities and multiple spawning events, krill require a large energy input (Miller and Hampton 1989; Nicol *et al*. 1995; Quetin and Ross 2001). The major energetic cost of reproduction for females is the accumulation of lipidic yolk for the mature oocytes in ovaries (Clarke 1980). The costs for males include spermatophore production and affixation to females (Pond *et al*. 1995; Virtue *et al*. 1996). Therefore, the energetically demanding reproductive processes, and thus, the strength of the whole population, are reliant on receiving enough energy, which is influenced by

environmental conditions such as food availability, sea-ice extent, photoperiod and temperature (Kawaguchi *et al*. 2007*a*).

The population size of krill seems to be driven by recruitment, rather than predation pressure, and recruitment is related to seasonal sea-ice dynamics (Siegel and Loeb 1995; Atkinson *et al*. 2004). The seasonal advance and retreat of sea-ice has a profound influence on the Antarctic ecosystem, affecting the productivity, food availability and the life cycle of krill, including larval survival and spawning success (Nicol and Allison 1997). The extent of sea-ice and the process of melting or retreat in spring influences the timing of the phytoplankton bloom, and consequently, the overall reproduction of krill (Quetin *et al*. 1994). An extensive sea-ice cover and extended duration during the preceding winter promotes early spawning, a longer spawning season, higher fecundities, multiple spawning events, increased larval survival and favours overall successful recruitment (Siegel and Loeb 1995; Loeb *et al*. 1997). On the other hand, delayed spawning and overall poor recruitment are correlated to years with low sea-ice extent (Loeb *et al*. 1997). Furthermore, a decline in sea-ice extent and in regions of warmer and low chlorophyll oceanic water, the abundance of the pelagic tunicate *Salpa thompsoni* (salps) increases (Loeb *et al*. 1997). Salps are opportunistic feeders and may compete with krill for limited food, and thus, have an adverse effect on krill abundance and their recruitment for the following year (Siegel 2000*b*).

From laboratory studies, it has been shown that krill undergo a winter regression in maturity stage followed by re-maturation in spring (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*). The Southern Ocean is dominated by extreme seasonal changes in day length and light intensity. Hirano *et al*. (2003) demonstrated that the initiation of maturation and spawning of krill can be controlled by photoperiod. However, the results of Thomas and Ikeda (1987) and Kawaguchi *et al*. (2007*a*) suggest that light is not an important factor and have experimentally induced krill maturation under conditions of constant food, temperature and darkness. This indicates that the seasonal cycle of maturation is controlled by an annual endogenous rhythm apparently not controlled by a changing photoperiod or food supply (Thomas and Ikeda 1987) and this rhythm is primarily driven by energy accumulation of krill (Kawaguchi *et al*. 2007*a*). Over a short 12-week experimental period, Teschke *et al*. (2008) showed sexual maturity of female krill advancing in a range of light conditions (continuous light - 200 lux max and 12 hrs light and 12 hrs dark - 50 lux max), while, in contrast, krill exposed to continuous darkness showed no changes in maturity. They conclude that maturity development of krill was affected either directly or indirectly by differing simulated light regimes. The influence of seasonal variation in light conditions on the seasonal maturation cycle could result

from one or a combination of the following scenarios from Teschke *et al*. (2008): (1) direct response: the onset of krill maturation is governed directly by changes of the absolute intensity of light, and reproduction may start when they are exposed to a certain level of light intensity, (2) signal response: the krill maturity cycle is triggered directly by changes of the light:dark ratio, (3) circannual rhythm: reproduction appears independent of direct control of light conditions and is driven by an endogenous clock, which is synchronised by an environmental factor, mainly the annual course of the photoperiod.

There is an obvious need to reconcile the conflicting theories about the relationship between maturity and photoperiod. This relationship was investigated further in this thesis considering a longer time frame, providing essential data to define further the krill maturation cycle.

1.5. Overwintering processes

The overall condition, recruitment and high biomass of krill are due to their ability to adapt to extreme seasonal changes in their environment, mainly food availability, sea-ice extent and light intensity and duration (Quetin and Ross 1991; Siegel and Loeb 1995). Currently, the physiological mechanisms which enable adult krill to survive during winter are relatively unknown, and have resulted in various theories and much speculation. This is mainly due to the limited research that has been undertaken during winter (i.e. Quetin and Ross 1991; Hagen *et al*. 2001, 2007). The reduced oceanic primary productivity, for up to eight months of the year, requires krill to adopt a range of strategies to survive or avoid periods of starvation (Quetin and Ross 1991; Virtue *et al*. 1993*a*; Hagen *et al*. 2001, 2007). Such strategies include overall body shrinkage and protein catabolism (Ikeda and Dixon 1982, Nicol *et al*. 1992), utilisation of lipid reserves (Clarke 1984; Quetin and Ross 1991; Virtue *et al*. 1996; Hagen *et al*. 1996, 2001), switching to a more omnivorous (Clarke 1980; Mauchline 1980; Price *et al*. 1988) and/or carnivorous (Huntley *et al*. 1994; Atkinson *et al*. 2002; Hagen *et al*. 2007) diet, as well feeding on ice-algae (Marschall 1988; Stretch *et al*. 1988) and sea-floor detritus (Kawaguchi *et al*. 1986), and suppression of metabolism (Kawaguchi *et al*. 1986; Quetin and Ross 1991; Torres *et al*. 1994).

Quetin and Ross (1991) concluded that a reduction in metabolic rate and low feeding activity are the most effective energy-saving mechanisms for adult krill during the ice covered winter, a theory also supported by Kawaguchi *et al*. (1986) and Torres *et al*. (1994). Overall, the physiological status of krill varies seasonally with high metabolic and feeding rates in summer and low and/or reduced rates in winter. A major question that is still debated is whether the observed decrease in

metabolism in winter is caused simply by reduced food concentration or whether there are other environmental factors involved such as photoperiod, temperature, or more complicated, an endogenous annual rhythm that is affected by a strong seasonal cue in the Southern Ocean. For example, an annual internal rhythm would allow krill to allocate metabolic capacity to the times of the year when food is abundant, while switching to an energy-saving mode in winter.

From laboratory experiments, Ikeda and Dixon (1984) concluded that respiration rates of non-feeding adult krill were 62.5% of those rates recorded from krill that were feeding at realistic food concentrations. Atkinson *et al*. (2002) showed that respiration rates of krill in April were 60% of those in summer and declined further during a starvation experiment. Additionally, ingestion and clearance rates were approximately one-third of those measured in summer and these rates failed to increase after 11 days of abundant food conditions. From the same experiment during summer, the metabolic activity of krill did respond positively to high concentrations of food (Atkinson and Snÿder 1997).

From starvation experiments, under constant temperature and continuous dim light, Ikeda and Dixon (1982) demonstrated a significant reduction in body size (32.1–56.1%, of initial wet weight) and oxygen consumption, compared to fed animals. They showed that krill can survive for 211 days without food and were the first to suggest shrinkage and utilisation of body protein as an alternative method of conserving energy and surviving the winter without food. Rapid growth occurs during spring and summer, when there is high food availability and the overall conditions are favourable (Atkinson *et al*. 2006; Kawaguchi *et al*. 2006), with growth rates observed above 5% in length per moult (Nicol *et al*. 1992).

Utilisation of stored lipid, in the form of triacylglycerols (TAG), has been examined in several studies in relation to winter survival (Clarke 1984; Quetin and Ross 1991; Quetin *et al*. 1994; Virtue *et al*. 1996; Hagen *et al*. 1996, 2001). It has been shown that the accumulation of lipid is maximal in autumn, while there is depletion of lipid during winter, before there are substantial phytoplankton blooms in the spring and summer (Clarke 1984; Hagen *et al*. 1996, 2001). Lipid reserves have also been shown to be utilised for important reproductive purposes during summer (Virtue *et al*. 1996).

Adult krill exploit many types of food resources and have a flexible diet in the wild so they may rarely actually encounter prolonged food shortages. This has led to speculation on the overwintering strategies of krill, and whether krill actually starve, undergo shrinkage, reduce metabolism or utilise lipid resources. This is considered by Huntley *et al*. (1994) who concluded that during times of reduced primary

productivity, krill can derive energy from other resources (i.e. undertaking carnivory) to sustain growth and even maintain metabolic rates during winter, which is contrary to the findings of Quetin and Ross (1991) in a similar region. With the use of new techniques, estimating growth rates in the field is improving substantially (as discussed in section 1.3) and analysis has shown small or negative growth across all length classes by autumn, confirming that shrinkage does occur in krill under natural conditions, particularly in winter (Candy and Kawaguchi 2006; Kawaguchi *et al*. 2006). Shrinkage is also accompanied by a regression of sexual characteristics (Thomas and Ikeda 1987), which further complicates the description of growth and ageing of krill.

Other environmental factors that have been shown to influence and reduce metabolism of krill during winter are temperature and light. McWhinnie and Marciniak (1964), Rakusa-Suszczewski and Opalinski (1978) and Segawa *et al*. (1979) have all concluded that temperature affects metabolic activity of krill, with respiration rates increasing at higher temperatures. As a result, a decrease in metabolic rates occurs during the cooler winter months. A study completed by Teschke *et al*. (2007) exposed krill to variable light intensities and duration to simulate the Southern Ocean in summer, autumn and winter to examine different light regimes on feeding activity and oxygen consumption in a short-term experiment. Respiration rates and feeding activity of krill increased during the experiment for krill exposed to summer and autumn light regimes. However, krill in the dark recorded no change, with significantly lower respiration rates than those under light conditions. Overall, irrespective of feeding conditions, Teschke *et al*. (2007) revealed that the Antarctic light cycle most likely triggers the changes in metabolic rates of krill, and thus, photoperiod (light duration, rather than actual intensity) is possibly the most important effect on the physiological parameters of krill.

All of these overwintering mechanisms are direct observations and have all been recorded at different times and places. This indicates that krill have an extremely flexible and versatile behaviour and can utilise a variety of strategies to cope with the winter and various environmental conditions (Atkinson *et al*. 2002). This may explain why krill have such a large biomass and are so successful in the Antarctic ecosystem. However, the relative importance of each overwintering strategy in the natural environment is yet to be determined, and thus, it is vital to understand when krill use one strategy opposed to another. This has led to a number of questions that have been addressed in this study:

- When a constant food supply is provided, do krill still undergo shrinkage and sexual regression during the winter period?

- At what time of year are growth and maturity resumed?
- Are metabolic rates and general energy requirements of krill reduced during the winter?
- Do krill utilise lipid reserves, even if there is constant food available?
- What are the key environmental variables that cue cycles of growth, maturation and general metabolic rates under controlled conditions?

1.6. Climatic change

The Southern Ocean is characterised by low and seasonally stable temperatures (Whitehouse *et al*. 2008) with slightly higher temperatures observed in the austral summer. Krill is considered to be stenothermal, with life cycles potentially sensitive to slight shifts in temperature (Wiedenmann *et al*. 2008). Temperature has been shown to influence the frequency of moulting in krill, and thus, affects the overall growth rates, which can vary considerably, even within the narrow temperature range observed in the Southern Ocean (Nicol 2000). Respiration rates have also been reported to be influenced by an increasing temperature (McWhinnie and Marciniak 1964; Rakusa-Suszczewski and Opalinski 1978; Segawa *et al*. 1979).

The Antarctic Peninsula is experiencing one of the fastest rates of regional climate change on Earth, resulting in the collapse of ice-shelves, the retreat of glaciers and the exposure of new terrestrial habitat (Clarke *et al*. 2007). Over the past 50 years there has been a significant warming trend over Antarctica, with surface air temperatures increasing by 5-6°C west of the Antarctic Peninsula during winter (Vaughan *et al*. 2003). In terms of the Southern Ocean, Meredith and King (2005) concluded rapid warming (>1°C) of summer surface waters west of the Antarctic Peninsula, but little to no warming to the east. In the ocean around South Georgia, Whitehouse *et al*. (2008) concluded that there had been rapid warming during the 20th Century, with a mean increase of ~0.9°C in January and ~2.3°C in August in the upper 100m of the water column. It is therefore clear that there is substantial evidence for long-term warming of the surface layer, the habitat of krill, in the Southern Ocean.

The warming climate in Antarctica is changing sea-ice dynamics in the region west of the Antarctic Peninsula, with sea-ice advance and retreat occurring earlier, shortening the duration of ice covered waters (Quetin *et al*. 2007). These environmental factors heavily impact the life cycle of krill, as reproduction and recruitment success is highly correlated with good sea-ice years (Siegel and Loeb 1995). As global warming continues, sea-ice cover is predicted to decrease (Arrigo

and Thomas 2004), which in turn, will likely have a negative impact on krill recruitment (Siegel and Loeb 1995; Wiedenmann *et al*. 2008). Whitehouse *et al*. (2008) concluded a significant negative relationship occurred between summer South Georgia water temperatures and mean summer density of krill across the south-west Atlantic sector of the Southern Ocean. This is also confirmed by Atkinson *et al*. (2004) who suggested that stocks of krill have declined significantly since the 1970s in the south-west Atlantic, which has been accompanied by an increase in water temperature, a decrease in sea-ice cover and a consequent lack of food for larvae during winter.

In areas of regional warming, phytoplankton communities may change from a diatom-dominated food for krill to phytoplankton species that are much less nutritious and smaller in size such as cryptophytes (Moline *et al*. 2004). As a consequence, this warming trend might favour competing macro- and mesozooplankton species, and may have a profound effect on the production and structure of the Southern Ocean food web (Atkinson *et al*. 2004; Whitehouse *et al*. 2008). Atkinson *et al*. (2006) examined the effects of food, temperature and sex on growth rates of krill in the south-west Atlantic and concluded that growth actually decreased above a temperature optimum of 0.5°C, which suggests that krill most likely experience thermal stress and may struggle to cope with predicted future warming at this location. However, krill are a versatile and flexible species, showing the ability to occupy a range of habitats, for example, the deep ocean, under the sea-ice and throughout the water column (Clarke and Tyler 2008). To date there has been limited long-term research on the effects of temperature on the physiological and biochemical parameters of krill such as lipid and fatty acid content and composition, metabolic rates, maturation and growth.

The vulnerability of krill to global warming amplifies the importance of understanding key aspects of their life history and biology. The research in this thesis examined krill growth, maturation and other physiological and biochemical parameters under controlled experimental conditions at varying temperatures. The main aim of this research was to better understand temperature-dependent growth, adaptability and survival of krill. These findings will assist in developing accurate model predictions of krill response to future climatic change.

1.7. Aims and objectives

The main purpose of this thesis was to test hypotheses brought about from key questions raised in field studies (sections 1.3 - 1.6) through a long-term laboratory experiment. Specifically, the key objectives of this thesis were:

1) To investigate the effects of temperature and sex on growth, physiology and maturity status over an annual cycle.

2) To investigate whether or not shrinkage and sexual regression occurs during the winter, even with a constant food supply.

3) To evaluate and reconcile conflicting theories pertaining to photoperiod control of maturity.

4) To test the effects of key environmental factors (light, food availability and temperature) on the metabolic activity of krill.

5) To examine seasonal respiration rates and lipid metabolism of krill incubated under a constant food supply.

1.8. Research approach

To achieve the objectives set out in this thesis, a series of experiments were conducted. Chapters 2 to 6 outline these experiments and are stand alone chapters, which have been published in, or submitted to, international scientific journals.

Chapter 2 explores the growth and maturation of individually maintained krill over a complete annual cycle. The growth increment, instantaneous growth rate, daily growth rate, intermoult period and maturity scores were determined over 14 months for male and female krill in three temperature treatments (-1°C, 1°C and 3°C). This chapter addresses the first and second objective.

Chapter 3 examines the third objective addressing whether light (timing, intensity and duration) is an important environmental cue for maturation.

Chapter 4 investigates the fourth and fifth objective evaluating the effects of light, diet and temperature on respiration rates of krill at various time points between the maturation and regression process.

Effects of temperature on total lipid, lipid class, fatty acid content and composition of krill are investigated in Chapters 5 and 6 (objectives one and five).

Chapter 5 examines lipid and fatty acid content of immature krill over a full year and addresses the question of lipid utilisation during the winter period, under constant food supply and different temperature regimes. Chapter 6 examines lipid and fatty acid content and composition of krill in summer with respect to temperature. Various tissue fractions are analysed and sex and maturity status are considered.

Finally, chapter 7 presents an overall integration of results. Major findings are discussed in the context of our current state of knowledge of krill biology and physiology. Recommendations for future research are also highlighted.

-- Chapter two --

Temperature effects on the growth and maturation of Antarctic krill (*Euphausia superba*)

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2.1. Abstract

This study describes growth and maturation of individual Antarctic krill for a complete annual cycle in an artificially controlled environment. Antarctic krill were kept individually and maintained under temperature regimes of -1°C, 1°C and 3°C, while light conditions simulated natural seasonal conditions for the 14 months of the experiment starting in April. The growth increment (GI), instantaneous growth rate (IGR), daily growth rate (DGR), intermoult period (IMP) and maturity scores (MS) were tracked by measuring the consecutively moulted exoskeletons and modelled using linear mixed models (LMMs). There was a clear seasonal trend in growth and maturity in the three different temperature treatments. Males generally reached peak growth rates and matured earlier than females, but females showed higher growth rates overall. Negative growth occurred towards the end of January for both males and females, coinciding with the regression of external sexual characteristics. There was, as expected, a significant decline in IMP with increasing temperature, which was moderated by increasing trends with total length (TL) and month for the -1°C treatment. For the 1°C and 3°C treatments there was no relationship with TL and the

season effect declined after October and December, respectively. For krill with a TL of 40mm in October, the predicted mean IMP values at -1°C, 1°C and 3°C were 28 days, 24 days and 19 days, respectively. There was some evidence to suggest that 1°C was optimum for growth, as the IMP was significantly lower than at -1°C, but the difference in GI between the two temperatures was not significantly different, while all growth variables were significantly lower at 3°C. For the first time, this study has been able to confirm, by fitting a bivariate LMM, that compensation mechanisms do exist between IMP and IGR and to quantify the degree of compensation at both the between- and within-animal levels. This will allow modelling of growth trajectories in the wild to quantify the distribution of length given age.

2.2. Introduction

Antarctic krill (*Euphausia superba*, hereafter krill) has been widely studied and recognised extensively as a primary prey species in the Antarctic marine ecosystem. Not only do krill play a major role in the food web (Mauchline and Fisher 1969; Miller and Hampton 1989; Everson 2000; Mangel and Nicol 2000; Alonzo *et al*. 2003*a*), but they are also attracting increasing interest as a target for commercial fishing (Everson and Goss 1991; Nicol and Endo 1999; Kawaguchi and Nicol 2007). Understanding characteristics of krill life history parameters, especially growth and reproduction, are essential to the development of an adequate management regime for its fishery under the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Constable and de la Mare 1996).

In the past, growth rates have been most commonly determined with field based length-frequency analysis on cohorts (Quetin *et al*. 1994). However, this method has uncertainties, relying on assumptions that the same population is repeatedly sampled and that mortality is not size dependent (Nicol 2000; Arnold *et al*. 2004). Also, length frequency data may not be transferable to other locations in the Southern Ocean and it is difficult to account for recruitment and mortality.

The instantaneous growth rate (IGR) technique measures growth directly from changes in length of uropods from individual live krill which have moulted (Quetin and Ross 1991). The IGR technique was later modified by Nicol *et al*. (1992) so as to provide a large amount of data on various sizes of krill from the whole single population. Another advantage of this method is that it can be modelled at an individual level and thus growth rates can easily be transferred to other locations and times, when growth is directly related to temperature, food availability and size (Ross *et al*. 2000).

Recently, krill daily growth rates (DGR) were estimated by using extensive datasets of field IGR experiments employing two different approaches. One estimating DGR from IGR and intermoult period (IMP), where IMP was predicted from temperature using published data on direct observations of IMP, and IGR was modelled as a function of sex, length, season and region (Kawaguchi *et al*. 2006). The other approach estimated IMP from proportions of observed moulting events during IGR experiments using data on individual moults that were classified by sex, maturity status, length and temperature variables (Tarling *et al*. 2006). Combining IGR with predicted IMP, DGR was modelled as a function of length, temperature and food concentration (Atkinson *et al*. 2006). The former approach allowed the generation of lifetime growth trajectories using seasonal temperature trends to determine IMP and monthly trends in average IGR by length class (Candy and Kawaguchi 2006). However, this approach was not able to quantify the direct effect of some seasonal variables that determine growth such as food availability and quality. The latter approach focussed on detailed evaluation of environmental effects on growth including chlorophyll and temperature as well as maturity stages (Atkinson *et al*. 2006), but was not used to generate growth trajectories.

Previous field and laboratory studies have revealed that the growth and maturation of krill undergo a seasonal cycle (Denys and McWhinnie 1982; Poleck and Denys 1982; Ikeda 1985; Thomas and Ikeda 1987; Siegel *et al*. 2004; Kawaguchi *et al*. 2007*a*) that are influenced by environmental conditions such as temperature and food availability (Kawaguchi *et al*. 2007*a*). However, it is not possible to delineate and understand the relative magnitudes of these effects by solely relying on field experiments. There are also mixed views as to whether or not krill show differential growth rates between sexes (e.g. Atkinson *et al*. 2006; Kawaguchi *et al*. 2007*b*).

In the present investigation, a long-term experiment was conducted using krill that were incubated in tanks subjected to different temperature regimes (-1°C, 1°C and 3°C) for 14-months under laboratory conditions. The purpose of this study was to investigate the effect of temperature on the growth and maturation of individual krill by measuring their consecutively moulted exoskeletons.

2.3. Materials and Methods

2.3.1. Sampling and experimental setup

Live Antarctic krill were collected on 3rd March 2006 (66°02'S, 79°32'E) using a rectangular mid-water trawl net (RMT 8) (Baker *et al*. 1973) on board the RSV *Aurora Australis*. The water temperature during krill capture was -1°C. Krill were transferred into 200-L tanks and maintained with a continuous supply of seawater in a cold laboratory (0°C, dim light and no food) on board the ship. Once north of the Polar Front, the continuous water supply was cut off and 50% of the tank water was exchanged with freshly pre-chilled seawater each day.

On return to the research aquarium at the Australian Antarctic Division in Kingston, Tasmania, krill were evenly distributed into three 600-L tanks (Appendix B) and acclimated to the aquarium conditions (King *et al*. 2003) on 30th March 2006. These stock populations of krill were maintained at temperatures of -1°C, 1°C and 3°C, under light conditions that were adjusted throughout the experimental period to mimic the natural Antarctic seasonal light cycle. Lighting was provided by twin fluorescent tubes. A personal computer controlled-timer system was used to set a natural photoperiod corresponding to that for the Southern Ocean (66°S at 30m depth). Continuous light and a maximum of 100 lux light intensity at the surface of the tank (assuming 1% light penetration to 30m depth) during summer midday (December), a sinusoidal annual cycle with monthly variations of photoperiod and daily variation of light intensity was calculated (Appendix C). At the start of each month, a new photoperiod was simulated by adjusting the timer system (Kawaguchi *et al*. 2010).

The experimental period ran from April 2006 to May 2007. A further two 600-L tanks were included in the experiment on 30th August 2006 to duplicate the -1°C and 3°C treatments. The stock population were fed daily throughout the experimental period (April 2006 to May 2007) with the following algal mixture; the cultured pennate diatom *Phaeodactylum tricornutum*, at a final concentration of approximately 4.3 x 10^4 cells mL^{-1}, and the diatom *Thallasasira* sp (1.8 x 10^3 cells mL^{-1}), the flagellate *Pavlova* sp. (6.7 x 10^3 cells mL^{-1}) and the flagellate *Isochrysis* sp. (2.6 x 10^4 cells mL^{-1}), which are a concentrated bulk feed of instant algae mixed with seawater. Water flow in the tank was closed for two hours per day to enable the krill to feed as much as possible, before the risk of disease and fouling. Minced clam, purchased from a local supplier, was also fed to the stock population (approximately 1g per 250 krill) when water was flowing into the system. Clam meat has successfully been used in previous laboratory experiments, providing krill with an additional protein source (Hirano *et al*. 2003).

2.3.2. Experimental procedure

Within each 600-L incubation tank, 14 individually isolated krill were contained in 750mL perforated jars to track their growth and maturity (a total of 70 jars throughout the temperature settings, Appendix B). All jars were checked for moults daily, and if observed, the moult was placed in a cryotube with 10% formalin seawater. Dead krill were replaced with similar sized animals from the stock population. Overall, 142 krill were replaced during the 14-month seasonal cycle, from either missing krill that "jumped" out of the jars or mortalities. This equates to about two krill being replaced a week, with a reasonable even distribution of mortalities across the three temperatures and individual jars. Of the original 70 individual krill, two individuals were observed for the entire experimental period, both of which were in the 1°C tank. Each krill was fed 15mL of the same mixture of algae made up for the stock population daily. The final concentrations were approximately 2.6 x 10^5 cells mL^{-1} of *Phaeodactylum tricornutum*, 10.8 x 10^4 cells $mL^{-1}$of *Thallasasira* sp., 4.0 x 10^4 cells mL^{-1} of *Pavlova* sp., 1.5 x 10^5 cells mL^{-1} of *Isochrysis* sp., and minced clam meat (approximately 1g per 70 krill) each day. The amount of food given was at saturating levels for both the stock population and individual krill, and it was calculated to be similar concentrations of food per krill for the varying volumes between the tanks and individual jars. The excess food remaining in the 600-L tanks and individual jars were flushed out of the system after 2 hours of feeding. Since food was in excess in both experimental setups, and approximately the same growth and maturity trends were observed between the stock populations and individually maintained krill (unpublished data, Appendix D) the replacement of krill from the stock population to an individual jar after mortality/escapement was justified.

The uropod growth increment was derived from the differences in length of both the left and right uropods from consecutively moulted exoskeletons using the Sigma Scan Pro 5.0® image analysis software. The uropod exopodite is measured from the distal tip to its external intersection with the basipodite (Appendix E). The measurement is made parallel to the axis of the uropod rather than from point to point. If one of the moulted uropods was damaged, then the measurement was only taken from the undamaged uropod. On occasions where both uropods were damaged, the moult was excluded from the analysis. There were 218 (30%) moulted exoskeletons that were measured using only one uropod, and in total, 50 moults were excluded from the analysis as both uropods were damaged. Total length (TL) (standard length 1, Appendix F) was calculated from the moulted uropod length (UL) using the following equation from Hollander (2001):

TL = (moulted UL – 0.1051)/0.1407

The daily growth rate (DGR, mm day^{-1}) was then calculated by dividing the mean growth increment in TL (GI, mm) by the intermoult period (IMP, days). The instantaneous growth rate (IGR, %) was determined by dividing the mean GI by the original calculated TL of the krill and multiplying it by 100.

Maturity stage (MS) was assigned a value between 1 and 5 for both males (M) and females (F), corresponding to the stages: 1=Juvenile; 2=2MA, 2FB; 3=2MB, 2FC; 4=2MC, 2FD; 5= 3M, 3FA. Higher numbers represent greater maturity. The maturity of males and females were staged according to the technique developed by Bargmann (1945), which was later modified by Makarov and Denys (1980) and Thomas and Ikeda (1987). The maturity staging system with corresponding scores is summarised in Appendix A. Since there is a transition between MS, there was often difficulty in determining whether the animal had progressed fully to the next stage or not. In these instances, the krill was identified as a 0.5 value (e.g. stage 3.5 or 4.5).

2.3.3. Statistical analysis

A total of 150 individual krill (83 females, 67 males) that were observed for at least two consecutive moults, in order to allow at least one observation of growth increment and IMP per animal, were used in this study. This corresponded to 735 exoskeletons overall. The continuous variable “month” used in the statistical analyses corresponds to integer values of 4 to 17, representing consecutive months starting from April 2006. The three temperature regimes were treated as a 3-level factor.

Since the data involved repeated observations of individual animals, each of the response variables; GI, IGR, DGR, IMP and MS were analysed using a linear mixed model (LMM) (Diggle *et al*. 1994), with random animal effects using the SAMM package (Butler *et al*. 2002) within Splus (Insightful 2002). In addition, IMP and IGR were analysed as a bivariate LMM by estimating the cross-correlation between IMP and IGR at both the between- and within-animal level, while simultaneously fitting the fixed effects. A LMM scheme was chosen above other alternatives, particularly circular statistics where time is categorised according to the sine or cosine of the data, is because the means plotted against month do not demonstrate a full cycle. Given the observed trends, the range of months observed were well fitted by the quadratic functions used in the LMM and there is little we can infer on the cyclical nature of these biological parameters of maturity score, growth rate and IMP. However, given the artificial rearing conditions, it could be argued that the true nature of any seasonal, and thus, cyclical variation in these parameters might not have been fully expressed under these experimental conditions.

Three fixed effects formulations were used. The first LMM excluded TL as a predictor variable and fitted all combinations of temperature by sex by month. This LMM was used to summarise that data in terms of these three factors and is called the "summary" LMM. In the second formulation, continuous values of TL and month, using linear terms in TL and both linear and quadratic terms in month, were fitted. Interactions of these "parametric" terms with sex and temperature, as well as the main effects and the interaction of these last two terms completed the fixed effect model. This "parametric" LMM was used to examine any smooth trends of the various response variables with TL and month and only included fixed effect terms that were statistically significant. To determine if these parametric terms and their interactions adequately modelled the data, the final "non-parametric" LMM formulation included, TL and month as factors (TL.f and month.f, respectively, where ".f" denotes the factor representation) with TL.f classes of <31,31-33,33-35,35-37,37-39, >39mm and the set of integer values for the month.f. The mean values of TL for the above TL.f classes were very close to 30, 32, 34, 36, 38, and 41mm, respectively, with corresponding numbers of observations in each class of 24, 63, 79, 72, 122, and 375, respectively. These values of class mean TL were used to compare predictions from the "parametric" LMM to the "non-parametric" LMM. The maximum level of interaction included in the LMMs was third order (e.g. sex:temperature:TL, sex:temperature:TL.f) and no interactions between month.f and TL.f were included due to the large number of degrees of freedom involved, and the high degree of imbalance in the number of observations in the TL.f classes. The bivariate LMM for IMP and IGR was constructed by linking these two variables and specifying separate fixed effect terms for each combination with covariance matrices at the between- and within-animal level. Significance levels were judged using sequential Wald tests (Welham and Thompson 1997) combined with t-statistics for individual parameter estimates. Normality of random effects and within-animal residuals was assessed using quantile-quantile plots.

2.4. Results

Means and ranges are summarised in Tables 2.1 and 2.2 for intermoult period (IMP), instantaneous growth rate (IGR), daily growth rate (DGR) and maturity stage (MS) for males and females in the three different temperature treatments within the main growth period (August to December) and also the non-growth period (January to July), respectively. There was a significant difference ($P<0.01$) between the means of all variables between the two periods, except for the IMP of both males and females in the -1°C treatment (Table 2.3).

2.4.1. Seasonal growth cycle

A clear pattern of seasonal growth was observed in the three different temperature treatments (Figure 2.1*a* and 2.1*b*), which was particularly evident for females. Krill generally showed low growth during the autumn and winter period, after which mean growth was positive in males during July and August, while female growth began later in August and September. The maximum mean growth rates occurred in September for males and October for females, with females generally showing slightly higher growth. Growth started to decrease after the main peak growth period in spring, and both males and females commenced shrinkage towards the end of January and February.

2.4.2. Seasonal maturity cycle

There was a general progression of the mean maturity score (MS) for all temperatures, with males reaching peak sexual maturity (October/November) before the females (November/December) (Figure 2.1*c*). MS was constant from October to January (~5) after which both sexes regressed. Even though general relationships were visually observed in the graphs between males and females throughout the 14-month cycle, there were no significant differences in the timing of maturity ($P>0.1$). A comparison of Figure 2.1*a* and 2.1*b* shows that under the three temperature treatments, all krill reached their peak growth rate as a sub-adult, and then growth decreased after a period of time once fully mature. Krill generally started to display negative growth when they were regressing in maturity.

Table 2.1. Mean and range values observed for intermoult period (IMP, days), instantaneous growth rates (IGR, %), daily growth rates (DGR, mm day^{-1}) and maturity stages (MS) for male and female krill in each of the three temperature -1ºC, 1ºC and 3ºC treatments during the main growth period (August to December).

Growth Season (August to December)									
-1ºC	**Male**			**Female**			**Male and Females**		
	N	**Range**	**Mean**	***N***	**Range**	**Mean**	***N***	**Range**	**Mean**
IMP (days)	33	19 to 35	28	64	20 to 34	28	97	19 to 35	28
IGR (%)	33	-1.33 to 4.39	1.21	64	-4.21 to 8.30	2.45	97	-4.21 to 8.30	2.03
DGR (mm day^{-1})	33	-0.02 to 0.07	0.02	64	-0.06 to 0.10	0.03	97	-0.06 to 0.10	0.03
Maturity Stage	32	3 to 5	4.52	64	3 to 5	4.58	96	3 to 5	4.56
1ºC	**Male**			**Female**			**Male and Females**		
	N	**Range**	**Mean**	***N***	**Range**	**Mean**	***N***	**Range**	**Mean**
IMP (days)	45	18 to 29	24	28	20 to 28	23	73	18 to 29	24
IGR (%)	45	-2.88 to 8.09	1.49	28	-1.97 to 5.73	2.22	73	-2.88 to 8.09	1.77
DGR (mm day^{-1})	45	-0.05 to 0.13	0.02	28	-0.03 to 0.08	0.03	73	-0.05 to 0.13	0.03
Maturity Stage	45	3 to 5	4.67	28	3 to 5	4.36	73	3 to 5	4.55
3ºC	**Male**			**Female**			**Male and Females**		
	N	**Range**	**Mean**	***N***	**Range**	**Mean**	***N***	**Range**	**Mean**
IMP (days)	72	14 to 26	19	79	15 to 28	19	151	14 to 28	19
IGR (%)	71	-5.85 to 5.85	0.96	79	-3.28 to 6.82	1.36	150	-5.85 to 6.82	1.17
DGR (mm day^{-1})	72	-0.12 to 0.11	0.02	79	-0.07 to 0.13	0.03	151	-0.12 to 0.13	0.02
Maturity Stage	73	3 to 5	4.92	79	3 to 5	4.74	152	3 to 5	4.83

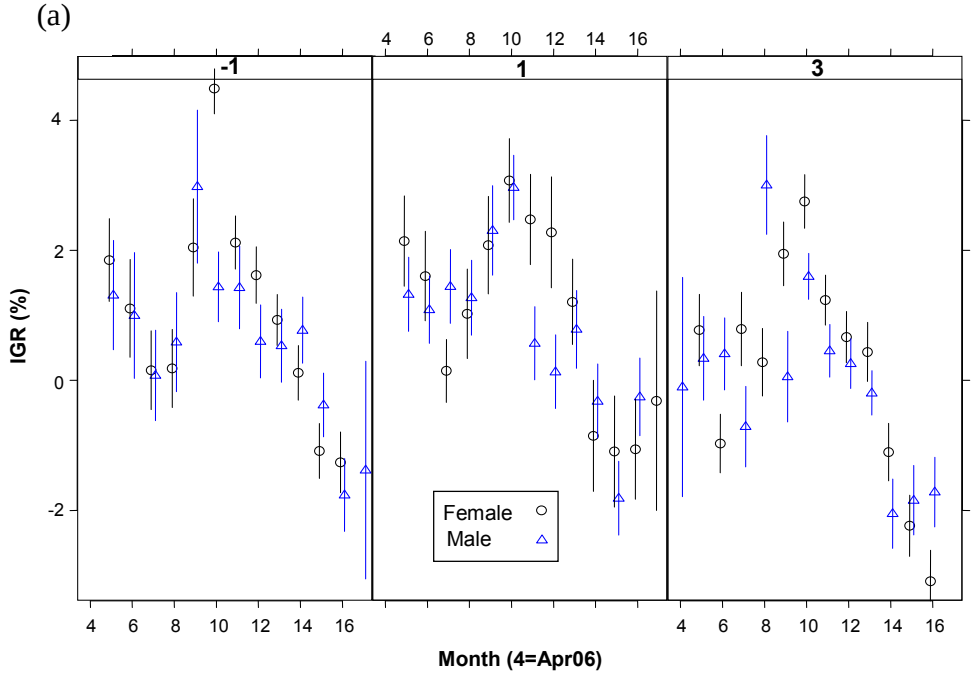
Table 2.2. Mean and range values observed for intermoult period (IMP, days), instantaneous growth rates (IGR, %), daily growth rates (DGR, mm day^{-1}) and maturity stages (MS) for male and female krill in each of the three temperature -1ºC, 1ºC and 3ºC treatments during the non-growth period (January to July).

Non-Growth Season (January to July)									
-1ºC	**Male**			**Female**			**Male and Females**		
	N	**Range**	**Mean**	***N***	**Range**	**Mean**	***N***	**Range**	**Mean**
IMP (days)	55	18 to 39	29	84	20 to 37	29	139	18 to 39	29
IGR (%)	55	-5.58 to 5.15	0.01	84	-3.33 to 5.40	0.08	139	-5.58 to 5.40	0.05
DGR (mm day^{-1})	55	-0.09 to 0.06	0.00	84	-0.05 to 0.07	0.00	139	-0.06 to 0.07	0.00
Maturity Stage	54	2 to 5	3.99	83	2 to 5	4.22	137	2 to 5	4.13
1ºC	**Male**			**Female**			**Male and Females**		
	N	**Range**	**Mean**	***N***	**Range**	**Mean**	***N***	**Range**	**Mean**
IMP (days)	63	17 to 24	21	46	18 to 23	21	109	17 to 24	21
IGR (%)	62	-6.50 to 6.53	0.44	46	-2.31 to 3.91	0.41	108	-6.51 to 6.53	0.43
DGR (mm day^{-1})	63	-0.13 to 0.10	0.01	46	-0.05 to 0.06	0.01	109	-0.13 to 0.10	0.01
Maturity Stage	63	2 to 5	3.67	45	2 to 5	3.69	108	2 to 5	3.68
3ºC	**Male**			**Female**			**Male and Females**		
	N	**Range**	**Mean**	***N***	**Range**	**Mean**	***N***	**Range**	**Mean**
IMP (days)	82	11 to 26	18	89	11 to 25	17	171	11 to 26	17
IGR (%)	81	-6.80 to 4.50	-0.70	87	-11.55 to 2.77	-0.95	168	-11.55 to 4.50	-0.83
DGR (mm day^{-1})	82	-0.19 to 0.09	-0.02	89	-0.23 to 0.07	-0.02	171	-0.23 to 0.09	-0.02
Maturity Stage	80	2 to 5	4.18	89	2 to 5	4.08	169	2 to 5	4.12

Table 2.3. Tests of differences for metrics calculated for the main growth period (August to December) and the non-growth period (January to July). T-values for intermoult period (IMP, days), instantaneous growth rate (IGR, %), daily growth rates (DGR, mm day^{-1}) and maturity stages (MS) for male and female krill in each of the three temperatures -1ºC, 1ºC and 3ºC treatments. The corresponding degrees of freedom is shown within the brackets (^{ns} represents a non-significant difference, all other values are significant to at least the P=0.01 level).

	-1ºC		1ºC		3ºC	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
IMP (days)	1.64 ^{ns} (234)	1.89 ^{ns} (234)	6.01 (180)	6.53 (180)	3.31 (320)	4.03 (320)
IGR (%)	3.27 (234)	7.17 (234)	2.67 (179)	4.19 (179)	5.07 (316)	7.52 (316)
DGR (mm day^{-1})	3.30 (234)	7.09 (234)	2.83 (180)	4.20 (180)	5.30 (320)	7.84 (320)
Maturity Stage	2.68 (232)	3.17 (232)	6.11 (179)	3.46 (179)	6.42 (319)	5.85 (319)

(a)



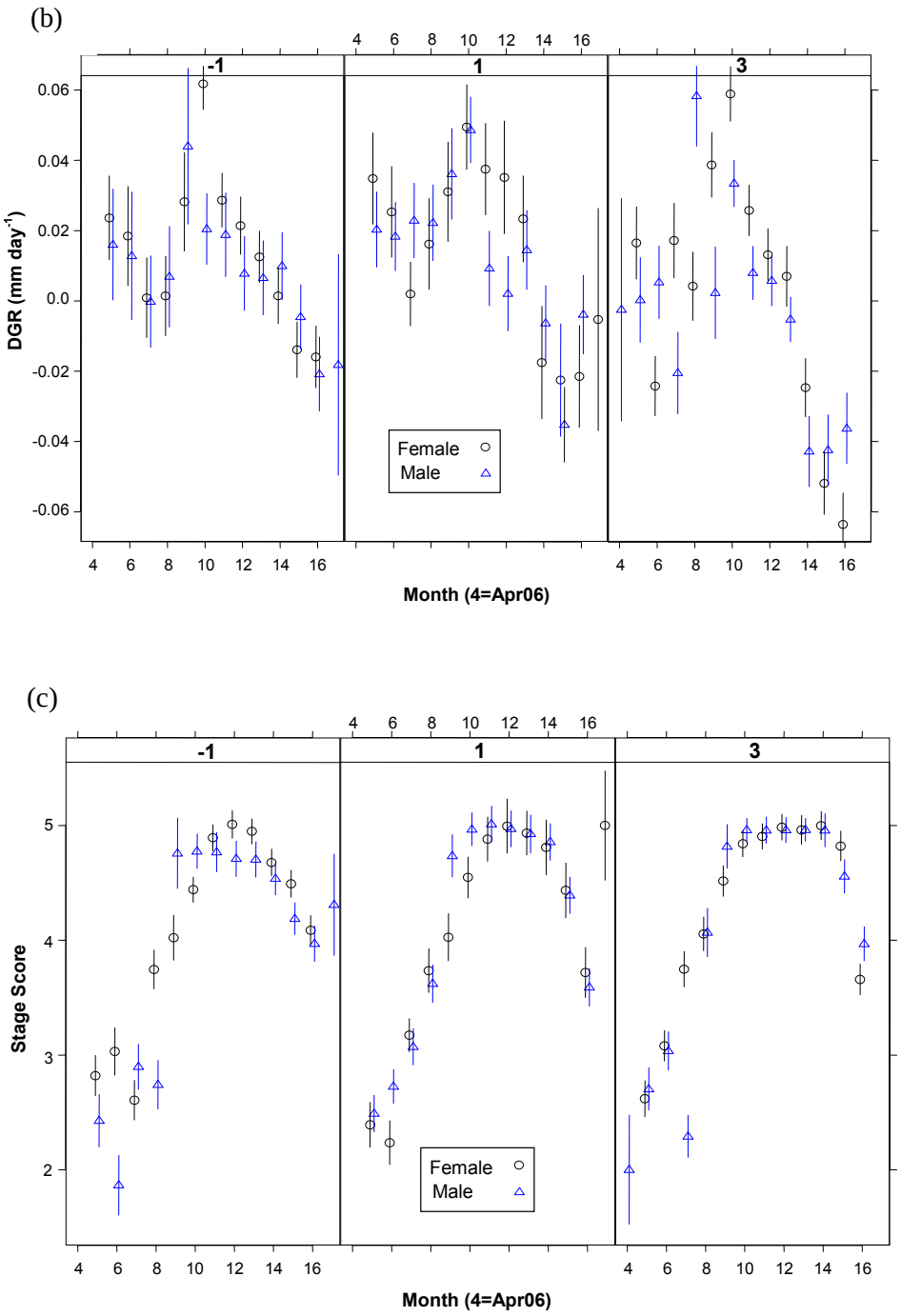


Figure 2.1. Predicted mean monthly (±1 SE) values for **(a)** instantaneous growth rate (IGR, %), **(b)** daily growth rate (DGR, mm day^{-1}) and **(c)** maturity score (MS) for males (triangles) and females (circles) obtained from the summary Linear Mixed Model (LMM) for each combination of temperature (-1ºC, 1ºC and 3ºC), month and sex (MS: 1=Juvenile; 2=2MA, 2FB; 3=2MB, 2FC; 4=2MC, 2FD; 5= 3M, 3FA, refer to Appendix A). Points for males and females have been slightly offset to improve clarity.

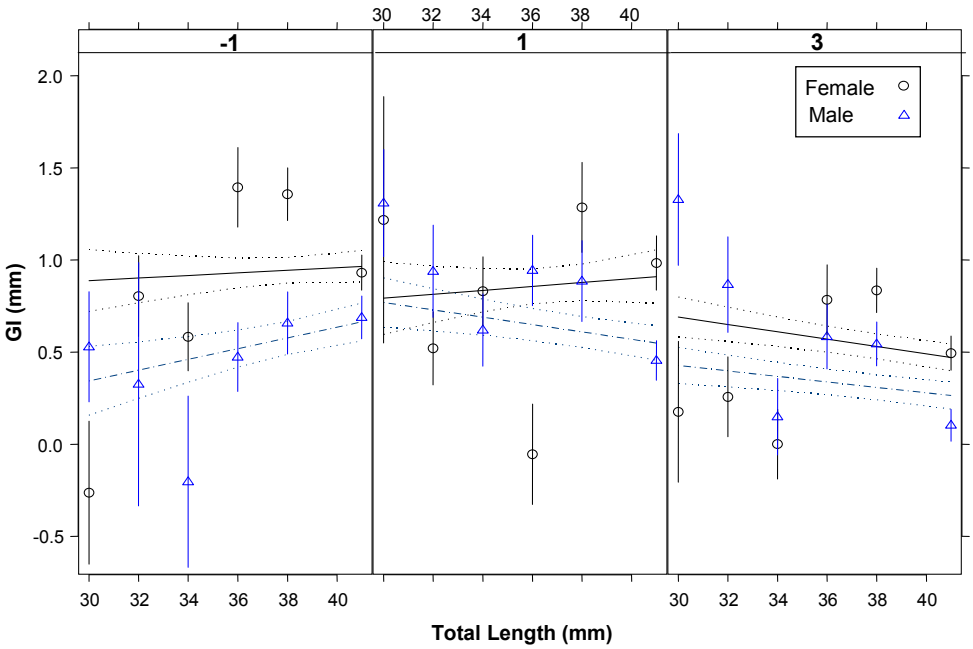
2.4.3. Linear mixed model predictions

Predictions from the LMMs were used in this study to compare means, trends and variability for each of the response variables (GI, IGR, DGR, IMP and MS). For all response variables, all third order interactions in the parametric LMM were not significant (P>0.1) so these terms were dropped. The set of graphs of predictions for each variable obtained from the parametric and non-parametric LMMs demonstrate significant (P<0.01) second order interactions.

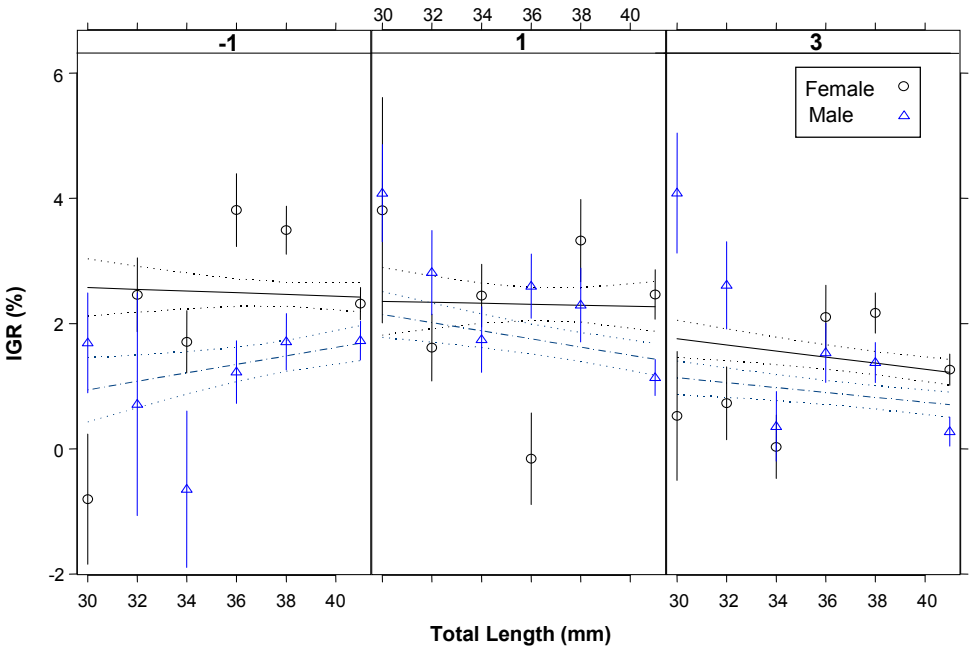
2.4.3.1. Effect of temperature and total length

The predicted mean values for GI, IGR and DGR, respectively, were obtained from the fit of the parametric (lines) and non-parametric (points) LMMs for the three temperatures (-1°C, 1°C and 3°C) across a range of total lengths (TL) of 30-41mm with month adjusted to October 06 (Figure 2.2). There were no significant differences in the slope of the regression on TL between each of the three temperatures or either sex for the growth variables (GI, IGR and DGR) (P>0.1). The standard errors of the predicted means were large for the lower TL classes, which consisted of considerably less data compared to the larger TL classes. When all terms involving interactions with TL were dropped from the parametric LMM, which allowed a simple comparison of temperature treatments, the -1°C and 1°C treatments were not significantly different for all growth variables (P>0.1). However, the predicted mean for the 3°C treatment was significantly lower than the -1°C treatment in each case (P<0.01), with a difference of 0.36mm (SE=0.06, t-value=5.53), 0.95% (SE=0.18, t-value=5.40) and 0.014mm day^{-1} (SE=0.003, t-value=4.37) for GI, IGR and DGR, respectively. The corresponding values for the difference calculated as the predicted mean for the -1°C treatment minus that of the 1°C treatment were 0.05mm (SE=0.08, t-value=0.61), 0.09% (SE=0.21, t-value=0.42) and 0.0002mm day^{-1} (SE=0.006, t-value=0.06) for GI, IGR and DGR, respectively. The TL slope coefficients obtained from this reduced parametric LMM were -0.012 (SE=0.006, t-value=-2.12) for GI, -0.037 (SE=0.016, t-value=-2.33) for IGR, and -0.00063 (SE=0.0003, t-value=-2.09) for DGR.

(a)


-1
1
3
Female
Male
GI (mm)
Total Length (mm)

(b)


-1
1
3
Female
Male
IGR (%)
Total Length (mm)

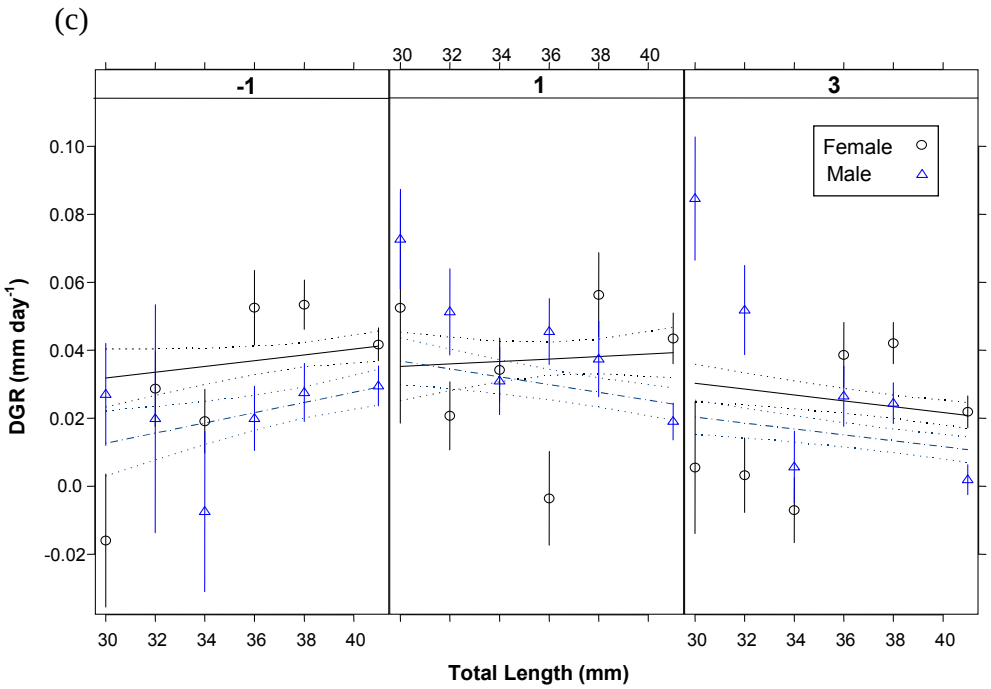


Figure 2.2. Linear mixed model (LMM) predictions of male (triangles) and female (circles) **(a)** growth increment (GI, mm), **(b)** instantaneous growth rate (IGR, %) and **(c)** daily growth rates (DGR, mm day^{-1}) versus total length (TL) at three temperatures (-1ºC, 1ºC and 3ºC). SE bars are shown for the predicted mean values and the fitted regression lines (solid for females; dot-dash for males) with one SE upper and lower bound (dotted lines) obtained from the parametric LMM.

The LMM for IMP, on the other hand, did show a significant difference between the three temperatures in the regression on TL ($P<0.01$) (Figure 2.3), however, sex did not significantly ($P>0.1$) affect the regression within temperature treatments. A strong positive relationship between IMP and TL was only observed for the -1°C treatment with a slope estimate of 0.299 (SE=0.095, t-value=3.15) for females and a slope for males obtained by adding 0.086 (SE=0.047, t-value=1.840) to the estimate for females. In other words, indicating a non-significant difference in the slopes between sexes for the -1°C treatment. For a TL of 40mm, when month was adjusted to October 06 and when averaged across levels of sex, predictions of the mean IMP values for -1°C, 1°C and 3°C were 28.1 days (SE=0.4), 23.6 days (SE=0.4) and 19.0 days (SE=0.3), respectively. For a TL of 30mm and month adjusted to June, the corresponding values were 23.4 days (SE=0.6), 20.3 days (SE=0.5) and 14.4 days (SE=0.4).

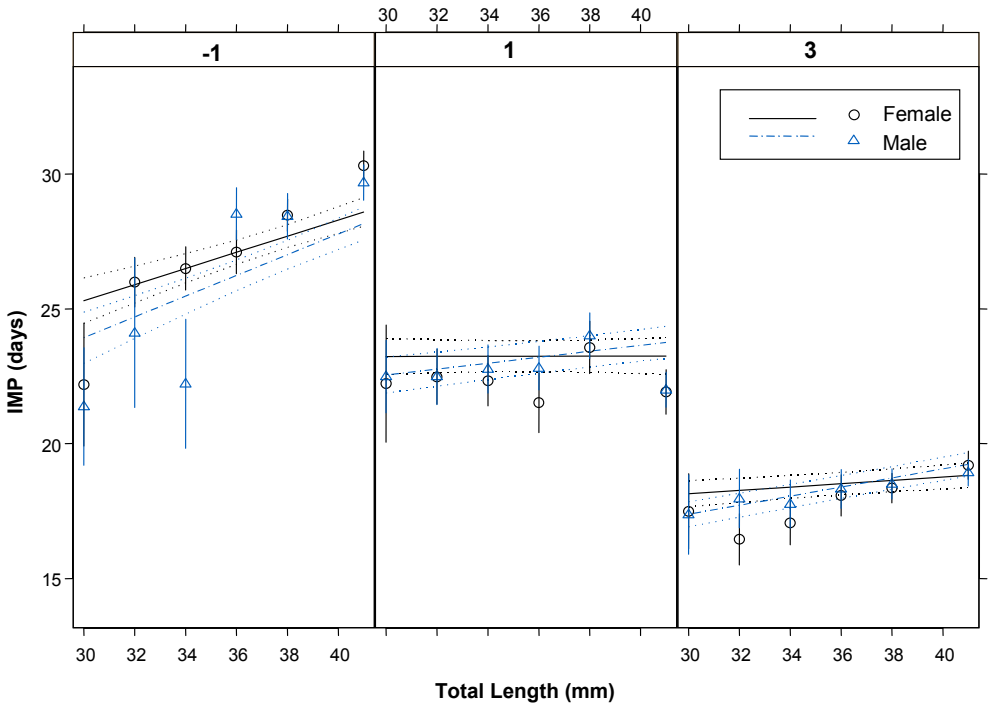
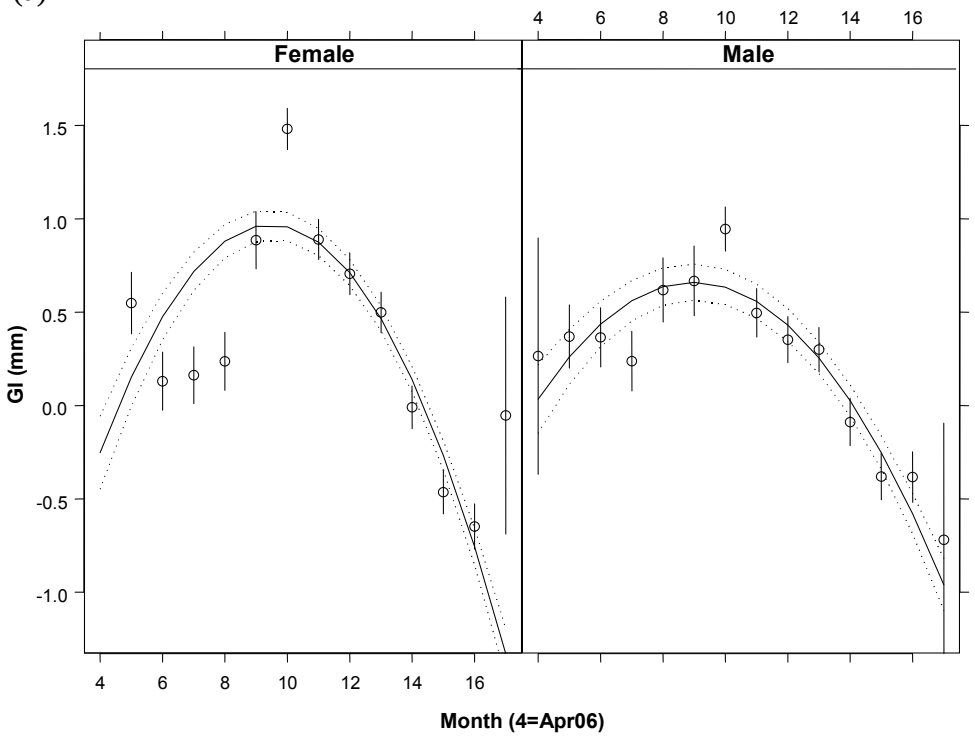


Figure 2.3. Linear mixed model (LMM) prediction of male (triangles) and female (circles) intermoult period (IMP, days) versus total length (TL) at three temperatures (-1ºC, 1ºC and 3ºC) (adjusted to October). SE bars are shown for the predicted mean values and the fitted regression lines (solid for females; dot-dash for males) with one SE upper and lower bound (dotted lines) obtained from the parametric LMM.

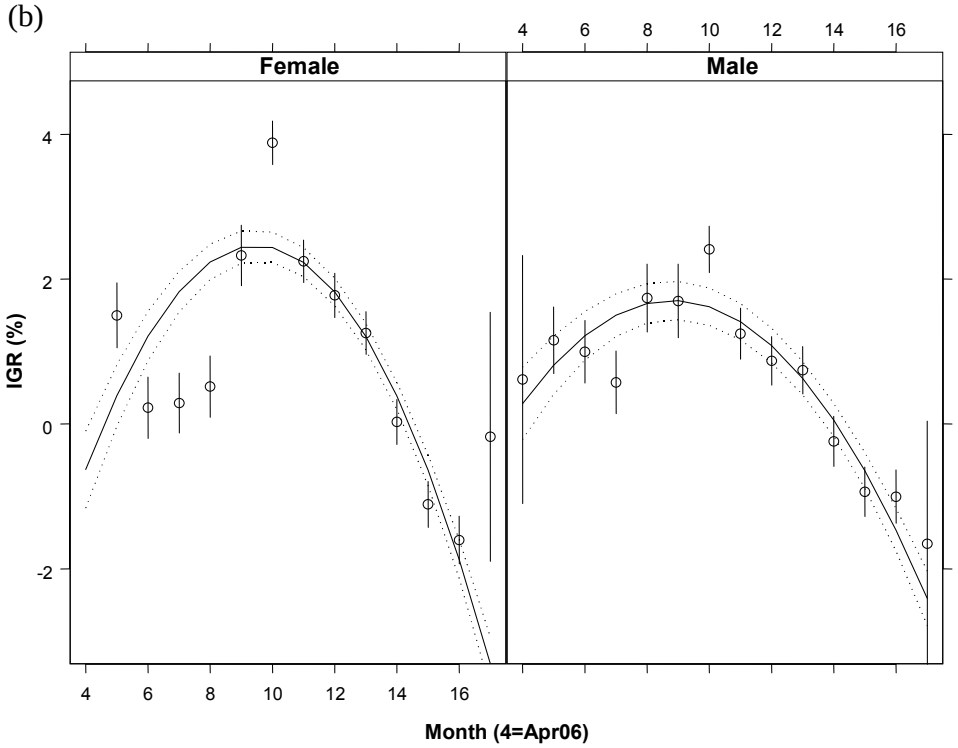
2.4.3.2. Effect of month

LMMs were also used to show predictions of GI, IGR and DGR (Figure 2.4) for sexes (adjusted to 40mm and -1°C) across months. All the growth variables showed similar trends with month and, in each case, sex significantly ($P<0.01$) affected the shape of this relationship. In contrast, the effect of temperature on the relationship with month was not significant ($P>0.1$) (graph not shown). Females reached higher GI, IGR and DGR compared to the males, and then growth rapidly decreased after the peak growth period, towards the end of spring. The decrease in male growth rate was slightly slower than females.

(a)


Female
Male
GI (mm)
1.5
1.0
0.5
0.0
-0.5
-1.0
4
6
8
10
12
14
16
Month (4=Apr06)

(b)


Female
Male
IGR (%)
4
2
0
-2
4
6
8
10
12
14
16
Month (4=Apr06)

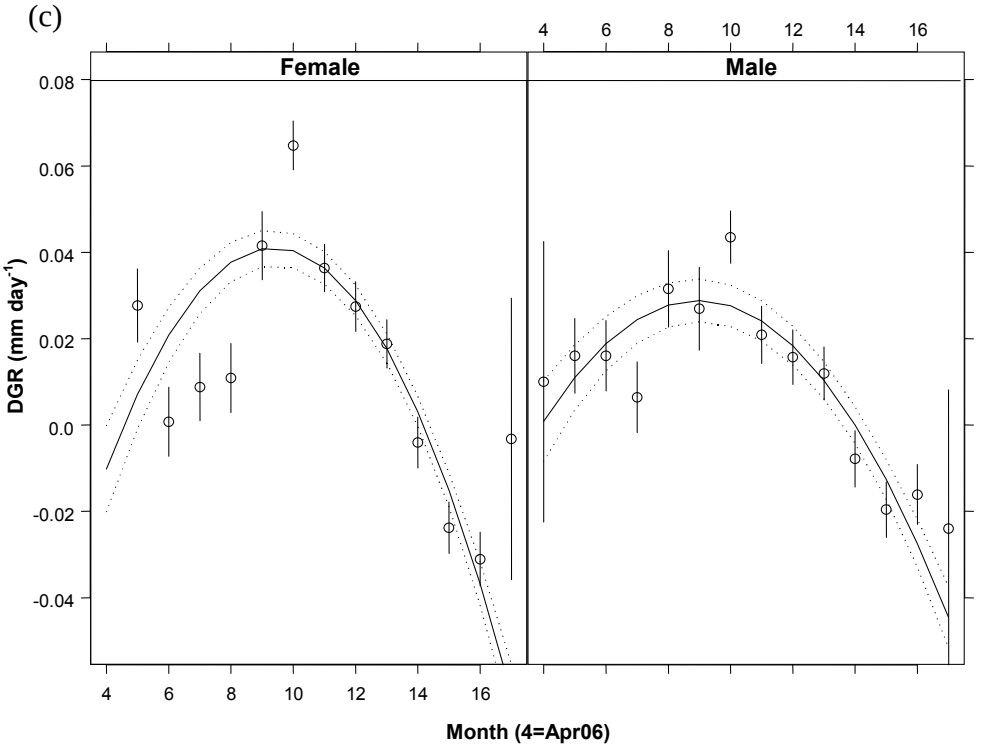


Figure 2.4. Linear mixed model (LMM) prediction of **(a)** growth increment (GI, mm) **(b)** instantaneous growth rate (IGR, %) and **(c)** daily growth rate (DGR, mm day^{-1}) for males and females against month (adjusted to -1ºC, 40mm). SE bars are also shown for the predicted mean values and the solid line is the fitted regression line showing single SE upper and lower bounds (dotted lines) obtained from the parametric LMM.

The LMM for IMP and MS showed no significant differences in the trend with month between males and females (graph not shown), but IMP did significantly differ ($P<0.01$) between temperature treatments. Predictions for IMP and MS versus month for each temperature treatment, averaged across the two sexes are shown in Figure 2.5. There was a general progression of IMP and MS throughout the year until November (11) to December (12) for 1°C and about December to January (13) for 3°C, in which IMP started to decrease. However, in the -1°C treatment, the IMP increased after December. The peak in MS was predicted to be between November and January for all temperatures. However, for the 3°C treatment the mean MS score was closer to 5 for these months than those for the 1°C treatment, while these means were lower again for the -1°C treatment (Figure 2.5*b*). There were no significant MS differences between temperatures ($P>0.1$).

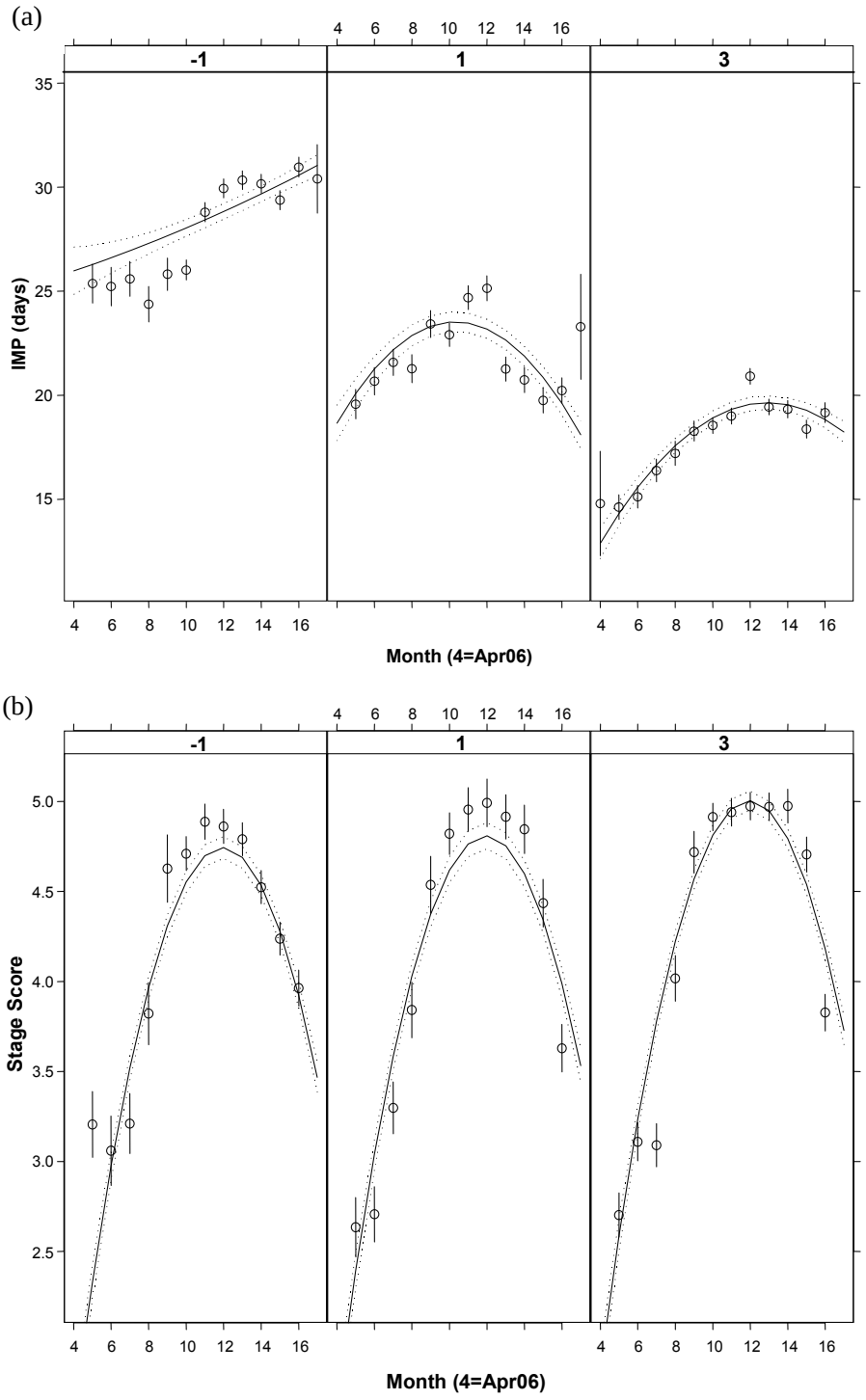


Figure 2.5. Linear mixed model (LMM) prediction of **(a)** intermoult period (IMP, days) and **(b)** maturity stage (MS) for the three temperature treatments (-1ºC, 1ºC and 3ºC) against month, averaged across the two sexes and for total length (TL) = 40mm. SE bars are also shown for the predicted mean values and the solid line is the fitted regression line showing single SE upper and lower bounds (dotted lines) obtained from the parametric LMM (1=Juvenile; 2=2MA, 2FB; 3=2MB, 2FC; 4=2MC, 2FD; 5= 3M, 3FA, refer to Appendix A).

2.4.3.3 Between- and within-animal variance

The between-animal variance (BAV) relative to the within-animal variance (WAV) was estimated to be small for the growth variables (i.e. GI: BAV=0.14, WAV=3.13; IGR: BAV=0.02, WAV= 0.42; DGR: BAV=0.05x10^{-3}, WAV=1.10x10^{-3}), but had a much higher relative value for IMP (BAV=4.11, WAV=3.16) and MS (BAV=0.07, WAV=0.21). The bivariate LMM estimates of individual animal random effects of IMP and IGR were obtained by fitting the model with fixed effects of sex, TL, temperature and month (Figure 2.6). This simultaneous estimation of fixed and random effects gives estimates of the between- and within-animal correlations that correspond to correlations obtained if all animals were of the same size and sex, were kept under the same temperature, and were observed within the same month. The between-animal correlation was estimated to be 0.52 (SE=0.25), while the within-animal correlation was weaker, but more precisely estimated as 0.15 (SE=0.04). Both male and female predicted means plus estimates of the random effects gave a range of short IMP/small IGR to long IMP/large IGR and similarly for the three temperature treatments (Figure 2.6).

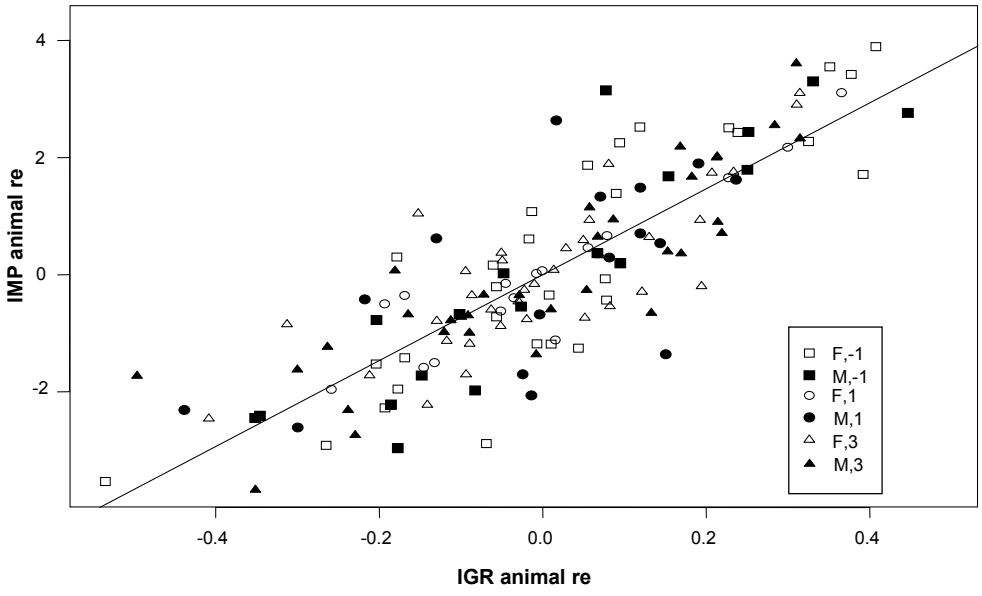


Figure 2.6. Multivariate linear mixed model (LMM) estimates of individual animal random effects for intermoult period (IMP, days) and instantaneous growth rate (IGR, %) showing the animal's sex and the temperature treatment. The line is the regression line fitted to the random effects estimates, with the IMP random effect as the response variable. Note that the correlation estimate of 0.52 was not obtained from this regression but from the fit of the LMM.

2.4.3.4. Assessment of tank effect

The parametric LMM was fitted with the extra fixed effect of tank as a factor to see whether there was any differential effect of the tanks for a given temperature on any of the variables. The data for krill from the 1°C temperature treatment were excluded from this analysis since there was only a single tank for this temperature. There was no significant effect (P>0.1) of tank in addition to temperature for any of the growth variables. However, the IMP for krill in the -1°C tank installed at the end of August had a predicted IMP significantly longer (P<0.01) than those in the other tank by 3.22 days (SE=0.651, t-value=4.95). Krill from the 3°C tank installed in late-August had a predicted IMP significantly shorter (P<0.01) than the other by 1.36 days (SE=0.56, t-value=2.48). This does not invalidate the conclusions about the effect of temperature on IMP since these differences are consistent with the effect of temperature on IMP. This can be concluded since the shorter of the predicted mean IMPs for the -1°C tanks (27.29 days, SE=0.43) was still significantly longer than the longer of the predicted mean IMPs for the 3°C tanks (18.87 days, SE=0.37), where these predictions are adjusted for October 06 and TL of 40mm. Removing the IMP data for the late installed -1ºC tank resulted in little change to the trend with TL shown in Figure 2.3. However, the trend with month was flatter than that in Figure 2.5*a*, but still quite different to the trends for the 1°C and 3°C treatments (graphs not shown). Therefore, despite the detection of a small but statistically significant effect of tank within temperature treatment for IMP, pooling the tanks was still considered justified.

2.4.3.5. Normality

Normal quantile-quantile plots for each variable and for each of the animal-level random effects estimates and within-animal residuals showed that these were well approximated by a normal distribution with this approximation worst, as expected, for the MS variable. A more statistically rigorous approach for the MS variable would be to treat the maturity scores as ordinal scale measurements, which would require analysis of numbers of individual moults for each integer score using a multinomial distribution. Given the small multinomial sample sizes for some combinations of the factors studied and the extra complexity of modelling random effects in this context, it was considered that the approach of considering the scores as a simple metric scale was adequate. However, confidence bounds for means, which go below 1 or exceed 5 obviously, in theory, should be truncated to 1 and 5, respectively. Very good approximations to

normality for both random effects and residuals were obtained for each of IMP and IGR from the fit of the bivariate LMM.

2.5. Discussion

2.5.1. Intermoult period

The mean intermoult period (IMP) of 28.1 days (-1°C), 23.6 days (1°C) and 19.0 days (3°C) for combined males and females for October and at a total length (TL) of 40mm, were all significantly different between the three temperature regimes. Additionally, for a TL of 30mm the corresponding IMP values for June were 23.4 days (-1°C), 20.2 days (1°C) and 14.4 days (3°C). In comparison, the predicted IMP derived from a model used by Kawaguchi *et al*. (2006) for krill in temperatures at -1°C, 1°C and 3ºC (with 95% confidence bounds in brackets) were 34.4 days (28.5, 41.4), 19.1 days (17.8, 20.5), and 14.5 days (13.2, 16.0), respectively. The lowest temperature used in the model by Kawaguchi *et al*. (2006) was -0.5°C so there is uncertainty using this prediction for IMP of krill in the current study at -1°C. Other than the -1°C treatment for which the predicted IMP is just below the lowest value in the confidence bound, the predicted IMP values from this experimental study fit within the confidence bounds for 1°C and 3°C for a 30mm krill.

Overall the IMP reported in the present study ranged from a minimum of 11 days (3°C) to a maximum of 39 days (-1°C) during the whole 14-month cycle, which covers the range of IMP estimates concluded from other laboratory studies and field based predictions using various temperatures (Clarke 1976; Murano *et al*. 1979; Poleck and Denys 1982; Morris and Keck 1984; Ikeda *et al*. 1985; Thomas and Ikeda 1987; Buchholz 1991; Quetin *et al*. 1994; Kawaguchi *et al*. 2006; Kawaguchi *et al*. 2007*a*). Interestingly, using a linear mixed model (LMM) to control for the effect of factors outside those in particular comparisons of interest involving a single factor or a two-factor interaction, the analysis revealed strong effect of month (generally longer IMP during summer) and temperature (shorter IMP in higher temperature) (Figure 2.5*a*), but no significant difference between sexes was observed throughout the 14-month seasonal cycle since the sex by month.f interaction was non-significant (P>0.1).

Tarling *et al*. (2006) concluded that the common pattern of decreasing IMP with increasing temperature was far from regular and may be influenced more by maturity- and sex-specific selective pressures. They predicted that the IMP decreased as temperature increased from -1°C to 2°C (using a logistic regression model fitted to field-

measured moult frequencies), however, this trend reversed above 2°C, with IMP increasing considerably as temperature increased. Tarling *et al*. (2006) further predicted that male IMP was 50% longer than those of equivalently sized females. Implementing the IMP model of Tarling *et al*. (2006) for 40mm animals at -1°C, 1°C and 3°C gives respective IMPs for male and females of 12 and 13 days, 16 and 11 days and 18 and 13 days. These values are much lower than those plotted in Figure 2.5 which, for January and February (the field season of Tarling *et al*. 2006), gives ~28 days, ~24 days and ~19 days, respectively. It appears therefore that IMP is comparatively higher in the incubations in this study. However, Tarling *et al*. (2006) did not give standard errors or confidence bounds on their predictions so it is difficult to compare results to those from the present study. Buchholz *et al*. (1996) also reported that gravid females moulted significantly less frequently than mature males and related this to the energy expenditure of reproduction. Although, they noted that such large differences between gender have not been observed from other laboratory studies.

Through previous studies, IMP has been demonstrated to be affected by temperature, as well as the seasonal maturity cycle for female krill (Thomas and Ikeda 1987: Kawaguchi *et al*. 2007*a*); however, to date no such information is available for males. As demonstrated in the present study, there was no significant difference in IMP between sexes. The pattern of IMP with standardised temperature showed a general increasing progression for all temperatures until around December and then IMP started to decrease. Since the maturity increased to November and December and then decreased after a summer plateau, a similar trend to IMP, it could be suggested that IMP depends directly on the state of maturity and only indirectly on season. The only exception was for krill in the -1°C treatment in which IMP kept increasing after December. This may have been due to the extra data points and longer mean IMP (by 3.22 days) towards the end of the seasonal cycle, resulting from the duplicate -1°C tank being installed in late-August. Since a LMM Scheme was chosen to analyse the data, we can only focus within the boundaries of our results (between April 06 and May 07). In April and May 2007 there was limited data causing the variability between the means to increase. It is expected that IMP would decrease again at a later date in the -1°C treatment and not increase with time indefinitely.

As indicated, in many of the field observations, krill have a longer IMP in winter (resting period) compared to the summer (reproductive period), and this is regarded as the effect of the seasonal temperature cycle (e.g. Siegel *et al*. 2004; Atkinson *et al.*

2006; Kawaguchi *et al*. 2006; Tarling *et al*. 2006). There are mixed views about krill's ability to reduce metabolism during winter. The increase in IMP during winter may be a consequence of krill entering an arrested state (Torres *et al*. 1994) or simply may be due to the effects of low temperature and low food availability, resulting in reduced feeding activity (Quetin *et al*. 1994). Our results indicated that if the temperature and food conditions are constant, krill have a relatively shorter IMP (higher moulting frequency) during the winter months compared to field observations. The reason for shorter IMP during the resting period is probably because they have minimal energy requirements (no reproduction and less growth). Therefore, if krill are supplied with the same amount of food as the summer season, then they might divert excess energy to driving the moulting cycle. A possible benefit for having a longer IMP in summer is that it will be advantageous for males to retain their spermatophores for an extended time to reduce the energy requirements to continually produce extra when they moult. As discussed further in **section 2.5.5**, krill can therefore compensate their growth with longer IMP and a higher growth increment. Immature krill moult more frequently, whereas IMP lengthens for mature krill. This underlines the importance of studying the energy budget and physiology in order to understand the process of krill growth.

2.5.2. Daily growth rates

2.5.2.1. Temperature effect

The minimum and maximum rates of daily growth (DGR) ranged from -0.06 to 0.1mm day^{-1} (-1°C), -0.05 to 0.13mm day^{-1} (1°C) and -0.12 to 0.13mm day^{-1} (3°C) for males and females combined during the peak growth season (August to December). Despite food being plentiful, during the non-growth season (January to July) the growth was significantly lower compared to the growth season for both males and females in the three different temperatures ($P<0.01$). The maximum DGR in this study was similar to those reported in the literature of 0.14mm day^{-1} at 3°C (Morris and Keck 1984) and 0.13mm day^{-1} at 2°C (Buchholz 1991). These rates, however, are two to three times higher than other reported growth rates from laboratory studies (e.g. Murano *et al*. 1979; Poleck and Denys 1982; Ikeda *et al*. 1985; Ikeda 1987; Ikeda and Thomas 1987), and in the present study, there were only a small number of krill that actually approached this maximum DGR. The actual mean values for each temperature in the growth period (Aug-Dec) (Table 2.1) of the present study were considerably lower for combined males and females, 0.03mm day^{-1} (-1°C), 0.03mm day^{-1} (1°C) and 0.02mm day^{-1} (3°C).

In general, data from laboratory studies on krill growth rates are considerably lower compared to those derived from field studies (Buchholz 1991). This is generally thought to be due to sub-optimal and unnatural laboratory conditions, and such effects on krill are difficult to assess (Morris and Keck 1984; Buchholz 1991). The overall mean DGR values are considerably lower in this study compared to various types of field based research (e.g. Clarke and Morris 1983; Rosenberg *et al*. 1986; Quetin and Ross 1991; Ross *et al*. 2000; Reid 2001; Atkinson *et al*. 2006; Kawaguchi *et al*. 2006). For example, the mean krill DGRs reported by Kawaguchi *et al*. (2006) for December for a 40mm krill were 0.13mm day^{-1} (SE=0.03) for females and 0.14mm day^{-1} (SE=0.03) for males, whereas in the present study, DGR rarely exceeded half these values, with peak mean values being in the order of 0.04mm day^{-1} (Figures 2.1*b*, 2.2*c* and 2.4*c*).

Differences in growth and overall condition and energy expenditure of krill can also be explained by possible variation in the size of the experimental chamber in the laboratory, with smaller chambers causing additional stress (Clarke and Morris 1983). However, in our study, similar growth and maturity trends were observed in both male and female krill between the stock population tanks (600-L) and individual 750mL experimental jars over the annual cycle (unpublished data, Appendix D).

In the present study, no significant differences were found in growth increment (GI) between krill from the -1°C and 1°C treatments, however, the IMP was significantly shorter in krill at 1°C compared to -1°C. This would suggest that the DGR will be higher in krill from the 1°C treatment, but due to the high degree of variability in DGR between moults within animals, this difference was not statistically significant. Krill in the 3°C treatment had a significantly lower IMP compared to those at -1°C and 1°C, and it appears that growth (GI) was also lower for these krill. The predicted mean DGR for krill at 3°C was significantly lower than that at -1°C. The substantially lower IMP for 1°C compared to the -1°C treatment, with both these treatments having a similar GI, combined with the lower DGR for the 3°C treatment, suggests that a 1°C temperature regime is possibly optimal for growth under the current experimental condition. This may show a dome shape relationship, but this still has to be confirmed and for this reason we still do not have a good handle on how and what kind of temperature-growth relationship krill generally have. This supports the findings by Atkinson *et al*. (2006), who suggested the existence of an optimal temperature (0.5°C) for krill growth in the south-west Atlantic sector of the Southern Ocean. The declining relationship of growth with total length (TL), an important feature of the IGR/IMP growth model of Candy and Kawaguchi (2006), was

evident in this study, but was weak. For example, the reduction in IGR from 30 to 40mm TL is only 0.37%. This is largely due to low growth rates and the inability to model the change in the slope of the relationship with TL across months (i.e. see Figure 1 of Candy and Kawaguchi 2006), due to the limited range in TL which was greatly intensified when disaggregated by month.

2.5.2.2. Effect of season and sex

The seasonal cycle of growth and maturation throughout this 14-month experiment may have been cued by the simulated light conditions. Light mimicked the natural seasonal pattern and all other environmental conditions were constant, including temperature and food. In the past, change in controlled photoperiod has been shown to induce maturation and spawning of krill (Hirano *et al*. 2003), as well as affecting feeding and metabolism (Teschke *et al*. 2007). In contrast, Thomas and Ikeda (1987) and Kawaguchi *et al*. (2007*a*) concluded that light is not necessary for maturation in krill and showed a seasonal cycle under constant darkness. As a consequence, the debate on whether the Antarctic light cycle is important for possibly triggering physiological parameters of krill, such as maturation, is still unclear. However, it underlines the importance of further developing an understanding on environmental influences, particularly the light conditions.

The growth in all temperature treatments was variable, but it appears males reached peak DGR slightly earlier than females (Figures 2.1*b* and 2.4*c*), which coincided with the period when krill were rapidly maturing corresponding mostly to the month of October (i.e. month 10, Figures 2.1*c* and 2.5*b*). The parametric LMM predicted that month and sex significantly ($P<0.01$) affected the shape of this relationship for each of the growth variables (Figure 2.4). Overall, females reached a higher GI, IGR and DGR compared to males, and then growth rapidly decreased after the peak growth period, towards the end of spring. Growth in males decreased at a considerably slower rate, but during a slightly earlier period. Both sexes commenced shrinkage in late January and February.

2.5.3. Differential growth rates between sexes

Currently there are alternative views about the growth rate between sexes. The parametric LMM in this study predicted higher GI, IGR and DGR in females compared to males. This is due to significantly higher peak growth for females (Figure 2.4) with no

significant IMP difference observed between sexes. Atkinson *et al*. (2006) predicted that there was no significant difference in DGR between males and females in the South Georgia and Scotia Sea regions by predicting DGR for males and females using GI and IMP derived from field IGR experiments during summer. Their conclusion was that lower GI in females compared to males tended to be compensated by shorter IMP, which led to DGR being similar between sexes. On the other hand, Kawaguchi *et al*. (2006) suggested higher growth rates in males by estimating DGR using IGR measurements together with published IMP-temperature functions. Further, recent analysis of the sex ratio using comprehensive length frequency data, model simulation, and the inter-annual pattern in sex ratio, concluded male krill generally grow fast and die young compared to females (Kawaguchi *et al*. 2007*b*).

It is difficult to reconcile the reported trends in differential growth rate between sexes. In fact, in each of the above studies, results appear to disagree. It is highly likely that this could be due to conclusions being made from data that were obtained on different timescales and from different environmental conditions. For example, the result by Atkinson *et al*. (2006) may represent the growth pattern during high summer. On the other hand, the result by Kawaguchi *et al.* (2007*b*) may represent the overall annual growth pattern.

2.5.4. Consideration of experimental effect

The field based model using data from krill in the south-west Atlantic sector showed there was a strong correlation between Chl *a* and growth (Atkinson *et al*. 2006). Not only is quantity, but food quality is also thought to be an important factor for growth (Schmidt *et al*. 1998). For example, diatoms supported higher growth rates for krill in comparison to *Phaeocycstis* species (Ross *et al*. 2000). Under laboratory conditions, it is impossible to reproduce the same diet as krill obtained in the field and it is certain that food quality is poorer. Inadequate diet may have caused the considerably lower growth rates towards the end of the experiment in April/May 2007 in the present study, compared to the start of the experiment in April 2006, which was approximately only a month after the krill were collected from the field, and therefore, still reflecting a more 'natural' condition.

2.5.5. Variance and compensation of growth

Kawaguchi *et al*. (2006) obtained an estimate of the sum of the between- and within-animal variance (since they observed a single moult per animal) for IGR on the log scale of 0.063. To compare this to the results here, taking a maximum mean IGR of 4% (Figure 2.1*a*), the Kawaguchi *et al*. (2006) estimate of variance of $0.063x4^2$, (i.e. approximately 1) can be compared to the corresponding value obtained here of 3.26. It appears from this comparison that the growth rates have been more variable under the experimental conditions in this study than those obtained from krill in the wild. Most of this extra variability has occurred between moults within animals. Unfortunately, the relative size of the between- to within-animal variance for IGR in the wild cannot be determined from the data described in Kawaguchi *et al*. (2006).

By increasing the GI, as IMP lengthens, an equivalent DGR can be obtained to that given by decreasing GI as IMP shortens (Buchholz *et al*. 1996; Atkinson *et al*. 2006; Kawaguchi *et al*. 2006), with this equivalence known as 'compensation.' The compensation between IMP and IGR shown by the random effects estimates in Figure 2.6 indicates that animals that tend to have longer IMPs compensate absolute growth by producing larger IGRs, and vice versa for animals that tend to have shorter IMPs, as quantified by the estimated correlation of 0.52. The correlation at the within-animal level was weaker, but more precisely estimated at 0.15 and indicates that this compensation effect can operate at different moults within the same animal.

The compensation effect of faster moult times corresponding to slower growth increments has been observed for crustaceans in general (Hartnoll 2001), but has never been examined for krill (Kawaguchi *et al*. 2006). This study has, for the first time, been able to confirm that there is such a compensation mechanism that operates at the between- and within-animal level in krill. Candy and Kawaguchi (2006) noted that confirmation and quantification of this compensation effect is required to generalise their IMP/IGR-based growth trajectory to allow length distributions at given ages (and seasons) to be predicted in addition to the mean trajectory they obtained.

2.6. Conclusion

The findings of this study further the integration between laboratory studies and field based investigations on the growth and maturation of krill. Generally, krill are difficult to maintain in close to natural conditions for long periods in a laboratory system. Due to the well acclimated aquarium conditions and evidence of krill behaving

reasonably naturally (Kawaguchi *et al*. 2010), this study provides important information on the seasonal cycle of growth and maturity, particularly during the relatively unknown winter and spring period, which enables these results to complement modelling work completed in the field. In addition, for the first time, this study has been able to confirm that compensation mechanisms do exist between IMP and IGR in individual krill and to quantify the degree of compensation at both the between- and within-animal levels. This will allow modelling of growth trajectories in the wild to quantify the distribution of length given age. Improved growth modelling of length trajectories with age and season, especially in relation to temperature, is critical for the work of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) for setting the precautionary catch limits for the krill fishery (Candy and Kawaguchi, 2006). Future studies will involve examining the energy budget and other physiological parameters to understand the *in situ* effect of temperature on growth and reproduction. Additionally, there is a need for improving the quality and/or quantity of food used in experiments so as to come up with growth rates that are closer to those observed in the field.

-- Chapter three --

Flexible adaptation of the seasonal krill maturation cycle in the laboratory

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3.1. Abstract

A long-term experiment was conducted under controlled laboratory conditions to investigate the influence of various light regimes on the maturation cycle of Antarctic krill (*Euphausia superba*). Based on external sexual characteristics (female - thelycum and male - petasma), krill exposed to a natural Antarctic light cycle, or to a fixed light/dark regime, progress through a natural maturation cycle. However, when krill were maintained in complete darkness following late summer light, at the timing of their sexual regression, the rate of regression accelerated, and as a result, re-maturation occurred three months earlier the following season. The ecological implications of this flexible maturation cycle in response to darkness means that krill can flexibly adjust their seasonal physiological cycle. Overall, krill must use a hierarchy of signals to help coordinate its biological cycles, but it appears that light is possibly one of the most important factors influencing the krill maturation cycle.

3.2. Introduction

Antarctic krill (*Euphausia superba*, hereafter krill) is a keystone species of the Antarctic ecosystem by playing a central role in the food chain and providing several top order predators a major food source (Mangel and Nicol 2000; Alonzo *et al.* 2003*a*). Despite this recognised importance and extensive research on krill, current knowledge of the ecology and biology is far from complete and much still remains to be studied (Nicol 2003). Reproduction of krill is one of the most important characteristics to understand, as reproductive output is a key factor influencing the overall population size. With such a large biomass in the Southern Ocean and the predicted high fecundities and multiple spawning events to support this population, krill require a large energy input (Miller and Hampton 1989; Nicol *et al*. 1995; Quetin and Ross 2001). The major energetic cost of reproduction for females is the accumulation of lipidic yolk for the mature oocytes in the ovaries (Clarke 1980), and for males, are the processes of spermatophore production and affixation to females (Pond *et al*. 1995; Virtue *et al*. 1996). Therefore, the energetically demanding reproductive processes and the strength of the whole population are reliant on receiving enough energy. This is thought to be influenced and regulated by environmental parameters such as food availability, sea-ice extent, photoperiod and temperature (Kawaguchi *et al*. 2007*a*).

Seasonal cycles of maturity in krill are apparently synchronised with seasonal cycles of food, sea-ice and light in the Antarctic environment (Quetin and Ross 1991). For example, following a summer spawning, females undergo a long gonadal resting period and re-organisation of their ovary throughout winter (Denys and McWhinnie 1982; Poleck and Denys 1982; Ikeda 1985; Thomas and Ikeda 1987; Siegel *et al*. 2004; Kawaguchi *et al*. 2007*a*). During the same period, the female external reproductive organ, thelycum, regresses to an immature stage, before a progression and re-maturation of sexual characteristics again during spring. This occurs in time for the spring and summer phytoplankton blooms (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*). Much less is known about the maturation of males, but it is thought that it occurs before the females and it happens at a faster rate (Ross and Quetin 2000).

Understanding the flexibility of the krill maturation cycle is highly important, particularly in relation to the adjustment and synchronisation to local environments, as krill are believed to travel great distances, both latitudinally and longitudinally, within their lifetime (Thorpe *et al*. 2007). However, to date there are a limited number of long-term studies that have examined maturation under controlled laboratory conditions, and the

mechanisms causing synchronisation between the seasonal development of krill maturity and the seasonal cycles of environmental parameters are still subject to speculation (Teschke *et al*. 2008).

The relationship between maturity development and environmental parameters is uncertain in many crustaceans (Hartnoll 1985; Nelson 1991), especially pelagic species because environmental factors are difficult to control for. The reproductive cycle of the ovary in most crustaceans, including krill, has a period of vitellogenesis and undergo spawning during favourable conditions, followed by a recovery period during low productivity (Nelson 1991). Understanding the factors, particularly environmental, that influence the timing and synchronisation of the reproductive cycles is a key area of research in crustacean biology.

Previous laboratory studies have shown the influence of different simulated light regimes on the maturation of krill (Hirano *et al*. 2003; Teschke *et al*. 2008). Both Hirano *et al*. (2003) and Teschke *et al*. (2008) observed maturation of krill shortly after an increase in photoperiod, demonstrating that maturity development of krill was either directly or indirectly affected by differing simulated light regimes, which cue their overall seasonal physiological cycle. Photoperiod has also been shown to influence the maturity cycle of the lobster *Homarus americanus*. A short-day photoperiod appears to promote primary maturation, but prevents secondary vitellogenisis, whereas, a long-day light regime can cue vitellogenic initiation at any time in the lobster moult cycle (Nelson 1986). However, Thomas and Ikeda (1987) and Kawaguchi *et al*. (2007*a*) experimentally induced krill maturation under conditions of constant food, temperature and darkness and suggested that light is not an important factor. Thus, the seasonal cycle of krill maturation may be controlled by an annual endogenous rhythm and not cued by a changing photoperiod or food supply.

This experiment was designed as a first step to reconcile the previous conflicting theories pertaining to photoperiod control of krill maturity under long-term laboratory conditions. This study particularly helps understand the importance of the timing of darkness to regulating their maturity/regression cycle and to provide further insights into the flexibility of krill maturation and how it may be seasonally adjustable.

3.3. Materials and methods

3.3.1 Collection of krill

Live Antarctic krill were collected on 3rd March 2006 (66°02'S, 79°32'E) using a rectangular mid-water trawl net (RMT 8) (Baker *et al*. 1973) on board the RSV *Aurora Australis*. Krill were transferred into 200-L tanks and maintained with a continuous supply of seawater in a cold laboratory (0°C, dim light and no food) on board the ship. Once north of the Polar Front, the continuous water supply was cut off and 50% of the tank water was exchanged with freshly pre-chilled seawater each day.

3.3.2. Aquaria conditions

On return to the research aquarium at the Australian Antarctic Division in Kingston, Tasmania, krill were kept in 1,170-L and 1,670-L holding tanks and acclimated to the aquarium conditions (King *et al*. 2003) on 12th March 2006. The tanks were maintained at 0.5°C, under light conditions that were adjusted to mimic the natural Antarctic seasonal light cycle. Lighting was provided by twin fluorescent tubes. A personal computer controlled-timer system was used to set a natural photoperiod corresponding to that for the Southern Ocean (66°S at 30m depth). Continuous light and a maximum of 100 lux light intensity at the surface of the tank (assuming 1% light penetration to 30m depth) during summer midday (December), a sinusoidal annual cycle with monthly variations of photoperiod and daily variation of light intensity was calculated (Appendix C). At the start of each month, a new photoperiod was simulated by adjusting the timer system (Kawaguchi *et al*. 2010).

For approximately two years after collection (March 2006 to February 2008), krill were maintained in good condition under a natural light regime. Feeding regimes varied during this time and between the two holding tanks. An algae mixture of the cultured pennate diatom *Phaeodactylum tricornutum*, the cultured flagellates *Gemingera* sp. and *Pyramimonas geldicola*, as well as *Pavlova* sp., *Isochrysis* sp. and *Thallasasira* sp., which are concentrated bulk feeds of instant algae mixed with seawater (Reed Mariculture, California), and a commercial larval food (EZ-Larva mixed with seawater) were fed to the krill daily with concentrations summarised in Table 3.1. Water flow into the tanks was closed for two hours per day to enable the krill to feed as much as possible, but avoid the risk of disease and fouling. During the two years of maintenance prior to the experiment, krill maintained a maturity cycle that was expected of a wild

population, i.e. maturation in spring/summer, regression in autumn and recovery and early maturation in winter (R. King, personal observation).

Table 3.1. A table summarising the experimental food concentrations.

		Cultured algae			Instant algae			
		Phaeodactylum (cells mL^{-1})	***Gemingera*** (cells mL^{-1})	***Pyramimonas*** (cells mL^{-1})	***Thallasasira*** (cells mL^{-1})	***Pavlova*** (cells mL^{-1})	***Isochrysis*** (cells mL^{-1})	**EZ-larva** (mL L^{-1})
Before Experimentation (Mar 06 - Feb 08)	**1,170-L Tank**	4.4×10^4	6.7×10^2	1.7×10^3	3.2×10^3	1.2×10^4	4.7×10^4	0.04
	1,580-L Tank	3.3×10^4	4.9×10^2	1.2×10^3	2.4×10^3	8.9×10^3	3.5×10^4	0.04
During Experimentation (Feb 08 - Jan 09)	**Without light**	1.3×10^5	1.9×10^3	4.9×10^3	-	-	-	-
	With light	1.3×10^5	1.9×10^3	4.9×10^3	7.6×10^3	2.8×10^4	1.1×10^5	0.04

3.3.3. Experimental procedure

Approximately 300 krill of mixed sex were distributed into three cylindrical 100-L tanks (~100 krill in each) situated within one 1,000-L rectangular container on February 15th 2008. An additional ~800 krill of mixed sex were distributed into a further four 100-L tanks (~200 krill in each) on May 31st 2008. The entire system was connected to a 5,000-L chilled seawater recirculation system with a variety of filtration devices, guaranteeing identical water quality and temperature (0.5°C) throughout the experiment.

Each tank was covered with a black lightproof plastic container with a sliding door at the front to create a separate light compartment (Appendix G). Lighting was provided by fluorescent tubes (Osram L18W/640 Cool White) covered with a filter film around the outside (ARRI, Marine Blue 131) to reproduce a light spectrum close to an underwater environment. Photoperiod and light intensity were controlled by a PC-controlled timer system. The tanks were divided into three groups (Light 1, Light 2 and Dark) and exposed to the following light regimes (Table 3.2; Appendix C).

The group 'Light 1' was comprised of three tanks starting on February 15th with complete darkness until the end of March, before the light intensity and duration increased to mid-summer conditions, which concluded at the end of August and then decreased until the end of the experiment in mid January 2009. The group 'Light 2' was

comprised of two tanks starting on May 31st 2008 with complete darkness until the end of September and then a light regime that simulated October in the Southern Ocean was exposed to the krill until the end of the experiment. Finally, the krill in group 'Dark' was kept in two tanks, which originated from the same population as 'Light 2' and kept under natural light regime in the stock population tank, and were then exposed to complete darkness from the start of the experiment on May 31st 2008 until mid January 2009. For one of the tanks in groups 'Light 2' and 'Dark,' night vision equipment were used during periods of complete darkness when the sliding door was opened. The use of night vision equipment allowed all experimental procedures to be undertaken in complete darkness. The reason for using night vision equipment on one tank was to check whether the light from the use of torches affect the maturity of krill compared to a tank which gets no light at all.

During periods of complete darkness, or when the light regime was simulated to June in the Southern Ocean, the krill in all three experimental groups were fed daily with *Phaeodactylum tricornutum*, *Gemingera* sp. and *Pyramimonas geldicola*. As soon as krill were exposed to experimental light, the daily ration was increased with the addition of instant algae; *Thallasasira* sp., *Pavlova* sp. and *Isochrysis* sp., as well as the commercial larval food (EZ-Larva, approx. 0.04mL L^{-1}) to the cultured algae diet with concentrations summarised in Table 3.1. This was to reproduce minimum food availability in mid winter in the field.

3.3.3.1 Rationale of light regimes

This experiment was designed to help understand the effects of light on krill maturation cycles, especially the importance of the timing of darkness for regulating their maturity/regression cycles. The photoperiod treatments were arbitrarily chosen and were based on conflicting studies that have suggested the cyclic maturation of krill is directly or indirectly induced by light (Hirano *et al*. 2003; Teschke *et al*. 2008), or light is not an important factor to experimentally induce krill maturation (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*). Due to large logistical demands, a wider range of photoperiodic regimes could not be implemented in further experiments and a gap between experiments starting in February and June was unfortunate, but could not be avoided. However, the krill that remained in the holding systems between February and June under a natural photoperiod would have likely maintained a maturity cycle that was expected of a wild population, i.e. a gradual regression that has been consistently

observed in the aquarium conditions at the Australian Antarctic Division (R. King, personal observation).

Table 3.2. A table showing the light regimes for the actual and perceived months for the three experimental groups of krill: 'Light 1' (krill from 1,170-L holding tank), 'Light 2' (krill from 1,580-L tank) and 'Dark' (krill from 1,580-L tank). In brackets is the maximum light intensity for that month, measured in lux, or whether the tanks were in complete darkness. NVE: use of night vision equipment on one of the tanks in 'Light 2' and 'Dark'.

	'Light 1' (x 3 100-L tanks)	**'Light 2' (x 2 100-L tanks; 1 with NVE)**	**'Dark' (x 2 100-L tanks; 1 with NVE)**
Actual Month	**Perceived Month**	**Perceived Month**	**Perceived Month**
January	Natural Antarctic light cycle	Natural	Natural
February	June (dark)	Antarctic	Antarctic
March	June (dark)	Light	Light
April	October (60 lux)	Cycle	Cycle
May	October (60 lux)		
June	November (80 lux)	June (dark)	June (dark)
July	December (100 lux)	June (dark)	June (dark)
August	December (100 lux)	June (dark)	June (dark)
September	January (80 lux)	June (dark)	June (dark)
October	February (60 lux)	October (60 lux)	June (dark)
November	March (40 lux)	October (60 lux)	June (dark)
December	April (20 lux)	October (60 lux)	June (dark)
January	April (20 lux)	October (60 lux)	June (dark)

3.3.4. Determination of sexual maturity stages

The tanks were checked daily for moults and mortality, and if observed, they were collected, grouped into each week and preserved in 70mL jars with 10% formalin seawater. The sexual maturity stages of males and females were determined by assessing the degree of the petasma or thelycum development according to the technique developed by Bargmann (1945), which was later modified by Makarov and Denys (1980) and Thomas and Ikeda (1987). Maturity stage was assigned a value between 1 and 5 for both males and females corresponding to the stages: 1=Juvenile; 2=2MA, 2FB; 3=2MB, 2FC; 4=2MC, 2FD; 5=3M, 3FA. Higher numbers represent greater maturity. The maturity staging system with corresponding scores is summarised in Appendix A. Since there is a transition between maturity scores, there was often difficulty

in determining whether the animal had progressed fully to the next stage or not. In these instances, the krill was identified as a 0.5 value (i.e. stage 3.5 or 4.5).

3.4. Results

There was no significant difference (P>0.1) in the maturation of krill between the tanks with or without the use of night vision equipment, which suggests the effect of exposure to torch lights during daily experimental handlings for the dark treatments is negligible. Therefore, the data were pooled together for each individual treatment. The mean maturity scores (±SD) of the individual light experiments are shown in Figure 3.1, which are plotted against the time of year for (a) female and (b) male krill. In total, 1,289 individual moulted exoskeletons were measured from all experimental treatments. The percentage of males was generally lower (29%) when compared to female krill (71%).

Female krill in experimental treatment 'Light 1' showed a sharp regression in maturity (Figure 3.1*a*), from full sexual maturity at stage 5 to a mean immature stage 3, when the photoperiod was changed from late summer conditions to complete darkness. After a short period of complete darkness, female krill in 'Light 1' were then provided with an increasing light regime (starting with October – 60 lux) in April 2008, and then re-maturation commenced, with overall full sexual maturity reached in June. Regression of maturity occurred in September 2008, after the mid-summer light conditions ceased at the end of August, and the strength and duration of light gradually declined until the end of the experiment. A similar trend was also observed for males in 'Light 1' (Figure 3.1*b*), however, the regression and re-maturation was not as pronounced.

Krill in experimental treatments 'Light 2' and 'Dark' began in complete darkness in June 2008, after being maintained under natural photoperiod. Krill maturity scores were predominantly 3 in June, which means that krill were still at a low maturity score compared to fully matured females and mostly matured males in the 'Light 1' treatment at the same time. Female and male krill in both 'Light 2' and 'Dark' progressed in maturity regardless of light conditions. Krill reached full maturation in approximately September 2008 and remained so until the end of the experiment in mid January 2009.

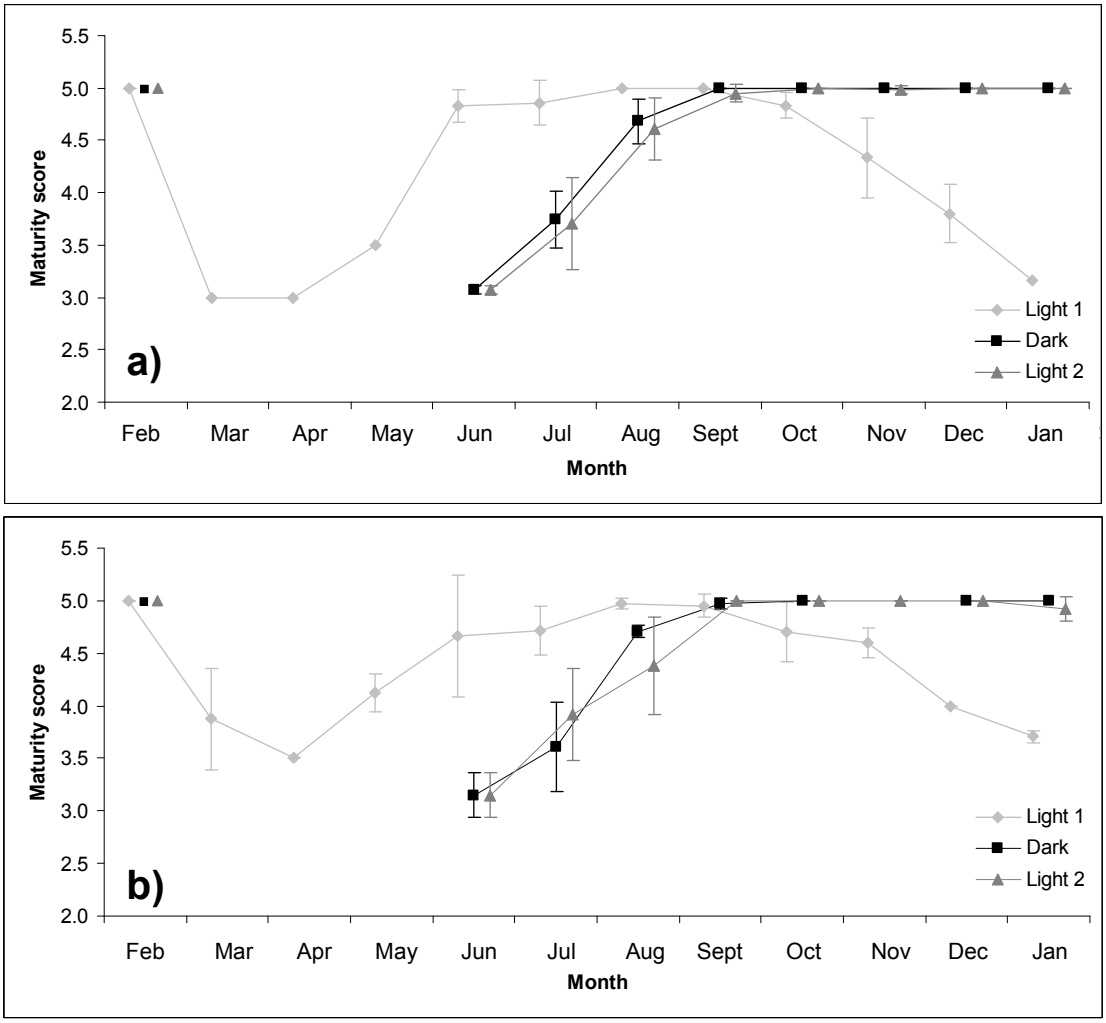

Figure 3.1. Mean maturity scores (±SD) for **a)** female and **b)** male krill in the individual light experiments across months (refer to Table 3.2 and Appendix C for detailed information about the actual light treatments for Light 1, Light 2 and Dark).

3.5. Discussion

The results from this current study exposed one of a number of mechanisms that are likely to link photoperiod to maturation cycles. In particular, this study demonstrated that complete darkness at the period when krill commence sexual regression rapidly accelerated the rate of maturity regression in both males and females ('Light 1'). This was followed by earlier re-maturation the following reproductive season compared to other groups of krill maintained in a fixed light regime ('Light 2' and 'Dark') or even under a natural Antarctic light cycle (Chapter 2). On the other hand, continuous darkness did not have any effect on the rate of progression in krill maturity. This strongly suggests that

the timing of specific light regimes, in this case the timing of darkness, is playing an important role in governing the rates of the krill maturation cycle and that krill can flexibly adjust their seasonal physiological cycle. These results are further presented in a conceptual model (Figure 3.2), which shows krill maturity scores under the various light regimes for each experimental treatment.

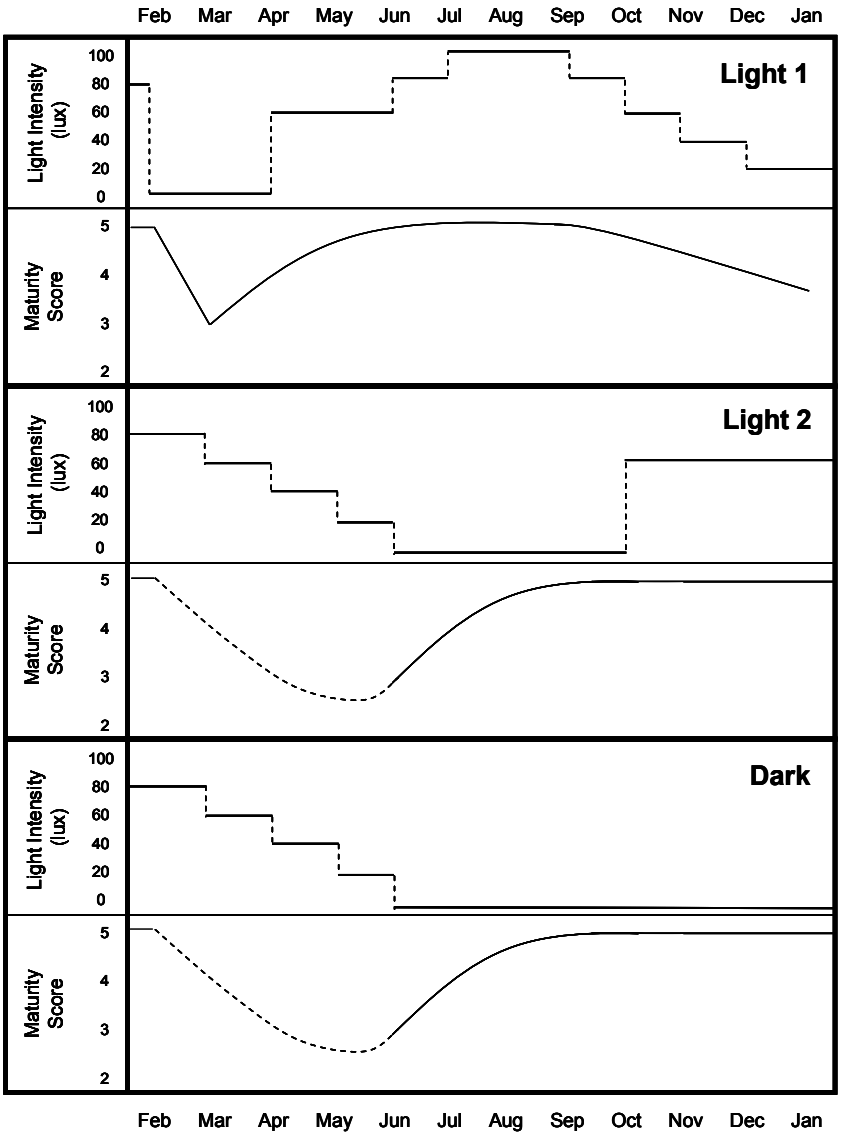

Figure 3.2. A schematic showing female maturity scores under the various light regimes across months; Light 1, Light 2 and Dark (Maturity scores: 1= Juvenile; 2=2FB; 3=2FC; 4=2FD; 5=3FA, sexually mature). Note the dotted line for the maturity scores in the Light 2 and Dark treatments represents the assumed maturity scores before commencement of experiment. A similar representation is expected for male maturation.

The seasonal maturation of krill involves a short summer spawning period when environmental conditions are favourable (Ross and Quetin 2000). During this period, females undergo successful cycles of maturation, vitellogenesis and spawning to produce multiple egg batches until the end of the reproductive season (Cuzin-Roudy 2000). Following the summer reproductive season, krill enter a long period of gonadal rest and ovary re-organisation, as well as a regression of external sexual characteristics for both females (thelycum) and males (petasma) during autumn and winter. At the beginning of the next reproductive season, the secondary sexual characteristics progressively develop into a fully mature form once again to repeat the reproductive processes (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*).

The habitat of krill is dominated by extreme seasonal changes in sea-ice extent, food availability and photoperiod, and thus, the timing of this maturation cycle is very important for krill's overall reproductive success (Kawaguchi *et al*. 2007*a*). The re-maturation process especially needs to be coupled with the spring phytoplankton blooms and overall higher food availability to satisfy the energetic demands for successful reproduction (Ross and Quetin 2000).

To help coordinate its maturation cycle, krill must use a hierarchy of signals. For example, day length and mean water temperature varies with latitude in the Southern Ocean (Figure 3.3). Krill inhabiting areas further south in the Southern Ocean experience a shorter summer period, as well as cooler water temperatures, due to longer-dark days occurring sooner compared to lower latitudes (Knox 1994). Since krill are capable of travelling great distances across many degrees of latitudes within a single season (Thorpe *et al*. 2007), an individual krill may receive different photoperiodic signals from one year to the next. Therefore, krill must synchronise its reproductive activity to spawn in periods of greatest productivity wherever it is. In higher latitudes, midwinter provides complete darkness and krill may need to rely on an internal clock system to be set so that physiologically processes can be performed at the correct time (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*; Teschke *et al*. 2007, 2008). On the other hand, krill need to adapt to a reduced mid-winter light at lower latitudes to control physiological processes. As a result, krill exhibit flexible physiological cycles to cope with varying environmental conditions, which need to be synchronised.

This experiment has shown that the timing of the krill maturation cycle is flexible and can be manipulated by inducing rapid regression with a period of darkness, and the overall seasonal maturational cycle can be re-scheduled. Previous laboratory studies

have shown the influence of different simulated light regimes on the maturation of krill (Hirano *et al*. 2003; Teschke *et al*. 2008). Both Hirano *et al*. (2003) and Teschke *et al*. (2008) observed maturation of krill shortly after an increase in photoperiod, demonstrating that maturity development of krill was either directly or indirectly affected by differing simulated light regimes, which cue their overall seasonal physiological cycle. The timing of photoperiod has also been shown to influence the maturation and moult cycles of the lobster *Homarus americanus*, and thus, photoperiod can cue whether or not lobsters commit to reproduction in that season (Nelson 1991). However, in our study, whether acceleration in regression was due to increased moulting frequency or larger increments of reduction per moulting event is yet to be solved. Overall, it appears that photoperiod is a key factor in controlling the timing of maturity development in all crustaceans.

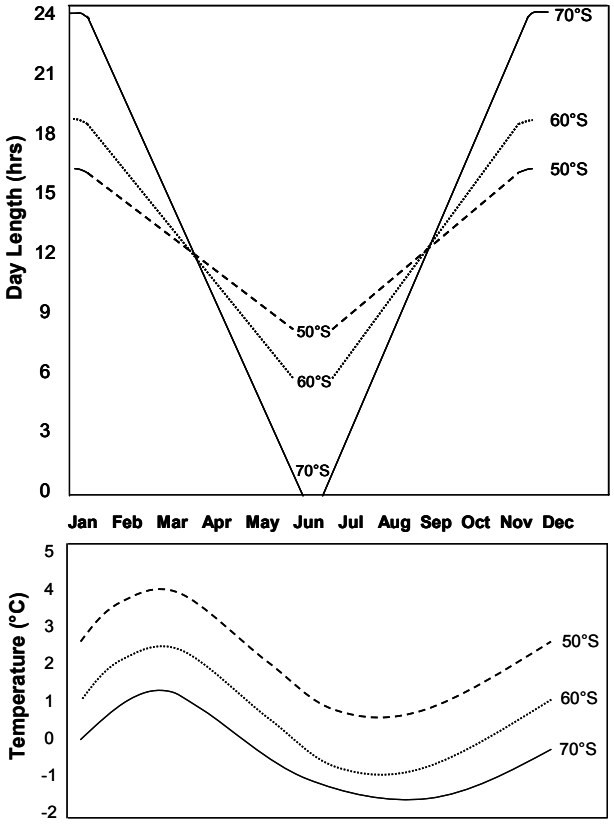

Figure 3.3. Latitudinal variation in day length and mean water temperature during the year between 50° and 70°S. Source of data (approximate) from: Knox (1994), Nicol *et al*. (2000) and Whitehouse *et al*. (2008).

3.6. Conclusion

Our findings demonstrated that an accelerated rate of maturity regression occurs when krill commence sexual regression under complete darkness. As a result, this experiment has exposed one of a number of mechanisms that are likely to link photoperiod to maturation cycles. Krill are capable of adjusting their seasonal biological cycles to cope with varying environmental conditions. To do so successfully, krill must be flexible and use a hierarchy of signals to help coordinate its physiology, and it appears the timing of specific light regimes, particularly the timing of darkness, plays an important role in the krill maturation cycle.

-- Chapter four --

Long-term effect of photoperiod, temperature and feeding regimes on the respiration rates of Antarctic krill (*Euphausia superba*) in the laboratory

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4.1. Abstract

Antarctic krill is thought to undergo an annual cycle of biological processes to cope with seasonal changes in their environment. The question of whether, and to what degree, seasonal environmental parameters such as photoperiod, food availability and temperature govern metabolism in krill is not clear. In this long-term laboratory study, respiration rates were determined in krill incubated under simulated natural light cycle or total darkness, subjected to fed or starved conditions and in krill kept at different temperatures (-1°C, 1°C and 3°C). There was a strong and significant increasing trend of respiration rates with month in all experimental treatments. In August (late winter), the

mean respiration rates ranged between 0.22 – 0.35 µL O_2 mg DW^{-1} hr^{-1} for krill in both simulated seasonal light and complete darkness, and 0.25 – 0.26 µL O_2 mg DW^{-1} hr^{-1} for both fed and starved krill. Mean maximal respiration rates were recorded in October and December for all light and feeding treatments (0.46 – 0.56 µL O_2 mg DW^{-1} hr^{-1}). Mean respiration rates for krill in the temperature treatments ranged between 0.24 – 0.30 µL O_2 mg DW^{-1} hr^{-1} in September reaching mean maximal rates in November and February (0.60 – 0.71 µL O_2 mg DW^{-1} hr^{-1}). The covariate total length of krill was found to be non-significant and there was no significant interaction of experimental treatment with month or generally the main effect of each treatment. Results here suggest that light, food availability and temperature may not be the dominant environmental variables to cue seasonal changes in metabolic activity.

4.2. Introduction

Antarctic krill, *Euphausia superba* (hereafter krill), is a dominant pelagic species in the Southern Ocean and has significant economic and ecological importance (Miller and Hampton 1989; Mangel and Nicol 2000; Alonzo *et al.* 2003*a*). Their overall success and high biomass is due to their ability to adapt to large seasonal changes in their environment; mainly sea-ice extent, food availability and light intensity and duration (Quetin and Ross 1991). Currently, there are various theories on the overwintering strategies of krill (Quetin and Ross 1991). The reduced primary productivity in the ocean, for up to eight months of the year, requires krill to adopt a range of strategies to survive and avoid periods of starvation (Quetin and Ross 1991; Virtue *et al*. 1993*a*; Hagen *et al*. 2001, 2007). Such strategies include overall body shrinkage and protein catabolism (Ikeda and Dixon 1982, Nicol *et al*. 1992), utilisation of lipid reserves (Clarke 1984; Quetin and Ross 1991; Hagen *et al*. 1996; Virtue *et al*. 1996; Hagen *et al*. 2001), switching to a more omnivorous (Clarke 1980; Mauchline 1980; Price *et al*. 1988) and/or carnivorous (Huntley *et al*. 1994; Atkinson *et al*. 2002; Hagen *et al*. 2007) diet, as well as feeding on ice-algae (Marschall 1988; Stretch *et al*. 1988) and sea-floor detritus (Kawaguchi *et al*. 1986), and suppression of metabolism (Kawaguchi *et al*. 1986; Quetin and Ross 1991; Torres *et al*. 1994).

Respiration rates of krill have been extensively studied in the past (i.e. Rakusa-Suszczewski and Opalinski 1978; Segawa *et al*. 1979, 1982; Ikeda and Mitchell 1982, Hirche 1983; Ikeda 1984*b*; Ikeda and Dixon 1984; Kawaguchi *et al*. 1986; Opalinski 1991; Quetin and Ross 1991; Atkinson *et al*. 2002; Swadling *et al*. 2005; Teschke *et al*.

2007), but understanding the physiological responses of krill to a changing environment is scarce. Information on respiration rates over a long period, spanning a complete maturity cycle, is therefore essential for a greater understanding of krill metabolism. A major question that is still unanswered is whether the observed decrease in metabolism in winter is caused simply by reduced food availability. Other important influential environmental factors may include photoperiod, temperature, or more complicated, an endogenous annual rhythm that is affected by a strong seasonal cue in the Southern Ocean.

Reduced metabolic rate and feeding activity are suggested to be the most effective energy-saving mechanisms for adult krill during the less productive months (Quetin and Ross 1991). Nevertheless, Huntley *et al*. (1994) suggests that starvation, body shrinkage and reduced metabolism are not common for krill in winter given they found winter krill feeding carnivorously and excreting at summer rates. This contrasts with Atkinson *et al*. (2002) who demonstrated that respiration and clearance rates of juvenile and adult krill during autumn were only one-third of summer rates, which even failed to increase after 11 days of abundant food. Whereas, during the summer season of the same study, Atkinson and Snÿder (1997) concluded that the metabolic and feeding activity of krill did respond positively to periods of high food concentrations. Irrespective of feeding conditions, Teschke *et al*. (2007) revealed for the first time that the environmental light regime triggers the changes in metabolic rates of krill, suggesting the Antarctic light cycle is possibly the most important effect on the physiological parameters of krill.

One of the main characteristics of the Southern Ocean is that water temperatures remain within a narrow range, with an annual variation rarely exceeding 5°C (Knox 1994). An increase in temperature increases growth rate and metabolic activity in crustaceans (Hartnoll 2001). Studies by McWhinnie and Marciniak (1964), Rakusa-Suszczewski and Opalinski (1978) and Segawa *et al*. (1979) all conclude that respiration rate increased with increasing temperature for krill. However, to date, no study has examined the effects of this narrow temperature range on krill physiology within the context of other environmental factors varying over a long-term.

In the present investigation, a long-term controlled experiment was conducted in the laboratory using krill that were incubated under different light, food and temperature regimes. The objectives were to test, and to determine, which of these key

environmental factors dictate krill respiration rates between the critical period of maturation in late winter/early spring and sexual regression in late summer/early autumn.

4.3. Materials and Methods

4.3.1. Collection of krill

Live Antarctic krill were collected on 7th February 2005 (66°15'S, 74°45'E) and 3rd March 2006 (66°02'S, 79°32'E) using a rectangular mid-water trawl net (RMT 8) (Baker *et al*. 1973) on board the RSV *Aurora Australis*. The water temperature during krill capture was -1°C. Krill were transferred into 200-L tanks and maintained with a continuous supply of seawater in a cold laboratory (0°C, dim light and no food) on board the ship. Once north of the Polar Front, the continuous water supply was cut off and 50% of the tank water was exchanged with freshly pre-chilled seawater each day.

4.3.2. Aquaria conditions

On return to the research aquarium at the Australian Antarctic Division in Kingston, Tasmania, krill were acclimated to the aquarium conditions (King *et al*. 2003). Krill were maintained under light conditions that were adjusted throughout the experimental period to mimic the natural Antarctic seasonal light cycle. Lighting was provided by twin fluorescent tubes. A personal computer controlled-timer system was used to set a natural photoperiod corresponding to that for the Southern Ocean (66°S at 30m depth). Continuous light and a maximum of 100 lux light intensity at the surface of the tank (assuming 1% light penetration to 30m depth) during summer midday (December), a sinusoidal annual cycle with monthly variations of photoperiod and daily variation of light intensity was calculated (Appendix C). At the start of each month, a new photoperiod was simulated by adjusting the timer system (Kawaguchi *et al*. 2010).

Krill were kept in eight different experimental conditions (natural light cycle or complete darkness, fed or starved conditions and temperatures ranging between -1°C and 3°C), as outlined in Table 4.1. An algae mixture of the cultured pennate diatom *Phaeodactylum tricornutum*, and the flagellates *Pavlova* sp. and *Isochrysis* sp., and diatom *Thallasasira* sp., which are concentrated bulk feeds of instant algae mixed with seawater (Reed Mariculture, California), and minced clam meat were fed at various regimes summarised in Table 4.1.

Table 4.1. Summary of the experimental conditions.

Tank	Experimental Period	Tank Size (L)	Photoperiod	Temperature (°C)	Diet (Feeding regime below)	Measurement dates and Symbols in Figure 4.4
H1	Feb-05 to Apr-06	1,670	Darkness	0.5	Phytoplankton + clam	Aug-05, Sept-05, Oct-05, Dec-05
H2	Feb-05 to Apr-06	1,670	Natural	0.5	Phytoplankton + clam	Aug-05, Sept-05, Oct-05, Dec-05
A	Jul-05 to Apr-06	100	Natural	0.5	Phytoplankton	Aug-05, Sept-05, Oct-05, Dec-05, Mar-06
B	Jul-05 to Apr-06	100	Natural	0.5	Phytoplankton + clam	Aug-05, Sept-05, Oct-05, Dec-05, Mar-06
C	Jul-05 to Apr-06	100	Natural	0.5	Starved	Aug-05, Sept-05, Oct-05, Dec-05, Mar-06
-1	Mar-06 to May-07	600	Natural	-1	Phytoplankton + clam	Sept-06, Nov-06, Feb-07, Apr-07
1	Mar-06 to May-07	600	Natural	1	Phytoplankton + clam	Sept-06, Nov-06, Feb-07, Apr-07
3	Mar-06 to May-07	600	Natural	3	Phytoplankton + clam	Sept-06, Nov-06, Feb-07, Apr-07

	Feeding regime (Approx. cells/ml)				
Tank	*Phaeodactylum*	*Thallasasira*	*Pavlova*	*Isochrysis*	Clam
H1	1.3×10^4	3×10^4	3×10^4	3×10^4	~ 1g per 200 krill
H2	1.3×10^4	3×10^4	3×10^4	3×10^4	~ 1g per 200 krill
A	1.3×10^4	3×10^4	3×10^4	3×10^4	~ 1g per 200 krill
B	1.3×10^4	3×10^4	3×10^4	3×10^4	~ 1g per 200 krill
C	Starved**	Starved**	Starved**	Starved**	Starved**
-1	4.3×10^4	1.8×10^3	6.7×10^3	2.6×10^4	~ 1g per 250 krill
1	4.3×10^4	1.8×10^3	6.7×10^3	2.6×10^4	~ 1g per 250 krill
3	4.3×10^4	1.8×10^3	6.7×10^3	2.6×10^4	~ 1g per 250 krill

** Although no food was given to Tank C, the entire tank system was setup in a single water circulation system, so we could not establish a completely food-free environment, and therefore, they may have been exposed to a low level of food.

4.3.3. Measurement of respiration rates

Respiration measurements were conducted in filtered seawater (0.1µm pore size). From each of the experimental tanks, four random krill were selected and incubated individually in 1.2-L glass reagent bottles filled with filtered seawater at the various time points. Krill were rinsed with filtered water that was acclimated to the correct temperature and added to the bottle. Two controls of the same volume without krill were also taken for each experimental treatment. The krill and control bottles were incubated for 24 hours. At the end of the incubation period, oxygen concentrations were measured after immediate fixing for Winkler titrations, as described in Omori and Ikeda (1984) and Meyer *et al*. (2002) by the use of a titrator; 716 DMS Titrino (METROHM). The total length (TL) of krill was measured (Standard Length 1, Appendix F) and the dry weight (DW) was calculated using the following equation:

DW = (POWER(1.082,TL))*4.009

4.3.4. Statistical analysis

The data was analysed using the GenStat package (Lawes Agricultural Trust 2007) to provide an ANOVA for each dataset and for the corresponding treatment applied in each. Initially, the TL was also included as a covariate in each ANOVA. A trend analysis with month was also determined by using a decomposition of the month factor sums of squares into linear and quadratic orthogonal polynomial components with the remainder degrees of freedom used to obtain a lack-of-fit statistic. To do this, a continuous version of the month factor was created as a variable using integers 1 (August) through to 9 (April), even though for each treatment only some of these months were measured. For the temperature treatment a similar orthogonal polynomial decomposition was applied, but restricted to linear and lack-of-fit terms since there were only three temperature levels tested. Residual plots and histograms combined with normal quantile-quantile plots for each variable were used to examine whether or not the assumptions of homogeneous variance and normal distribution for the residuals were reasonable.

4.4. Results

Respiration rates were not significantly different ($P>0.05$) between the randomly selected males and females at each of the measured time points so the data was combined for sex in all experimental conditions. There was a significant and strong

increasing trend with month, which showed an increase in respiration rate from late winter to summer in all experimental treatments (Table 4.2, Figures 4.1, 4.2 and 4.3). Mean respiration rates for krill on a DW basis in the treatments natural light versus complete darkness were 0.22 and 0.35 µL O_2 mg DW^{-1} hr^{-1}, respectively, in August (Figure 4.1) and gradually increased to December (0.46 and 0.56 µL O_2 mg DW^{-1} hr^{-1}, respectively). Mean respiration rates for krill in the treatments fed versus starved in August ranged between 0.25 – 0.26 µL O_2 mg DW^{-1} hr^{-1} (Figure 4.2), showing a gradual increase into summer. Krill in Tank A (phytoplankton plus clam) reached mean maximal rates in December (0.51 µL O_2 mg DW^{-1} hr^{-1}), with similar rates measured at the end of the experiment in March. Krill in Tank B (phytoplankton only) reached mean maximal respiration rates in October (0.51 µL O_2 mg DW^{-1} hr^{-1}), and the mean respiration was stabilised at approximately these rates throughout summer until March. Krill in Tank C (starved), however, reached maximal respiration rates in October (0.53 µL O_2 mg DW^{-1} hr^{-1}), which then decreased in December and March (0.39 and 0.43 µL O_2 mg DW^{-1} hr^{-1}, respectively). Under natural photoperiod, mean respiration rates of krill under varying temperature regimes (-1°C, 1°C and 3°C) increased from September (means ranging between 0.24 – 0.30 µL O_2 mg DW^{-1} hr^{-1}) to reach mean maximal rates in November and February (0.60 – 0.71 µL O_2 mg DW^{-1} hr^{-1}), before a slight decrease in April (0.50 – 0.57 µL O_2 mg DW^{-1} hr^{-1}), under constant food conditions (Figure 4.3).

Table 4.2. ANOVA table for each of the experimental treatments with the linear and quadratic orthogonal polynomial decomposition. The covariate total length (TL) was non-significant and therefore not included in this table.

Photoperiod Treatment					
Source of Variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Month	3	0.223508	0.074503	8.85	<.001
Lin	1	0.216683	0.216683	25.75	<.001
Quad	1	0.006545	0.006545	0.78	0.387
Deviations	1	0.00028	0.00028	0.03	0.857
Treat	1	0.076238	0.076238	9.06	0.006
Month.Treat	3	0.005521	0.00184	0.22	0.882
Lin.Treat	1	0.000176	0.000176	0.02	0.886
Quad.Treat	1	0.000735	0.000735	0.09	0.77
Deviations	1	0.00461	0.00461	0.55	0.466
Residual	24	0.201966	0.008415		
Total	31	0.507233			
Feeding Treatment					
Source of Variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Month	4	0.471441	0.11786	27.84	<.001
Lin	1	0.284949	0.284949	67.3	<.001
Quad	1	0.13034	0.13034	30.78	<.001
Deviations	2	0.056152	0.028076	6.63	0.003
Treat	2	0.007535	0.003767	0.89	0.418
Month.Treat	8	0.116958	0.01462	3.45	0.126
Lin.Treat	2	0.018341	0.00917	2.17	0.768
Quad.Treat	2	0.002251	0.001126	0.27	0.77
Deviations	4	0.096366	0.024092	5.69	<.001
Residual	45	0.190528	0.004234		
Total	59	0.786462			
Temperature Treatment					
Source of Variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Month	3	1.098843	0.366281	88.34	<.001
Lin	1	0.339628	0.339628	81.91	<.001
Quad	1	0.679799	0.679799	163.96	<.001
Deviations	1	0.079415	0.079415	19.15	<.001
Treat	2	0.012389	0.006195	1.49	0.238
Lin	1	0.011074	0.011074	2.67	0.111
Deviations	1	0.001315	0.001315	0.32	0.577
Month.Treat	6	0.036872	0.006145	1.48	0.212
Lin.Lin	1	0.000287	0.000287	0.07	0.794
Quad.Lin	1	0.00015	0.00015	0.04	0.85
Lin.Dev	1	0.004281	0.004281	1.03	0.316
Dev.Lin	1	0.17545	0.17545	4.23	0.047
Quad.Dev	1	0.010527	0.010527	2.54	0.12
Deviations	1	0.004082	0.004082	0.98	0.328
Residual	36	0.149261	0.004146		
Total	47	1.297366			

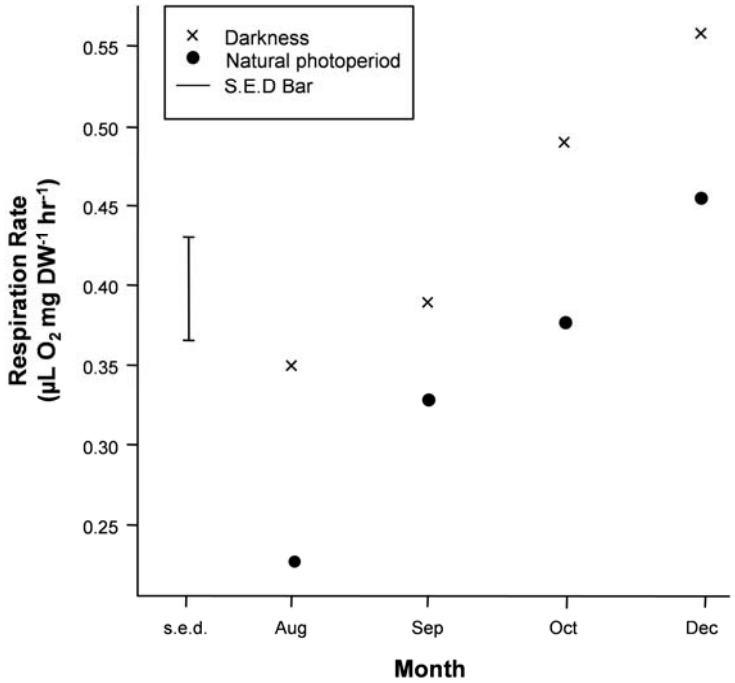

Figure 4.1. Mean respiration rates on a dry weight (DW) basis (µL O_2 mg DW^{-1} hr^{-1}) for the photoperiod treatment (natural light versus complete darkness). The Standard Error of Difference bar (s.e.d) is based on the residual mean square (m.s.) given in Table 4.2 and the sample of four krill in each treatment by month combination and gives the standard error of the pairwise differences in means for a given month. Any differences that are more than twice the length of the s.e.d. bar based on a t-statistic of 2.0 can be considered significant at the 95% level.

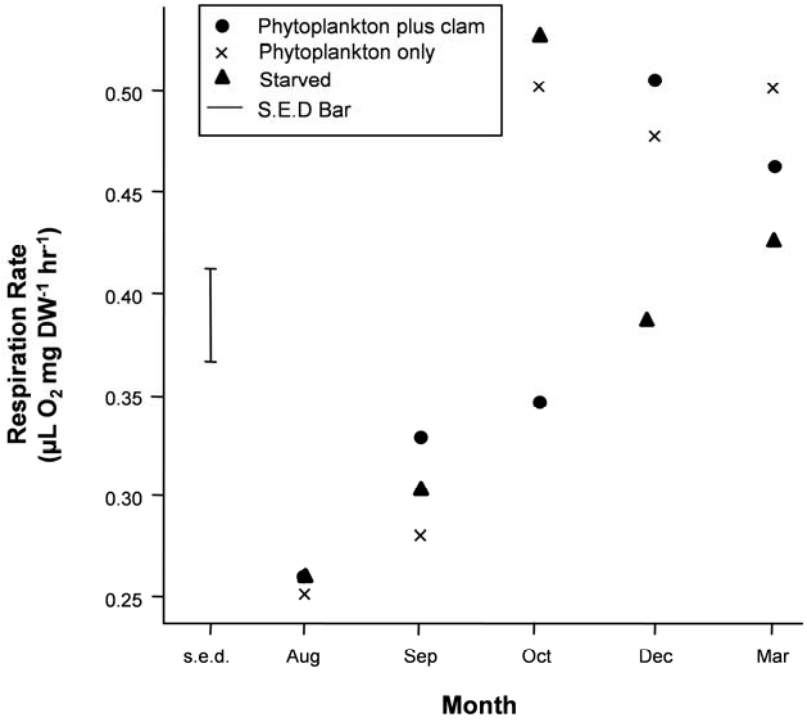

Figure 4.2. Mean respiration rates on a dry weight (DW) basis (µL O_2 mg DW^{-1} hr^{-1}) for the feeding regime treatment (fed versus starved krill). The Standard Error of Difference bar (s.e.d) is based on the residual mean square (m.s.) given in Table 4.2 and the sample of four krill in each treatment by month combination and gives the standard error of the pairwise differences in means for a given month. Any differences that are more than twice the length of the s.e.d. bar based on a t-statistic of 2.0 can be considered significant at the 95% level.

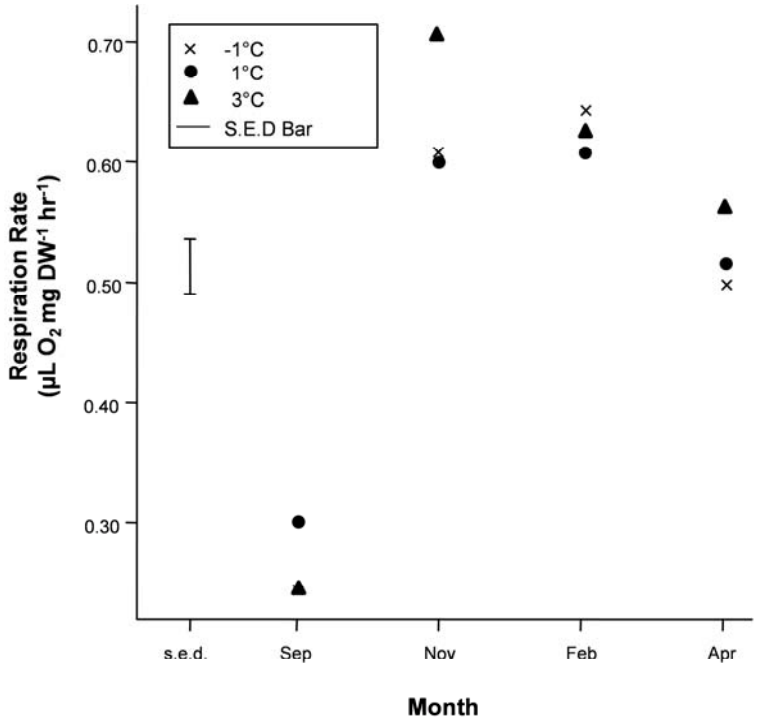

Figure 4.3. Mean respiration rates on a dry weight (DW) basis (µL O_2 mg DW^{-1} hr^{-1}) for the temperature treatment (-1°C, 1°C and 3°C). The Standard Error of Difference bar (s.e.d) is based on the residual mean square (m.s.) given in Table 4.2 and the sample of four krill in each treatment by month combination and gives the standard error of the pairwise differences in means for a given month. Any differences that are more than twice the length of the s.e.d. bar based on a t-statistic of 2.0 can be considered significant at the 95% level.

The covariate TL was non-significant and there was no significant interaction of treatments with the above trend of month. Residual plots and histograms combined with normal quantile-quantile plots (graphs not shown) showed that the assumptions of homogeneous variance and normal distribution for the residuals were reasonable for each dataset. The mean respiration rates were lower in the natural light treatment compared to complete darkness, and the same trend with month was evident for both treatments (Figure 4.1). This was determined from the ANOVA since a significant interaction between treatments in this trend was not detected. However, the main effect of treatment was significant ($P<0.01$) (Table 4.2), as expressed in the lower respiration rates for the natural light regime (Figure 4.1). The corresponding mean difference detected by the main effect term (i.e. averaged over months) was 0.097 with SE of 0.023.

For the food treatments, despite the general increasing trend in respiration rate with month for all treatments (significant “lin” and “quad” terms in Table 4.2, Figure 4.2), there was a somewhat strange result for the food treatments in October, which showed

respiration rates for animals fed with phytoplankton and clam were significantly lower than those krill fed with phytoplankton only and the starved treatment (Figure 4.2). This caused the trend analysis (lin+quad) to have a significant lack-of-fit ("Deviations" terms in Table 4.2) for both the month main effects and month by treatment interaction. However, there is little in the way of useful inferences that can be drawn from this since, overall, there was no significant interaction of month with treatment or main effect of treatment (Table 4.2).

There was a general increasing trend in respiration rate with month for all temperature treatments, but no significant interaction with treatment or main effect of treatment (Table 4.2). The significant lack-of-fit term (i.e. "Deviations") for the month decomposition for the temperature treatment is due to the very sharp increase in respiration rate between September and November, which is difficult to model using a 2^{nd} degree polynomial. The "Dev.Lin" term in the decomposition of the month by treatment interaction represents the difference in the linear trend with temperature of each month's deviations from the fitted 2^{nd} degree polynomial and is only just significant at the 5% probability level. This is due to the reversal of the order of treatments with respect to respiration rate for September for which the 1°C regime gave the highest rate which was not the case for the other months. However, considering the length of the S.E.D bar in Figure 4.3 relative to the differences between temperatures for each month considered separately indicates a lack of statistical significance and combined with this, the marginally significant "Dev.Lin" term, makes any strong inferences drawn from the significance of this term unjustified.

Individual respiration rates, as a function of total length, were compared to a field based study by Quetin and Ross (1991) (Figure 4.4). Laboratory data from this study was generally observed to fall between Quetin and Ross' (1991) winter (July -1.5°C – not feeding) and summer (January 2°C – feeding) trend lines, which represented the relationship between total length and oxygen consumption from the wild in Bransfield Strait.

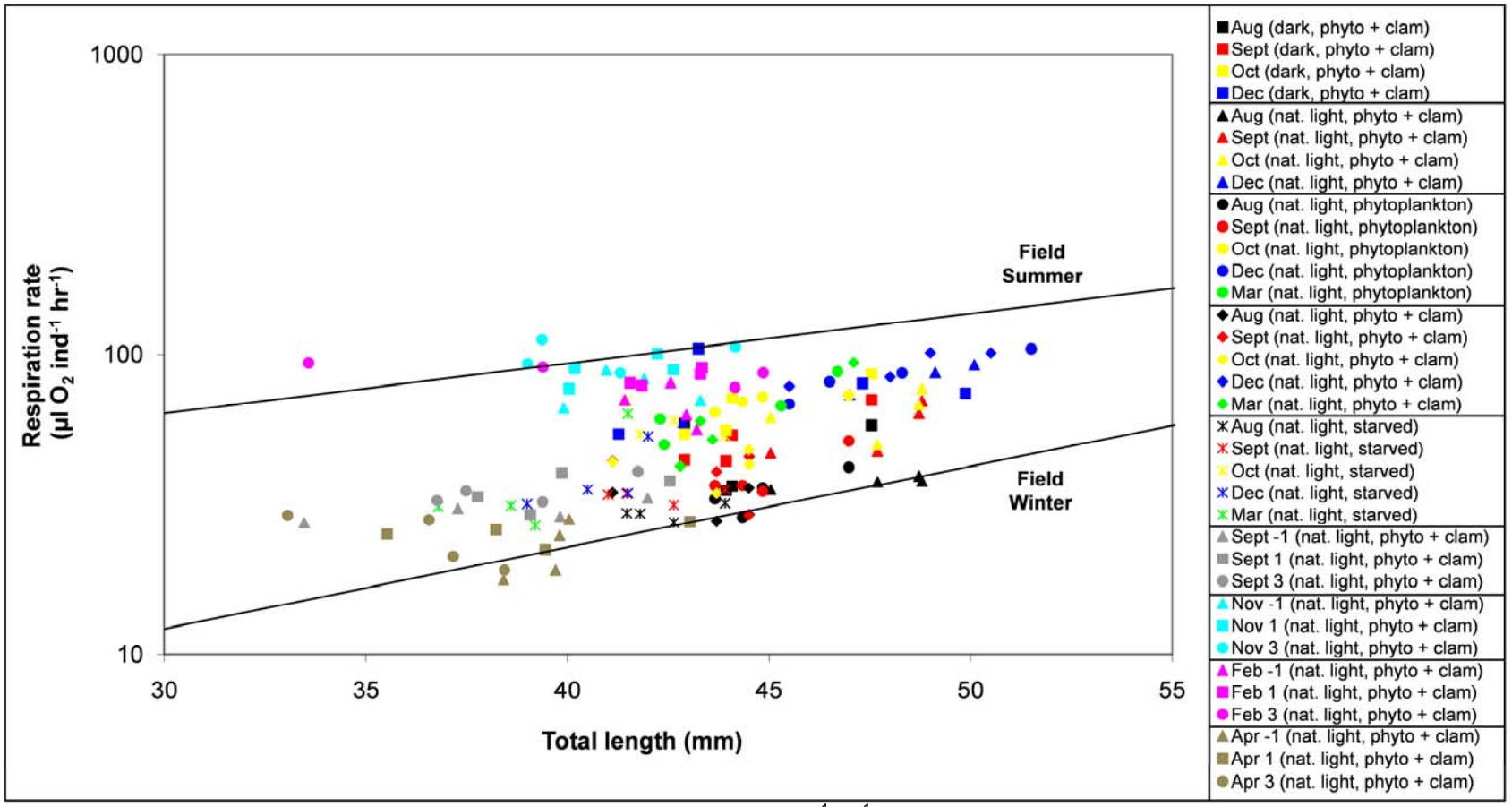

Figure 4.4. Laboratory and field measurements of respiration rates (µL O_2 ind^{-1} hr^{-1}, on a logarithmic scale) of Antarctic krill as a function of total length (mm). The two trend lines are an approximate representation of Quetin and Ross (1991) field respiration measurements in the summer (January 2°C) and winter (July -1.5°C) at Bransfield Strait for krill between 30 and 55mm in length. Laboratory measurements are represented by the coloured symbols at various time points (combined sex) and experimental conditions (natural light versus complete darkness, fed versus starved krill and temperatures between -1°C and 3°C). Refer to Table 4.1 for details on experimental conditions.

4.5. Discussion

There was a strong increasing trend of respiration rate with month, with respiration increasing from late winter/early spring to late summer/early autumn, during the important and energy consuming maturation and regression process of krill, under all experimental conditions; natural Antarctic light cycle versus complete darkness, fed versus starved krill and krill subjected to different temperature regimes (-1°C, 1°C and 3°C). The covariate total length of krill was found to be non-significant and there was no significant interaction of experimental treatment with month or main effect of each treatment. Overall, although the power to detect a difference was low, the results from this study indicate that the three experimental parameters (light, food and temperature) were not directly involved in governing the respiration rates of krill.

4.5.1. Food availability

Recruitment and population size of krill are strongly influenced and dictated by the survival success during the dark and less productive winter (Quetin and Ross 1991; Siegel and Loeb 1995; Siegel 2000*b*; Atkinson *et al*. 2002). Krill undertake a variety of strategies to avoid starvation during reduced primary productivity. Krill are known to exploit many types of food resources in the wild so the chances they will actually encounter overall food shortages may be small (Siegel and Nicol 2000). Krill almost certainly exploit whatever particulate matter is in the water column including elements of the microbial loop and marine snow (Clarke and Tyler 2008), as well as ice-algae (Marschall 1988; Stretch *et al*. 1988) and sea-floor detritus (Kawaguchi *et al*. 1986). Krill also feed carnivorously (Huntley *et al*. 1994; Atkinson *et al*. 2002; Hagen *et al*. 2007). Lowering overall feeding activity and metabolism are also suggested as overwintering strategies (Kawaguchi *et al*. 1986; Quetin and Ross 1991; Torres *et al*. 1994). It is difficult to recognize and interpret whether metabolic rates are the result of changes in feeding activity, or vice versa, feeding activity is reflected by the changes in metabolic rates (Teschke *et al*. 2007). According to Ikeda and Dixon (1984), an increase in metabolic activity in krill was reported to follow the ingestion of food.

Quetin and Ross (1991) showed that oxygen consumption in a large size range of adult krill from Bransfield Strait was 33% higher in a summer experimental setup (January, 2°C) compared to winter (July, -1.5°C). They suggested that adult krill do not feed in winter, and have considerably lowered metabolic rates and negative or zero growth rates. Huntley *et al*. (1994) examined krill west of the Antarctic Peninsula during winter (July-August) and the preceding summer (December-January) in a region similar to that of Quetin and Ross (1991). Huntley *et*

al. (1994) demonstrated that krill fed on a carnivorous diet during winter, particularly copepods, which enabled them to sustain growth comparable to that of summer. They further suggested that starvation, body shrinkage and reduced metabolism is not common for krill during winter, but instead, carnivory is sufficient for growth and to meet energy requirements until phytoplankton blooms occur in spring.

Under constant food supply in this study, krill were observed to be feeding year round. The digestive glands were dark green and healthy looking during the experiment (personal observations), indicating that krill were continuously feeding (Kawaguchi *et al*. 1999; Nicol *et al*. 2004). Total lipid and fatty acid content and composition analysis of the digestive gland (Chapter 6) and the whole body of krill (Chapter 5) also showed that krill were actively feeding year round.

Our results may help interpret respirations rates reported in winter krill by Quetin and Ross (1991). Despite constant year round feeding, a reduction in respiration rates was observed in the current study in all experimental krill during the non-summer months on a dry weight basis. Respiration rates also increased in krill with month in both fed and starved krill. At an individual level, the recorded respiration from krill in the laboratory was distributed fully within the whole range of those measured from summer (feeding) and winter (non-feeding) field experiments by Quetin and Ross (1991), as shown in Figure 4.4. This demonstrated the fact that respiration rates of well fed krill under simulated winter conditions were similar to those non-feeding winter observations from the field. These krill in Bransfield Strait, despite the fact there may be limited sea-ice, may in fact be feeding to some extent and not starving as it has been suggested (Quetin and Ross 1991). Their conclusions came about from observing that both the faecal pellet production and ingestion of phytoplankton in winter were less than 3% of summer rates, suggesting that adult krill did not derive a large proportion of their energy from carnivory. From our observations, we conclude that when food density is low, krill tend to retain their gut content and do not excrete at the same rate as when they are fully fed, which could have also been the case in wild krill from Quetin and Ross (1991).

4.5.2. Temperature

Krill is considered to be stenothermal and is sensitive to slight changes in temperature and even other environmental change (Wiedenmann *et al*. 2008). Temperature has been shown to influence the frequency of moulting in krill, and thus, affect overall growth rates, which can vary considerably, even within a narrow annual temperature range that is observed in the Southern Ocean (Nicol 2000; Chapter 2). McWhinnie and Marciniak (1964), Rakusa-Suszczewski and Opalinski (1978) and

Segawa *et al*. (1979) have also suggested that temperature is a key environmental factor influencing the metabolism of krill, with increasing respiration rates with increasing temperature (0°C to 5°C, -1°C to 2.4°C, and -1.5°C to 5°C, respectively). This is generally true up to 5°C, but above this temperature, krill are probably thermally stressed (Mayzaud *et al*. 2000; Atkinson *et al*. 2006). This relationship between temperature and respiration has been reported for another euphausiid species. Respiration rates in *Thysanoessa longipes* in the Japan Sea increased exponentially with an increase of temperature from 0°C to 8°C (Iguchi and Ikeda 2005).

In our study, however, there was no significant interaction with the temperature treatments (-1°C to 3°C) and month or main effect of temperature, indicating that temperature was not a key environmental variable to cue changes in metabolic activity.

4.5.3. Photoperiod

Light intensity and duration has strong seasonality in the Southern Ocean, from near-constant light in December to near-constant darkness in June. Photoperiod has been suggested as the likely trigger and possibly has the most important effect on the changes in metabolic activity of krill (Teschke *et al*. 2007). The respiration of krill in the starved and fed treatments, as well as krill in the various temperature regimes in our study, increased with month during the stages of maturation and sexual regression under a natural light period. These results may suggest that photoperiod also has a significant influence on krill metabolic rates. However, the respiration rates of krill were higher in the complete darkness compared to a natural light treatment, with the same increasing trend with month. There was no significant interaction between treatments, but when all data was pooled, to increase the power of the test, the main effect of treatment was significantly different ($P<0.01$). These results oppose those by Teschke *et al*. (2007) who reported a significant decrease in respiration rates in krill incubated under complete darkness compared to light. Experimental conditions were different between these studies, with a tank size of 100-L used by Teschke *et al*. (2007), and 1,670-L in the present study. A larger tank may have facilitated an increase in swimming activity inducing higher respiration rates in this study. Additionally, the abrupt changes in the light regime, and also variation in timing and duration of the experiments in Teschke *et al*. (2007), may account for the differences observed. Our study is closer to “natural” conditions in the wild.

4.5.4. Endogenous annual rhythm

It is becoming more apparent that krill possibly have a more complicated endogenous annual rhythm, an internal clock, to control biological cycles, which is influenced by photoperiod or independent of direct control (Teschke *et al*. 2007, 2008). However, this concept of an internal clock mechanism is still not fully proven yet and it is far from clear whether krill enter a state of winter quiescence in the wild. There is evidence on the one hand of continuous feeding throughout the year (Huntley *et al*. 1994) and, on the other, of reduced respiration and lack of response to increase food (Atkinson *et al*. 2002). The different conditions experienced by sampled populations undoubtedly underlie these contrasting observations. Published winter based respiration measurements from the field (i.e. Kawaguchi *et al*. 1986; Quetin and Ross 1991; Huntley *et al*. 1994) and long-term controlled experiments in the laboratory (this study), were completed during the late winter/early spring months so the seasonal timings of krill were in a winter state and comparable. Therefore, reductions in respiration levels were irrespective of effects of photoperiod and feeding conditions during this period. The increasing respiration rates observed from winter to summer in this study, even in starved krill and those kept in complete darkness, were possibly caused by krill changing endogenously to a summer state. This idea of an endogenous annual rhythm is also evident from the growth and maturation results in Chapter 2. A clear seasonal cycle of growth and maturation for both males and females in the three temperature treatments (-1°C, 1°C and 3°C) were observed under constant and high food concentrations. This is also in agreement with Thomas and Ikeda (1987) who showed female krill underwent a regression of external sexual characteristics after spawning, which was accompanied by negative growth and an increase in intermoult period, in both starved and fed krill.

Many biological cycles operate through a combination of endogenous cycles and external environmental cues. If devoid of any external stimuli, free-running endogenous cycles eventually become unsynchronised from the ideal cycle since the mechanism that provide the periodicity are often imperfect (Gaten *et al*. 2008). A coordinating cue, or *zeitgeber*, is required to resynchronise the endogenous cycle. For example, it is believed that Northern krill (*Meganyctiphanes norvegica*) receive a *zeitgeber* from the dawn sun immediately prior to their descent to depths that are devoid of any further light cues (Tarling *et al*. 1999). Although the results from our study show that the three experimental parameters (light, food and temperature) were not directly involved in dictating the respiration rates of krill, there is still no solid evidence to suggest that an endogenous annual rhythm was actually at work. Future experiments will need to incorporate measurements over a whole cycle (over one

year) to determine when an observed respiration cycle starts to become unsynchronised from the ideal annual pattern.

4.6. Conclusion

Krill use a variety of strategies to survive the less productive winter, which highlights the fact that krill is a versatile species and is a contributing explanation for its high biomass in the Southern Ocean (Hagen 1999). It has been widely accepted that a reduction in metabolic activity can occur during winter, with varying environmental variables considered to be responsible, which can differ between different regions of the Southern Ocean. Under controlled conditions, this long-term study has shown that the environmental variables; light, food availability and temperature, may not necessarily be the major cues in governing the metabolic cycles. Although it is suspected that an endogenous rhythm was operating in krill, the existence cannot be confirmed in this study. The significance of an 'internal clock' mechanism is still relatively unknown and warrants further investigation, particularly examining photoperiod as the possible cue for dictating internal processes.

-- Chapter five --

Effects of temperature and constant food supply on immature Antarctic krill (*Euphausia superba*) over a full year: Lipid and fatty acid content and composition

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5.1. Abstract

Changes in total lipid and fatty acid content and composition of immature Antarctic krill over a full year were examined. Krill were maintained in controlled laboratory conditions under temperature regimes of -1°C, 1°C and 3°C, with a constant food supply and a photoperiod simulating the natural Antarctic light cycle. Although a decreasing trend was observed as the season went on, there were no significant differences in total lipid and major fatty acid content and composition between krill sampled throughout the year, except for February. Lipid and fatty acid contents showed depletion at the end of summer in February, suggesting that under constant and plentiful food conditions krill did not store lipid reserves for use during winter. The utilisation of stored lipid by February was possibly for reproduction purposes during spring and summer. The relative (%) levels of the main

polyunsaturated fatty acids (PUFA) increased as the season progressed, particularly 22:6ω3 (DHA), reaching a maximum in February. Accumulation of PUFA during the summer months is likely in relation to maturation processes. Within the 4°C range examined, there was no significant temperature effect on the total lipid and fatty acid content and composition of krill throughout the year.

5.2. Introduction

Antarctic krill, *Euphausia superba* (hereafter krill), is an important species in the Southern Ocean due to its high biomass and the role it plays in food web dynamics (Laws 1985; Miller and Hampton 1989; Mangel and Nicol 2000; Alonzo *et al.* 2003*a*). Krill can cope with a number of extreme environmental changes throughout a full seasonal cycle including sea-ice extent, food availability and photoperiod (Siegel and Loeb 1995). The recruitment and population size of krill are strongly influenced and dictated by the survival success during the less productive and studied winter (Quetin and Ross 1991; Siegel and Loeb 1995; Siegel 2000*a*). It has been suggested that krill use a variety of overwintering mechanisms at different times and regions, which include being able to utilise sea-ice algal communities and/or carnivory (i.e. copepods) as an alternative food source and energy supply (Kawaguchi *et al*. 1986; Marschall 1988; Stretch *et al*. 1988; Daly 1990; Daly and Macaulay 1991; Huntley *et al*. 1994; Hagen *et al*. 1996; Atkinson *et al*. 2002), body shrinkage and protein catabolism (Ikeda and Dixon 1982; Quetin and Ross 1991; Nicol *et al*. 1992), metabolic reduction (Kawaguchi *et al*. 1986; Quetin and Ross 1991; Torres *et al*. 1994) and the utilisation of lipid reserves (Clarke 1984, Quetin and Ross 1991; Hagen *et al*. 1996; Virtue *et al*. 1996; Hagen *et al*. 2001).

Utilisation of stored lipid, in the form of triacylglycerols (TAG), has been examined in relation to winter survival (Clarke 1984; Quetin and Ross 1991; Quetin *et al*. 1994; Hagen *et al*. 1996, 2001; Virtue *et al*. 1996; O'Brien *et al*. in press). Despite krill being able to store significant amount of lipids during the spring and summer when phytoplankton is abundant (Clarke 1984; Hagen *et al*. 1996), it is thought unlikely that krill meet all their energy requirements and sustain themselves throughout winter with these reserves alone (Clarke 1980, 1984; Quetin and Ross 1991; Quetin *et al*. 1994; Ju and Harvey 2004). Accumulation of lipid is maximal in autumn, while there is depletion of lipid during winter, before there are substantial phytoplankton blooms in the spring and summer (Clarke 1984; Hagen *et al*. 1996, 2001).

To date, investigations of lipid and fatty acid content and composition of krill have concentrated mostly on various diet and feeding regimes (for example: Virtue *et*

al. 1993*a*; Virtue *et al*. 1997; Cripps and Hill 1998; Cripps *et al*. 1999; Ju and Harvey 2004; Hagen *et al*. 2007). There has been far less attention to the effects of temperature alone on lipid metabolism in krill, and other pelagic marine species, particularly over a full year. Previous investigations on temperature have examined seasonal changes in lipid concentrations of various crustaceans including the lobster *Homarus americanus* (Ouellet and Allard 2002) and the amphipod *Hyalella curvispina* (Dutra *et al*. 2008). However, it is difficult to account for differences between other factors such as trophic changes in these studies. Investigating the effects of temperature requires maintenance under independently controlled systems, which can be difficult to undertake for many marine animals (Mayzaud *et al*. 2000).

Temperature is known to cause gel/liquid phase transformations of cell structure, which affect cell membrane fluidity (McElhaney 1984; Neidleman 1987). Farkas (1979) concluded that there is an accumulation of polyunsaturated fatty acid (PUFA) at low temperatures in crustaceans, which is an adaptation to maintain physicochemical properties and membrane function in the cold. This is supported by Sellner and Hazel (1982) who also showed this adaptation in the gills of rainbow trout. The opossum shrimp (*Mysis relicta*), which undergoes diel vertical migration in deep thermally stratified lakes, contained lowest lipid content in a 14°C treatment compared to at 4°C. The overall energy conversion efficiency was significantly higher in the 4°C treatment, regardless of prey density (Chess and Stanford 1999). Additionally, phosphatidylcholine and phosphatidylethanolamine are structural phospholipids, and levels are known to increase with decreasing temperature in the euphausiid *Meganyctiphanes norvegica* (Mayzaud *et al*. 2000), in the membranes of rainbow trout gills (Hazel and Carpenter 1985), in freshwater planktonic crustaceans (Farkas *et al*. 1984) and in crabs (Brichon *et al*. 1980).

Northern krill (*Meganyctiphanes norvegica*) survives in waters from the Arctic Ocean to the Mediterranean Sea and has adapted to a natural temperature range from 1ºC to 16ºC (Mauchline 1980; Mayzaud *et al*. 2000). This physiological adaptation to temperature regime is not only shown from lipid changes in Northern krill (Mayzaud *et al*. 2000), but also oxygen consumption (Saborowski *et al*. 2002). Respiration rates increased exponentially with experimental temperature, which ranged from 4ºC to 16ºC. During summer, krill from the Clyde Sea, the Kattegat and Ligurian Sea showed similar respiration rates when incubated at the ambient temperatures found in their respective environments (5ºC, 9ºC and 12ºC, respectively). Overall, this indicates that Northern krill adjust their overall metabolic rates to the local ambient thermal conditions (Saborowski *et al*. 2002). Antarctic krill,

on the other hand, inhabits an isothermal environment, limited to the Southern Ocean, with annual temperature amplitudes <5°C (Clarke and Morris 1983). Despite a small temperature range, temperature has previously been shown to affect the metabolic activity of krill, with increasing respiration rates associated with increasing temperature (McWhinnie and Marciniak 1964; Rakusa-Suszczewski and Opalinski 1978; Segawa *et al*. 1979). However, in Chapter 4, no significant differences in krill respiration rates were recorded between temperature treatments of -1°C, 1°C and 3°C. Temperature alone was therefore not considered critical in influencing the metabolic activity. Potential differences in total lipid, lipid class and fatty acid content and composition under various temperature conditions for krill have not been examined. It is expected there will be minimal changes in structural lipids, which reflects evolutionary adaptation to cold waters (Mayzaud *et al*. 2000).

In the present investigation, a long-term experiment was conducted using krill incubated in tanks subjected to different temperature regimes (-1°C, 1°C and 3°C) for 14-months under controlled laboratory conditions. The objectives were to examine whether krill utilise lipid as an energy source during winter under constant and plentiful food supply, and also whether temperature affects the lipid and fatty acid content and composition over an annual cycle. Overall, this study tested the hypothesis that krill undertake minimal changes in lipid utilisation under constant diet and various temperature conditions. Immature whole krill were used to eliminate any effects of sex and maturity, as well as variation between body tissues.

5.3. Materials and Methods

5.3.1. Sampling and experimental setup

Krill were collected on 3rd March 2006 (66°02'S, 79°32'E) using a rectangular mid-water trawl net (RMT 8) (Baker *et al*. 1973) on board RSV *Aurora Australis*. The water temperature during krill capture was -1°C.

On return to the research aquarium at the Australian Antarctic Division in Kingston, Tasmania, krill were acclimated to aquarium conditions (King *et al*. 2003) and then evenly distributed into three 600-L tanks on 30th March 2006 (Appendix B). These stock populations of krill were maintained at temperatures of -1°C, 1°C and 3°C, under light conditions that were adjusted throughout the experimental period to mimic the natural Antarctic seasonal light cycle. Lighting was provided by twin fluorescent tubes. A controlled-timer system was used to set a natural photoperiod corresponding to that for the Southern Ocean (66°S at 30m depth). Continuous light and a maximum of 100 lux light intensity at the surface of the tank (assuming 1% light penetration to 30m depth) during summer midday (December), a sinusoidal

annual cycle with monthly variations of photoperiod and daily variation of light intensity was calculated (Appendix C). At the start of each month, a new photoperiod was simulated by adjusting the timer system (Kawaguchi *et al*. 2010).

Krill were fed daily throughout the experimental period (April 2006 to May 2007) with the following algal mixture: the cultured pennate diatom *Phaeodactylum tricornutum*, at a final concentration of approximately 4.3 x 10^4 cells mL^{-1}, and the diatom *Thallasasira* sp. (1.8 x 10^3 cells mL^{-1}), the flagellates *Pavlova* sp. (6.7 x 10^3 cells mL^{-1}) and *Isochrysis* sp. (2.6 x 10^4 cells mL^{-1}), which are concentrated bulk feeds of instant algae mixed with seawater (Reed Mariculture, California). These algal densities are representative of spring bloom conditions in the Southern Ocean. Water flow in the tank was closed daily for two hours each day to enable krill to feed. Minced clam, purchased from a local supplier, was also fed to the krill (approximately 1g per 250 krill) when water was flowing into the system. Clam meat has been used successfully in previous laboratory experiments, providing krill with an additional protein source (Hirano *et al*. 2003). Lipid and fatty acid composition of the provided diet is summarised in Appendix H.

From each of the three 600-L tanks (-1°C, 1°C and 3°C), three immature krill were randomly sampled in September 2006, November 2006, February 2007 and at the end of the experiment in April 2007. Three immature krill were also sampled from the field (early March 2006) and at time zero of the experiment starting at the end of March 2006 (n=42). Immature males were defined as having a developing petasma, although it was not yet swollen or fully developed (Appendix A). Immature females contained a small and developing thelycum, which showed no red pigmentation (Makarov and Denys 1980). The immature krill analysed in February and April were predominately krill that had regressed from full sexual maturity earlier in the summer. Samples were wrapped in aluminium foil and stored in a -80°C freezer.

While frozen, each krill was dissected into three sections: abdomen (cut vertically between the carapace and abdominal segments), digestive gland and the thoracic components (containing reproductive tissue) and placed on pre-extracted and pre-weighed glass fibre filters to ensure all tissue and fluids were collected. Although the body of each krill was divided into three segments and analysed separately, the data were pooled for analytical purposes and results reported here are for whole krill to eliminate the variation of body tissue. Samples were frozen at -80°C until analysed.

5.3.2 Lipid extraction

Dissected samples were freeze-dried and weighed, and extracted overnight using a modified Bligh and Dyer (1959) single phase methanol-chloroform-water extraction (2:1:0.8, by volume). Phases were separated by addition of chloroform-water. The final solvent ratio was 1:1:0.9, by volume, methanol-chloroform-water. The total solvent extract (TSE) was concentrated using rotary evaporation at 40°C, transferred to glass vials and stored in chloroform at -20°C.

5.3.3. Lipid class analysis

Individual lipid classes were analysed using an Iatroscan MK V TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Iatron Laboratories, Japan). An aliquot of the TSE was spotted onto silica gel SIII chromarods (5μm particle size) with 1μL micropipettes and developed in a glass tank lined with pre-extracted filter paper. The solvent system used for lipid separation was hexane-diethyl ether-acetic acid (60:17:0.1 by volume). After 25 minutes development, the chromarods were oven dried and analysed immediately to minimise absorption of atmospheric contaminants. The FID was calibrated for each compound class: phosphatidylcholine, cholesterol, oleic acid, hydrocarbon (HC, squalene), triacylglycerol (TAG, derived from fish oil), wax ester (WE, derived from orange roughy, *Hoplostethus atlanticus*, oil) and diacylglyceryl ether (DAGE, derived from shark liver oil). Lipid classes were quantified on an IBM compatible computer using DAPA Scientific software (Kalamunda, Western Australia, Australia). The results from the TLC-FID are generally reproducible to ±10% of individual lipid class abundances (Volkman and Nichols, 1991).

5.3.4. Fatty acid analysis

An aliquot of the TSE was trans-methylated in methanol–chloroform–hydrochloric acid (10:1:1, by volume) for two hours at ~90°C. The mixture was cooled, milli-Q water added and fatty acid methyl esters (FAME) extracted with hexane:chloroform (4:1, by volume, 3x). FAME were dried under a stream of nitrogen, and an internal injection standard (C19 FAME) added.

Samples were analysed by gas chromatography (GC) using an Agilent Technologies 7890A GC (Palo Alto, California, USA) equipped with an Equity™-1 fused silica capillary column (15 mm × 0.1 mm i.d., 0.1μm film thickness), an FID, a split/splitless injector and an Agilent Technologies 7683B Series auto sampler and injector. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 120°C. After injection, the oven temperature was raised to

270°C at 10°C min^{-1} and finally to 310°C at 5°C min^{-1}. Peaks were quantified with Agilent Technologies ChemStation software (Palo Alto, California, USA).

Individual components were identified using mass spectral data and by comparing retention time data with those of authentic and laboratory standards. GC results are subject to an error of up to 5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector using Thermoquest Xcalibur software (Austin, Texas, USA). The GC was fitted with an HP-5 cross-linked methyl silicone fused silica capillary column of similar polarity to that described above.

5.3.5. Statistical analysis

To determine whether there was any significant differences in lipid and fatty acid content between months and temperatures, the linear ('lm') method in the statistical package 'R' (version 2.8.0) was used to fit linear models to carry out a straight forward analysis of variance (ANOVA). The maximum level of interaction included in the linear model was second order (e.g. month:temperature).

5.4. Results

5.4.1. Total lipid content and class composition

Mean total lipid content of immature krill and mean absolute content of the two major lipid classes, triacylglycerol (TAG) and polar lipid (PL), showed a seasonal effect (Figure 5.1). Total lipid content and individual lipid classes were low in field krill collected in early March and increased substantially when maintained under laboratory conditions with plentiful food for approximately one month. After this, lipid content generally decreased throughout the experiment with lowest values recorded in February, the end of summer. In April, the lipid content increased again, before the winter period.

Total lipid content and the absolute concentrations of TAG and PL in krill sampled at 1°C and 3°C in February were significantly lower compared to krill under the same temperatures in September, November and April ($P<0.05$). Total lipid and TAG content were significantly lower in krill sampled at -1°C in February compared to the same treatment in September and November ($P<0.05$), but there were no significant differences in PL for the -1°C treatment throughout the different time periods ($P>0.05$). For each individual month, September, November, February and April, there were no significant differences between the three temperature treatments (-1°C, 1°C and 3°C) for total lipid, TAG and PL content, indicating no temperature

effect on lipid accumulation. The only exception was total lipid and PL content were significantly higher at -1°C compared to 1°C in February ($P<0.05$).

The means and standard errors for the relative levels (% of total lipid) of individual lipid classes for immature krill, in relation to month and temperature (Table 5.1), show that PL was the dominant lipid class, which increased as the season progressed from winter to summer, reaching a mean maximum of 85% at the end of February (-1°C). The next dominant lipid class, TAG, decreased with seasonal progression with a mean maximum of 41% in March, at the start of the experiment, and the lowest mean maximum recorded was 3% in February (1°C), consistent with depletion of lipid stores by the end of summer.

Relative levels (% of total lipid) of the main lipid classes, PL and TAG, were significantly different in February compared to other months for all three temperatures ($P<0.05$). PL was significantly higher in February, whereas TAG was significantly lower. The only exception was PL in the 3°C February treatment, which was not significantly different to the 3°C treatment in the other measured months ($P>0.05$).

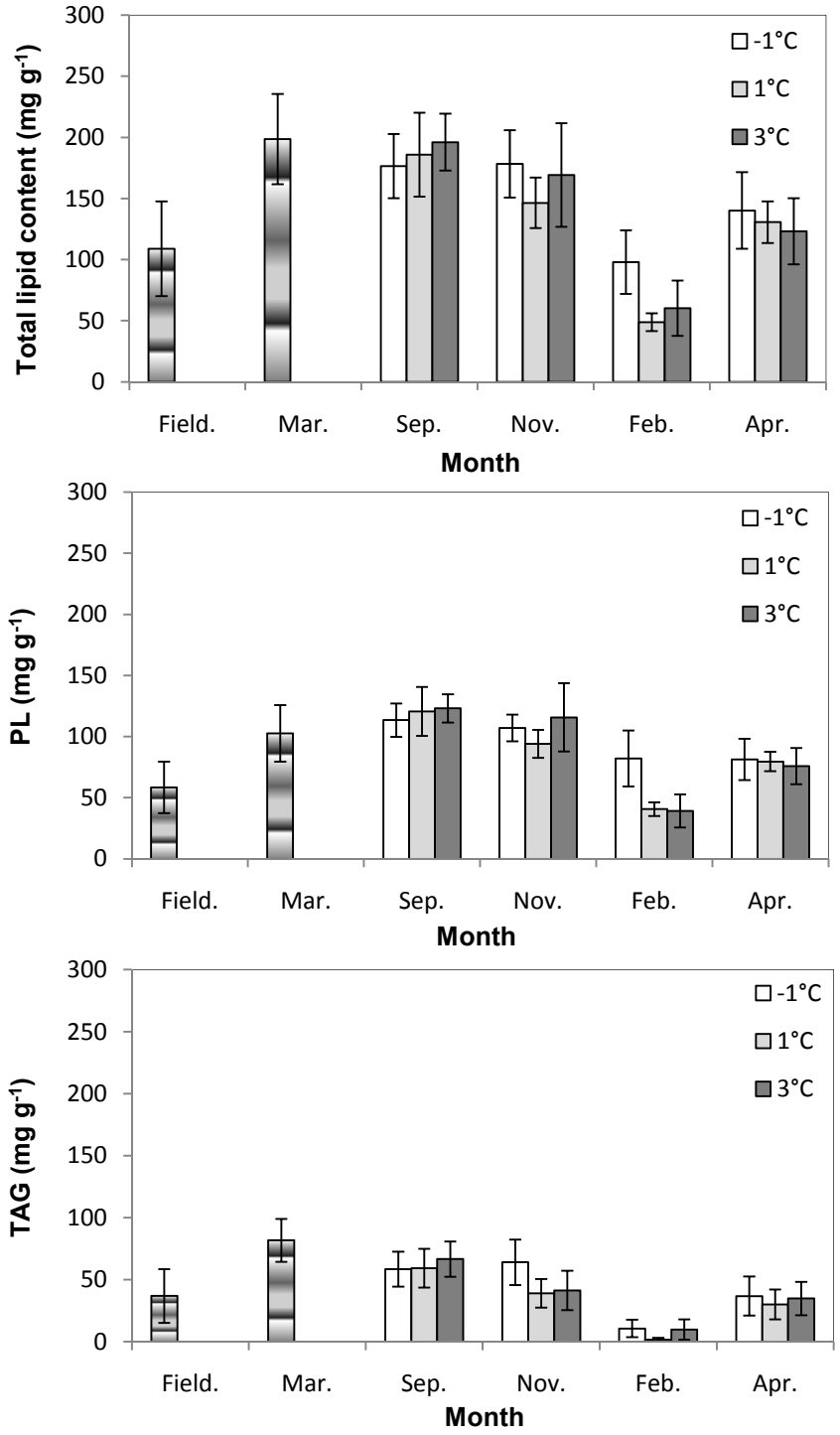

Figure 5.1. Changes in mean (±1 SE): **(a)** total lipid, **(b)** polar lipid (PL) and **(c)** triacylglycerol (TAG) content, per body weight, in relation to month and temperature (-1°C, 1°C and 3°C). Krill from the field and those sampled in March were not under temperature treatment.

Table 5.1. Mean (±1 SE) lipid class composition (as % of total lipid) of krill in relation to month and temperature (-1°C, 1°C and 3°C). Lipid classes: HC – Hydrocarbon, TAG – Triacylglycerol, FFA – Free fatty acid, ST/DG – Sterol/Diaclglycerol and PL – Polar lipid.

		HC			TAG			FFA			ST/DG			PL		
		Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE
Field		0.0	±	0.0	24.8	±	18.1	20.1	±	15.9	3.8	±	2.6	51.3	±	14.7
March		0.0	±	0.0	41.1	±	10.4	4.2	±	5.1	3.6	±	1.5	51.2	±	9.2
September	**-1°C**	0.0	±	0.0	31.9	±	8.7	0.6	±	0.6	1.8	±	0.6	65.6	±	9.0
	1°C	0.0	±	0.0	31.0	±	7.2	1.2	±	1.3	2.1	±	0.7	65.7	±	6.7
	3°C	0.0	±	0.0	33.2	±	8.2	1.0	±	0.7	2.2	±	0.6	63.6	±	7.9
November	**-1°C**	1.2	±	2.3	33.7	±	10.9	1.0	±	0.8	2.3	±	0.6	61.9	±	8.7
	1°C	0.0	±	0.0	26.2	±	9.8	5.1	±	12.3	2.6	±	1.1	66.1	±	13.4
	3°C	0.8	±	0.8	23.2	±	13.0	1.7	±	1.8	4.2	±	3.0	70.1	±	10.8
February	**-1°C**	1.6	±	0.6	9.88	±	8.99	0.5	±	0.4	3.7	±	2.5	84.5	±	8.6
	1°C	2.3	±	1.1	2.8	±	4.4	5.7	±	2.9	5.2	±	2.7	84.0	±	6.1
	3°C	4.9	±	3.0	10.4	±	12.4	14.0	±	9.1	4.2	±	1.3	66.5	±	8.3
April	**-1°C**	1.7	±	0.5	22.1	±	13.3	13.9	±	12.6	3.8	±	0.9	58.5	±	5.5
	1°C	3.4	±	1.3	21.1	±	12.2	9.9	±	7.4	3.6	±	1.2	62.0	±	9.8
	3°C	2.4	±	1.5	24.5	±	1.6	7.1	±	1.8	2.7	±	0.4	63.3	±	0.4

5.4.2. Fatty acid content and composition

General trends of the mean fatty acid content of immature krill also showed seasonal variation (Figure 5.2), from a maximum content recorded in March and a general decrease throughout the season until a minimum in February. However, krill sampled in September, November and April were not significantly different ($P>0.05$) for each temperature treatment (-1°C, 1°C and 3°C). Generally, the fatty acid content was substantially lower in February. The amount of 16:1ω7c in all February temperature treatments were significantly lower than in March and September ($P<0.05$). In comparison to November and April, however, concentrations of 16:1ω7c were only lower in krill sampled in February at -1°C and 1°C ($P<0.05$). Only krill in the 1°C and 3°C treatments in February had significantly lower content of 20:5ω3 (EPA) than September and April ($P<0.05$), but not November ($P>0.05$), and krill in the -1°C treatment in February was only significantly lower than the same temperature in November ($P<0.05$). This result of krill in February containing lower values was not clear cut for all fatty acids. For example, 22:6ω3 (DHA) (Figure 5.2*c*) and 18:2ω6 (LA) were less depleted in February compared to other fatty acids, which indicates sparing (preferential storage) is occurring of these essential PUFA.

Fatty acid content was not significantly different for each temperature against all months measured ($P>0.1$). Krill sampled in September, February and April had no significant differences in fatty acid content between the three temperature treatments

(-1°C, 1°C and 3°C), indicating no clear temperature effect. However, in November, krill at -1°C had significantly higher amounts of 14:0, 18:1ω7c and EPA than krill in both the 1°C and 3°C treatments, but only significantly higher than krill at 1°C for 16:0, 16:1ω7c, 18:1ω9c, LA and DHA ($P<0.05$).

The means and standard errors for the relative (%) levels of fatty acid for immature krill, in relation to month and temperature, are shown in Table 5.2. The saturated fatty acid (SFA) 16:0 was dominant, ranging between 18-22% throughout all months and temperatures. Generally, the main PUFA, 20:4ω6 (AA), LA, EPA and DHA increased as the season progressed, reaching maximum levels in February before decreasing in autumn. The ratio of ω3/ω6 decreased throughout the season, while the ratios of AA/EPA and DHA/EPA increased until the end of the summer. The sum of SFA decreased, while the sum of PUFA increased from March until the end of the following summer, February.

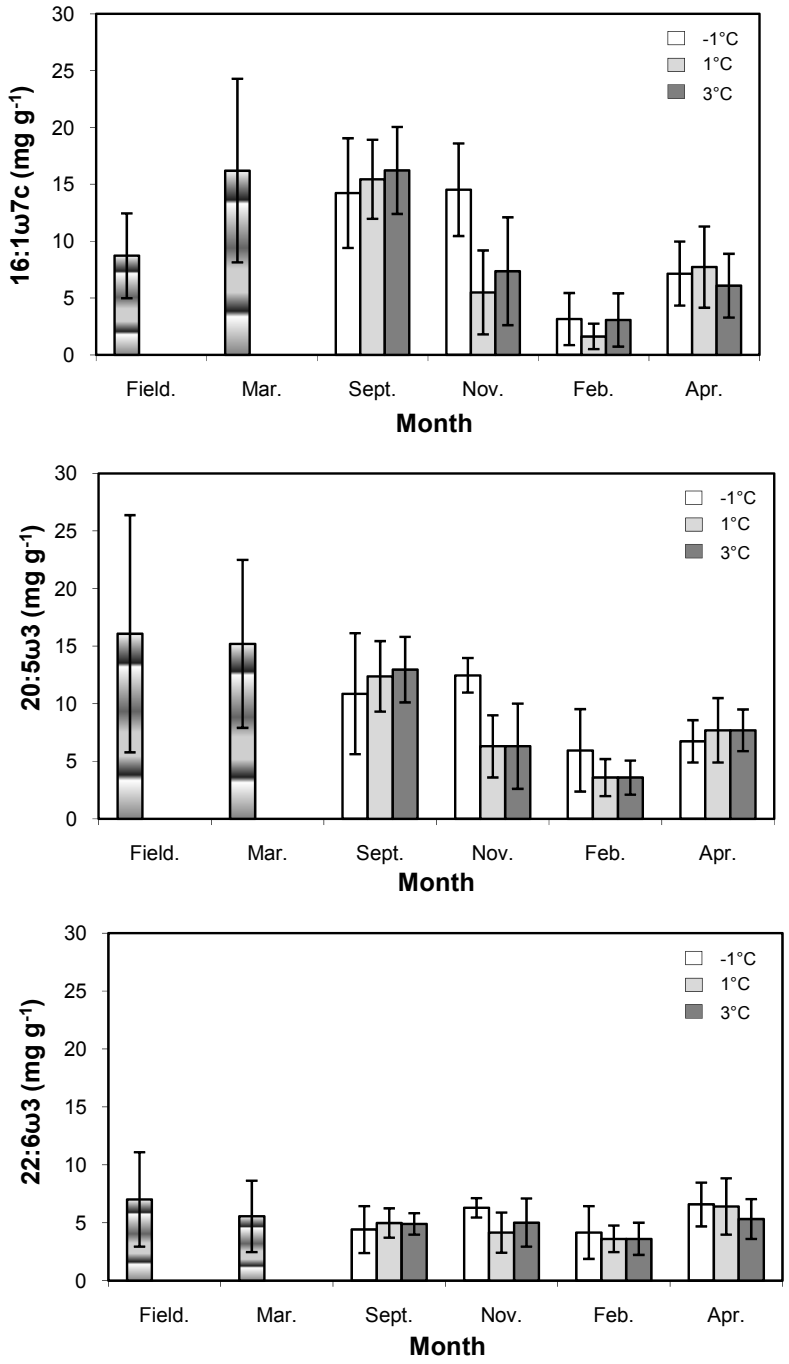

Figure 5.2. Changes in the mean (±1 SE) major fatty acid content: **(a)** 16:1ω7c, **(b)** 20:5ω3 (EPA) and **(c)** 22:6ω3 (DHA), per body weight, in relation to month and temperature (-1°C, 1°C and 3°C). Krill from the field and those sampled in March were not under temperature treatment.

Table 5.2. Mean (±1 SE) fatty acid composition (as % of total fatty acid) of krill in relation to month and temperature (-1°C, 1°C and 3°C).

	Field		March		September			November		
					-1°C	1°C	3°C	-1°C	1°C	3°C
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
14:0	9.9 ± 1.9	10.0 ± 0.9	8.6 ± 1.8	7.8 ± 1.1	7.1 ± 0.9	6.8 ± 0.8	4.4 ± 1.0	4.5 ± 1.7
15:0	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.3	0.8 ± 0.1
16:0	21.4 ± 2.2	22.4 ± 1.2	22.7 ± 3.9	20.9 ± 1.0	21.2 ± 0.4	19.9 ± 0.4	21.7 ± 3.9	20.0 ± 0.6
18:0	1.8 ± 1.3	1.4 ± 0.4	1.2 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.4 ± 0.4	1.1 ± 0.1
phytanic acid	0.7 ± 0.3	0.7 ± 0.1	1.0 ± 0.3	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.0	1.0 ± 0.2	1.1 ± 0.1
16:1ω7c	8.1 ± 1.3	14.3 ± 4.3	16.3 ± 1.4	17.6 ± 2.2	17.8 ± 1.6	16.3 ± 2.5	12.4 ± 2.7	13.2 ± 1.8
18:1ω9c	11.4 ± 4.1	10.7 ± 1.0	12.3 ± 1.5	11.4 ± 0.4	12.3 ± 0.7	12.6 ± 1.3	10.8 ± 3.4	13.8 ± 2.1
18:1ω7c	8.2 ± 1.5	8.0 ± 0.6	6.2 ± 0.5	5.8 ± 0.3	5.9 ± 0.8	5.7 ± 0.7	5.1 ± 1.0	5.5 ± 0.7
20:1ω9	1.4 ± 2.9	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1
16:4	1.1 ± 0.3	1.1 ± 0.1	0.6 ± 0.3	0.6 ± 0.2	0.6 ± 0.1	0.4 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
18:4ω3	2.6 ± 1.0	2.6 ± 0.4	1.6 ± 0.6	1.8 ± 0.3	1.6 ± 0.1	1.3 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
18:2ω6 (LA)	2.6 ± 0.7	2.3 ± 0.3	2.6 ± 0.5	2.7 ± 0.2	2.8 ± 0.2	3.2 ± 0.2	3.8 ± 0.5	3.8 ± 0.2
20:4ω6 (AA)	0.4 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	1.1 ± 0.2	1.1 ± 0.1
20:5ω3 (EPA)	14.4 ± 4.6	13.1 ± 3.5	12.2 ± 4.5	14.0 ± 1.7	14.2 ± 0.8	14.4 ± 1.9	15.4 ± 1.9	15.1 ± 1.3
22:5ω6	0.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.4	0.7 ± 0.4	0.6 ± 0.1	0.6 ± 0.1	1.5 ± 1.0	1.2 ± 0.1
22:6ω3 (DHA)	6.3 ± 2.2	4.8 ± 2.0	5.0 ± 2.0	5.6 ± 1.0	5.4 ± 0.6	7.3 ± 1.6	10.4 ± 2.0	9.4 ± 1.5
Other**	8.8	6.9	7.1	7.1	6.4	6.8	7.9	7.3
Sum SFA	35.7 ± 2.7	36.1 ± 1.7	35.7 ± 5.6	33.2 ± 1.3	32.8 ± 1.0	31.2 ± 1.1	31.0 ± 5.2	29.0 ± 1.5
Sum MUFA	33.9 ± 7.8	37.0 ± 5.3	38.9 ± 2.7	38.7 ± 1.6	39.8 ± 0.6	38.9 ± 2.6	32.5 ± 6.2	36.8 ± 2.9
Sum PUFA	30.4 ± 9.3	26.9 ± 6.1	25.4 ± 7.8	28.1 ± 2.6	27.4 ± 1.3	29.9 ± 3.6	36.5 ± 4.0	34.2 ± 3.0
Total ω3	24.4 ± 8.4	21.3 ± 6.3	19.6 ± 7.6	22.4 ± 3.3	21.9 ± 1.9	23.8 ± 4.0	27.6 ± 4.5	26.3 ± 3.4
Total ω6	4.6 ± 1.9	4.1 ± 1.2	4.6 ± 2.0	4.6 ± 1.3	4.3 ± 0.6	5.2 ± 0.6	7.6 ± 3.9	6.5 ± 0.8
Ratio ω3/ω6	5.3	5.1	4.2	4.9	5.1	4.5	3.6	4.0
Ratio AA/EPA	0.03	0.03	0.04	0.04	0.03	0.06	0.07	0.07
Ratio DHA/EPA	0.43	0.37	0.41	0.40	0.38	0.51	0.68	0.62

**** Other fatty acids (≤ 0.1%) include**: 4,8,12TMTD, i15:0, a15:0, C16PUFA, 16:1ω9c, 16:1ω5c, 16:1ω13t, i17:0, 17:1ω8c, 17:1, 17:0, 18:3ω6, 18:1ω7t, 18:1ω5c, 18:1, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω7c, 20:1ω5c, 20:0, 21:5ω3, 21:0, 22:4ω6, 22:5ω3, 22:4ω3, 22:2ω6, 22:1ω11c, 22:1ω9c, 22:1ω7c, 22:0, C24PUFA, 24:1ω11c, 24:1ω9c, 24:1ω7c, 24:0.

Table 5.2 *continued*. Mean (±1 SE) fatty acid composition (as % of total fatty acid) of krill in relation to month and temperature (-1°C, 1°C and 3°C).

	February									April								
	-1°C			1°C			3°C			-1°C			1°C			3°C		
	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE
14:0	3.2	±	2.0	1.1	±	0.8	4.5	±	1.7	3.6	±	1.8	3.8	±	1.7	3.1	±	1.8
15:0	0.4	±	0.3	0.4	±	0.1	0.8	±	0.1	0.6	±	0.1	0.7	±	0.1	0.6	±	0.3
16:0	19.9	±	1.9	18.9	±	1.6	20.0	±	0.6	18.8	±	1.0	18.8	±	1.9	19.8	±	0.7
18:0	1.4	±	0.6	1.4	±	0.8	1.1	±	0.1	1.1	±	0.3	1.1	±	0.2	1.1	±	0.4
phytanic acid	0.9	±	0.3	1.0	±	0.3	1.1	±	0.1	1.1	±	0.1	1.0	±	0.1	0.9	±	0.1
16:1ω7c	10.0	±	4.1	6.2	±	2.7	13.2	±	1.8	11.4	±	1.9	12.4	±	1.5	11.5	±	2.5
18:1ω9c	11.5	±	4.5	13.0	±	2.4	13.8	±	2.1	15.1	±	2.0	14.5	±	3.1	18.1	±	1.2
18:1ω7c	6.2	±	1.3	6.8	±	0.8	5.5	±	0.7	6.1	±	0.9	6.1	±	0.4	5.9	±	1.4
20:1ω9	0.9	±	0.4	1.0	±	0.3	0.8	±	0.1	1.3	±	0.2	1.1	±	0.2	1.3	±	0.4
16:4	0.5	±	0.4	0.4	±	0.3	0.7	±	0.2	0.4	±	0.2	0.8	±	0.2	0.6	±	0.2
18:4ω3	0.8	±	0.2	0.6	±	0.3	0.8	±	0.2	0.6	±	0.3	0.7	±	0.1	0.4	±	0.2
18:2ω6 (LA)	4.5	±	0.4	5.0	±	0.4	3.8	±	0.2	5.9	±	0.5	5.0	±	0.6	5.3	±	0.4
20:4ω6 (AA)	2.5	±	1.1	2.6	±	0.9	1.1	±	0.1	1.4	±	0.2	1.4	±	0.3	1.4	±	0.3
20:5ω3 (EPA)	17.5	±	3.6	15.8	±	2.0	15.1	±	1.3	11.6	±	2.5	13.0	±	2.3	11.6	±	1.6
22:5ω6	1.1	±	0.4	2.0	±	0.6	1.2	±	0.1	1.3	±	0.3	1.5	±	0.6	1.3	±	0.3
22:6ω3 (DHA)	13.1	±	3.6	17.3	±	4.7	9.4	±	1.5	11.3	±	2.1	10.8	±	2.7	11.0	±	2.3
Other**	5.7			6.4			7.3			8.2			7.3			6.0		
Sum SFA	27.2	±	3.0	24.2	±	2.0	26.1	±	5.0	26.8	±	2.2	27.1	±	3.6	27.0	±	2.0
Sum MUFA	31.3	±	6.1	30.4	±	5.7	34.2	±	4.8	38.6	±	3.3	37.5	±	6.2	39.4	±	2.5
Sum PUFA	41.5	±	7.2	45.3	±	7.1	39.7	±	8.0	34.6	±	5.1	35.4	±	5.3	33.7	±	3.8
Total ω3	32.2	±	8.0	34.6	±	7.6	29.0	±	8.4	24.6	±	5.6	25.4	±	5.7	23.7	±	4.7
Total ω6	8.5	±	2.2	10.2	±	2.2	9.7	±	3.4	9.2	±	1.3	8.7	±	2.3	9.0	±	1.7
Ratio ω3/ω6	3.8			3.4			3.0			2.7			2.9			2.6		
Ratio AA/EPA	0.14			0.17			0.07			0.12			0.11			0.13		
Ratio DHA/EPA	0.74			1.09			0.62			0.97			0.84			0.95		

** **Other fatty acids (≤ 0.1%) include**: 4,8,12TMTD, i15:0, a15:0, C16PUFA, 16:1ω9c, 16:1ω5c, 16:1ω13t, i17:0, 17:1ω8c, 17:1, 17:0, 18:3ω6, 18:1ω7t, 18:1ω5c, 18:1, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω7c, 20:1ω5c, 20:0, 21:5ω3, 21:0, 22:4ω6, 22:5ω3, 22:4ω3, 22:2ω6, 22:1ω11c, 22:1ω9c, 22:1ω7c, 22:0, C24PUFA, 24:1ω11c, 24:1ω9c, 24:1ω7c, 24:0.

5.5. Discussion

Lipids are a critical biochemical component of all living organisms (Ju and Harvey 2004). In the past, there have been a number of intensive studies on total lipid and fatty acid content and composition of krill. Important factors that have been shown to influence the physiological variability of lipid structure and content, as well as fatty acid composition, include sex and maturity stage (Clarke 1980; Kolakowska 1991; Pond *et al*. 1995; Virtue *et al*. 1996; Mayzaud *et al*. 1998), diet (Virtue *et al*. 1993*a*; Cripps *et al*. 1999; Cripps and Atkinson 2000; Hagen *et al*. 2007) and seasonality (Quetin and Ross 1991; Hagen *et al*. 1996; 2001). Our study examined the influence of seasonality and temperature on immature krill under constant diet conditions. The hypothesis tested here was that krill undertake minimal changes in lipid utilisation under constant dietary conditions incubated within the natural range of temperature variations (-1°C to 3°C). Results show that krill storage lipid levels varied throughout the year and krill did not utilise lipid as an overwintering source under these experimental conditions. A clear pattern was seen in lipid storage and utilisation which varied very little between the three incubation temperatures indicating no effect of temperature on lipid levels.

5.5.1. Seasonal variation in total lipid content and composition

The reliance on lipid reserves during winter has been questioned in the past for krill (Clarke 1980; Quetin and Ross 1991). TAG has been identified as the principal storage lipid for krill, which accumulates during periods of high food supply and is metabolised during food shortages (Clarke 1980, 1984; Pond *et al*. 1995; Hagen *et al*. 1996). This would suggest that lipids of krill in the wild would be most depleted in early spring and reach a maximum in autumn before the onset of winter (Clarke 1980; Virtue *et al*. 1997; Hagen *et al*. 1996, 2001). Krill are known to be capable of surviving more than 200 days, the entire winter, without food by utilising their lipid reserves and therefore, tolerate periods of starvation (Hagen *et al*. 1996, 2001; Atkinson *et al*. 2002; O'Brien *et al*. in press). In contrast, Quetin and Ross (1991) concluded that krill are unlikely to meet energy requirements and sustain themselves throughout the whole winter when phytoplankton levels are low with these lipid reserves alone. Additionally, Virtue *et al*. (1997) suggested that lipids are an important energy source during short-term starvation, but over the longer term, lipid reserves are essentially being conserved. Krill are known to be flexible and exploit many types of food resources in the wild year round, from elements of the microbial loop and marine snow to phytoplankton and heterotrophic organisms such as copepods, so they may rarely actually encounter prolonged food shortages and

may not actually require extensive lipid reserves (Clarke 1980, 1984; Atkinson and Snÿder 1997; Siegel and Nicol 2000).

Krill were provided with a constant diet in this study which is an unrealistic scenario compared to field conditions. Previous laboratory experiments within these aquarium facilities have shown that constant food year round and the actual concentrations provided are required for the good health of krill and also to sustain biological cycles of growth and maturation (Kawaguchi *et al*. 2010). Under constant food conditions, krill showed evidence of seasonal changes in mean total lipid content, as well as absolute content of the two major lipid classes, TAG and PL. Mean lipid content increased after approximately one month under laboratory conditions and then generally decreased throughout the experiment from March, with the lowest values recorded at the end of summer (February), before the lipid content increased in April. Although these general seasonal trends were observed, the variance in means was substantial. Lipid content of krill sampled in February were generally significantly lower, while there were no significant differences of total lipid, TAG and PL content observed between the other months; March, September, November and April.

Despite the general decrease in storage lipid throughout our experiment, there were no significant differences between months (except for krill sampled in February) suggesting krill did not rely on TAG solely as an energy reserve to survive the experimental winter with constant food. It is expected that the storage lipids would have been reduced significantly if krill were subjected to periods of starvation, similarly to the experimental results recorded by Yoshida (2009). Although, during periods of reduced food availability and consequent starvation in the wild, a decrease in metabolic rate and low feeding activity are the most effective energy-saving mechanisms for adult krill during winter (Quetin and Ross 1991). Quetin and Ross (1991) calculated that 71% of energy is saved by a reduction of metabolism during winter, while utilisation of lipids (11%) and even body shrinkage (4%) is thought to be a considerably lesser saving. Chapter 4 also showed a reduction in krill metabolism during the non-summer months under a constant food supply. Although it was expected that the reduced metabolic rate was a result of endogenous rhythmic processes rather than food availability or any other environmental variable, the existence could not be confirmed in that study

The significantly lower TAG content in krill in February in the present study is consistent with utilisation of this storage lipid as an energy source. With TAG content virtually depleted in February, the PL content was also significantly lower for the 1°C and 3°C treatments compared to other months. PL, in the form of

phosphatidylcholine, is an essential component for biomembranes, and Hagen *et al*. (1996) further proposed that it may also serve as a storage lipid, which would further increase the potential for energy reserves in krill. The result of TAG depletion and a substantial decrease of PL content in krill (at 1°C and 3°C) in February in this study supports the hypothesis that phospholipids may also play a storage role in krill.

Another explanation for the general lipid decrease throughout the experiment is food quality/quantity. Krill were actively feeding as indicated by full and healthy digestive glands (personal observations), and reasonable total lipid and fatty acid content and composition of the digestive gland of krill (Chapter 6). However, clearance rates measured in April 2007 were between 0.35 and 1.02-L of water filtered ind^{-1} hr^{-1} (unpublished data), which are considerably lower than rates estimated from the field (Morris 1984). Food concentration was supposedly plentiful throughout the experiment (recognised by food remaining in tanks), however, it appears krill may not be able to utilise all the food resources, as they would be expected in the wild, due to possible confinement under laboratory conditions.

The higher lipid contents in spring compared to summer was also reported in Virtue *et al*. (1997), who suggested that lipid reserves are primarily used for reproduction rather than as an overwintering energy source. This finding can be supported by our results, as krill were sexually mature in early- to mid-summer and regressed to an immature form by the time they were analysed in February (Chapter 2). Even though only immature krill were analysed in our study, the depletion of lipid at the end of summer may have been caused by krill utilising lipid resources required for reproductive purposes, during the spring/summer period leading up to the February sampling time. However, the interaction between lipids and reproductive activity is difficult to assess here, as immature krill were used specifically to avoid this.

Since it is not presently possible to reproduce the same diet as krill obtained in the field, food quality is therefore likely to be poorer in laboratory based studies. It is therefore possible that krill were unable to maintain an adequate energy budget required for normal physiological development, particularly reproduction. Krill may have preferentially utilised most of their lipid reserves during the summer period for metabolic rather than reproductive efforts. In April, after these demanding summer processes were complete, lipid reserves increased again. Food quality in relation to reproduction in krill under incubation is further discussed in Chapter 6 (section 6.5.1.1). In this companion study, krill completed pre-vitellogenesis and not full ovarian maturation, probably a result of dietary conditions. However, total lipid contents of krill sampled during spring/summer (November) in this study were

comparable to that of wild krill (Hagen *et al*. 1996, 2001), which undergo growth, vitellogenesis and spawning.

5.5.2. Seasonal variation in fatty acid content and composition

Fatty acid signatures provide trophic information and can be used as indices of feeding and metabolism for krill (Virtue *et al*. 1996; Hagen *et al*. 2001). The krill diet used in this study was made up of the diatoms *Phaeodactylum tricornutum* and *Thallasasira* sp., flagellates *Pavlova* sp. and *Isochrysis* sp., and minced clam meat. Even though food supply was constant, there were differences in fatty acid content and composition in immature krill throughout the experiment. The dominant fatty acids were 16:0, 16:1ω7c, 18:1ω9c and EPA and the majority of fatty acid showed lowest contents in February.

From controlled feeding experiments and field studies, it has been shown that 16:1ω7c and EPA are the principal fatty acid markers for diatoms, while 18:4ω3 and DHA are markers for flagellates (Alonzo *et al*. 2005*a*; Lee *et al*. 2006). Levels of the flagellate marker 18:4ω3 and even DHA were low in comparison to the diatom markers 16:1ω7c and EPA, suggesting that krill fed mostly on diatoms, and the flagellates provided did not contribute significantly to the diet of krill in our study despite being abundant. This selective feeding on diatoms was also seen by O'Brien *et al*. (in press) for wild krill during the winter and spring months. However, the flagellate species (*Pavlova* and *Isochysis* sp.) fed to the krill in our study are smaller in size than diatoms, and thus, contain less lipid which could also explain the differences.

The relative percentage levels of the main PUFA, AA, LA, EPA and DHA, increased in krill as the experiment progressed, peaking in February. Cripps and Atkinson (2000) suggested that an accumulation of PUFA reflects a carnivorous diet. The observed increase in PUFA levels towards the end of the experiment in this present study may be due to krill preferably feeding on the clam meat, than earlier in the experiment. Clam meat is known as an effective food to enhance the reproductive success of laboratory krill (Hirano *et al*. 2003), and the consequent levels of PUFA is believed to play a crucial role in the embryo hatching success (Yoshida *et al*. in press). This was also recognised by Harrison (1990) who suggested that ω3 PUFA is an important nutrient for ovarian maturation in crustaceans. Despite only immature krill being analysed in our study, the observed increase in PUFA during the summer months may be correlated to the build up and potential reproductive processes of krill. To date, there are no studies that have investigated fatty acid compositions of krill relative to their maturity stages. Further

studies are needed to understand the diet and nutritional transfer of fatty acids in krill during the maturation process.

5.5.3. Temperature effects

Krill is considered to be stenothermal and is sensitive to slight changes in temperature and other environmental changes (Wiedenmann *et al*. 2008). Over the past 50 years there has been a significant warming trend over parts of Antarctica, with surface air temperatures increasing by 5-6°C, west of the Antarctic Peninsula during winter (Vaughan *et al*. 2003). In terms of the Southern Ocean, both Meredith and King (2005) and Whitehouse *et al*. (2008) have concluded that there had been rapid warming (>1°C) of summer surface waters west of the Antarctic Peninsula and the ocean around South Georgia, respectively. It is therefore clear that there is substantial evidence for long-term warming of the surface layer, the habitat of krill, in the Southern Ocean.

As global warming continues, sea-ice cover is predicted to decrease (Arrigo and Thomas 2004), which in turn, may have a negative impact on krill recruitment (Siegel and Loeb 1995; Wiedenmann *et al*. 2008). Whitehouse *et al*. (2008) calculated a significant negative relationship between summer water temperatures at South Georgia and mean summer density of krill across the south-west Atlantic sector of the Southern Ocean. This can also be related to a study by Atkinson *et al*. (2004) who suggested that stocks of krill have declined significantly since the 1970s in the south-west Atlantic, which has increased in water temperature, and decreased in sea-ice cover with a consequent lack of food for larvae during the winter period. Atkinson *et al*. (2006) examined the effects of food, temperature and sex on growth rates of krill in the south-west Atlantic and concluded that growth actually decreased above a temperature optimum of 0.5°C, which suggests that krill may experience thermal stress and struggle to cope with future climate change at this location.

Temperature has been found to influence polar lipid structure and fatty acid composition (Farkas 1979), as well as cause gel/liquid phase transformations that result in changes to membrane fluidity (McElhaney 1984). Mayzaud *et al*. (2000) compared changes in polar lipids and corresponding fatty acid composition of immature female Northern krill (*Meganyctiphanes norvegica*) from two sites with extreme temperature regimes (Kattegat: 4°C and Ligurian Sea: 13°C). They showed a significantly lower content of phosphatidylethanolamine and lysophosphatidylcholine in the warmer Ligurian Sea population, as well as significantly higher percentages of DHA and lower content in saturated and monoenoic acids. This suggests some degree of adaptation to environmental

temperature, although, the effect of diet cannot be separated easily. Farkas (1979) concluded that there is an accumulation of PUFA at low temperatures in crustaceans, which is an adaptation to maintain physicochemical properties and membrane function in the cold. However, clear temperature effects have only been observed with differences of at least 15°C for marine animals (Mayzaud *et al*. 2000). Below this range of temperature, the expected changes in lipid structure and composition would be minimal with Mayzaud *et al*. (2000) proposing that Antarctic krill do not need to adapt to higher temperatures, as the annual variation rarely exceeds 5°C in the Southern Ocean. Thus, the metabolism and lipid composition of krill have naturally adapted to this variation (Clarke and Morris 1983). Instead, seasonality and regional differences in food supply will be more of a constraint on krill and will be the most important factor influencing lipid structure, in comparison to temperature (Clarke 1988).

Our study has provided the first experimental information on changes in the total lipid and fatty acid content and composition of krill over a full year under three constant temperature regimes. For the months of September, November, February and April, the means of total lipid, TAG and PL content and composition were variable and showed no significant differences between the three temperature treatments (-1°C, 1°C and 3°C). This was also generally the case for individual fatty acids. Overall, with a temperature range of only 4°C, it generally appears that temperature alone did not affect the total lipid and fatty acid content and composition of krill in our study. The variability of the means for all temperature treatments and months was substantial and further demonstrates that krill is a versatile and flexible species, which can survive and adapt to a range of temperature conditions below a natural maximum amplitude of 5°C. In terms of krill survival with future climatic change, conclusive results cannot be obtained from this study alone, due to a multitude of other factors and interactions in the Southern Ocean, which will also be affected during global warming (i.e. sea-ice, ocean acidification, competing species, and changes in predation and diet). These interactions with future climatic change need to be examined to better understand the vulnerability of krill.

5.6. Conclusion

It is unclear whether the hypothesis of krill undertaking minimal changes in lipid utilisation under constant diet was upheld since lipid storage levels did vary throughout the year within reasonable bounds. Despite this general decreasing trend of mean lipid content and major fatty acid throughout the year in krill, there were no overall significant differences between months. The only exception was in krill

sampled in February for which lipid and fatty acid content were significantly lower, indicative of lipid depletion at the end of summer. Our results indicate that lipid storage is possibly used primarily for reproduction in krill rather than an actual overwintering energy source. However, since only immature krill were examined in this study, the interaction between lipids and reproductive activity should be minimised. Temperatures of -1°C to 3°C generally resulted in no effect on total lipid and fatty acid content and composition in krill, indicating that immature krill have adapted to this relatively small environmental temperature range in the Southern Ocean. However, water temperature alone is only a small component of a multitude of interactions that will affect krill in light of future climatic change. Understanding the yearly cycle of krill, particularly during winter, is also important since it represents a vital stage of the life cycle. Further research into the relatively unknown winter period is fundamental to understanding the overall success of krill, particularly with various light, food and temperature scenarios.

-- Chapter six --

Effects of temperature and constant food supply on lipid and fatty acid content and composition with respect to sex and body tissue of Antarctic krill (*Euphausia superba*) in summer

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6.1. Abstract

Antarctic krill is a keystone species in the Southern Ocean, however, information on the effects of temperature on growth, maturity and condition is limited, hampering understanding of the effects of a changing environment on these fundamental parameters. This study examines the changes in total lipid and fatty acid content and composition of immature, mature male and female krill in November and February. Under temperature regimes of -1°C, 1°C and 3°C, krill were maintained in a controlled environment with a constant food supply and a photoperiod that simulated natural summer Antarctic light. Krill were divided into three body fractions; abdomen, digestive gland and the thoracic components (containing reproductive tissue). Lipid and fatty acid content for the tissues abdomen and thoracic components showed similar trends between sex and temperature, while digestive

gland revealed complex differences. Total lipid, triacylglycerol (TAG) and polar lipid (PL) content and major fatty acids; 16:1ω7c, 20:5ω3 (EPA) and 22:6ω3 (DHA), were generally not significantly different between immature, male and female krill for each individual temperature and tissue fraction in November and February. Mated females were only observed at -1°C in November, and total lipid, TAG and PL content were significantly lower in mated females compared to un-mated females. 16:1ω7c, EPA and DHA were also lower in the thoracic components and digestive gland for mated females, suggesting utilisation of lipids and these essential polyunsaturated fatty acid (PUFA) during mating. The effects of temperature were complex for sex and tissue. Krill at the cooler temperature, -1°C, generally contained higher mean lipid and fatty acid contents. The relative levels of TAG decreased with temperature for each sex and tissue, with near depletion in the digestive gland at 3°C in February. Overall, the observed lipid and fatty acid content and composition were highly variable between individual krill under the same experimental condition, highlighting the flexible and versatile physiology of krill.

6.2. Introduction

In the Southern Ocean, Antarctic krill (*Euphausia superba*, hereafter krill) is a crucial link in the transfer of energy between primary producers and large predators, including many species of baleen whales, seals, penguins, seabirds and fish (Laws 1985; Miller and Hampton 1989; Mangel and Nicol 2000). The success of maintaining such a high biomass is attributed to their ability to survive and adapt to extreme seasonal changes in their environment. Krill can also feed efficiently and opportunistically on whatever food is available (Schnack 1985) including phytoplankton (Hopkins *et al*. 1993; Quetin *et al*. 1994), detritus (Kawaguchi *et al*. 1986) and/or carnivory (Price *et al*. 1988; Huntley *et al*. 1994; Atkinson and Snÿder 1997; Atkinson *et al*. 2002; Hagen *et al*. 2007).

Lipid analysis, particularly changes in triacylglycerol (TAG) and fatty acid composition, has been used to describe feeding habits and condition of krill from controlled experiments (Virtue *et al*. 1993*a,b*; Cripps and Atkinson 2000; Alonzo *et al*. 2005*a*,*b*) and in the field (Mayzaud 1997; Phleger *et al*. 1998; Cripps *et al*. 1999). Changes in lipid class and fatty acid composition have been observed in the digestive gland (Virtue *et al*. 1993*a*; Alonzo *et al*. 2003*b*, 2005*a*), which is one of the main sites for lipid synthesis and storage in crustaceans (Dall *et al*. 1992). Analysing lipid content and composition in the whole body of krill (as previously commonly analysed) can be of less use for obtaining trophic information, due to the fact that somatic growth, sex and maturity stages may influence the profiles (Clarke 1984;

Kolakowska 1991; Pond *et al*. 1995; Virtue *et al*. 1996). Lipid content and composition has been analysed within the ovary of females (Clarke 1980; Mayzaud *et al*. 1998), but other studies have also examined the distribution of lipids in a variety of tissues; including abdomen (Clarke 1980; Saether *et al*. 1985; Mayzaud *et al*. 1998), digestive gland (Virtue *et al*. 1993*a*; Alonzo *et al*. 2003*b*, 2005*a*; O'Brien *et al*. submitted) and thorax (Saether *et al*. 1985).

Mature and gravid females contain high levels of neutral storage lipids (e.g. TAG) during the demanding yolk accumulation period, particularly in the ovary or thorax (Clarke 1980; Pond *et al*. 1995; Mayzaud *et al*. 1998), but much less is known about mature males. Males accumulate considerably less neutral lipids compared to females during the reproductive development in summer, while immature krill contain intermediate amounts (Clarke 1980, 1984; Saether *et al*. 1985; Mayzaud 1997; Hagen *et al*. 2001). However, according to Virtue *et al*. (1996), the energetic cost of frequent sperm production and transfer for males is most likely to be high.

In terms of fatty acid composition, there has been variation observed with maturity and sex, mainly a higher proportion of polyunsaturated fatty acid (PUFA) 20:5ω3 (EPA) in adult males (Clarke 1980; Virtue *et al*. 1996). These results are also affected by diet and spatial distribution of krill (Cripps *et al*. 1999), so changes in fatty acid content and composition can be obscured by sexual maturity alone (Cripps and Atkinson 2000). Outside the summer period, there are no sex-dependent differences in lipid and fatty acid levels recorded (Hagen *et al*. 2001).

There has been far less attention on the effects of temperature on total lipid and fatty acid content and composition, particularly whether there is any variation between sexes. Although, it is thought that temperature, within the natural range in the Southern Ocean, does not influence lipid and fatty acid concentrations (Mayzaud *et al*. 2000).

The objectives of the present investigation were to determine temperature related effects on lipid and fatty acid content and composition of krill during the summer period under controlled laboratory conditions. We also tested whether there was any variation in the distribution of lipids with respect to sex between the body tissues: abdomen, digestive gland and the remaining thoracic components (containing reproductive tissue) under different temperature regimes.

6.3. Materials and Methods

6.3.1. Sampling and experimental setup

Krill were collected on 3rd March 2006 (66°02'S, 79°32'E) using a rectangular mid-water trawl net (RMT 8) (Baker *et al*. 1973) on board RSV *Aurora Australis*. The water temperature during krill capture was -1°C.

On return to the research aquarium at the Australian Antarctic Division in Kingston, Tasmania, krill were acclimated to aquarium conditions (King *et al*. 2003) and then evenly distributed into three 600-L tanks on 30th March 2006 (Appendix B). These stock populations of krill were maintained at temperatures of -1°C, 1°C and 3°C. Lighting was provided by twin fluorescent tubes. A controlled-timer system was used to set a natural photoperiod corresponding to that for the Southern Ocean (66°S at 30m depth). Continuous light and a maximum of 100 lux light intensity at the surface of the tank (assuming 1% light penetration to 30m depth) during summer midday (December), a sinusoidal annual cycle with monthly variations of photoperiod and daily variation of light intensity was calculated (Appendix C). At the start of each month, a new photoperiod was simulated by adjusting the timer system (Kawaguchi *et al*. 2010).

Krill were fed daily throughout the experimental period (April 2006 to May 2007) with the following algal mixture: the cultured pennate diatom *Phaeodactylum tricornutum*, at a final concentration of approximately 4.3 x 10^4 cells mL^{-1}, and the diatom *Thallasasira* sp (1.8 x 10^3 cells mL^{-1}), the flagellates *Pavlova* sp. (6.7 x 10^3 cells mL^{-1}) and *Isochrysis* sp. (2.6 x 10^4 cells mL^{-1}), which are concentrated bulk feeds of instant algae mixed with seawater (Reed Mariculture, California). These algal densities are representative of spring bloom conditions in the Southern Ocean. Water flow in the tank was closed daily for two hours each day to enable krill to feed. Minced clam, purchased from a local supplier, was also fed to the krill (approximately 1g per 250 krill) when water was flowing into the system. Clam meat has been used successfully in previous laboratory experiments, providing krill with an additional protein source (Hirano *et al*. 2003). Lipid and fatty acid composition of the provided diet is summarised in Appendix H.

From each of the three 600-L tanks (-1°C, 1°C and 3°C), three random immature, mature male and mature female krill were sampled in the summer months of November 2006 (~8 months after incubation) and February 2007 (~11 months after incubation). Immature krill were defined as either having a small and developing petasma (not swollen or fully developed) or thelycum (small and still developing, with no signs of red pigmentation) (Appendix A). Mature males were identified as having a fully developed, complex and swollen petasma, and mature females had a large and

fully developed thelycum that was brightly pigmented (Makarov and Denys 1980). An additional three mated females were sampled from the -1°C treatment in November, which were identified as having spermatophores attached to the thelycum. The overall number of krill analysed was 57. The samples were wrapped in aluminium foil and stored in a -80°C freezer.

While frozen, each krill was dissected into three sections: abdomen (cut vertically between the carapace and abdominal segments), digestive gland and the remaining tissue ('thoracic component') of the body (containing reproductive tissue), and placed on pre-extracted and pre-weighed glass fibre filters to ensure all tissue and fluids were collected. Samples were dissected in order to examine the distribution of lipids in various tissues and frozen until analysed.

6.3.2. Lipid extraction

Dissected samples were freeze-dried and weighed, and extracted overnight using a modified Bligh and Dyer (1959) single phase methanol-chloroform-water extraction (2:1:0.8, by volume). Phases were separated by addition of chloroform-water. The final solvent ratio was 1:1:0.9, by volume, methanol-chloroform-water. The total solvent extract (TSE) was concentrated using rotary evaporation at 40°C, transferred to glass vials and stored in chloroform at -20°C.

6.3.3. Lipid class analysis

Individual lipid classes were analysed using an Iatroscan MK V TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Iatron Laboratories, Japan). An aliquot of the TSE was spotted onto silica gel SIII chromarods (5μm particle size) with 1μL micropipettes and developed in a glass tank lined with pre-extracted filter paper. The solvent system used for lipid separation was hexane-diethyl ether-acetic acid (60:17:0.1 by volume). After 25 minutes development, the chromarods were oven dried and analysed immediately to minimise absorption of atmospheric contaminants. The FID was calibrated for each compound class: phosphatidylcholine, cholesterol, oleic acid, hydrocarbon (HC, squalene), triacylglycerol (TAG, derived from fish oil), wax ester (WE, derived from orange roughy, *Hoplostethus atlanticus*, oil) and diacylglyceryl ether (DAGE, derived from shark liver oil). Lipid classes were quantified on an IBM compatible computer using DAPA Scientific software (Kalamunda, Western Australia, Australia). The results from the TLC-FID are generally reproducible to ±10% of individual lipid class abundances (Volkman and Nichols, 1991).

6.3.4. Fatty acid analysis

An aliquot of the TSE was trans-methylated in methanol–chloroform–hydrochloric acid (10:1:1, by volume) for two hours at ~90°C. The mixture was cooled, milli-Q water added and fatty acid methyl esters (FAME) extracted with hexane:chloroform (4:1, by volume, 3x). FAME were dried under a stream of nitrogen, and an internal injection standard (C19 FAME) added.

Samples were analysed by gas chromatography (GC) using an Agilent Technologies 7890A GC (Palo Alto, California, USA) equipped with an Equity™-1 fused silica capillary column (15 mm × 0.1 mm i.d., 0.1µm film thickness), an FID, a split/splitless injector and an Agilent Technologies 7683B Series auto sampler and injector. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 120°C. After injection, the oven temperature was raised to 270°C at 10°C min^{-1} and finally to 310°C at 5°C min^{-1}. Peaks were quantified with Agilent Technologies ChemStation software (Palo Alto, California, USA).

Individual components were identified using mass spectral data and by comparing retention time data with those of authentic and laboratory standards. GC results are subject to an error of up to 5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector using Thermoquest Xcalibur software (Austin, Texas, USA). The GC was fitted with an HP-5 cross-linked methyl silicone fused silica capillary column of similar polarity to that described above.

6.3.5. Statistical analysis

To determine whether there was any significant differences in lipid and fatty acid content between months, temperatures, sex and tissue the linear ('lm') method in the statistical package 'R' (version 2.8.0) was used to fit linear models to carry out a straight forward analysis of variance (ANOVA). The maximum level of interaction included in the linear model was fourth order (e.g. month:temperature:sex:tissue). However, only second order interactions were found to be significant ($P<0.05$).

6.4. Results

6.4.1. Total lipid content and class composition

Total lipid, triacylglycerol (TAG) and polar lipid (PL) content in the tissue fractions abdomen and the thoracic components for all sexes resulted in similar trends in November and February, and thus, only the tissues thoracic components and digestive gland are compared in Figures 6.1, 6.2 and Table 6.1.

6.4.1.1. November

6.4.1.1.1. Lipid content in thoracic components and digestive gland

There were generally no significant differences (P>0.05) for total lipid, TAG and PL content in the body fractions thoracic components and digestive gland between sexes (immature, males and females) of krill for each individual temperature (-1°C, 1°C and 3°C) (Figure 6.1). However, the mean values for males were generally lower than for females, particularly at 3°C. For male digestive glands at 3°C, total lipid and TAG content were significantly lower than females (P<0.05). Mated females were only observed in the -1°C treatment, and total lipid, TAG and PL content for the tissues thoracic components and digestive gland were significantly lower than the un-mated females. The only exception was PL in the digestive gland, with the mated females containing lower concentrations, but there were no significant differences (P>0.05).

For the thoracic components, there were lower concentrations of total lipid, TAG and PL for all sexes at higher temperatures. A step-wise decreasing mean trend in lipid content with temperature was observed for males, but the 1°C treatment was lowest for immature krill and similar mean concentrations were observed for the 1°C and 3°C treatments for females. Overall, there were significant differences for males between -1°C and 3°C treatments for total lipid, TAG and PL content (P>0.05) and all lipid contents for immature and female krill at -1°C were significantly higher than 1°C, but not 3°C (P<0.05).

There were no significant differences in total lipid, TAG and PL content between temperatures for the digestive gland for each sex (P>0.05). Immature krill contained higher total lipid and PL content at increasing temperature, whereas, the mean trends for males and females showed no clear temperature effect.

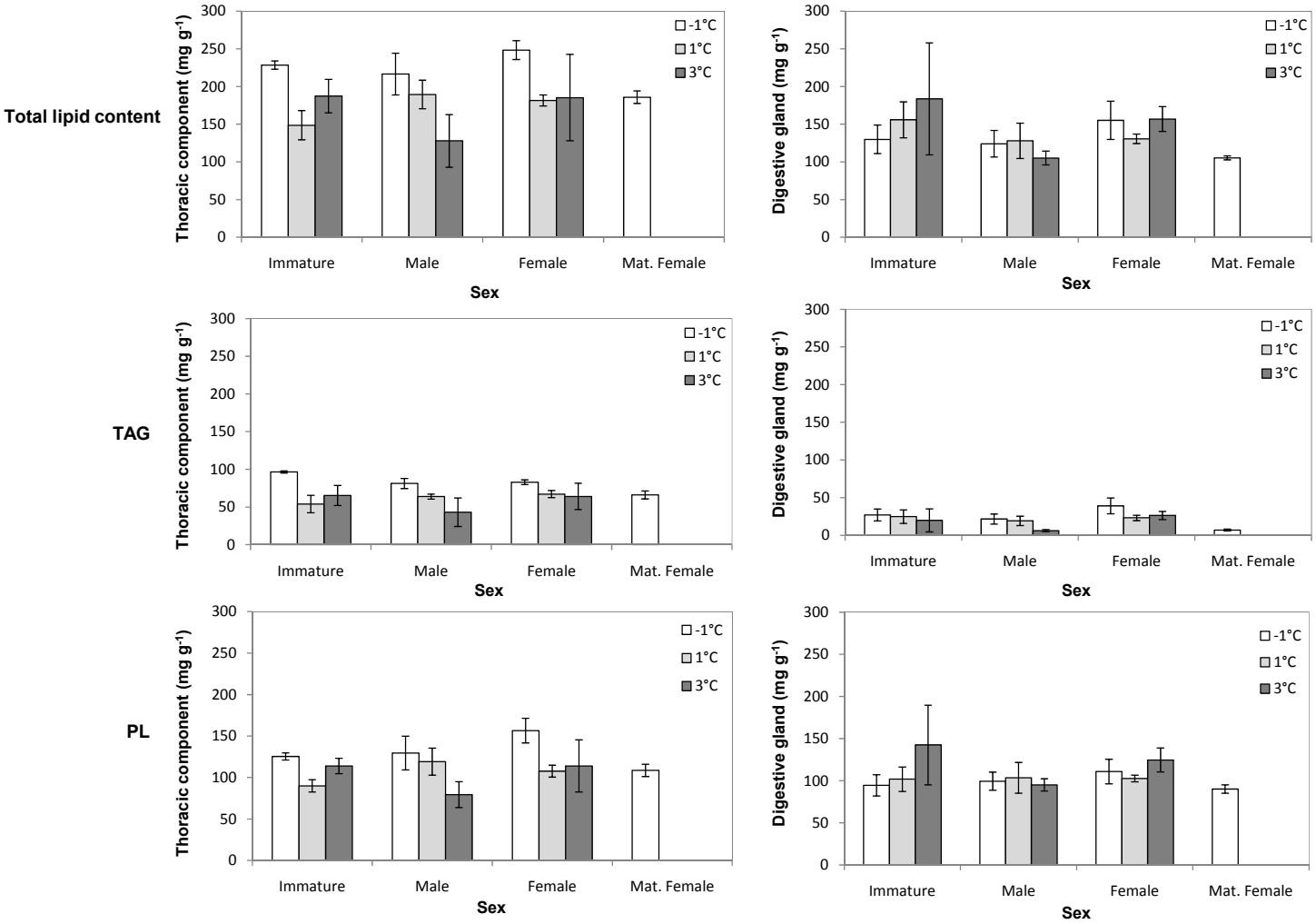


Figure 6.1. Changes in mean (±1 SE) total lipid and lipid class content (TAG – triacylglycerol; PL –polar lipid) for two segments of krill (thoracic components and digestive gland) in November in relation to sex (immature, male and female) and temperature (-1°C, 1°C and 3°C). Mated females (Mat. Female) only in the -1°C treatment.

6.4.1.1.2. Relative levels of lipid classes

The means and standard errors for the relative levels (as % of total lipid) of individual lipid classes for the thoracic components and digestive gland of krill in November, in relation to sex and temperature, are shown in Table 6.1. Overall, PL was the dominant lipid class and generally increased with temperature for each sex, reaching a mean maximum of 91% in male digestive glands at 3°C. The next dominant lipid class, TAG, generally decreased with temperature for each sex and tissue of krill. This was particularly evident for immature and male digestive glands in the 3°C treatment, which contained significantly lower TAG values of 8% and 7%, respectively, ($P<0.05$) compared to 17% for females.

6.4.1.2. February

6.4.1.2.1. Lipid content in the thoracic components

The general trends in February for mean total lipid, TAG and PL content (Figure 6.2) were different to those observed in November (Figure 6.1). Total lipid, TAG and PL content in the female tissue fraction thoracic component were significantly higher than for immature krill at -1°C and 1°C ($P<0.05$), but not at 3°C. The mean male lipid content was higher than immature krill with significance recorded solely at 1°C ($P<0.05$). There were no significant differences between male and female lipid content ($P>0.05$). However, the general trends showed that mean female lipid content was higher at -1°C, while mean male content was higher at 1°C and similar means were recorded at 3°C.

The 1°C treatment was significantly higher for males compared to -1°C and 3°C for total lipid, TAG and PL content ($P<0.05$) and immature krill in the -1°C treatment was significantly higher than at 1°C ($P<0.05$) for all lipid content. There were no significant differences between temperatures for female krill ($P>0.05$).

Table 6.1. Mean (±1 SE) lipid class composition (as % of total lipid) for two segments of krill (thoracic components and digestive gland) in November and February in relation to sex (immature, male and female) and temperature (-1°C, 1°C and 3°C). (Lipid classes: HC – Hydrocarbon, TAG – Triacylglycerol, FFA – Free fatty acid, ST/DG – Sterol/Diaclglycerol and PL – Polar lipid).

		November																					
		-1°C						1°C						3°C									
		Immature			Male			Female			Immature			Male			Female			Immature			Male			Female		
		Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE
Thoracic components	HC	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	1.0	±	1.0	0.6	±	1.0	0.0	±	0.0
	TAG	42.3	±	0.7	37.8	±	4.4	33.6	±	4.4	35.5	±	5.1	34.1	±	4.3	37.0	±	4.3	34.0	±	6.0	29.5	±	14.3	34.6	±	3.4
	FFA	0.5	±	0.1	0.4	±	0.2	0.6	±	0.2	0.5	±	0.3	0.6	±	0.3	0.7	±	0.3	0.6	±	0.5	0.8	±	0.3	0.4	±	0.3
	ST/DG	2.4	±	0.3	2.4	±	0.2	3.0	±	0.3	2.9	±	0.1	2.9	±	0.3	3.1	±	0.3	2.9	±	0.3	3.1	±	0.7	3.6	±	0.4
	PL	54.9	±	0.9	59.4	±	4.2	62.8	±	4.8	61.2	±	5.0	62.4	±	4.8	59.2	±	4.1	61.5	±	5.1	66.0	±	14.6	61.4	±	4.1
Digestive gland	HC	3.4	±	3.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.8	±	0.7	0.4	±	0.4	0.5	±	0.9
	TAG	20.0	±	6.4	16.5	±	6.7	24.2	±	4.9	23.9	±	20.7	14.3	±	5.2	17.6	±	3.6	7.9	±	6.5	5.8	±	1.0	16.8	±	4.7
	FFA	1.8	±	1.0	0.8	±	0.1	1.6	±	0.8	9.8	±	13.4	1.6	±	1.0	1.6	±	1.0	3.7	±	1.8	0.9	±	0.3	1.1	±	0.4
	ST/DG	1.6	±	0.2	1.6	±	0.4	1.9	±	0.2	1.5	±	1.2	2.8	±	1.4	2.2	±	0.5	5.8	±	5.4	2.3	±	0.4	2.1	±	0.1
	PL	73.2	±	2.7	81.2	±	7.0	72.3	±	4.3	64.7	±	32.8	81.3	±	5.4	78.7	±	2.4	81.9	±	9.4	90.6	±	1.6	79.4	±	4.9
		February																					
		-1°C						1°C						3°C									
		Immature			Male			Female			Immature			Male			Female			Immature			Male			Female		
		Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE	Mean		SE
Thoracic components	HC	1.2	±	0.7	1.6	±	0.2	1.3	±	0.4	1.4	±	0.6	1.7	±	1.0	1.7	±	1.2	3.0	±	1.4	2.7	±	0.6	2.1	±	0.5
	TAG	10.0	±	11.7	29.1	±	10.5	36.2	±	4.9	0.9	±	1.2	37.5	±	3.9	22.9	±	15.2	17.4	±	17.8	28.5	±	6.9	22.8	±	9.7
	FFA	0.6	±	0.5	3.0	±	1.6	1.5	±	0.8	3.9	±	2.7	1.6	±	0.3	2.3	±	0.4	6.6	±	3.2	6.0	±	0.9	3.7	±	2.4
	ST/DG	3.2	±	2.9	4.0	±	0.4	4.5	±	0.6	4.7	±	0.3	4.2	±	0.3	4.7	±	0.1	4.6	±	0.8	4.0	±	0.6	14.0	±	16.7
	PL	85.0	±	10.5	62.3	±	9.4	56.6	±	3.6	89.1	±	3.0	54.9	±	3.0	68.3	±	14.4	68.4	±	15.2	58.9	±	5.2	57.4	±	8.5
Digestive gland	HC	1.4	±	0.2	1.2	±	1.1	3.0	±	0.5	3.3	±	0.6	2.2	±	0.2	2.8	±	0.4	7.5	±	4.0	4.3	±	1.2	4.8	±	2.6
	TAG	9.9	±	9.3	9.9	±	10.2	9.6	±	1.2	1.8	±	0.2	7.5	±	3.7	6.6	±	3.9	3.2	±	4.5	3.2	±	3.7	5.6	±	4.8
	FFA	0.1	±	0.2	7.4	±	6.7	16.9	±	5.6	7.7	±	4.3	18.8	±	8.8	15.8	±	5.7	21.7	±	7.9	20.8	±	10.0	21.1	±	15.3
	ST/DG	2.3	±	1.8	3.7	±	1.8	5.1	±	1.1	2.9	±	1.0	4.6	±	1.9	3.2	±	0.8	3.3	±	1.8	4.0	±	1.8	3.0	±	0.6
	PL	86.2	±	11.2	77.8	±	17.1	65.4	±	6.9	84.3	±	6.1	66.9	±	14.5	71.5	±	7.8	64.4	±	9.2	67.8	±	15.7	65.4	±	14.1

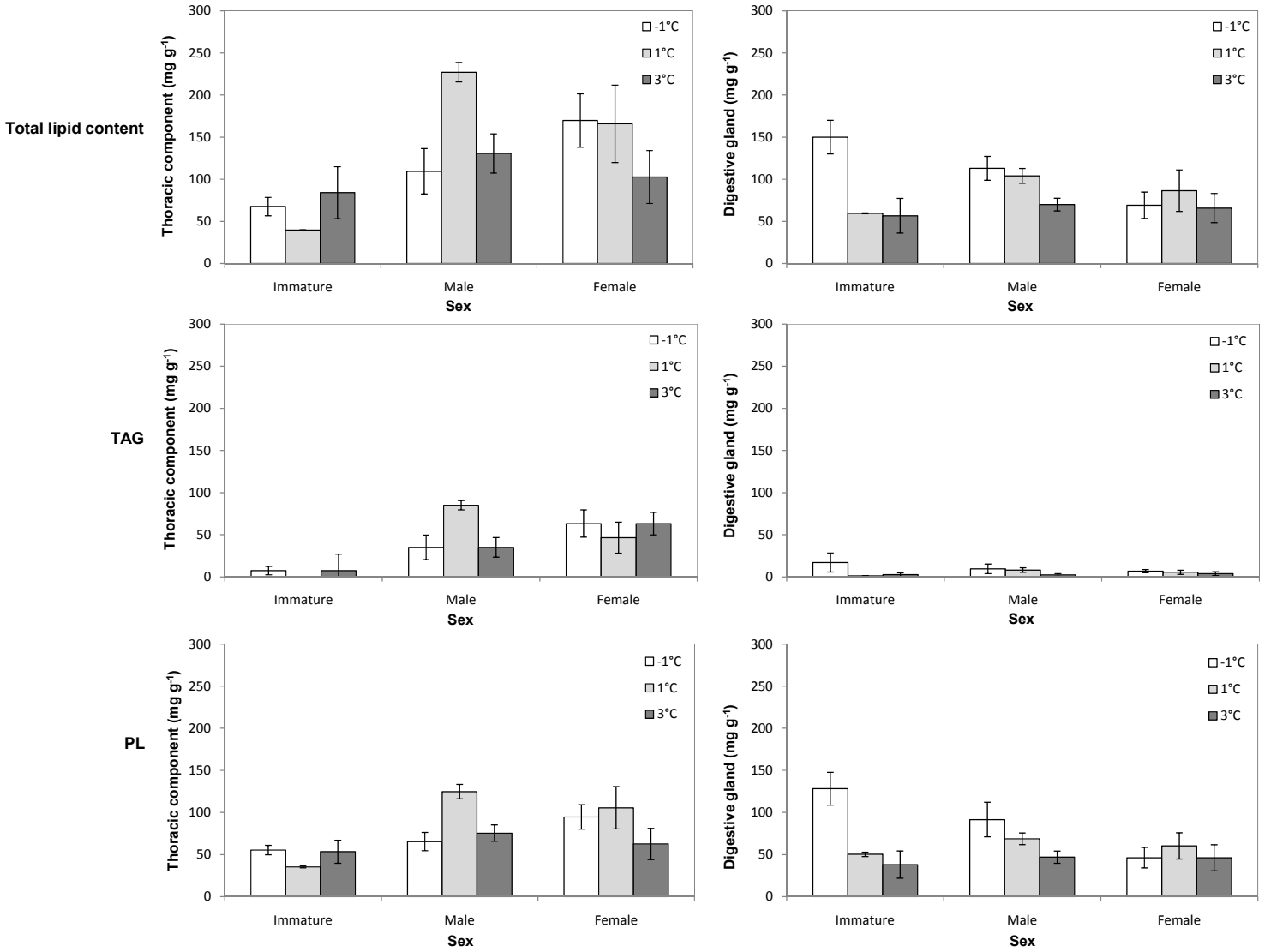


Figure 6.2. Changes in mean (±1 SE) total lipid and lipid class content (TAG – triacylglycerol; PL –polar lipid) for two segments of krill (thoracic components and digestive gland) in February in relation to sex (immature, male and female) and temperature (-1°C, 1°C and 3°C).

6.4.1.2.2. Lipid content in digestive gland

Total lipid and PL content in the digestive glands of immature krill were significantly higher than in females at -1°C ($P<0.05$), but not significantly different to males ($P>0.05$). Concentrations of total lipid and PL of mature males were significantly higher than immature krill in the 1°C treatment ($P<0.05$), but were not different for females ($P>0.05$). There were no significant differences in total lipid and PL content between sexes for the 3°C treatment ($P>0.05$). There was a general step-wise decrease in total lipid and PL content in digestive glands of immature krill and mature males with increasing temperature, while concentrations were higher at 1°C in females. Overall, the absolute values for TAG were low for immature, male and female krill in all temperature treatments and not significantly different between treatments ($P>0.05$).

Total lipid and PL content of immature krill at -1°C were significantly higher than at both 1°C and 3°C ($P>0.05$). Only the total lipid and PL content in males at -1°C treatment was statistically higher than the 3°C treatment. Total lipid and PL content across the temperatures for females were not statistically different ($P>0.05$), but the means were higher at 1°C.

6.4.1.2.3. Relative levels of lipid class

The means and standard errors for the lipid class composition (as % of total lipid) for the thoracic components and digestive gland of krill in February, in relation to sex and temperature, are shown in Table 6.1. PL and TAG were again the two dominant classes. TAG was highest for males in the warmer 1°C and 3°C temperatures, reaching a mean maximum of 38% in the 1°C treatment (thoracic components), whereas TAG was highest at -1°C for females (36%, thoracic components). TAG levels were substantially lower in the digestive gland for all sexes of krill in the 3°C treatment, ranging between 3% and 6%.

6.4.2. Fatty acid content and composition

General trends of fatty acid profiles in our study showed various relationships with temperature, sex and tissue fractions. As a consequence, changes in the mean content of the major fatty acid, 16:1ω7c, 20:5ω3 (EPA) and 22:6ω3 (DHA) are only shown in the results and focused on in the Discussion. The tissue fractions abdomen and the thoracic components for all sexes and temperatures also showed similar trends in November and February, and thus, only thoracic components and digestive gland are shown (Figures 6.3, 6.4 and Tables 6.2, 6.3, 6.4).

6.4.2.1 November

6.4.2.1.1. Sex related differences in fatty acid content

For each temperature treatment (-1°C, 1°C and 3°C), there were no significant differences between sexes for 16:1ω7c, EPA and DHA in the tissues thoracic components and digestive gland (P>0.05) (Figure 6.3). Mean contents of 16:1ω7c, EPA and DHA in mated females were lower than un-mated females for thoracic components and digestive gland at -1°C, however, they were not significantly different (P>0.05).

6.4.2.1.2. Temperature related differences in fatty acid content

Mean 16:1ω7c, EPA and DHA content in the -1°C treatment of all sexes and tissues were higher than for the warmer 1°C and 3°C treatments. For mature males and females there was a step-wise decrease in major fatty acid content with increasing temperature, whereas, for immature krill the 1°C treatment was lowest. Content of 16:1ω7c and EPA in the tissue thoracic components at -1°C were significantly higher than only 1°C for immature krill and statistically higher than only 3°C for males and females (P<0.05). In digestive gland, 16:1ω7c and EPA content for the -1°C treatment was significantly higher than both 1°C and 3°C for immature krill and only EPA for males (P<0.05). There were no statistical differences in 16:1ω7c and EPA between temperatures for female digestive gland. DHA content showed similar means between each temperature treatment for all sexes and tissues.

6.4.2.2. February

6.4.2.2.1. Sex related differences in fatty acid content

Males contained significantly higher 16:1ω7c, EPA and DHA content (P<0.05) in the tissue thoracic components at 1°C compared to immature krill, but no other statistical differences were observed between sexes and temperatures (P>0.05, Figure 6.4). However, immature krill generally contained lower values than males and females. Males contained significantly higher 16:1ω7c content in digestive gland at 1°C compared to immature krill (P<0.05), with no significant differences for EPA and DHA (P>0.05). At -1°C, females contained significantly higher 16:1ω7c, EPA and DHA content compared to males, but not statistically different to immature krill (P>0.05). No significant differences were observed between sexes for the 3°C treatment (P>0.05).

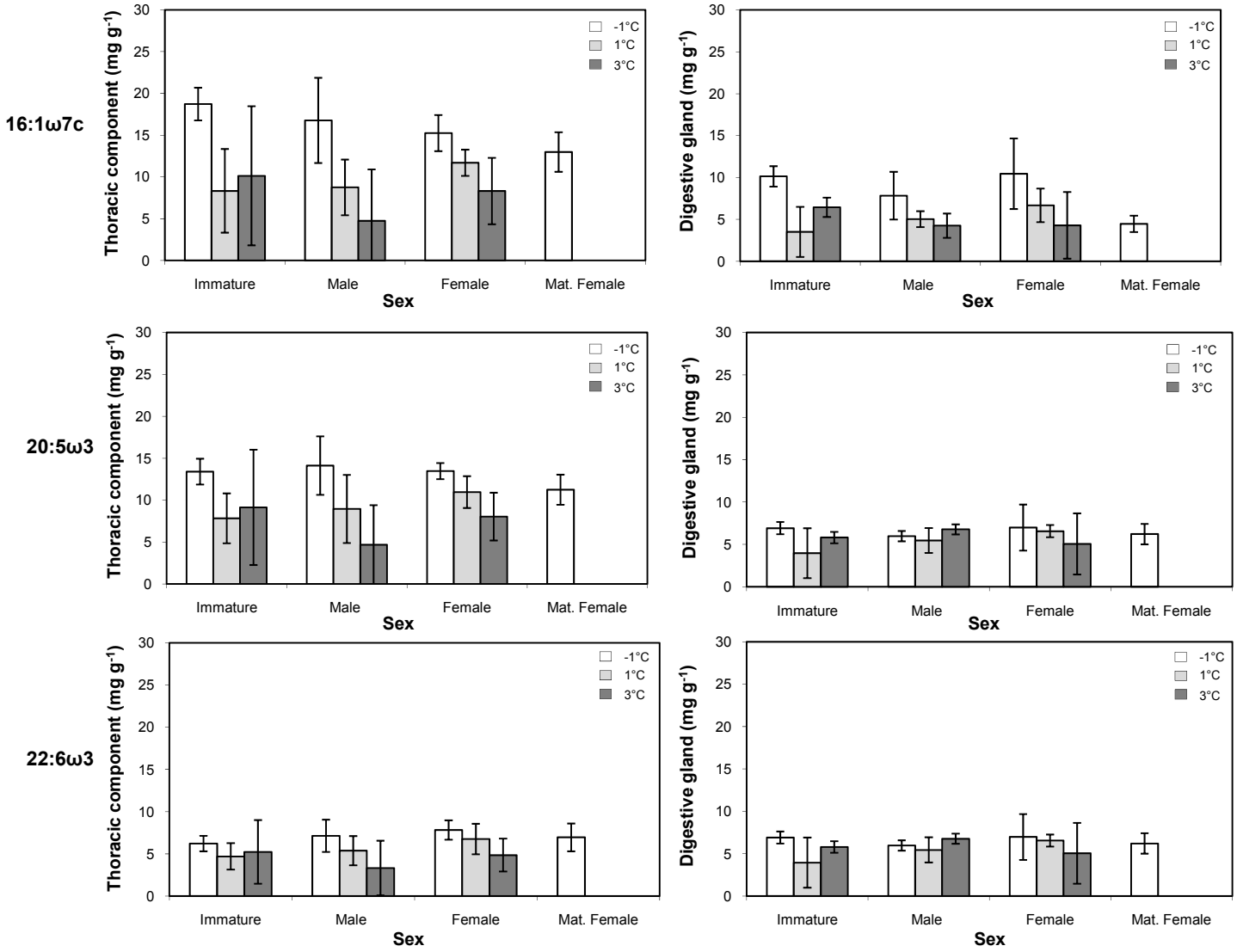


Figure 6.3. Changes in mean (±1 SE) fatty acid content (16:1ω7c, 20:5ω3 [EPA] and 22:6ω3 [DHA]) for two segments of krill (thoracic components and digestive gland) in November in relation to sex (immature, male and female) and temperature (-1°C, 1°C and 3°C).

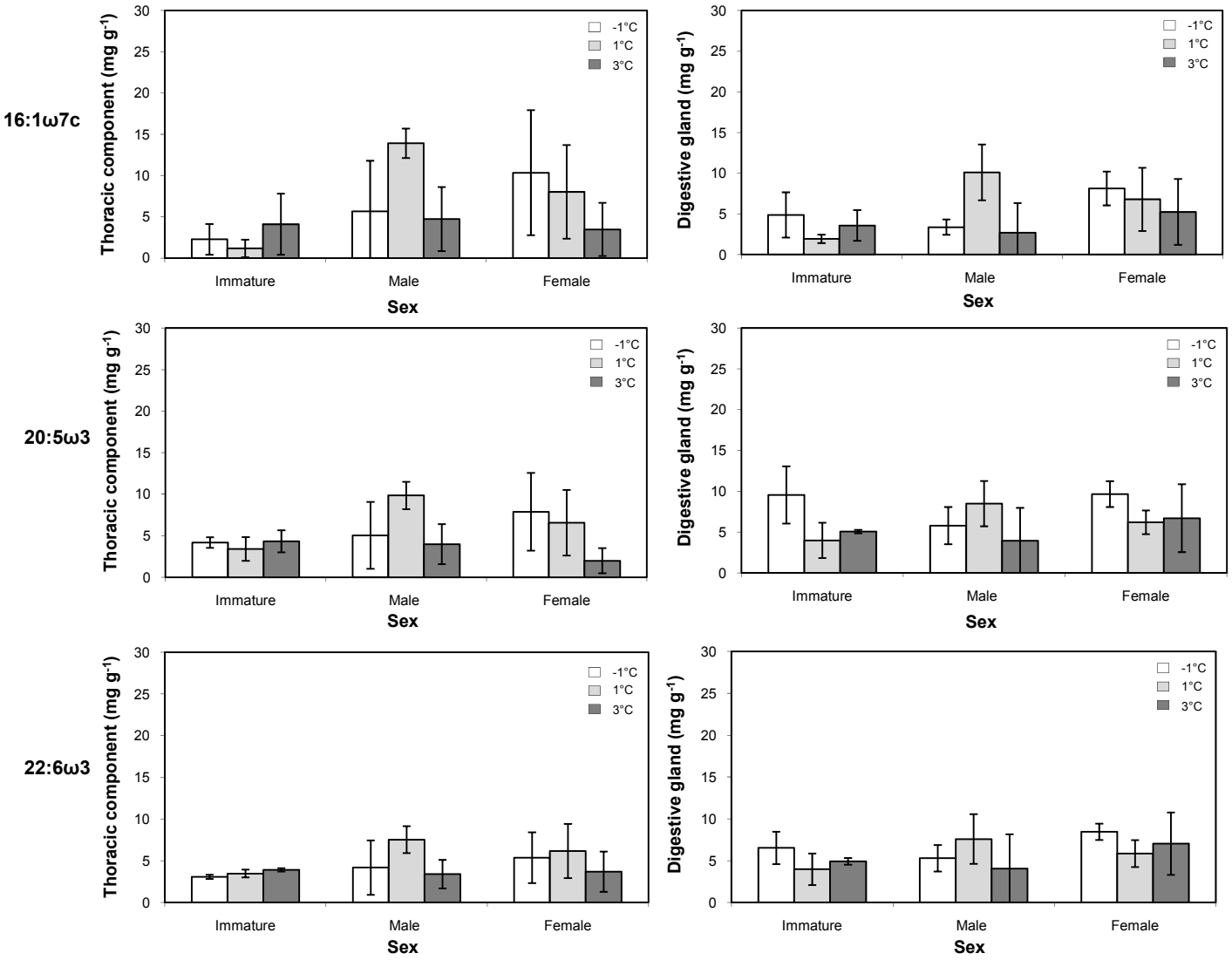


Figure 6.4. Changes in mean (±1 SE) fatty acid content (16:1ω7c, 20:5ω3 [EPA] and 22:6ω3 [DHA]) for two segments of krill (thoracic components and digestive gland) in February in relation to sex (immature, male and female) and temperature (-1°C, 1°C and 3°C).

6.4.2.2.2. Temperature related differences in fatty acid content

The general mean trends of 16:1ω7c, EPA and DHA between temperatures varied between sexes. There was a general step-wise decrease in content with increasing temperature for females, which is particularly evident for 16:1ω7c and EPA for both the thoracic components and digestive gland. Males at 1°C contained higher 16:1ω7c, EPA and DHA content in both tissue fractions, whereas the tissue thoracic components in immature krill showed similar fatty acid content at all three temperatures. However, mean fatty acid content in immature krill at -1°C contained higher values in the digestive gland. Overall, the only significant differences for the tissue thoracic components were observed for males at 1°C and both the -1°C and 3°C treatments for 16:1ω7c, and 1°C and just 3°C for EPA and DHA ($P<0.05$). For the digestive gland, significant differences were only observed between 1°C and both the -1°C and 3°C treatments for 16:1ω7c in males, -1°C and 3°C for EPA in immature krill, and -1°C and 1°C for EPA and DHA in females ($P<0.05$).

6.4.2.3. Relative levels of fatty acids for November and February

6.4.2.3.1. Sex related differences

Means and standard errors for fatty acid composition (as % of total fatty acid), as well as the sums of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and the ratios of ω3/ω6, AA/EPA and DHA/EPA, in relation to tissue and sex, for November and February, are shown in Table 6.2 (-1°C), Table 6.3 (1°C) and Table 6.4 (3°C). For the major PUFA; EPA, DHA, sum PUFA and DHA/EPA, there were no substantial differences between sexes for the thoracic components and digestive gland in November for the three temperature treatments. Males contained higher mean values at -1°C and 3°C, while immature krill contained higher values at 1°C. The only exceptions were males which contained higher relative levels of EPA and DHA in the digestive gland at 1°C.

In February, immature krill generally contained highest mean relative levels of EPA, DHA, sum PUFA and DHA/EPA values in the -1°C and 1°C treatments, while females had the lowest for tissues thoracic components and digestive gland. Exceptions were males which had highest mean values for the ratio DHA/EPA in the thoracic components at -1°C, and DHA and DHA/EPA in digestive gland at -1°C. At 3°C, immature krill contained higher relative EPA levels in the thoracic components and digestive gland, while females had higher DHA, sum PUFA and DHA/EPA in thoracic components and DHA/EPA in digestive gland. Males contained highest mean DHA levels and sum PUFA in digestive gland.

6.4.2.3.2. Temperature related differences

For the tissues thoracic components and digestive gland, relative mean EPA, DHA, sum PUFA and DHA/EPA values in November were highest at 1°C for immature krill and highest at 3°C for males and females. However, relative mean values of EPA were highest at -1°C for all sexes in digestive gland. In February, the relative mean values of EPA, DHA, sum PUFA and DHA/EPA varied more with temperature. At -1°C, higher mean values of EPA were in the tissue thoracic components for immature and male krill, and EPA was also highest in digestive gland for immature and female krill. At 1°C, DHA, sum PUFA and DHA/EPA was highest in immature krill, and also, EPA, DHA, sum PUFA was highest at 1°C in the tissue thoracic components for female krill. In the digestive gland, mean relative DHA levels and DHA/EPA were highest at 1°C for immature krill. At 3°C, the ratio of DHA/EPA was highest in male and female krill in the thoracic components, and in digestive gland, EPA, DHA, sum PUFA and DHA/EPA were highest at 3°C for male and female krill (except for EPA in females).

Table 6.2. Mean (±1 SE) fatty acid composition (as % of total fatty acid) for two segments of krill (thoracic components and digestive gland) in the -1°C treatment in November and February in relation to sex (immature, male and female).

	November -1°C												February -1°C											
	Immature				Male				Female				Immature				Male				Female			
	Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	7.5 ± 0.2		5.8 ± 0.4		8.4 ± 0.2		5.9 ± 0.6		7.5 ± 0.2		5.8 ± 0.4		2.8 ± 2.4		2.5 ± 1.8		5.8 ± 1.6		3.3 ± 2.4		6.2 ± 0.9		5.2 ± 0.3	
15:0	0.9 ± 0.1		0.8 ± 0		0.8 ± 0.1		0.7 ± 0.1		0.9 ± 0.1		0.8 ± 0		0.3 ± 0.3		0.5 ± 0.2		0.7 ± 0.2		0.5 ± 0.1		0.7 ± 0.1		0.7 ± 0.0	
16:0	20 ± 0		19.5 ± 0.3		20.4 ± 0.3		20.1 ± 0.6		20 ± 0		19.5 ± 0.3		19.6 ± 1.5		19.0 ± 1.8		20.4 ± 1.3		18.2 ± 0.9		19.4 ± 0.9		18.3 ± 1.1	
18:0	1.1 ± 0.3		1.4 ± 0.1		1.1 ± 0.1		1.3 ± 0.2		1.1 ± 0.3		1.4 ± 0.1		1.2 ± 0.2		1.9 ± 0.9		1.2 ± 0.1		1.4 ± 0.2		0.9 ± 0.1		1.0 ± 0.2	
phytanic acid	1.1 ± 0		1.1 ± 0		1.1 ± 0.1		1 ± 0.1		1.1 ± 0		1.1 ± 0		0.7 ± 0.6		0.9 ± 0.1		1.1 ± 0.1		1.0 ± 0.0		1.1 ± 0.1		1.0 ± 0.1	
16:1ω7c	18 ± 1.9		13.7 ± 1.6		15.9 ± 1.8		12.5 ± 2.5		18 ± 1.9		13.7 ± 1.6		8.6 ± 5.9		8.6 ± 2.4		12.8 ± 1.7		9.2 ± 2.3		14.5 ± 1.8		11.4 ± 1.4	
18:1ω9c	13.4 ± 0.7		11.1 ± 0.7		13 ± 1		11.4 ± 0.4		13.4 ± 0.7		11.1 ± 0.7		12.1 ± 3.5		13.4 ± 2.8		8.7 ± 5.8		12.4 ± 1.2		16.3 ± 1.1		13.7 ± 0.8	
18:1ω7c	5.4 ± 0.5		6.3 ± 0.7		5.3 ± 0.6		6 ± 0.6		5.4 ± 0.5		6.3 ± 0.7		6.8 ± 1.9		5.7 ± 0.8		5.8 ± 0.9		6.3 ± 0.4		5.3 ± 0.7		6.1 ± 0.5	
20:1ω9	0.8 ± 0.1		0.9 ± 0.1		0.7 ± 0.1		0.9 ± 0.1		0.8 ± 0.1		0.9 ± 0.1		0.6 ± 0.5		1.1 ± 0.2		1.2 ± 0.2		1.5 ± 0.4		0.9 ± 0.2		1.2 ± 0.3	
16:04	0.4 ± 0.3		0.4 ± 0.1		0.5 ± 0.1		0.3 ± 0.1		0.4 ± 0.3		0.4 ± 0.1		0.6 ± 0.5		0.3 ± 0.3		0.6 ± 0.1		0.4 ± 0.1		0.4 ± 0.1		0.4 ± 0.1	
18:4ω3	1.4 ± 0.1		1 ± 0.1		1.3 ± 0.1		0.9 ± 0.1		1.4 ± 0.1		1 ± 0.1		0.8 ± 0.3		0.8 ± 0.1		1.1 ± 0.3		0.9 ± 0.1		1.0 ± 0.2		0.9 ± 0.1	
18:2ω6 (LA)	3 ± 0.1		3.4 ± 0.1		3 ± 0.2		3.6 ± 0.4		3 ± 0.1		3.4 ± 0.1		4.2 ± 0.4		4.6 ± 0.3		5.1 ± 0.6		5.4 ± 0.2		4.6 ± 0.0		4.9 ± 0.2	
20:4ω6 (AA)	0.7 ± 0.1		1 ± 0.1		0.8 ± 0.1		1 ± 0.2		0.7 ± 0.1		1 ± 0.1		3.5 ± 1.0		1.8 ± 0.1		2.1 ± 0.9		1.6 ± 0.3		1.4 ± 0.2		1.4 ± 0.1	
20:5ω3 (EPA)	12.9 ± 0.1		16.8 ± 1.1		13.5 ± 0.6		17.3 ± 2.4		12.9 ± 0.1		16.8 ± 1.1		18.5 ± 3.4		18.4 ± 1.2		13.8 ± 2.6		15.2 ± 2.3		11.8 ± 0.5		13.7 ± 0.2	
22:5ω6	0.6 ± 0		0.7 ± 0.1		0.6 ± 0.1		0.8 ± 0.1		0.6 ± 0		0.7 ± 0.1		1.4 ± 0.6		0.9 ± 0.2		1.2 ± 0.5		1.1 ± 0.1		0.8 ± 0.0		1.0 ± 0.0	
22:6ω3 (DHA)	6 ± 0.3		9.3 ± 0.4		6.8 ± 0.4		9.9 ± 1.1		6 ± 0.3		9.3 ± 0.4		14.2 ± 5.6		13.0 ± 2.4		11.5 ± 3.2		14.2 ± 1.6		8.2 ± 0.9		12.1 ± 0.6	
Other**	6.7		7.1		6.7		6.4		6.7		7.1		4.0		6.6		6.8		7.3		6.6		7.3	
Sum SFA	32 ± 0.4		29.9 ± 0.4		33.4 ± 0.4		30.4 ± 1.3		32 ± 0.4		29.9 ± 0.4		25.6 ± 4.4		26.3 ± 0.9		30.9 ± 2.8		26.2 ± 0.6		29.7 ± 0.8		27.6 ± 1.2	
Sum MUFA	41.2 ± 0.8		35.6 ± 0.9		38.3 ± 1.3		34.4 ± 2.4		41.2 ± 0.8		35.6 ± 0.9		30.0 ± 5.2		32.3 ± 4.6		31.9 ± 7.2		32.9 ± 3.6		40.7 ± 1.6		36.1 ± 0.2	
Sum PUFA	26.8 ± 0.5		34.5 ± 1.3		28.3 ± 1		35.2 ± 3.4		26.8 ± 0.5		34.5 ± 1.3		44.4 ± 9.1		41.4 ± 3.7		37.2 ± 7.8		40.8 ± 4.1		29.6 ± 1.0		36.3 ± 0.9	
Total ω3	21.1 ± 1		28 ± 1.8		22.3 ± 1.5		28.9 ± 3.9		21.1 ± 1		28 ± 1.8		34.1 ± 10.1		33.0 ± 4.2		27.2 ± 6.5		31.4 ± 4.1		21.7 ± 1.7		27.6 ± 1.4	
Total ω6	4.9 ± 0.4		5.8 ± 0.5		5 ± 0.6		5.7 ± 0.9		4.9 ± 0.4		5.8 ± 0.5		9.4 ± 2.4		7.8 ± 0.8		9.0 ± 2.3		8.8 ± 0.8		7.1 ± 0.4		8.0 ± 0.5	
Ratio ω3/ω6	4.3		4.8		4.4		5.1		4.3		4.8		3.6		4.2		3.0		3.6		3.0		3.4	
Ratio AA/EPA	0.06		0.06		0.06		0.06		0.06		0.06		0.19		0.10		0.15		0.10		0.12		0.10	
Ratio DHA/EPA	0.46		0.56		0.5		0.57		0.46		0.56		0.77		0.71		0.83		0.93		0.70		0.88	

** **Other fatty acids (≤ 0.1%) include**: 4,8,12TMTD, i15:0, a15:0, C16PUFA, 16:1ω9c, 16:1ω5c, 16:1ω13t, i17:0, 17:1ω8c, 17:1, 17:0, 18:3ω6, 18:1ω7t, 18:1ω5c, 18:1, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω7c, 20:1ω5c, 20:0, 21:5ω3, 21:0, 22:4ω6, 22:5ω3, 22:4ω3, 22:2ω6, 22:1ω11c, 22:1ω9c, 22:1ω7c, 22:0, C24PUFA, 24:1ω11c, 24:1ω9c, 24:1ω7c, 24:0.

Table 6.3. Mean (±1 SE) fatty acid composition (as % of total fatty acid) for two segments of krill (thoracic components and digestive gland) in the 1°C treatment in November and February in relation to sex (immature, male and female).

	November 1°C												February 1°C											
	Immature				Male				Female				Immature				Male				Female			
	Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	4.8 ± 0.8		4.6 ± 1.3		6.9 ± 0.3		5.2 ± 0.7		7.8 ± 0.7		5.3 ± 1.9		1.5 ± 1.5		0.7 ± 0.7		5.3 ± 3.2		4.1 ± 3.0		2.8 ± 3.0		2.8 ± 2.1	
15:0	0.8 ± 0.1		0.7 ± 0.4		0.8 ± 0.1		0.8 ± 0.1		0.9 ± 0.0		0.8 ± 0.0		0.5 ± 0.0		0.5 ± 0.2		0.8 ± 0.1		0.6 ± 0.1		0.5 ± 0.4		0.5 ± 0.3	
16:0	20.6 ± 0.5		23.3 ± 7.3		20.2 ± 0.6		19.2 ± 0.4		20.2 ± 0.1		19.0 ± 0.1		18.1 ± 0.7		18.3 ± 2.4		19.2 ± 0.6		18.5 ± 1.5		20.2 ± 1.3		18.8 ± 0.9	
18:0	1.4 ± 0.2		1.8 ± 0.5		1.7 ± 0.6		1.4 ± 0.2		1.2 ± 0.2		1.3 ± 0.2		0.9 ± 0.2		2.2 ± 1.0		0.8 ± 0.1		1.0 ± 0.1		1.1 ± 0.2		1.4 ± 0.7	
phytanic acid	1.1 ± 0.1		0.9 ± 0.2		1.0 ± 0.1		0.9 ± 0.1		1.1 ± 0.0		1.0 ± 0.0		1.4 ± 0.4		0.9 ± 0.0		1.1 ± 0.1		1.1 ± 0.0		1.1 ± 0.2		1.0 ± 0.0	
16:1ω7c	14.5 ± 1.7		9.8 ± 2.9		13.3 ± 0.7		11.1 ± 0.1		13.9 ± 1.0		11.3 ± 1.5		5.3 ± 3.3		7.5 ± 0.8		15.7 ± 0.1		13.8 ± 0.7		8.6 ± 6.1		11.4 ± 2.3	
18:1ω9c	13.0 ± 1.9		9.2 ± 2.8		13.7 ± 1.8		11.9 ± 1.5		13.4 ± 1.1		11.8 ± 0.3		12.0 ± 1.3		13.0 ± 0.9		15.9 ± 1.3		15.5 ± 3.1		14.2 ± 2.9		14.7 ± 2.2	
18:1ω7c	5.0 ± 0.6		4.9 ± 1.7		5.5 ± 0.7		6.3 ± 0.8		5.6 ± 0.2		6.5 ± 0.4		6.4 ± 0.4		7.8 ± 0.0		5.5 ± 0.2		6.5 ± 1.2		6.7 ± 1.1		7.3 ± 0.5	
20:1ω9	0.8 ± 0.2		0.8 ± 0.2		0.8 ± 0.2		0.8 ± 0.2		0.7 ± 0.0		0.8 ± 0.1		0.9 ± 0.0		1.4 ± 0.1		0.9 ± 0.1		1.2 ± 0.0		0.9 ± 0.2		1.5 ± 0.1	
16:04	0.8 ± 0.0		0.6 ± 0.1		0.7 ± 0.1		0.5 ± 0.1		0.8 ± 0.1		0.5 ± 0.1		0.3 ± 0.3		0.2 ± 0.3		0.8 ± 0.1		0.5 ± 0.1		0.4 ± 0.4		0.4 ± 0.1	
18:4ω3	0.9 ± 0.1		0.7 ± 0.2		1.0 ± 0.1		0.8 ± 0.2		1.0 ± 0.2		0.9 ± 0.1		0.5 ± 0.1		1.0 ± 0.2		1.0 ± 0.2		1.1 ± 0.4		0.5 ± 0.4		1.1 ± 0.4	
18:2ω6 (LA)	3.7 ± 0.2		3.8 ± 0.8		3.2 ± 0.0		3.6 ± 0.0		3.5 ± 0.2		4.0 ± 0.4		4.5 ± 0.1		5.4 ± 0.1		4.5 ± 0.5		5.1 ± 0.4		4.4 ± 0.3		5.2 ± 0.3	
20:4ω6 (AA)	1.2 ± 0.3		1.0 ± 0.1		0.9 ± 0.2		0.9 ± 0.1		0.9 ± 0.1		0.9 ± 0.0		3.5 ± 0.7		1.8 ± 0.3		1.0 ± 0.2		1.0 ± 0.1		2.5 ± 1.7		1.2 ± 0.3	
20:5ω3 (EPA)	14.5 ± 1.9		14.9 ± 2.0		13.5 ± 1.6		16.7 ± 1.7		13.0 ± 0.1		15.2 ± 0.6		17.5 ± 0.1		14.6 ± 2.9		11.1 ± 0.4		11.6 ± 0.4		14.9 ± 5.0		11.7 ± 2.9	
22:5ω6	1.0 ± 0.1		2.3 ± 1.5		0.9 ± 0.2		1.0 ± 0.2		0.9 ± 0.2		1.1 ± 0.2		2.3 ± 0.8		1.8 ± 0.5		0.9 ± 0.1		1.2 ± 0.2		1.7 ± 0.8		1.3 ± 0.4	
22:6ω3 (DHA)	8.8 ± 1.7		11.4 ± 2.4		8.3 ± 0.1		11.9 ± 0.9		7.9 ± 1.2		11.5 ± 1.9		18.9 ± 5.4		14.7 ± 1.8		8.5 ± 1.2		10.3 ± 1.6		14.4 ± 5.7		11.5 ± 2.0	
Other**	7.2		9.4		7.5		7.0		7.3		8.0		5.6		8.2		6.9		6.9		5.3		8.0	
Sum SFA	30.3 ± 1.6		33.3 ± 9.8		32.3 ± 0.3		29.0 ± 0.8		32.8 ± 0.8		28.9 ± 1.8		23.4 ± 1.8		24.6 ± 3.5		28.7 ± 3.4		26.5 ± 2.2		26.9 ± 2.9		26.0 ± 2.3	
Sum MUFA	36.8 ± 2.3		27.8 ± 8.4		37.3 ± 1.7		33.4 ± 1.0		37.2 ± 2.2		34.5 ± 1.0		27.2 ± 4.6		34.5 ± 0.7		41.8 ± 1.6		40.3 ± 3.7		32.9 ± 8.8		38.7 ± 4.2	
Sum PUFA	32.8 ± 3.9		38.9 ± 1.4		30.4 ± 1.5		37.6 ± 0.7		30.0 ± 1.8		36.6 ± 2.3		49.4 ± 6.4		40.9 ± 4.2		29.5 ± 1.9		33.2 ± 2.2		40.2 ± 11.7		35.3 ± 6.1	
Total ω3	25.1 ± 4.0		28.0 ± 4.8		23.5 ± 2.4		30.6 ± 3.4		22.7 ± 1.7		28.7 ± 3.1		37.9 ± 6.4		31.2 ± 5.6		21.3 ± 1.9		24.2 ± 3.0		30.3 ± 11.6		26.1 ± 5.8	
Total ω6	6.4 ± 0.8		9.6 ± 6.0		5.7 ± 0.8		6.2 ± 0.8		5.8 ± 0.8		6.9 ± 1.3		11.0 ± 1.8		9.4 ± 1.3		6.9 ± 0.9		8.1 ± 1.6		9.3 ± 3.5		8.2 ± 1.3	
Ratio ω3/ω6	3.9		2.9		4.1		5.0		3.9		4.2		3.4		3.3		3.1		3.0		3.2		3.2	
Ratio AA/EPA	0.08		0.07		0.07		0.06		0.07		0.06		0.20		0.12		0.09		0.08		0.2		0.10	
Ratio DHA/EPA	0.61		0.76		0.62		0.71		0.61		0.76		1.08		1.01		0.76		0.89		0.96		0.98	

** **Other fatty acids (≤ 0.1%) include**: 4,8,12TMTD, i15:0, a15:0, C16PUFA, 16:1ω9c, 16:1ω5c, 16:1ω13t, i17:0, 17:1ω8c, 17:1, 17:0, 18:3ω6, 18:1ω7t, 18:1ω5c, 18:1, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω7c, 20:1ω5c, 20:0, 21:5ω3, 21:0, 22:4ω6, 22:5ω3, 22:4ω3, 22:2ω6, 22:1ω11c, 22:1ω9c, 22:1ω7c, 22:0, C24PUFA, 24:1ω11c, 24:1ω9c, 24:1ω7c, 24:0.

Table 6.4. Mean (±1 SE) fatty acid composition (as % of total fatty acid) for two segments of krill (thoracic components and digestive gland) in the 3°C treatment in November and February in relation to sex (immature, male and female).

	November 3°C												February 3°C											
	Immature				Male				Female				Immature				Male				Female			
	Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland		Thoracic components		Digestive gland	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	4.2	± 3.0	4.6	± 0.6	4.5	± 3.0	3.2	± 2.4	3.7	± 2.4	2.4	± 2.6	4.2	± 3.0	4.6	± 0.6	3.4	± 1.7	1.1	± 1.4	3.2	± 2.0	3.6	± 0.8
15:0	0.9	± 0.1	0.9	± 0.0	0.8	± 0.2	0.7	± 0.2	0.7	± 0.2	0.7	± 0.1	0.9	± 0.1	0.9	± 0.0	0.5	± 0.4	0.4	± 0.4	0.6	± 0.2	0.6	± 0.1
16:0	20.0	± 1.0	19.7	± 0.4	20.7	± 0.7	19.6	± 0.7	20.4	± 0.2	18.7	± 0.3	20.0	± 1.0	19.7	± 0.4	18.8	± 0.6	16.0	± 4.6	19.8	± 0.2	19.1	± 0.9
18:0	1.1	± 0.2	1.2	± 0.0	1.2	± 0.2	1.6	± 0.1	1.1	± 0.1	1.3	± 0.1	1.1	± 0.2	1.2	± 0.0	1.0	± 0.1	2.4	± 2.0	1.1	± 0.2	1.2	± 0.2
phytanic acid	1.1	± 0.1	1.0	± 0.1	1.2	± 0.1	1.1	± 0.0	1.1	± 0.1	1.1	± 0.1	1.1	± 0.1	1.0	± 0.1	1.1	± 0.1	0.7	± 0.6	1.1	± 0.1	1.0	± 0.1
16:1ω7c	14.8	± 1.6	11.9	± 0.4	12.4	± 3.0	8.9	± 2.1	13.9	± 1.6	9.8	± 1.7	14.8	± 1.6	11.9	± 0.4	12.3	± 2.2	6.2	± 4.0	9.2	± 2.8	9.5	± 1.6
18:1ω9c	15.9	± 2.0	11.8	± 0.2	13.5	± 1.1	10.0	± 0.6	15.3	± 1.2	12.7	± 1.5	15.9	± 2.0	11.8	± 0.2	19.1	± 0.9	15.3	± 2.8	16.3	± 2.0	13.9	± 2.1
18:1ω7c	4.8	± 0.5	6.3	± 0.2	6.1	± 1.2	6.5	± 1.1	6.3	± 0.1	7.3	± 0.9	4.8	± 0.5	6.3	± 0.2	5.4	± 0.6	7.8	± 1.0	6.3	± 0.8	6.3	± 0.7
20:1ω9	0.8	± 0.1	0.8	± 0.1	0.8	± 0.0	1.1	± 0.2	0.8	± 0.2	1.0	± 0.1	0.8	± 0.1	0.8	± 0.1	1.2	± 0.3	2.0	± 1.3	1.1	± 0.2	1.2	± 0.2
16:04	0.8	± 0.2	0.6	± 0.0	0.6	± 0.0	0.3	± 0.1	0.6	± 0.4	0.4	± 0.2	0.8	± 0.2	0.6	± 0.0	0.7	± 0.1	0.2	± 0.1	0.6	± 0.2	0.5	± 0.1
18:4ω3	0.9	± 0.2	0.7	± 0.1	0.9	± 0.2	0.7	± 0.1	0.9	± 0.1	0.8	± 0.1	0.9	± 0.2	0.7	± 0.1	0.8	± 0.2	0.4	± 0.4	0.7	± 0.1	0.8	± 0.3
18:2ω6 (LA)	3.7	± 0.2	4.0	± 0.2	3.8	± 0.2	4.6	± 0.2	3.5	± 0.2	4.4	± 0.4	3.7	± 0.2	4.0	± 0.2	4.8	± 0.2	6.1	± 1.1	4.7	± 0.4	5.4	± 0.2
20:4ω6 (AA)	1.1	± 0.1	1.0	± 0.0	1.6	± 0.8	1.4	± 0.6	1.1	± 0.0	1.1	± 0.2	1.1	± 0.1	1.0	± 0.0	1.7	± 0.5	1.6	± 0.4	2.5	± 1.0	1.6	± 0.2
20:5ω3 (EPA)	13.6	± 0.3	16.3	± 0.3	14.2	± 2.6	16.9	± 2.2	14.1	± 1.1	16.7	± 1.2	13.6	± 0.3	16.3	± 0.3	11.2	± 0.6	16.3	± 3.9	12.0	± 2.4	12.9	± 1.0
22:5ω6	1.1	± 0.0	1.3	± 0.1	1.3	± 0.4	1.7	± 0.3	0.9	± 0.0	1.4	± 0.1	1.1	± 0.0	1.3	± 0.1	1.3	± 0.2	1.5	± 0.2	1.8	± 0.5	1.5	± 0.1
22:6ω3 (DHA)	7.9	± 0.7	10.8	± 0.3	10.2	± 2.5	14.4	± 2.2	8.5	± 1.1	13.3	± 1.8	7.9	± 0.7	10.8	± 0.3	10.0	± 1.6	17.1	± 4.4	12.8	± 2.5	14.0	± 1.5
Other**	7.3		7.3		6.4		7.2		6.9		7.0		7.3		7.3		6.8		4.9		6.4		7.1	
Sum SFA	29.0	± 2.0	28.6	± 0.2	29.9	± 2.7	27.8	± 3.3	28.6	± 2.5	25.7	± 2.7	26.7	± 3.1	26.5	± 10.3	26.2	± 2.5	21.6	± 5.9	27.1	± 2.3	27.0	± 1.3
Sum MUFA	40.1	± 2.5	34.2	± 0.5	36.0	± 3.2	29.8	± 1.7	40.0	± 1.5	33.9	± 0.8	37.0	± 6.0	33.0	± 0.2	41.4	± 1.2	33.9	± 2.8	36.0	± 3.5	34.4	± 3.0
Sum PUFA	30.9	± 1.1	37.2	± 0.5	34.1	± 5.9	42.4	± 4.9	31.4	± 1.7	40.4	± 2.4	36.3	± 9.1	40.6	± 10.1	32.5	± 3.3	44.5	± 8.1	36.9	± 5.7	38.6	± 2.3
Total ω3	23.3	± 1.6	29.2	± 1.2	26.2	± 5.8	33.1	± 4.7	24.3	± 2.4	32.2	± 3.8	26.3	± 8.6	29.5	± 10.5	22.9	± 2.8	34.6	± 9.4	26.7	± 5.6	28.8	± 3.1
Total ω6	6.1	± 0.6	6.8	± 0.6	7.0	± 1.7	8.5	± 1.3	6.0	± 0.4	7.3	± 0.9	8.8	± 2.0	10.4	± 3.7	8.2	± 0.9	9.7	± 2.3	9.5	± 2.4	9.0	± 0.7
Ratio ω3/ω6	3.8		4.3		3.7		3.9		4.0		4.4		3.0		2.8		2.8		3.6		2.8		3.2	
Ratio AA/EPA	0.08		0.06		0.11		0.09		0.07		0.06		0.08		0.06		0.15		0.10		0.21		0.12	
Ratio DHA/EPA	0.58		0.66		0.71		0.86		0.60		0.79		0.58		0.66		0.89		1.05		1.07		1.08	

** **Other fatty acids (≤ 0.1%) include**: 4,8,12TMTD, i15:0, a15:0, C16PUFA, 16:1ω9c, 16:1ω5c, 16:1ω13t, i17:0, 17:1ω8c, 17:1, 17:0, 18:3ω6, 18:1ω7t, 18:1ω5c, 18:1, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω7c, 20:1ω5c, 20:0, 21:5ω3, 21:0, 22:4ω6, 22:5ω3, 22:4ω3, 22:2ω6, 22:1ω11c, 22:1ω9c, 22:1ω7c, 22:0, C24PUFA, 24:1ω11c, 24:1ω9c, 24:1ω7c, 24:0

6.5. Discussion

The body tissues, with the exception of digestive gland in February, generally showed no major differences between total lipid and fatty acid content and composition. The relationship of total lipid and fatty acid content and composition between the body tissues thoracic components and digestive gland in February was more complicated with varying trends between sex and temperature. Although there was some evidence of krill in the cooler temperatures containing elevated lipid stores, there were generally no significant differences with respect to temperature in krill sampled in November and February. Additionally, total lipid, TAG and PL content, as well as the major fatty acids; 16:1ω7c, EPA and DHA, were generally not significantly different between immature, male and female krill, despite past studies suggesting there are sex related differences in summer.

6.5.1. Sex related differences

Sex and maturity stage are known factors influencing lipid composition in krill (Clarke 1980; Saether *et al*. 1985; Kolakowska 1991; Pond *et al*. 1995; Virtue *et al*. 1996; Mayzaud *et al*. 1998; Phleger *et al*. 1998). These studies have concluded that sex related differences were mostly related to changes in TAG accumulation (Mayzaud 1997), which is an important energy reserve for krill that increases during feeding and is depleted during starvation (Virtue *et al*. 1996). Mature female krill can contain twice as much lipid as males (Saether *et al*. 1985; Kolakowska 1991), due mainly to oocyte production (Pond *et al*. 1995), and they can lose approximately half their body lipids (Clarke 1980), as well as one third of their body mass (Nicol *et al*. 1995), during one spawning event. Female reproduction, therefore, uses substantial amounts of energy and resources and is a major influence on total lipid and fatty acid content and composition (Quetin *et al*. 1994; Lee *et al*. 2006). The reproductive investment of male krill is far less known.

It has been shown that the accumulation of oocytes in the ovaries of females contains high levels of neutral lipids including TAG (Pond *et al*. 1995), while it appears that males do not require high levels of neutral lipids for the production of spermatophores (Virtue *et al*. 1996). Females can shed several spermatophores during one moulting episode (Bargmann 1937; Watkins *et al*. 1992). Since moulting continually occurs throughout the reproductive season (Nicol 1989), males are required to continually produce sperm mass, and therefore, attach spermatophores regularly to females. This suggests that the energy cost of continual spermatophore production and re-mating for males would be considerably high, and accounts for depletion of lipid content (Pond *et al*. 1995; Virtue *et al*. 1996). The energetic costs

between males and females vary over different timescales. For example, females accumulate a substantial and constant amount of lipid reserves, particularly TAG, for spawning during summer (Pond *et al*. 1995; Virtue *et al*. 1996). The reproductive effort of males, on the other hand, is invested in a series of events over the season, each of which has a lower overall lipid cost (Pond *et al*. 1995).

The results from our study differed from others reporting higher lipid content in females, notably TAG in summer, in comparison to males, probably because female krill were not gravid in this study. The mature females contained small pre-vitellogenic ovaries throughout the summer period, and the thorax was not swollen with extra-cellular space observed between the ovary and carapace. Despite no significant differences of lipid and fatty acid content and composition between sexes, the general trends observed in November suggest that mean total lipid, TAG and PL content were generally higher in females, particularly for the warmer 3°C treatment, in comparison to male and immature krill. The mean relative levels of the major PUFA, EPA and DHA were generally higher in males compared to females, albeit only slightly, for all temperature treatments and tissues in November, while mean 16:1ω7c was generally higher in females. Likewise in past studies, relative levels of EPA and DHA are substantially higher in males compared to female and juvenile krill (Virtue *et al*. 1996; Phleger *et al*. 1998; Cripps *et al*. 1999). Relative levels of MUFA, particularly 16:1ω7c, and SFA have also been shown to be significantly lower in male krill (Virtue *et al*. 1996). However, in February, relative levels of EPA and DHA were generally only higher for males in -1°C, and females contained higher levels in the warmer 1°C and 3°C treatments (except for in male digestive gland at 3°C). The general lipid and fatty acid trends in krill sampled in February were complex and there were no clear differences between sexes.

Mated females (spermatophores attached) were only observed in the cooler -1°C treatment in November, but the ovaries were still pre-vitellogenic. However, mated females contained total lipid, TAG and PL levels that were significantly lower than in mature females without spermatophores attached suggesting utilisation of lipids during the energetic mating process. Mean contents of 16:1ω7c, EPA and DHA in mated females were also lower than un-mated females. Although there are studies that have examined the energetic costs of general reproduction and oocyte production for females (Clarke 1980, 1984; Saether *et al*. 1985; Kolakowska 1991; Quetin *et al*. 1994; Pond *et al*. 1995; Virtue *et al*. 1996), there is a lack of literature concerning the actual energetic costs of mating. It has been suggested that the substantial lipid expenditure observed in males is accounted for by the general cost of spermatophore production and affixation to females, as well as the energy

involved in searching for un-mated and mature females (Pond *et al*. 1995; Phleger *et al*. 1998). Our study has shown that there is evidence that females also undergo some degree of energy expenditure in mating, due to utilisation of lipids in mated females. This expenditure may be involved in the attachment of spermatophores to the thelycum, or in the swimming costs of searching for, or being chased by, reproductive males.

6.5.1.1. Effect of experimental condition

Krill did not undergo full ovarian maturation in this study. They did, however, enter pre-vitellogenesis, which can occur prior to mating and the reproductive season, and is known as the first plateau of the ovarian maturation cycle involving minimal energy requirements (Quetin and Ross 2001). The second plateau during ovarian maturation involves the energetically demanding yolk accumulation, which will only proceed if sufficient resources are available, particularly food quality and quantity at the right time (Ross and Quetin 2000; Quetin and Ross 2001). Despite plentiful food concentrations provided throughout this study, female krill did not proceed through vitellogenesis, suggesting inadequate food quality and overall energy resources. Even though krill were actively feeding, as indicated by full and healthy digestive glands (personal observations) and reasonable total lipid and fatty acid content of the digestive gland, feeding rates were low. Clearance rates measured in April 2007 were between 0.35 and 1.02-L of water filtered ind^{-1} hr^{-1} (unpublished data), and thus, were substantially lower than rates estimated from the field (Morris 1984). Therefore, it appears krill may not be able to utilise all available food resources, as they would be expected to in the wild, due to possible confinement under laboratory conditions.

Mean total lipid content of the thoracic components of mature male and female krill sampled in November ranged from 13-25% of dry mass. The commencement of reproductive processes such as mating has been observed during this time in November in the laboratory (R. King, personal communication). This total lipid range is comparable to reported whole krill levels of 10.5% (spring/November) and 28.2% (summer/February) in the Weddell Sea (Hagen *et al*. 1996, 2001). Relative levels of TAG observed in krill from the Weddell Sea were also similar to those found in our study. During spring/summer in the Weddell Sea, krill undergo cycles of vitellogenesis and multiple spawning events (Quetin *et al*. 1994), utilizing lipid reserves. Therefore, it can be assumed that krill in our study, with similar lipid levels, had sufficient reserves to undergo vitellogenesis and even spawning.

Laboratory incubation can obviously never simulate natural conditions. Various factors of confinement may have influenced the reproductive processes of krill in our study. For example, in the present study, translucent acrylic tanks were used. However, it appears krill behave more naturally (swarming episodes and annual breeding observed) in a neutral/white walled tank that removes contrast from the visual environment (Kawaguchi *et al*. 2010). Results from controlled laboratory studies such as this one can help us optimise conditions, so as to provide further physiological data on krill.

6.5.2. Distribution of lipids

Sex and maturity stages markedly influence the variability (Clarke 1984; Kolakowska 1991; Pond *et al*. 1995; Virtue *et al*. 1996) and the distribution of lipid throughout a range of tissues (Mayzaud *et al*. 1998). The distribution of lipid has been reported in the abdomen (Clarke 1980; Saether *et al*. 1985; Mayzaud *et al*. 1998), ovary (Clarke 1980; Mayzaud *et al*. 1998), digestive gland (Virtue *et al*. 1993*a*; Mayzaud *et al*. 1998; Alonzo *et al*. 2003*b*, 2005*a*; O'Brien *et al*. in press) and various other body fractions, such as the thorax (Saether *et al*. 1985). Generally, total lipid concentration is higher (up to 60%) in the thoracic region for females compared to the abdomen, while the opposite is true for males (Saether *et al*. 1985). This is mainly due to very high levels of TAG accumulation in the ovary of females, and the abdomen of male krill accounting for a larger fraction of the total weight in comparison to the thorax. However, in our study, there were no major differences between the abdomen and the thoracic components for total lipid, TAG, PL and the major fatty acid content for November and February with no clear trends for each sex. This finding suggests that ovaries of females were at a very early stage of development and did not contribute to the overall results. The ovaries were too small to be dissected out for analysis.

Changes in lipid class and fatty acid composition have been observed in the digestive gland (Virtue *et al*. 1993*a*; Alonzo *et al*. 2003*b*, 2005*a*), which is one of the main sites for lipid metabolism and storage in crustaceans (Dall *et al*. 1992). The digestive gland plays a major role in providing essential energy during periods of short-term starvation (Virtue *et al*. 1993*b*). Analysing digestive gland lipid and fatty acid content and composition provides reliable indices of nutritional condition as well recent changes in diet activity (Virtue *et al*. 1993*a,b*). Our study has shown that there were no major differences or relationships in TAG, PL and the major fatty acid content within the digestive gland for each sex in November and February. Other than lipid and fatty acid content generally being lower in the digestive gland

compared to the thoracic components, there were no clear differences or trends between the sexes. The one major exception was the TAG contents for both mature male and female krill were significantly lower for all temperature treatments in the digestive gland compared to the thoracic components in November and February.

TAG concentrations in digestive gland were virtually depleted in krill sampled in February suggesting that lipids were utilised rapidly as an energy source. This result was also observed in immature whole krill (Chapter 5). This utilisation of energy stores was more likely for reproductive purposes earlier in the summer (including mating and spermatophore production), rather than being depleted through actual starvation. This finding also supports the theory by Virtue *et al*. (1993*a*) who concluded that lipids accumulate in the digestive gland for energetic purposes and are transferred to the thoracic region (i.e. ovary) for reproduction. However, there is a possibility that low TAG content was due to an inadequate diet or stress resulting from incubation, influencing overall reproduction.

6.5.3. Temperature related differences

Krill is considered to be stenothermal and is sensitive to slight changes in temperature (Wiedenmann *et al*. 2008). Temperature has been found to influence PL structure and fatty acid composition (Farkas 1979), as well as to cause gel/liquid phase transformations that result in changes to membrane fluidity (McElhaney 1984). However, clear temperature effects have only been observed with differences of at least 15°C for marine animals (Mayzaud *et al*. 2000). Below this range of temperatures, the expected changes in lipid structure and composition would be minimal (Mayzaud *et al*. 2000). This is particularly true for krill, which are probably thermally stressed above about 5°C, and thus through evolution, they have physiologically adapted to temperatures below 5°C (Clarke and Morris 1983).

Total lipid, TAG, PL and generally fatty acid content and composition of whole immature krill showed no clear temperature effect between -1°C and 3°C at various time points over a year (Chapter 5). During the summer period in this study, the effects of temperature between sex and among tissues fractions were again complex, even though krill were incubated under a constant supply of food. At -1°C in November, immature, male and female krill generally contained higher mean total lipid, TAG and PL content in the thoracic components compared to the warmer temperatures. The major fatty acid content at -1°C in the thoracic components and digestive gland were also higher than at 1°C and 3°C. Overall, the warmer 1°C and 3°C treatments contained lower lipid stores, which were generally similar in mean concentrations. There was no clear temperature relationship observed in the

digestive gland for total lipid, TAG and PL content for each sex. However, lipid stores in the digestive gland at 3°C were higher than at -1°C and 1°C. In terms of the relative levels of TAG and PL, the storage lipid TAG generally decreased with increasing temperature, while PL was dominant and increased with increasing temperature.

In February, immature krill contained higher mean lipid and fatty acid content in the thoracic components at 3°C. Lipid and fatty acid content in the female thoracic components and digestive gland was generally higher in the cooler temperatures (-1°C and 1°C), while highest values were recorded at 1°C for males.

Overall, there is some evidence to suggest that krill in the cooler temperatures, particularly at -1°C, have elevated lipid stores in the summer months (November and February), and thus are in better condition compared to krill at 3°C. This is also supported by the fact that female krill only mated in the -1°C treatment in November, suggesting that krill may have been more stressed and their energy resources were reduced below a threshold that is required to undertake the reproductive processes in warmer temperatures. However, the variation of mean total lipid and fatty acid content and composition in krill was substantial under controlled light and feeding conditions, resulting in no significant relationships between the sexes and temperatures during summer.

There is growing interest in the role of inter-individual variability in structuring populations and providing resilience to environmental change. This is highlighted by Goodall-Copestake *et al*. (2010) who examined the relative levels of variability in the population genetic marker *cox-1* of Antarctic krill. They concluded that krill has one of the most diverse genomes of any metazoan and this diversity is seen as much within swarms as between swarms. A possibility of such diversity is probably a result of the relatively unrestricted mixing of krill within its large Southern Ocean habitat and its large population size, which prevents the overall loss of alleles through drift (Goodall-Copestake *et al*. 2010). What the present work demonstrates is a phenotypic expression of this described diversity, in the range of capacities in the storage and utilisation of lipids.

This high individual variation highlights krill's ability to adapt and survive under various environmental conditions, within a natural temperature range in the Southern Ocean. Understanding the effects of temperature on the physiology of krill is only part of the equation. Survival and adaptation to future climatic change is contingent on a range of biotic and abiotic parameters. Future research investigating environmental factors such as sea-ice thickness and extent, effects of ocean

acidification and possible alterations to the Southern Ocean trophic structure are needed to assess climate change impacts on krill.

6.6. Conclusion

Overall, there were relatively few significant differences in lipid composition between temperatures and between maturity stages of krill. There was a general trend of decreasing TAG with increasing temperature, and higher lipid content in females compared to males, but generally this was not statically significant. Interestingly, mated females in the -1ºC treatment contained lower lipid and fatty acid content, compared to un-mated females, suggesting utilisation of lipids during mating. Even though mating was observed, ovaries of females were only in pre-vitellogenesis, which likely explains why there were no major differences in lipid and fatty acid content between sexes. A depletion of lipids was observed in krill sampled in February for all sexes and temperatures, particularly TAG in digestive gland, which implies krill were possibly utilising their energy stores for reproductive purposes. However, the incubation influences such as diet and confinement cannot be quantified. We show possible evidence of elevated lipid stores in krill at cooler temperatures. Variability in krill lipid levels, particularly with temperature, demonstrates a physiological plasticity necessary in an environment where, although temperature is reasonably stable, trophic resources are highly seasonal.

-- Chapter seven --

General Discussion

Antarctic krill (*Euphausia superba*) is an ecologically significant species in the Southern Ocean. Not only do krill play a major role in the food web (Mangel and Nicol 2000; Alonzo *et al*. 2003*a*), but they are also attracting increasing interest as a target for commercial fishing (Kawaguchi and Nicol 2007). Over-exploitation of krill could have major effects on the whole Antarctic ecosystem and it is therefore imperative to have sound management procedures in place before the krill fishery expands. A key to adequately managing this important and expanding fishery, and to protect the overall ecosystem, is to understand the life history traits of krill, particularly growth, maturation and physiology, which are the driving factors influencing the variability in krill biomass. Despite the recognised importance and extensive research on krill, current knowledge of the ecology and biology is far from complete and much still remains to be studied (Nicol 2003).

The central aim of this thesis was to test hypotheses brought about from key questions raised in field studies and to evaluate the effects of environmental parameters (light, food availability and temperature) on the growth, maturation and physiology in krill. In addressing the main aims of this study, krill were incubated under controlled conditions for a complete annual cycle. Information on growth, maturation and physiology under various light, diet and temperature regimes throughout a full year is limited, hampering understanding of the effects a changing environment may have on krill. It is crucial to understand how krill adapts and survives under extreme and changing environmental conditions.

7.1. Temperature effects on growth and maturity

A clear seasonal cycle of growth and maturity was evident for both male and female krill in all temperature treatments (-1°C, 1°C and 3°C), under a natural Antarctic light cycle and plentiful food concentrations (Chapter 2). An observed progression of maturity and rapid growth occurred during spring, and shrinkage commenced towards the end of January when krill were undertaking sexual regression. The analysis of linear mixed models (LMM) developed and used in this thesis revealed strong effects of month sampled (generally longer IMP during summer) and temperature (shorter IMP in higher temperature) on intermoult period

(IMP). In the wild, krill generally have a longer IMP in winter compared to summer, which is regarded as the effect of the seasonal temperature cycle (Atkinson *et al*. 2006; Kawaguchi *et al*. 2006; Tarling *et al*. 2006). Our results indicated that if the temperature and food conditions are constant, krill have a higher moulting frequency during winter compared to the field. This is probably because krill have minimal energy requirements (no reproduction and less growth) in winter. If supplied with the same amount of food all year, krill might divert excess energy to driving the moulting cycle. This underlines the importance of further studying the energy budget and physiology in order to understand the processes of krill growth. It also showcases the importance of regular moulting to the condition of krill.

Temperature is the major influence on the rate of moulting and possibly growth in krill, with a rise in temperature resulting in shorter IMP (Hartnoll 2001). From this study, krill incubated under the highest temperature (3°C) did not result in the greatest growth. Under the current experimental condition, 1°C was considered optimum for krill growth. IMP was significantly shorter at 1°C than -1°C, but the difference in growth increment between the two temperatures was not significantly different. Although not statistically demonstrated, this should result in higher daily growth rate (DGR) at 1°C. IMP and all growth variables were significantly lower for krill in the 3°C treatment. Our findings support those from Atkinson *et al*. (2006), who suggested the existence of an optimal temperature (~0.5°C) for krill growth in the south-west Atlantic sector of the Southern Ocean. Furthermore, for the first time, this study has confirmed that compensation mechanisms do exist between IMP and instantaneous growth rate (IGR) in individual krill (faster moult times corresponding to slower growth increment). Compensation mechanisms had previously been reported for crustaceans in general, however, they had not been examined in krill (Kawaguchi *et al*. 2006). Understanding compensation effects is important for modelling growth trajectories of wild krill using IGR to quantify the distribution of size (age) frequencies with respect to season. My results will facilitate more accurate and robust model predictions for temperature-dependent growth for krill.

7.2. Effect of various light regimes on the maturation cycle

The effect of temperature and constant food was negligible on the actual timing of the krill maturity cycle; however results presented in Chapter 3, show clear evidence that the timing of specific light regimes is playing an important role in governing the krill maturation cycle. Overall, it appears that krill maturation will undergo a normal seasonal cycle of regression and re-maturation under a range of light conditions; a simulated Antarctic light cycle (Chapter 2), fixed photoperiod (i.e.

light:dark – 14:10 hrs; Hirano *et al*. 2003, Chapter 3) or darkness (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*; Chapter 3). This suggests that there is an endogenous annual rhythm underlying the maturation cycle and it is not solely controlled by light. However, when krill were subjected to complete darkness following late summer light, at a time when krill were commencing sexual regression, the rate of regression rapidly accelerated, which resulted in earlier re-maturation (by three months) the following season. The ecological implications of this flexible maturation cycle in response to darkness means that krill can flexibly adjust their seasonal physiological cycle. Overall, krill must use a hierarchy of signals to help coordinate its biological cycles, but it appears that the timing of specific light regimes, particularly the timing of darkness, is possibly one of the most important factors influencing the krill maturation cycle

7.3. Seasonal variation in respiration rates

Respiration rates in krill showed an increasing trend with month between the commencement of maturation in late winter and sexual regression in late summer under all controlled experimental conditions (Chapter 4). Results here suggest that the environmental variables; light, food availability and temperature, may not be the dominant factors influencing seasonal changes in krill metabolic activity. Our results may help interpret respiration rates reported in field winter krill, in which a reduction in metabolic and feeding activity has been widely accepted as a strategy to survive periods of food scarcity (Quetin and Ross 1991). Respiration rates of well fed krill under simulated winter conditions in this study were similar to non-feeding winter observations of krill from the Bransfield Strait (Quetin and Ross 1991). Quetin and Ross (1991) considered that krill in that environment were starving, but my results suggest that rates are as expected for that time of year irrespective of feeding condition. Instead, seasonal changes in metabolic rates may be controlled by an endogenous rhythm, which is independent of environmental variables. However, there was no solid evidence to suggest that an endogenous annul rhythm was actually at work in this study, as an experiment needs to run for greater than one year. More information about an endogenous rhythm is described further in Section 7.6, but the significance of this ‘internal clock’ mechanism warrants further investigation, particularly examining photoperiod as the possible cue for dictating internal processes.

7.4. Trends in lipid content and composition

The first experimental results on changes in total lipid and fatty acid content and composition of krill over a full year under constant food and three temperature regimes has been established (Chapters 5 and 6). Combining information from immature krill over a full year (Chapter 5) and mature adults during summer (Chapter 6), there was some evidence to suggest that krill in cooler temperatures, particularly at -1°C, had elevated lipid stores, and thus, were in better condition compared to krill at 3°C. However, the results were highly variable between individuals under the same experimental conditions, generally resulting in no significant trends between sex and temperature. Female krill only mated in the -1°C treatment in November, suggesting that krill may have been more stressed and their energy resources were reduced below a threshold that is required to undertake the reproductive processes in warmer temperatures.

It has commonly been observed from field studies that the accumulation of lipid is maximal in autumn, while there is a depletion of lipid during winter, before there are substantial phytoplankton blooms in the following spring and summer (Hagen *et al*. 1996, 2001). This utilisation of lipid, in the form of triacylglycerols (TAG), during winter is related to surviving the low food availability during this period (Hagen *et al*. 1996, 2001; Virtue *et al*. 1996). TAG accumulates during periods of high food supply and is metabolised during food shortages (Clarke 1980, 1984; Pond *et al*. 1995; Hagen *et al*. 1996). With constant food provided during this 14 month experiment, there was a gradual decrease in lipid storage as the season progressed, with significantly lower lipid and fatty acid content at the end of summer in February. Depletion of lipid content, as the experiment progressed, can either be explained by krill utilising lipid resources for reproductive purposes, a process previously reported by Virtue *et al*. (1997), or due to possible limitations of the laboratory environmental conditions used.

7.5. Consideration of the effects of experimental conditions

Undertaking laboratory based research can provide limitations and uncertainties, especially when trying to extrapolate key findings to wild populations (Nicol 2000). In their natural pelagic environment, krill are unlikely to be constrained by any physical barriers. However, in the laboratory, krill are confined to small experimental chambers, tanks and/or jars, which cause stress, restrict movement and can ultimately alter overall behaviour (Clarke and Morris 1983). Since it is not presently possible to reproduce the same diet as krill obtained in the field, food quality (and feeding ability) may likely be poorer in laboratory based studies.

Therefore, there remains a need for improving the quality and/or quantity of food used in krill experiments so as to approximate conditions in the field.

Regression of external sexual characteristics and shrinkage commenced at the end of January when there was still a natural summer light regime and high food concentration. The environmental conditions were still favourable to maintain growth and reproductive processes during this period, however, krill still underwent regression and shrinkage, further suggesting that krill had limited resources, possibly indicating an inadequate diet and/or reduced feeding ability due to confinement. Inadequate laboratory diet may have caused the considerably lower growth rates towards the end of the experiment in April/May 2007 in the present study, compared to the start of the experiment in April 2006. April 2006 was approximately one month after the krill were collected from the field, and therefore, still reflecting a more 'natural' condition. The clearance rates and feeding activity of krill in this experiment, and in laboratory based studies in general, are considerably lower than rates estimated from the field (Morris 1984). The biological cycle of krill, however, may have also been under endogenous control. Under the experimental conditions used, an internal clock mechanism may have triggered the timing of regression and shrinkage in this study, regardless of the diet and light conditions.

It is presently difficult to clarify the precise reason(s) why female krill were only pre-vitellogenic and did not undergo full ovarian maturation in this study. Sufficient nutrients, quality and quantity, are required at the right time for krill to fuel reproduction and initiate the energetically demanding accumulation of yolk (Ross and Quetin 2000; Quetin and Ross 2001; Kawaguchi *et al*. 2007*a*). Krill may not have received an adequate diet to show vitellogenic development in the ovaries so they could have abandoned full ovary maturation. However, mean total lipid content in percent of dry mass in November was comparable to the reported levels in whole krill in the Weddell Sea during spring and summer (Hagen *et al*. 1996, 2001). During this time of year in the Weddell Sea, krill undergo cycles of vitellogenesis and multiple spawning events (Quetin *et al*. 1994), utilizing their lipid reserves. Therefore, it can be assumed that krill in our study, with similar lipid levels, had sufficient reserves to undergo vitellogenesis and even spawning.

Laboratory incubation can never fully simulate natural conditions. At the time of this experiment, krill reproduction and breeding success in the aquarium facilities were limited. Confinement may have influenced the reproductive processes of krill in this study. For example, translucent acrylic tanks were used to maintain krill, but it appears krill behave more naturally (swarming episodes and annual breeding observed) in a neutral/white walled polyethylene tank that removes contrast from the

visual environment (Kawaguchi *et al*. 2010). However, the timing and the actual trends of the growth and maturity cycles observed from this study (in clear acrylic tanks) are comparable to those krill maintained in white polyethylene tanks (personal observations). Results from controlled laboratory studies such as this one can help us optimise conditions, so as to provide further physiological data on krill.

7.6. Endogenous annual rhythm

It is becoming more apparent that krill can utilise a strategy of quiescence (Hirche 1996), a type of dormancy, in which life processes (i.e. feeding activity, metabolism and growth) are retarded in the winter in response to limiting conditions (Seear *et al*. 2009). Internal clocks have also been proposed to dictate the physiological mechanisms of krill (Thomas and Ikeda 1987; Kawaguchi *et al*. 2007*a*; Teschke *et al*. 2007, 2008; Gaten *et al*. 2008), which are frequently synchronised by environmental cues, or *zeitgebers* (Buchholz 1991). The presence of an endogenous circadian clock is extremely likely, but has not yet been fully resolved (Gaten *et al*. 2008; Teschke *et al*. 2008). There was some further evidence in this study for the existence of an endogenous annual rhythm, however, the importance of such to krill biology was only indirectly inferred. Teschke *et al*. (2007, 2008) suggested that krill switch between an active and quiescent physiological state, which is cued by photoperiodic cycles. A follow up study showed that key processes of krill responded at the molecular level after just seven days of exposure to a changed photoperiodic cycle with a significant change in the transcription of genes (Seear *et al*. 2009). The future approach is to investigate the molecular mechanisms of a circadian and/or circannual rhythm in krill and the rhythmic expression of genes encoding for particular proteins. This analysis will be crucial to further understand the processes of synchronisation between the seasonal development of krill and the seasonal cycles of environmental variables in the Southern Ocean (Teschke *et al*. 2008).

The overall condition, recruitment and high biomass of krill are a result of their ability to successfully adapt to patchy and extreme seasonal changes in their environment, mainly food availability, sea-ice extent and light intensity and duration (Quetin and Ross 1991; Siegel and Loeb 1995; Quetin and Ross 2001). As a consequence, the natural biological rhythm of krill is synchronised to make use of the seasonal environmental conditions so that the appropriate biological activity is correctly scheduled at the right time and place for overall survival. For example, published winter based respiration measurements from the field (i.e. Kawaguchi *et al*. 1986; Quetin and Ross 1991; Huntley *et al*. 1994) and long-term controlled experiments in the laboratory (Chapter 4), were completed during the winter months

so the seasonal mode of krill were in a winter resting state, when finding adequate food is limited. The increasing metabolic rates observed from winter to summer, even in starved krill, at various temperatures and in those krill kept in complete darkness in this study, were caused by krill changing endogenously to a summer state. Additionally, the onset and timing of krill maturation is crucial for overall reproductive success (Kawaguchi *et al*. 2007*a*). Female krill need to restore and re-organise their ovary in sufficient time and be physiologically prepared for the following reproductive period (Ross and Quetin 2000; Kawaguchi *et al*. 2007*a*). Thelycum and petasma maturation must begin during early winter regardless of environmental conditions so as to coincide with vitellogenic maturation of the ovaries when abundant spring food resources are available (Kawaguchi *et al*. 2007*a*). Following reproduction in summer, krill reduce their metabolism and maturity corresponding to a decrease in light.

The cycle of maturation and regression allows krill to make the most of the highly seasonal changes in environmental conditions. This study, however, has shown that the maturation cycle of krill can be rescheduled by manipulating the light conditions, from late summer light to constant darkness, resulting in earlier re-maturation (by three months) in the following season. Therefore, it is possible to alter the biological activity of krill by manipulating their environment under controlled conditions. If this can be done with changes in the seasonal light regime, will other environmental conditions, such as increasing temperature, have similar effects on krill? This study examined the effect of water temperature on the growth, maturation and physiology in krill ranging from -1°C to 3°C, but each of these temperatures remained constant throughout the experimental period. The effect of changing these temperatures throughout the experiment, for example from 1°C to 3°C, might alter the natural biological activity of krill.

The timing of spring/summer phytoplankton blooms has generally been relatively predictable in the past (Clarke 1988). Krill appear to have optimised their biological cycles to make the most of these conditions. Food availability affects the overall rate of maturation and reproductive output of krill (Quetin and Ross 2001), which is correlated with good sea-ice years at the Antarctic Peninsula (Siegel and Loeb 1995). Since air and water temperature, as well as wind force, influences sea-ice dynamics and the changes in sea-ice extent and duration (Clarke *et al*. 2007; Quetin *et al*. 2007), this in turn will influence the timing and degree of ice-edge phytoplankton blooms (Quetin and Ross 2001). Therefore, krill must be able to adapt to these changes and reschedule and alter their natural biological cycles. Since krill is the dominant prey item for many Antarctic predators and a target of a developing

fishery, it is vital to understand the susceptibility of krill to global warming (Wiedenmann *et al*. 2008).

7.7. Evidence of climate change

Currently, the Southern Ocean is characterised by low and seasonally stable temperatures (Whitehouse *et al*. 2008) with slightly higher temperatures observed in the austral summer (Clarke 1988). However, in terms of year-to-year variation, the Antarctic Peninsula is experiencing one of the fastest rates of regional climate change on Earth, resulting in the collapse of ice-shelves, the retreat of glaciers and the exposure of new terrestrial habitat (Clarke *et al*. 2007). Meredith and King (2005) reported rapid warming (>1°C) of summer surface waters west of the Antarctic Peninsula, while in the ocean around South Georgia, Whitehouse *et al*. (2008) concluded that there had been rapid warming during the 20th Century, with a mean increase of ~0.9°C in January and ~2.3°C in August in the upper 100m of the water column. It is therefore becoming clear that there is substantial evidence for long-term warming of the surface layer, the habitat of krill, in the Southern Ocean.

The Southern Ocean is considered to be an isothermal environment with an annual temperature range <5°C (Clarke and Morris 1983). Invertebrates, therefore, tend to be stenothermal, sensitive to even slight changes in temperature in the Southern Ocean (Peck *et al*. 2004). It was predicted that a 2°C rise in sea temperature would cause population or species removal from the Southern Ocean due to the onset of anaerobic metabolism (Peck *et al*. 2004). Based on other Antarctic ectotherms, it is expected that krill will also demonstrate this extreme sensitivity to environmental change (Atkinson *et al*. 2006; Wiedenmann *et al*. 2008), as part of their life cycle is dependent upon sea-ice concentration and the resultant algal blooms at critical times of the year will be influenced by climate variability (Quetin and Ross 2001; Hofmann and Murphy 2004).

7.8. Optimal temperature for krill growth, maturation and condition

This thesis has provided some insights into the condition of krill from a range of temperatures in the laboratory over an annual cycle. Temperature alone (-1°C, 1°C and 3°C) was not shown to be a major influence on the timing of the maturation cycle or respiration rates of krill throughout the year. Although the results were highly variable between individuals under the same experimental conditions, there was some evidence to suggest that krill at -1°C were in better condition than those at warmer temperatures. Krill generally contained elevated mean lipid stores and mating only occurred at -1°C, suggesting conditions for utilisation of energy

resources were optimal. Additionally, at 1°C, daily growth rates in krill were highest. These results provide the foundation for further studies on temperature-growth relationships in krill.

7.9. Other parameters influenced by global warming

The vulnerability of krill to global warming will involve many factors, not just water temperature alone. The life cycle of krill relies on interactions with other biotic and abiotic components within the ecosystem that will also be influenced by future climate change. A warming climate (air and water temperature) in Antarctica will change sea-ice dynamics (Arrigo and Thomas 2004), with sea-ice advance and retreat occurring earlier and a shorter duration of ice covered waters (Quetin *et al*. 2007). A decrease in sea-ice cover is reported to have a negative impact on krill recruitment, as larvae are dependent on sea-ice for survival (i.e. habitat and food) (Daly 1990), and gonadal development and spawning success of adults will be reduced (Siegel and Loeb 1995; Nicol 2006; Wiedenmann *et al*. 2008). Sea-ice extent and the timing of retreat are therefore considered to be responsible for determining the timing, length and intensity of krill reproduction, as sea-ice facilitates ice-edge phytoplankton blooms, which is currently a vital energy resource (Siegel and Loeb 1995; Quetin and Ross 2001). A significant decline in krill density has been reported for the south-west Atlantic sector of the Southern Ocean since the 1970s, which is associated with increased water temperature and a decrease in sea-ice cover (Atkinson *et al*. 2004; Whitehouse *et al*. 2008). However, it is unsure whether this is a more widespread phenomenon (Smetacek and Nicol 2005).

In areas of regional warming, phytoplankton communities may change from a diatom-dominated food for krill to phytoplankton species that are much less nutritious and smaller in size such as cryptophytes (Moline *et al*. 2004). As a consequence, this warming trend and a decline in sea-ice extent might favour competing macro- and mesozooplankton species, such as the pelagic tunicate *Salpa thompsoni* (salps) (Loeb *et al*. 1997). Salps are opportunistic feeders and compete with krill for limited food, and thus, have an adverse effect on krill abundance and recruitment for the following year (Siegel and Loeb 1995; Siegel 2000*b*). Unlike krill, salps are scarce following winters of extensive sea-ice cover and they are not a major dietary source for Antarctic vertebrate predators (Foxton 1966). Global warming, and the subsequent changes in sea-ice dynamics, may have a profound effect on the production and structure of the Southern Ocean food web (Atkinson *et al.* 2004; Hofmann and Murphy 2004).

Studying the effects of water temperature alone is only a small component in understanding the susceptibility of krill to future warming. There are many uncertainties with future climatic change and the impacts that global warming may have on krill populations. Therefore, future temperature related experiments will also need to take into account environmental factors such as sea-ice thickness and extent, effects of ocean acidification and possible alterations to the Southern Ocean trophic structure in order to predict the overall impacts on krill. Additionally, measuring natural responses to temperature of krill at their northern distributional limit, i.e. South Georgia, will be valuable in predicting the effects of climate change, as warming will be more pronounced at this location (Atkinson *et al*. 2006; Wiedenmann *et al*. 2008).

7.10. Comparison studies to krill from the warmer waters at South Georgia

Over half of the krill stocks are predicted to be located within the south-west Atlantic sector of the Southern Ocean, which is also the location where the krill fishery is currently concentrated (Atkinson *et al*. 2004). South Georgia is at the north eastern edge of the Scotia Sea in this Atlantic sector, the only krill habitat near the Antarctic Polar Front, in a relatively ice-free region (Ward *et* al. 1990; Siegel 2005; Tarling *et al*. 2007; Atkinson *et al*. 2008; Whitehouse *et al*. 2008). As a result, South Georgia contains the warmest temperatures that krill inhabit (Tarling *et al*. 2007; Whitehouse *et al*. 2008; Wiedenmann *et al*. 2008). In the past, krill populations at South Georgia have generally been assumed to have maximal growth rates, due to warmer temperatures and plentiful food (Hofmann and Lascara 2000; Atkinson *et al*. 2001). However, this was later disproved by the use of statistical empirical models (Atkinson *et al*. 2006). They examined the effects of food, temperature and sex on growth rates of krill in the south-west Atlantic in summer and concluded that growth rates were highest in the more modest ice-edge blooms of the southern Scotia Sea. Growth actually decreased above a temperature optimum of ~0.5°C, which is similar to the optimum temperature (1°C) for growth proposed here (Chapter 2). Overall, mean growth increased with rising temperature in the colder waters off the Antarctic Peninsula and decreased with increasing temperature in the warmer waters at South Georgia (Wiedenmann *et al*. 2008). The differential predictions within regions is described by a decreasing IMP with increasing temperature (Kawaguchi *et al*. 2006; Chapter 2) and the growth increment model by Atkinson *et al*. (2006) follows a parabolic relationship with temperature (maximum at ~0.5°C). The growth measurements from warm water stations were all examined at South Georgia, and Atkinson *et al*. (2006) showed that the decrease in growth was a real effect of

temperature and invalidated other possible causes such as a distinct diet, predation, and reallocation of energy sources.

South Georgia is a highly productive region with phytoplankton blooms regularly producing chlorophyll *a* concentrations above 10 mg m^{-3} (Korb *et al*. 2004), which supports a rich food web (Atkinson *et al*. 2001). Krill habitat at South Georgia has been suggested to be viable because of the costs of growth at high temperatures is offset by the regions enhanced food supply (Atkinson *et al*. 2006). With water temperature at South Georgia being as high as 5°C in recent years (i.e. Georgia Basin, Korb *et al*. 2005; Whitehouse *et al*. 2008), the slow growth rates in warmer temperature (Atkinson *et* al. 2006; Chapter 2) reflect the possibility that South Georgia is now becoming thermally suboptimal and krill may struggle to cope with future climatic change at this location (Atkinson *et al*. 2006). This is further compounded by the reduced biomass estimates with long-term temperature data showing declining habitat suitability for krill (Atkinson *et al*. 2004; Whitehouse *et al*. 2008; Wiedenmann *et al*. 2008). Wiedenmann *et al*. (2008) proposed that if waters off South Georgia warm by 1°C, an increase in food of approximately 40% will be needed to keep biomass per recruit the same.

The future size of the krill standing stock is primarily influenced by the recruitment success (Siegel 2005). However, there is a general consensus that the warmer waters at South Georgia do not support a self-sustaining krill population, as there are limited observations of hatched larvae and skewed length frequency distributions (Ward *et al*. 1990; Quetin *et al*. 2007; Tarling *et al*. 2007). Instead, krill populations are proposed to originate in waters near the Antarctic Peninsula or in the Weddell Sea (Brierley *et al*. 1999), and are transported to South Georgia via the prevailing Antarctic Circumpolar Current (Murphy *et al*. 1998; Hofmann and Murphy 2004; Murphy *et al*. 2004). Although there are no direct observations (Nicol 2006), it appears that krill spend the winter under sea-ice in the southern Scotia Sea, in a potentially more favourable environment, and following the austral spring when sea-ice recedes, krill are transported to South Georgia (Murphy *et al*. 1998; Hofmann and Murphy 2004, Murphy *et al*. 2004; Siegel *et al*. 2004). The reason krill are unable to maintain local recruitment at South Georgia is not well understood.

Recently, Tarling *et al*. (2007) was the first to examine the relationship between the reproductive capacity of female krill and the presence of larvae in the South Georgia area. They showed that females were capable of successfully completing a full reproductive cycle around South Georgia releasing an average of 12,343 eggs $year^{-1}$. These fecundity estimates are amongst the largest ever recorded for krill; however, observed estimates of larval concentrations were well below

predicted concentrations and the majority of measured stations contained no larvae at all (Tarling *et al*. 2007). The discrepancy between predicted and observed larvae concentrations was potentially likely due to predation and advection, but also the possibility of physiological stress with high temperatures (Tarling *et al*. 2007). Even though krill only mated and contained elevated lipid stores at -1°C in this study (suggesting optimal utilisation of energy resources at lower compared to warmer temperatures), krill at South Georgia have been shown to undergo successful reproduction and early stage development in high temperatures (up to 5°C). Ross *et al*. (1988) and Quetin and Ross (1989) have examined the effect of temperature ranging between -1°C and 2°C on various factors of larval survival, however, there has been no examinations of the physiological response of krill larvae at temperatures greater than 2°C. This is vital to understanding the local recruitment failure of krill at South Georgia, but also the general reproductive success in the Southern Ocean in light of the predicted future climatic change (Tarling *et al*. 2007).

Overall, it is difficult to predict whether krill have the ability to adapt their behaviour and physiology to increasing water temperatures and a changing habitat (Quetin *et al*. 2007). Studying krill at their northern distribution and temperature limit, South Georgia, has been proposed to be valuable in predicting krill's survival and response to climate change (Atkinson *et al*. 2006). However, since krill at South Georgia are thought to be a product of immigration (Tarling *et al*. 2007), understanding changing environmental factors (i.e. sea-ice and temperature) on krill upstream is also vital for this population. Climate change could also produce small changes in the controlling factors of the current system in the Antarctic Peninsula, which is responsible for transporting krill to South Georgia (Hofmann and Murphy 2004). For example, surface heating may affect the speed of Antarctic Circumpolar Current, the location of fronts and the distribution of Circumpolar Deep Water (Hofmann and Murphy 2004). This may influence the location and immigration of krill populations, and subsequently, affect predator survival that depend on these krill populations (Siegel 2005).

7.11. Physiological and behavioural plasticity of other euphausiids

Euphausiids, in general, have a wide geographic range throughout the global oceans (Mauchline and Fisher 1969; Mauchline 1980). Two other dominant species of euphausiids are *Meganyctiphanes norvegica* and *Euphausia pacifica*. *M. norvegica* is found in many areas of the North Atlantic and survives in waters from the Arctic Ocean to the Mediterranean Sea (Mauchline and Fisher 1969). *E. pacifica* occurs typically in the Yellow Sea, China Sea, Sea of Japan, Gulf of Alaska and in

the Californian Current System (Brinton 1962). Within this wide distributional range, populations of *M. norvegica* and *E. pacifica* are exposed to significant regional differences in temperature, salinity, bathymetric, hydrographic, seasonal and trophic conditions (Mauchline and Fisher 1969; Mauchline 1980). As a result, each species of krill can live in a wide range of environments and that the timing of their key life cycle events (spawning intensity and season, growth rates and physiology) can be completely different depending on the habitat (Iguchi and Ikeda 1995; Tarling *et al*. 1998, 1999; Mayzaud *et al*. 2000; Yoon *et al*. 2000; Saborowski *et al*. 2002).

As part of their life cycle, both *M. norvegica* and *E. pacifica* perform diel vertical migration, exhibiting ascent into the upper layers at dusk and descent at dawn (Tarling *et al*. 1999; Endo and Yamano 2006). Therefore, not only do these krill have varying tolerances to regional temperature differences, but also vertical temperature changes (Tarling *et al*. 1999). The entire temperature range which *M. norvegica* and *E. pacifica* experience during its lifetime (and in the case of *M. norvegica*, on a daily basis) is estimated to be as wide as 1-16°C (Lindley 1982; Mayzaud *et al*. 2000) and 1-28°C (Brinton 1962; Yoon *et al*. 2000), respectively. Unlike Antarctic krill, *M. norvegica* and *E. pacifica* reside in both warm and cold waters. Therefore, these krill exhibit specific behavioural and physiological adaptations that allow them to inhabit a wide range of temperatures (Tarling *et al*. 1998). Physiological adaptation to this wide temperature regime is shown from both lipid changes (Mayzaud *et al*. 2000) and oxygen consumption (Saborowski *et al*. 2002) in *M. norvegica*. Tarling *et al*. (1999) and Endo and Yamano (2006) also investigated the vertical migration of *M. norvegica* and *E. pacifica*, respectively, in relation to moult and reproductive processes. These studies concluded that moulting occurred in deep, cool waters at night, and that spawning females were most evident in the warmer uppermost layer (Tarling *et al*. 1999; Endo and Yamano 2006). The warmer temperatures are expected to accelerate oocyte maturation in the ovary through speeding up final meiosis (Tarling *et al*. 1999). Therefore, it is possible that these krill can utilise the temperature gradient to enhance physiological processes such as moulting and spawning (Tarling *et al*. 1999).

In light of evidence of plasticity of *M. norvegica* and *E. pacifica* life history patterns, Antarctic krill, like other euphausiids, might also be capable of adapting to changing environmental conditions, and overall, survive predicted future climatic change.

7.12. Conclusion

Antarctic krill is a highly successful species in the Southern Ocean ecosystem, with an extremely large biomass and production, thus playing an important and under-pinning role in regional food web dynamics. The overall recruitment and population size of krill are strongly influenced and dictated by the survival success and the ability to exploit and cope with a highly seasonal environment (Quetin and Ross 1991; Siegel and Loeb 1995). The present study focused on environmental effects (light, diet and temperature) on the growth, maturity and physiology in krill over an annual cycle. It appears that krill can flexibly adjust their biological cycles (growth, maturity and metabolism) to cope with various environmental conditions. This thesis forms a solid basis in terms of understanding changing environmental conditions on the biological and physiological cycles of krill.

For the first time this study has provided new insights into the life history and biology of krill over an annual cycle, which will enable more robust modelling for accurate assessments and management for the krill fishery. It is crucial to expand the capacity and to build up more of a detailed knowledge on the mechanisms that drive the life cycle of krill, particularly during the relatively unknown winter period. The research in this thesis has emphasised the urgency to further understand krill adaptations and survival to its seasonal environment, as well as the effects of a changing habitat in the Southern Ocean, in light of future climatic change.

-- Literature cited --

Alonzo, S.H., Switzer, P.V. and Mangel, M. (2003*a*). An ecosystem-based approach to management: using individual behaviour to predict the indirect effects of Antarctic krill fisheries on penguin foraging. *Journal of Applied Ecology*. **40**, 692-702.

Alonzo, F., Nicol, S. Virtue, P., and Nichols, P.D. (2003*b*). Lipids as trophic markers in Antarctic krill, I. Validation under controlled laboratory conditions. ***In:*** A.H.L. Huiskes., W.W.C. Gieskes., J. Rozema., R.M.L. Schorno, L., S.M. van der Vies. And W.J. Wolff. (Eds). *Antarctic Biology in a Global Context*. Backhuys Publishers, Leiden, The Netherlands, pp. 121-128.

Alonzo, F., Virtue, P., Nicol, S. and Nichols, P.D. (2005*a*). Lipids as trophic markers in Antarctic krill. II. Lipid composition of the body and digestive gland of *Euphausia superba* in controlled conditions. *Marine Ecology Progress Series*. **296**, 65-79.

Alonzo, F., Virtue, P., Nicol, S. and Nichols, P.D. (2005*b*). Lipids as trophic markers in Antarctic krill. III. Temporal changes in digestive gland lipid composition of *Euphausia superba* in controlled conditions. *Marine Ecology Progress Series*. **296**, 81-91.

Arnold, K.H., Shreeve, R.S., Atkinson, A. and Clarke, A. (2004). Growth rates of Antarctic krill, *Euphausia superba*: Comparison of the instantaneous growth rate method with nitrogen and phosphorous stoichiometry. *Limnology and Oceanography*. **49(6)**, 2152-2161.

Arrigo, K.R. and Thomas, D.M. (2004). Large scale importance of sea ice biology in the Southern Ocean. *Antarctic Science*. **209**, 203-217.

Atkinson, A. and Snÿder, R. (1997). Krill-copepod interactions at South Georgia Antarctica, I. Omnivory by *Euphausia superba*. *Marine Ecology and Progress Series.* **160**, 63-76.

Atkinson, A., Siegel, V., Pakhomov, E.A. and Rothery, P. (2004). Long-term decline in krill stock and increase in salps within the Southern Ocean. *Nature*. **432**, 100-103.

Atkinson, A., Siegel, V., Pakhomov, E.A., Jessopp, M.J. and Loeb, V. (2009). A re-appraisal of the total biomass and annual production of Antarctic krill. *Deep-Sea Research I*. **56**, 727-740.

Atkinson, A., Whitehouse, M.J., Priddle, J., Cripps, C., Ward, P. and Brandon, M.A. (2001). South Georgia, Antarctica: A productive, cold water, pelagic ecosystem. *Marine Ecology Progress Series*. **216**, 279-308.

Atkinson, A., Meyer, B., Stübing, D., Hagen, W., Schmidt, K. and Bathmann, U.V. (2002). Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter - II. Juveniles and adults. *Limnology and Oceanography*. **47(4)**, 953-966.

Atkinson, A., Shreeve, R.S., Hirst, A.G., Rothery, P., Tarling, G.A., Pond, D., Korb, R.E., Murphy, E.J. and Watkins, J.L. (2006). Natural growth rates in Antarctic krill (*Euphausia superba*): II. Predictive models based on food, temperature, body length, sex, and maturity stage. *Limnology and Oceanography*. **51(2)**, 973-987.

Atkinson, A., Siegel, V., Pakhomov, E.A., Rothery, P., Loeb, V., Ross, R.M., Quetin, L.B., Schmidt, K., Fretwell, P., Murphy, E.J., Tarling, G.A. and Fleming, A.H. (2008). Oceanic circumpolar habitats of Antarctic krill. *Marine Ecology and Progress Series*. **362**, 1-23.

Baker, A.d.C., Clarke, M.R. and Harris, M.J. (1973). The NIO combination net (RMT) 1+8 and further developments of Rectangular Midwater Trawls. *Journal of Marine Biological Association of the United Kingdom*. **53**, 167-184.

Baker, A.d.C., Boden, B.P. and Brinton, E. (1990). *A Practical Guide to the Euphausiids of the World*. The Natural History Museum, London.

Bakun, A. (2006). *Wasp-waist* populations and marine ecosystem dynamics: Navigating the "*predator pit*" topographies. *Progress in Oceanography*. **68**, 271-288.

Bargmann, H.E. (1937). The reproductive system of *Euphausia superba*. *Discovery Reports*. **14**, 325-350.

Bargmann, H.E. (1945). The development and life-history of adolescent and adult krill, *Euphausia superba*. *Discovery Reports*. **23**, 103-176.

Bligh, E.G. and Dyer, W.M. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemical Physiology.* **35**, 911-917.

Brichon, G., Chapelle, S. and Zwingelstein. G. (1980). Phospholipid composition and metabolism in the hemolymph of *Carcinus maenas* (Crustacea, Decapoda) – effect of temperature. *Comparative Biochemistry and Physiology, Part B*. **67**, 647-652.

Brierley, A.S., Watkins, J.L. and Murray, A.W.A. (1997). Inter-annual variability in krill abundance at South Georgia. *Marine Ecology Progress Series*. **150**, 87-98.

Brierley, A.S., Demer, D.A., Watkins, J.L. and Hewitt, R.P. (1999). Concordance of interannual fluctuations in acoustically estimated densities of Antarctic krill around South Georgia and Elephant Island: biological evidence of same-year teleconnections across the Scotia Sea. *Marine Biology*. **134**, 675-681.

Brinton, E. (1962). The distribution of Pacific euphausiids. *Bull. Scripps. Inst. Oceanogr. Univ. Calif.* **8**, 51-270.

Brinton, E. (1976). Population biology of *Euphausia pacifica* off southern California. *Fish. Bull. US.* **74**, 733-762.

Buchholz, F. (1985). Moult and growth in euphausiids. ***In*:** W.R. Siegfried., P. Condy. and R.M. Laws. (Eds). *Antarctic Nutrient Cycles and Food Webs.* Springer-Verlag, Berlin, pp 339-345.

Buchholz, F. (1991). Moult cycle and growth of Antarctic krill *Euphausia superba* in the laboratory. *Marine Ecology Progress Series*. **69**, 217-229.

Buchholz, F., Watkins, J.L., Priddle, J., Morris, D.J. and Ricketts, C. (1996). Moult in relation to some aspects of reproduction and growth in swarms of Antarctic krill, *Euphausia superba*. *Marine Biology*. **127**, 201-208.

Butler, D., Cullis, B.R., Gilmour, A.R. and Gogel, B.J. (2002). Spatial analysis mixed models: samm reference manual. Training series QE02001, Queensland Department of Primary Industries, Toowoomba, Australia.

Candy, S.G. and Kawaguchi, S. (2006). Modelling growth of Antarctic krill. II. Novel approach to describing the growth trajectory. *Marine Ecology Progress Series*. **306**, 17-30.

CCAMLR (2008). Schedule of Conservation Measures in Force 2008/09. Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR). Hobart, Australia. Retrieved from: http://www.ccamlr.org/pu/e/e_pubs/cm/08-09/toc.htm Viewed on 15/06/09.

Chess, D.W. and Stanford, J.A. (1999). Experimental effects of temperature and prey assemblage on growth and lipid accumulation by *Mysis relicta* loven. *Hydrobiologia*. **412**, 155-164.

Clarke, A. (1976). Some observations on krill (*Euphausia superba* Dana) maintained alive in the laboratory. *Br. Antarct. Surv. Bull.* **43**, 111-118.

Clarke, A. (1980). The biochemical composition of krill, *Euphausia superba* Dana from South Georgia. *Journal of Experimental Marine Biology and Ecology*. **43**, 221-236.

Clarke, A. (1984). Lipid content and composition of Antarctic krill, *Euphausia superba* Dana. *Journal of Crustacean Biology*. **4(1)**, 285-294.

Clarke, A. (1985). Energy flow in the Southern Ocean food web. ***In*:** W.R. Siegfried., P. Condy. and R.M. Laws (Eds). *Antarctic Nutrient Cycles and Food Webs.* Springer-Verlag, Berlin, pp 339-345.

Clarke, A. (1988). Seasonality in the Antarctic marine environment. *Comparative Biochemistry and Physiology, Part B*. **90**, 461-473.

Clarke, A. and Morris, D.J. (1983). Towards an energy budget for krill: The physiology and biochemistry of *Euphausia superba* Dana. *Polar Biology*. **2**, 69-86.

Clarke, A. and Tyler, P.A. (2008). Adult Antarctic krill feeding at abyssal depths. *Current Biology*. **18**, 282-285.

Clarke, A., Murphy, E.J., Meredith, M.P., King, J.C., Peck, J.M., Barnes, D.K.A. and Smith, R.C. (2007). Climate change and the marine ecosystem of the western Antarctic Peninsula. *Phil. Trans. R. Soc. B*. **362**, 149-166.

Constable, A.J. and de la Mare, W.K. (1996). A generalized model for evaluating yield and the long-term status of fish stocks under conditions of uncertainty. *CCAMLR Science*. **3**, 1564-1576.

Constable, A.J., de la Mare, W.K., Agnew, D.J., Everson, I. and Miller, D. (2000). Managing fisheries to conserve the Antarctic marine ecosystem: practical implementation of the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR). *ICES Journal of Marine Science*. **57**, 778-791.

Cripps, G.C. and Hill, H.J. (1998). Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. *Deep-Sea Research I*. **45**, 1257-1381.

Cripps, G.C. and Atkinson, A. (2000). Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. *Canadian Journal of Fisheries and Aquatic Science*. **57(Suppl. 3)**, 31-37.

Cripps, G.C., Watkins, J.L., Hill, H.J. and Atkinson, A. (1999). Fatty acid content of Antarctic krill *Euphausia superba* at South Georgia related to regional populations and variations in diet. *Marine Ecology Progress Series*. **181**, 177-188.

Cuzin-Roudy, J. (2000). Seasonal reproduction, multiple spawning, and fecundity in northern krill, *Meganyctiphanes norvegica*, and Antarctic krill, *Euphausia superba*. *Canadian Journal of Fisheries and Aquatic Science*. **57(Suppl. 3),** 6-15.

Cuzin-Roudy, J. and Amsler, M.O. (1991). Ovarian development and sexual maturity staging in Antarctic krill, *Euphausia superba* Dana (Euphausiacea). *Journal of Crustacean Biology*. **11(2),** 236-249.

Dall, W., Chandumpai, A. and Smith, D.M. (1992). Fatty acid composition organs and tissues of the tiger prawn *Paenaeus esculentus* during the moulting cycle and during starvation. *Marine Biology*. **113**, 45-55.

Daly, K.L. (1990). Overwintering development, growth and feeding of larval *Euphausia superba* in the Antarctic marginal ice edge zone. *Limnology and Oceanography*. **35(7),** 1564-1576.

Daly, K.L. and Macaulay, M.C. (1991). Influence of physical and biological mesoscale dynamics on the seasonal distribution and behaviour of *Euphausia superba* in the Antarctic marginal ice zone. *Marine Ecology Progress Series*. **79**, 37-66.

Denys, C.J. and McWhinnie, M.A. (1982). Fecundity and ovarian cycles of the Antarctic krill *Euphausia superba* (Crustacea: Euphausiacea). *Canadian Journal of Fisheries and Aquatic Science*. **60**, 2414-2423.

Diggle, P.J., Liang, K.Y. and Zeger, S.L. (1994). *Analysis of Longitudinal Data*. Clarendon Press, London.

Dutra, B.K., Santos, R.B., Bueno, A.A.P. and Oliveira, G.T. (2008). Seasonal variations in the biochemical composition and lipoperoxidation of *Hyalella curvispina* (Crustacea, Amphipoda). *Comparative Biochemistry and Physiology, Part A*. **151**, 322-328.

El-Sayed, S.Z. (1994). History, organization and accomplishments of the BIOMASS program. ***In*:** S.Z. El-Sayed (Ed). *Southern Ocean Ecology: the BIOMASS Perspective*. Cambridge University Press, Cambridge. pp 1-8.

Endo, Y. and Yamano, F. (2006). Diel vertical migration of *Euphausia pacifica* (Crustacea, Euphausiacea) in relation to molt and reproductive processes, and feeding activity. *Journal of Oceanography*. **62**, 693-703.

Ettershank, G. (1984). A new approach to the assessment of longevity in the Antarctic krill *Euphausia superba*. *Journal of Crustacean Biology*. **4**, 295-305.

Everson, I. (2000). Biological observations. ***In*****:** I. Everson (Ed). *Krill: Biology, Ecology and Fisheries*. Blackwell Science, London, pp 33-39.

Everson, I. and Goss, C. (1991). Krill fishing activity in the southwest Atlantic. *Antarctic Science*. **3(4)**, 351-358.

Everson, I. and Miller, D.G.M. (1994). Krill mesoscale distribution and abundance: results and implications of research during the BIOMASS Programme. ***In*****:** S. El-sayed. (Ed). *Southern Ocean Ecology: The BIOMASS Perspective*. Cambridge University Press, Cambridge, pp 129-144.

Everson, I. and de la Mare. (1996). Some thoughts on precautionary measures for the krill fishery. *CCAMLR Science*. **3**, 1-11.

Farkas, T. (1979). Adaptation of fatty acid compositions to temperature - A study on planktonic crustaceans. *Comparative Biochemistry and Physiology, Part B*. **64**, 71-76.

Farkas, T., Nemecz, G. and Csengeri, I. (1984). Differential response of lipid metabolism and membrane physical state by an actively and passively overwintering planktonic Crustacean. *Lipids*. **19**, 436-442.

Foxton, P. (1966). The distribution and life-history of *Salpa thompsoni* Foxton with observations on a related species, *Salpa gerlachei* Foxton. *Discovery Reports*. **34**, 1-116.

Frazer, F.C. (1936). On the development and distribution of the young stages of krill (*Euphausia superba*). *Discovery Reports*. **14**, 1-192.

Gaten, E., Tarling, G., Dowse, H., Kyriacou, C. And Rosata, E. (2008). Is vertical migration in Antarctic krill (*Euphausia superba*) influenced by an underlying circadian rhythm? *Journal of Genetics*. **87**, 473-483.

Gibbons, M.J., Vassily, A., Spiridonov, V.A. and Tarling, G.A. (1999). Euphausiacea. ***In*****:** D. Boltovskoy. (Ed)**.** *South Atlantic Zooplankton Volume 2*. Backhuys Publishers, Leiden, pp 1241-1279.

Goodall-Copestake, W.P., Espona-Perez, S., Clark, M.S., Murphy, E.J. Seear, P.J. and Tarling, G.A. (2010). Swarms of diversity at the gene *cox1* in Antarctic krill. *Heredity*. **104**, 513-518.

Gutt, J. and Siegel, V. (1994). Benthopelagic aggregations of krill (*Euphausia superba*) on the deeper shelf of the Weddell Sea (Antarctica). *Deep Sea Research Part I: Oceanographic Research Papers*. **41**, 169-178

Hagen, W. (1999). Reproductive strategies and energetic adaptations of polar zooplankton. *Invertebrate Reproduction and Development*. **36**, 25-34.

Hagen, W., Van Vleet, E.S. and Kattner, G. (1996). Seasonal lipid storage as overwintering strategy of Antarctic krill. *Marine Ecology Progress Series*. **134**, 85-89.

Hagen, W., Kattner, G., Terbruggen, A. and Van Vleet, E.S. (2001). Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. *Marine Biology*. **139**, 95-104.

Hagen, W., Yoshida, T., Virtue, P., Kawaguchi, S., Swadling, K.M., Nicol, S. and Nichols, P.D. (2007). Effect of a carnivorous diet on the lipids, fatty acids and condition of Antarctic krill, *Euphausia superba*. *Antarctic Science*. **19**, 183-188.

Hamner, W.M. and Hamner, P.P. (2000). Behaviour of Antarctic krill (*Euphausia superba*): schooling, foraging and antipredatory behaviour. *Canadian Journal of Fisheries and Aquatic Science*. **57**(**Suppl. 3),** 192-202.

Hamner, W.M., Hamner, P.P., Strand, S.W. and Gilmer, R.W. (1983). Behaviour of Antarctic krill, *Euphausia superba*: chemoreception, feeding, schooling and moulting. *Science*. **220**, 433-435.

Harrison, K.E. (1990). The role of nutrition in maturation, reproduction and embryonic development of Decapod Crustaceans: A review. *Journal of Shellfish Research*. **9**, 1-28.

Hartnoll, R.G. (1985). Growth, sexual maturity and reproductive output. ***In*:** A. Wenner (Ed). *Crustacean Issues*. **3**, 101-128.

Hartnoll, R.G. (2001). Growth in Crustacea – twenty years on. *Hydrobiologia*. **449**, 111-122.

Hazel, J.R. and Carpenter, R. (1985). Rapid changes in the phospholipid composition of gill membranes during thermal acclimation of the rainbow trout, *Salmo gairdneri*. *Journal of Comparative Physiology, Part B*. **155**, 597-602.

Hewitt, R.P. and Linen low, E.H. (2000). The fishery on Antarctic krill: defining an ecosystem approach to management. *Reviews in Fisheries Science*. **8**, 235-298.

Hill, H.J., Trathan, P.N., Croxall, J.P. and Watkins, J.L. (1996). A comparison of Antarctic krill *Euphausia superba* caught by nets and taken by Macaroni penguins *Eudyptes chrysolophus*: evidence for selection? *Marine Ecology Progress Series*. **140**, 1-11.

Hirano, Y., Matsuda, T. and Kawaguchi, S. (2003). Breeding Antarctic krill in captivity. ***In*:** D.L. Macmillan (Ed). *Marine and Freshwater Behaviour and Physiology*. **36(4),** pp 259-269.

Hirche, H.J. (1983). Excretion and respiration of the Antarctic krill *Euphausia superba*. *Polar Biology*. **1**, 205-209.

Hirche, H.J. (1996). Diapause in the marine copepod, *Calanus finmarchicus* – a review. *Ophelia*. **44**, 129-143.

Hofmann, E.E. and Lascara,C. (2000). Modelling the growth dynamics of Antarctic krill *Euphausia superba*. *Marine Ecology Progress Series*. **194**, 219-231.

Hofmann, E.E. and Murphy, E.J. (2004). Advection, krill and Antarctic marine ecosystems. *Antarctic Science*. **16**, 487-499.

Hofmann, E.E., Capella, J.E., Ross, R.M. and Quetin, L.B. (1992). Models of the early life history of *Euphausia superba*. Time and temperature dependence during the decent-ascent. *Deep-Sea Research I*. **39**, 1177-1200.

Hollander, E. (2001). 'Investigation of Growth Measurements for Antarctic Krill, *Euphausia superba*.' Honours Thesis, University of Tasmania.

Hopkins, T.L., Ainley, D.G., Torres, JJ. and Lancraft, T.M. (1993). Trophic structure in open waters of the marginal ice-zone in the Scotia-Weddell confluence region during spring 1983. *Polar Biology*. **13**, 389-297.

Horst, M. and Freeman, J.A. (1993). (Eds). *The Crustacean Integument – Morphology and Biochemistry*. CRC Press Inc. Boca Raton, Florida.

Huntley, M.E., Nordhausen, W. and Lopez, M.D.G. (1994). Elemental composition, metabolic activity and growth of Antarctic krill *Euphausia superba* during winter. *Marine Ecology Progress Series*. **107**, 23-40.

Iguchi, N. and Ikeda, T. (1995). Growth, metabolism and growth efficiency of a euphausiid crustacean *Euphausia pacifica* in the southern Japan Sea, as influenced by temperature. *Journal of Plankton Research*. **17**, 1757-1769.

Iguchi, N. and Ikeda, T. (2005). Effects of temperature on metabolism, growth and growth efficiency of *Thysanoessa longipes* (Crustacea: Euphausiacea) in the Japan Sea. *Journal of Plankton Research*. **27(1)**, 1-10.

Ikeda, T. (1984*a*). Development of the larvae of the Antarctic krill (*Euphausia superba* Dana) observed in the laboratory. *Journal of Experimental Marine Biology and Ecology*. **75**, 107-117.

Ikeda, T. (1984*b*). Sequence in metabolic rates and elemental composition (C, N, P) during the developmental of *Euphausia superba* Dana and estimated food requirements during its life span. *Journal of Crustacean Biology*. **4(1)**, 273-284.

Ikeda, T. (1985). Life history of Antarctic krill *Euphausia superba*: A new look from an experimental approach. *Bulletin of Marine Science*. **37(2)**, 599-608.

Ikeda, T. (1987). Mature Antarctic krill (*Euphausia superba* Dana) grown from eggs in the laboratory. *Journal of Plankton Research*. **9**, 565-569.

Ikeda, T. and Dixon, P. (1982). Body shrinkage as a possible overwintering mechanism of the Antarctic krill (*Euphausia superba* Dana). *Journal of Experimental Marine Biology and Ecology*. **62**, 143-151.

Ikeda, T. and Mitchell, A.W. (1982). Oxygen uptake, ammonia excretion and phosphate excretion by krill and other Antarctic zooplankton in relation to their body size and chemical composition. *Marine Biology*. **71**, 283-298.

Ikeda, T. and Dixon, P. (1984). The influence of feeding on the metabolic activity of Antarctic krill (*Euphausia superba* Dana). *Polar Biology*. **3**, 1-9.

Ikeda, T. and Thomas, P.G. (1987). Moulting interval and growth of juvenile Antarctic krill (*Euphausia superba*) fed different concentrations of the diatom *Phaeodactylum tricornutum* in the laboratory. *Polar Biology*. **7**, 339-343.

Ikeda, T., Dixon, P. and Kirkwood, J. (1985). Laboratory observations of moulting, growth and maturation in Antarctic krill (*Euphausia superba* Dana). *Polar Biology*. **4**, 1-8.

Insightful (2002). S-plus 6 Supplement for Windows. Insightful Corporation Seattle, Washington, pp. 1-126.

Ivanov, B.G. (1970). On the biology of Antarctic krill *Euphausia superba*. *Marine Biology*. **7**, 340-3531.

Ju, S. and Harvey, R. (2004). Lipids as markers of nutritional condition and diet in the Antarctic krill *Euphausia superba* and *Euphausia crystallorophias* during austral winter. *Deep-Sea Research II*. **51**, 2199-2214.

Kanda, K., Takagi, K. and Seki, Y. (1982). Movement of the lager swarms of Antarctic krill *Euphausia superba* off Enderby Land during 1976-77 season. *Transactions of the Tokyo University of Fisheries*. **68**, 24-42.

Kawaguchi, S. and Nicol, S. (2007). Learning about Antarctic krill from the fishery. *Antarctic Science*. **19(2)**, 219-230.

Kawaguchi, K., Ishikawa, S. and Matsuda, T. (1986). The overwintering strategy of Antarctic krill (*Euphausia superba* Dana) under the coastal fast ice off the Ongul Islands in Lützol-Holm Bay, Antarctica. *Mem. Natl. Inst. Polar. Res. Spec. Iss.* **44**, 67-85.

Kawaguchi, S. Ichii, T. and Naganobu, M. (1999). Green krill, the indicator of micro- and nano-size phytoplankton availability to krill. *Polar Biology*. **22**, 133-136.

Kawaguchi, S., Candy, S.G., King, R., Naganobu, M. and Nicol, S. (2006). Modelling growth of Antarctic krill. I. Growth trends with sex, length, season, and region. *Marine Ecology Progress Series*. **306**, 1-15.

Kawaguchi, S., Yoshida, T., Finley, L., Cramp, P. and Nicol, S. (2007*a*). The krill maturity cycle: a conceptual model of the seasonal cycle in Antarctic krill. *Polar Biology*. **30**, 689-698.

Kawaguchi, S., Finley, L., Jarman, S.N., Candy, S.G., Ross, R.M., Quetin, L.B., Siegel, V., Trivelpiece, S., Naganobu, M. and Nicol, S. (2007*b*). Male krill grow fast and die young. *Marine Ecology and Progress Series*. **345**, 199-210.

Kawaguchi, S., King, R.A., Meijers, R., Osborn, J.E., Swadling, K.M., Ritz, D.A. and Nicol, S. (2010). An experimental aquarium for observing the schooling behaviour of Antarctic krill. *Deep-Sea Research II*. **57**, 683-292.

King, R., Nicol, S., Cramp, P. and Swadling, K.M. (2003). Krill maintenance and experimentation at the Australian Antarctic Division. ***In*:** D.L. Macmillan (Ed). *Marine and Freshwater Behaviour and Physiology*. **36**, 271-283.

Knox, G.A. (1994). *Biology of the Southern Ocean*. Cambridge University Press, Cambridge.

Kock, K.H., Reid, K., Croxall, J.P. and Nicol, S. (2007). Fisheries in the Southern Ocean: an ecosystem approach. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **362**, 2333-2349.

Kolakowska, A. (1991). The lipid composition of some Antarctic Crustacea. *Polish Polar Research*. **12**, 79-89.

Korb, R.E., Whitehouse, M.J. and Ward, P. (2004). SeaWiFS in the Southern Ocean: spatial and temporal variability in phytoplankton biomass around South Georgia. *Deep-Sea Research II*. **51**, 99-116.

Korb, R.E., Whitehouse, M.J., Thorpe, S.E. and Gordon, M. (2005). Primary production across the Scotia Sea in relation to the physico-chemical environment. *Journal of Marine Systems*. **57**, 231-249.

Lawes Agricultural Trust (2007). GenStat Release 10.2 (PC/Windows) (Rothamsted, UK), VSN International Ltd., Hemel Hempstead, UK.

Laws, R.M. (1985). The ecology of the Southern Ocean. *American Scientist*. **73**, 26-40.

Lee, R.F., Hagen, W. and Kattner, G. (2006). Lipid storage in marine zooplankton. *Marine Ecology Progress Series*. **307**, 273-306.

Lindley, J.A. (1982). Population dynamics and production of euphausiids. III. *Meganyctiphanes norvegica* and *Nyctiphanes couchi* in the North Atlantic Ocean and the North Sea. *Marine Biology*. **66**, 37-46.

Loeb, V., Siegel, V., Holm-Hansen, O., Hewitt, R., Fraser, W., Trivelpiece, W. and Trivelpiece, S. (1997). Effects of sea ice extent and krill or salp dominance on the Antarctic food web. *Nature*. **387**, 897-900.

Mackintosh, N.A. (1972). Life cycle of the Antarctic krill in relation to ice and water conditions. *Discovery Reports.* **36**, 1-94

Mackintosh, N.A. (1973). Distribution of post-larval krill in the Antarctic. *Discovery Reports*. **36**, 95-156.

Makarov, R.R. and Denys, C.J. (1980). Stages of sexual maturity of *Euphausia superba* Dana. *Biomass Handbook*. **11**, 1-13.

Mangel, M. and Nicol, S. (2000). Krill and the unity of biology. *Canadian Journal of Fisheries and Aquatic Science*. **57(Suppl. 3)**, 1-5.

Marr, J.W.S. (1962). The natural history and geography of the Antarctic krill (*Euphausia superba* Dana). *Discovery Reports*. **32**, 33-464.

Marschall, H.P. (1988). The overwintering strategy of Antarctic krill under the pack ice of the Weddell Sea. *Polar Biology*. **9**, 129-135.

Mauchline, J. (1980). The biology of mysids and euphausiids. *Advanced Marine Biology*. **18**, 373-595.

Mauchline, J. and Fisher, L.R. (1969). The biology of euphausiids. *Advanced Marine Biology*. **7**, 1-454.

Mayzaud, P. (1997). Spatial and life-cycle changes in lipid and fatty acid structure of the Antarctic euphausiid *Euphausia superba*. ***In*:** B. Battaglia, J. Valencia and D.W.H. Walton (Eds), *Antarctic Communities: Species, structure and survival*. Cambridge University Press, Cambridge, pp 284-294.

Mayzaud, P., Albessard, E. and Cuzin-Roudy, J. (1998). Changes in lipid composition of the Antarctic krill *Euphausia superba* in the Indian Ocean sector of the Antarctic Ocean: influence of geographical location, sexual maturity stage and distribution among organs. *Marine Ecology Progress Series*. **173**, 149-162.

Mayzaud, P., Albessard, E., Virtue, P. and Boutoute, M. (2000). Environmental constraints on the lipid composition and metabolism of euphausiids: the case of *Euphausia superba* and *Meganyctiphanes norvegica*. *Canadian Journal of Fisheries and Aquatic Science*. **57(Suppl. 3)**, 91-103.

McElhaney, R.N. (1984). The relationship between membrane lipid fluidity and phase state and the ability of bacteria and mycopasmas to grow and survive at different temperatures. ***In*:** M. Kates, L.A. Manson. (Eds) *Biomembranes, Membrane Fluidity*. Plenum Press, New York, pp 249-278.

McWhinnie, M.A. and Marciniak, P. (1964). Temperature responses and tissue respiration in Antarctic Crustacea with particular reference to the krill *Euphausia superba*. *Antarctic Research Series*. **1**, 63-72.

Meredith, M.P. and King, J.C. (2005). Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th Century. *Geophysical Research Letters*. **32**.

Meyer, B., Atkinson, A., Stübing, D., Oettl, B., Hagen, W. and Bathmann, U.V. (2002). Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter-I. Furcilia III larvae. *Limnology and Oceanography*. **47(4)**, 943-952.

Miller, D.G.M. and Hampton, I. (1989). Biology and ecology of the Antarctic krill (*Euphausia superba* Dana): a review. *Biomass Handbook*. **9**, 1-166.

Moline, M.A., Claustre, H., Frazer, T.K., Schofield, O. and Vernet, M. (2004). Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. *Global Climate Biology*. **10**, 1973-1980.

Morris, D.J. (1984). Filtration rates of *Euphausia superba* Dana: Under- or overestimates? *Journal of Crustacean Biology*. **4**, 185-197.

Morris, D.J. and Keck, A. (1984). The time course of the moult cycle and growth of *Euphausia superba* in the laboratory. A preliminary study. *Meeresforsch*. **30**, 94-100.

Murano, M., Segawa, S. and Kato, M. (1979). Moult and growth of the Antarctic krill in laboratory. *Transactions of the Tokyo University of Fisheries*. **3**, 99-106.

Murphy, E.J., Thorpe, S.E., Watkins, J.L. and Hewitt, R. (2004). Modelling the krill transport pathways in the Scotia Sea: spatial and environmental connections generating the seasonal distribution of krill. *Deep-Sea Research II*. **51**, 1435-1456.

Murphy, E.J., Watkins, J.L., Reid, K., Trathan, P.N., Everson, I., Croxall, J.P., Priddle, J., Brandon, M.A., Brierley, A,S. and Hofmann, E. (1998). Inter-annual variability of the South Georgia marine ecosystem: biological and physical sources of variation in the abundance of krill. *Fisheries Oceanography*. **7(3/4),** 381-390.

Neidleman, S.L. (1987). Effects of temperature on lipid unsaturation. *Biotechnology and Genetic Engineering Reviews*. **5**, 245-268.

Nelson, K. (1986). Photoperiod and reproduction in lobsters (*Homarus*). *American Zoologist*. **26**, 447-457.

Nelson, K. (1991). Scheduling of reproduction in relation to moulting and growth in malacostracan crustaceans. ***In*:** A. Wenner and A. Kuris (Eds). *Crustacean Issues*. **7**, 77-117.

Nicol, S. (1989). Apparent independence of the spawning and moulting cycles in female Antarctic krill (*Euphausia superba* Dana). *Polar Biology*. **9**, 371-375.

Nicol, S. (2000). Understanding krill growth and ageing: the contribution of experimental studies. *Canadian Journal of Fisheries and Aquatic Science*. **57(Suppl. 3)**, 168-177.

Nicol, S. (2003). Living krill, zooplankton and experimental investigations: A discourse on the role of krill and their experimental study in marine ecology. ***In*:** D.L. Macmillan (Ed). *Marine and Freshwater Behaviour and Physiology*. **36**, 191-205.

Nicol, S. (2006). Krill, currents, and sea ice: *Euphausia superba* and its changing environment. *Bioscience*. **56(2)**, 111-120.

Nicol, S. and Allison, I. (1997). The frozen skin of the Southern Ocean. *American Scientist*. **85**, 426-440.

Nicol, S. and Endo, Y. (1999). Krill fisheries: development, management and ecosystem implications. *Aquatic Living Resources*. **12**, 105-120.

Nicol, S. and Foster, J. (2003). Recent trends in the fishery for Antarctic krill. *Aquatic Living Resources*. **16**, 42-45.

Nicol, S., de la Mare, W.K. and Stolp, M. (1995). The energetic cost of egg production in Antarctic krill (*Euphausia superba* Dana). *Antarctic Science*. **7(1)**, 25-30.

Nicol, S., Forster, I. and Spence, J. (2000*a*). Products derived from krill. ***In*:** Everson, I. (editor). *Krill: Biology, Ecology and Fisheries.* Blackwell Science, London, pp 262-283.

Nicol, S., Pauly, T., Bindoff, N.L. and Strutton, P.G. (2000*b*). "BROKE" a biological/oceanographic survey off the coast of East Antarctic (80-150°E) carried out in January-March 1996. *Deep-Sea Research II*. **47**, 2281-2298.

Nicol, S., Stolp, M., Cochran, T.G., Geijsel, P. and Marshall, J. (1992). Growth and shrinkage of Antarctic krill *Euphausia superba* from the Indian Ocean Sector of the Southern Ocean during summer. *Marine Ecology Progress Series*. **89**, 175-181.

Nicol, S., Virtue, P., King, R., Davenport, S.R., McGaffin, A.F. and Nichols, P.D. (2004). Condition of *Euphausia crystallorophias* off East Antarctica in winter in comparison to other seasons. *Deep-Sea Research II*. **51**, 2215-2224.

O'Brien, C., Virtue, P., Kawaguchi, S. and Nichols P.D. (in press). Aspects of krill growth and condition during the late winter-early spring off East Antarctica between 110°E and 130°E during the SIPEX expedition (Sea Ice Physics & Ecosystem eXperiment, September- October 2007). *Deep-Sea Research II*.

Omori, M. and Ikeda, T. (1984). *Methods in Marine Zooplankton Ecology*. John Wiley and Sons, New York.

Opalinski, K.W. (1991). Respiratory metabolism and metabolic adaptations of Antarctic krill *Euphausia superba*. *Polskie Archiwum Hydrobiologii*. **38(2)**, 183-263.

Ouellet, P. and Allard, J.P. (2002). Seasonal and interannual variability in larval lobster *Homarus americanus* size, growth and condition in the Magdalen Islands, southern Gulf of St. Lawrence. *Marine Ecology Progress Series*. **230**, 241-251.

Peck, L.S. Webb, K.E. and Baily, D.M. (2004). Extreme sensitivity of biological function to temperature in Antarctic marine species. *Functional Ecology*. **18**, 625-630.

Phleger, C.F., Nichols, P.D. and Virtue, P. (1998). Lipids and trophodynamics of Antarctic zooplankton. *Comparative Biochemistry and Physiology, Part B*. **120**, 311-323.

Poleck, T.P. and Denys, C.J. (1982). Effect of temperature on the moulting, growth and maturation of the Antarctic krill *Euphausia superba* (Crustacea: Euphausiacea) under laboratory conditions. *Marine Biology*. **70**, 255-265.

Pond, D., Watkins, J.L., Priddle, J. and Sargent, J. (1995). Variation in the lipid content and composition of Antarctic krill *Euphausia superba* at South Georgia. *Marine Ecology Progress Series*. **117**, 49-57.

Price, H.J., Boyd, K.R., and Boyd, C.M. (1988). Omnivorous feeding behaviour of the Antarctic krill *Euphausia superba*. *Marine Biology*. **97**, 67-77.

Quetin, L.B. and Ross, R.M. (1984). Depth and distribution of developing *Euphausia superba* embryos, predicted from sinking rates. *Marine Biology*. **79**, 47-53.

Quetin, L.B. and Ross, R.M. (1989). Effect of oxygen, temperature and age on the metabolic rate of the embryos and early larval stages of the Antarctic krill *Euphausia superba* Dana. *Journal of Experimental Marine Biology and Ecology*. **125**, 43-62.

Quetin, L.B. and Ross, R.M. (1991). Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. *American Scientist*. **31**, 49-63.

Quetin, L.B. and Ross, R.M. (2001). Environmental variability and its impact on the reproductive cycle of Antarctic krill. *American Scientist*. **41**, 74-89.

Quetin, L.B., Ross, R.M. and Clarke, A. (1994). Krill energetics: seasonal and environmental aspects of the physiology of *Euphausia superba*. ***In*:** S.Z. El-Sayed (Ed), *Southern Ocean Ecology. The BIOMASS Perspective*. Cambridge University Press, Cambridge, pp 165-184.

Quetin, L.B., Ross, R.M., Frazer, T.K. and Haberman, K.L. (1996). Factors affecting distribution and abundance of zooplankton, with an emphasis on Antarctic krill, *Euphausia superba*. ***In:*** R.M. Ross, E.E. Hofmann and L.B. Quetin (Eds). *Foundations for Ecological Research West of the Antarctic Peninsula*, American Geophysical Union, Washington, DC, pp. 357-371.

Quetin, L.B., Ross, R.M., Fritsen, C.H. and Vernet, M. (2007). Ecological responses of Antarctic krill to environmental variability: can we predict the future? *Antarctic Science*. **19**, 253-266.

Quetin, L.B., Ross, R.M., Frazer, T.K., Amsler, M.O., Wyatt-Evens, C. and Oakes, S.A. (2003). Growth of larval krill, *Euphausia superba*, in fall and winter west of the Antarctic Peninsula. *Marine Biology.* **143**, 833-843.

Rakusa-Suszczewski, S. and Opalinski, K.W. (1978). Oxygen consumption in *Euphausia superba*. *Polskie Archiwum Hydrobiologii*. **25(3)**, 633-641.

Reid, K. (2001). Growth of Antarctic krill *Euphausia superba* at South Georgia. *Marine Biology*. **138**, 57-62.

Reid, K., Murphy, E.J., Loeb, V. Hewitt, R.P. (2002). Krill population dynamics in the Scotia Sea: variability in growth and mortality within a single population. *Journal of Marine Systems*. **36**, 1-10.

Rosenberg, A., Beddington, J.R. and Bassom, M. (1986). Growth and longevity of krill during its first decade of pelagic whaling. *Nature*. **334**, 152-154.

Ross, R. and Quetin, L. (2000). Reproduction in Euphausiacea. ***In*:** Everson, I. (editor). *Krill: Biology, Ecology and Fisheries*. Blackwell Science, London, pp 150-181.

Ross, R., Quetin, L. and Kirsch, E. (1988). Effect of temperature on developmental times and survival of early larval stages of *Euphausia superba* Dana. *Journal of Experimental Marine Biology and Ecology*. **121**, 55-71.

Ross, R.M., Quetin, L.B., Baker, K.S., Vernet, M. and Smith, R.C. (2000). Growth limitation in young *Euphausia superba* under field conditions. *Limnology and Oceanography*. **45(1)**, 31-43.

Ruud, J.T. (1932). On the biology of the southern Euphausiidae. *Hvalradets Skrifter*. **2**, 5-105.

Saborowski, R., Bröhl, S., Tarling, G.A. and Buchholz, F. (2002). Metabolic properties of Northern krill, *Meganyctiphanes norvegica*, from different climatic zones. I. Respiration and excretion. *Marine Biology*. **140**, 547-556.

Saether, O., Ellingsen, T.E. and Mohr, V. (1985). The distribution of lipid in the tissues of Antarctic krill *Euphausia superba*. *Comparative Biochemistry and Physiology, Part B*. **81(3)**, 609-614.

Schmidt, K., Kähler, P. and von Bodungen, B. (1998). Copepod egg production rates in the Pomeranian Bay (southern Baltic Sea) as a function of phytoplankton abundance and taxonomic composition. *Marine Ecology Progress Series*. **174**, 183-195.

Schnack, S.B. (1985). Feeding by *Euphausia superba* and copepod species in response to varying concentrations of phytoplankton. ***In:*** W.R. Siegfried., P.R. Condy. and R.M. Laws. (Eds). *Antarctic Nutrient Cycles and Food Webs*. Springer-Verlag, Heidelburg, pp 311-323.

Seear, P., Tarling, G., Teschke, M., Meyer, B., Thorne, M. Clark, M. Gaten, E. and Rosata, E. (2009). Effects of simulated light regimes on gene expression in Antarctic krill (*Euphausia superba* Dana). *Journal of Experimental Marine Biology and Ecology*. **381**, 57-64.

Segawa, S., Kato, M. and Murano, M. (1979). Oxygen consumption of the Antarctic krill. *Transactions of the Tokyo University of Fisheries*. **3**, 113-119.

Segawa, S., Kato, M. and Murano, M. (1982). Respiration and ammonia excretion rates of the Antarctic krill, *Euphausia superba* Dana. *Transactions of the Tokyo University of Fisheries*. **5**, 177-187.

Sellner, P.A. and Hazel, J.R. (1982). Time course of changes in fatty acid composition of gills of rainbow trout (*Salmo gairdneri*) during thermal acclimation. *Journal of Experimental Zoology*. **221**, 159-168.

Shin, H. (2000). 'Condition Indicators for Antarctic Krill, *Euphausia superba*.' PhD Thesis. University of Tasmania, Australia.

Shin, H., Nicol, S. King, R. (2003). Nucleic acid content as a potential growth rate estimator of Antarctic krill; results from field-caught krill from the Indian Sector of the Southern Ocean. ***In*:** D.L. Macmillan (Ed). *Marine and Freshwater Behaviour and Physiology*. **36**, 295-305.

Siegel, V. (2000*a*). Krill (Euphausiacea) life history and aspects of population dynamics. *Canadian Journal of Fisheries and Aquatic Science*. **57(Suppl. 3)**, 130-150.

Siegel, V. (2000*b*). Krill (Euphausiacea) demography and variability in abundance and distribution. *Canadian Journal of Fisheries and Aquatic Sciences*. **57(Suppl. 3),** 151-167.

Siegel, V. (2005). Distribution and population dynamics of *Euphausia superba*: summary of recent findings. *Polar Biology*. **29**, 1-22.

Siegel, V. and Loeb, V. (1995). Recruitment of Antarctic krill *Euphausia superba* and possible causes for its variability. *Marine Ecology Progress Series*. **123**, 45-56.

Siegel, V. and Nicol, S. (2000). Population parameters. ***In*:** I. Everson. (Ed). *Krill: Biology, Ecology and Fisheries*. Blackwell Science, London, pp 102-149.

Siegel, V., de la Mare, W.K. and Loeb, V. (1997). Long-term monitoring of krill recruitment and abundance indices in the Elephant Island area (Antarctic Peninsula). *CCAMLR Science* **4**, 19-35.

Siegel, V., Bergstrom, B., Stromberg, J.O. and Schalk, P.H. (1990). Distribution, size frequencies and maturity stages of krill, *Euphausia superba*, in relation to sea ice in the northern Weddell Sea. *Polar Biology*. **10**, 549-557.

Siegel, V., Loeb, V., Bergstrom, B., Scholing, S., Haraldsson, M., Kitchener, J. and Vortkamp, M. (2004). Demography of Antarctic krill in the Lazarez Sea (Subarea 48.6) and the Elephant Island area (Subarea 48.1) in 2004. SC-CAMLR WG-EMM-04/23.

Smetacek, V. and Nicol, S. (2005). Polar ocean ecosystems in a changing world. *Nature*. **437**, 362-368.

Stretch, JJ., Hamner, PP., Hamner, W.M., Michel, W.C., Cook, J. and Sullivan, C.W. (1988). Foraging behaviour of Antarctic krill *Euphausia superba* on sea ice microalgae. *Marine Ecology Progress Series*. **44**, 131-139.

Swadling, K.M., Ritz, D.A., Nicol, S., Osborn, J.E. and Gurney, L.J. (2005). Respiration rate and cost of swimming for Antarctic krill, *Euphausia superba*, in large groups in the laboratory. *Marine Biology*. **146**, 1169-1175.

Tarling, G.A., Cuzin-Roudy, J and Buchholz, F. (1999). Vertical migration behaviour in the northern krill *Meganyctiphanes norvegica* is influenced by moult and reproductive processes. *Marine Ecology and Progress Series*. **190**, 253-262.

Tarling, T.A., Matthews, J.B.L., Saborowski, R. and Buchholz, F. (1998). Vertical migratory behavior of the euphausiid, *Meganyctiphanes norvegica*, and its dispersion in the Kattegat Channel. *Hydrobiologia*. **375/376**, 331-341.

Tarling, G.A., Cuzin-Roudy, J., Thorpe, S.E., Shreeve, R.S., Ward, P. and Murphy, E.J. (2007). Recruitment of Antarctic krill *Euphausia superba* in the South Georgia region: adult fecundity and the fate of larvae. *Marine Ecology Progress Series*. **331**, 161-179.

Tarling, G.A., Shreeve, R.S., Hirst, A.G., Atkinson, A., Pond, D., Murphy, E.J. and Watkins, J.L. (2006). Natural growth rates in Antarctic krill (*Euphausia superba*): I. Improving methodology and predicting intermoult period. *Limnology and Oceanography*. **51(2)**, 959-972.

Teschke, M., Kawaguchi, S. and Meyer, B. (2007). Simulated light regimes affect feeding and metabolism of Antarctic krill, *Euphausia superba*. *Limnology and Oceanography*. **52(3)**, 1046-1054.

Teschke, M., Kawaguchi, S. and Meyer, B. (2008). Effects of simulated light regimes on maturity and body composition of Antarctic krill, *Euphausia superba*. *Marine Biology*. **154(2)**, 315-324.

Thomas, P.G. and Ikeda, T. (1987). Sexual regression, shrinkage, re-maturation and growth of spent female *Euphausia superba* in the laboratory. *Marine Biology*. **95**, 357-363.

Thorpe, S.E., Murphy, E.J. and Watkins, J.L. (2007). Circumpolar connections between Antarctic krill (*Euphausia superba* Dana) populations: Investigating the roles of ocean and sea ice transport. *Deep-Sea Research I*. **54**, 792-810.

Torres, J.J., Donnelly, J., Hopkins, T.L., Lancraft, T.M., Aarset, A.V. and Ainley, D.G. (1994). Proximate composition and overwintering strategies of Antarctic micronektonic Crustacea. *Marine Ecology Progress Series.* **113**, 221-232.

Vaughan, D.G., Marshall, G.J., Connolley, W.M., Parkinson, C., Mulvaney, R., Hodgson, D.A., King, J.C., Pudsey, C.J. and Turner, J. (2003). Recent rapid regional climate warming on the Antarctic Peninsula. *Climate Change*. **60**, 243-274.

Virtue, P., Nicol, S. and Nichols, P.D. (1993*a*). Changes in the digestive gland of *Euphausia superba* during short-term starvation: lipid class, fatty acid and sterol content and composition. *Marine Biology*. **117**, 441-448.

Virtue, P., Nichols, P.D., Nicol, S., McMinn, A. and Sikes, E.L. (1993*b*). The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. *Antarctic Science*. **5(2)**, 169-177.

Virtue, P., Nichols, P.D. and Nicol, S. (1997). Dietary-related mechanisms of survival in *Euphausia superba*: biochemical changes during long-term starvation and bacteria as a possible source of nutrition. ***In*:** B. Battaglia., J. Valencia and D.W.H. Walton (Eds). *Antarctic Communities: Species, Structure and Survival*. Cambridge University Press, Cambridge, pp 193-201.

Virtue, P., Nichols, P.D., Nicol, S. and Hosie, G.W. (1996). Reproductive trade-off in male Antarctic krill, *Euphausia superba*. *Marine Biology*. **126**, 521-527.

Volkman, J.K. and Nichols, P. (1991). Applications of thin layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine and environmental samples. *Journal of Planar Chromatography*. **4**, 19-26.

Ward, P., Atkinson, A., Peck, J.M. and Wood, A.G. (1990). Euphausiid life cycles and distribution around South Georgia. *Antarctic Science*. **2**, 43-52.

Watkins, J.L., Macaulay, M. Everson, I. (2000). *Sampling krill*. ***In:*** I. Everson. (Ed). Krill: Biology, Ecology and Fisheries. Blackwell Science, London, pp 8-39.

Watkins, J.L., Buchholz, F., Priddle, J., Morris, D.J. and Ricketts, C. (1992). Variation in reproductive status of Antarctic krill swarms; evidence for a size-related sorting mechanism. *Marine Ecology Progress Series*. **82**, 163-174.

Weber, L.H. and El-Sayed, S.Z. (1987). Contributions of net, nano- and picoplankton to the phytoplankton standing crop and primary productivity in the Southern Ocean. *Journal of Plankton Research*. **9**(5), 973-994.

Welham, S.J. and Thompson, R. (1997). Likelihood Ratio Tests for Fixed Model Terms Using Residual Maximum Likelihood. *Journal of the Royal Statistical Society. Series B (Methodological).* **59**: 701-714.

Whitehouse, M.J., Meredith, M.P., Rothery, P., Atkinson, A., Ward, P. and Korb, R.E. (2008). Rapid warming of the ocean around South Georgia, Southern Ocean, during the 20th Century: Forcings, characteristics and implications for lower trophic levels. *Deep-Sea Research II*. **55**, 1218-1228.

Wiedenmann, J., Cresswell, K. and Mangel, M. (2008). Temperature-dependent growth of Antarctic krill: predictions for a changing climate from a cohort model. *Marine Ecology and Progress Series*. **358**, 191-202.

Yoon, W.D., Cho, S.H., Lim, D., Choi, Y.K. and Lee, Y. (2000). Spatial distribution of *Euphausia pacifica* (Euphausiacea: Crustacea) in the Yellow Sea. *Journal of Plankton Research*. **22**, 939-949.

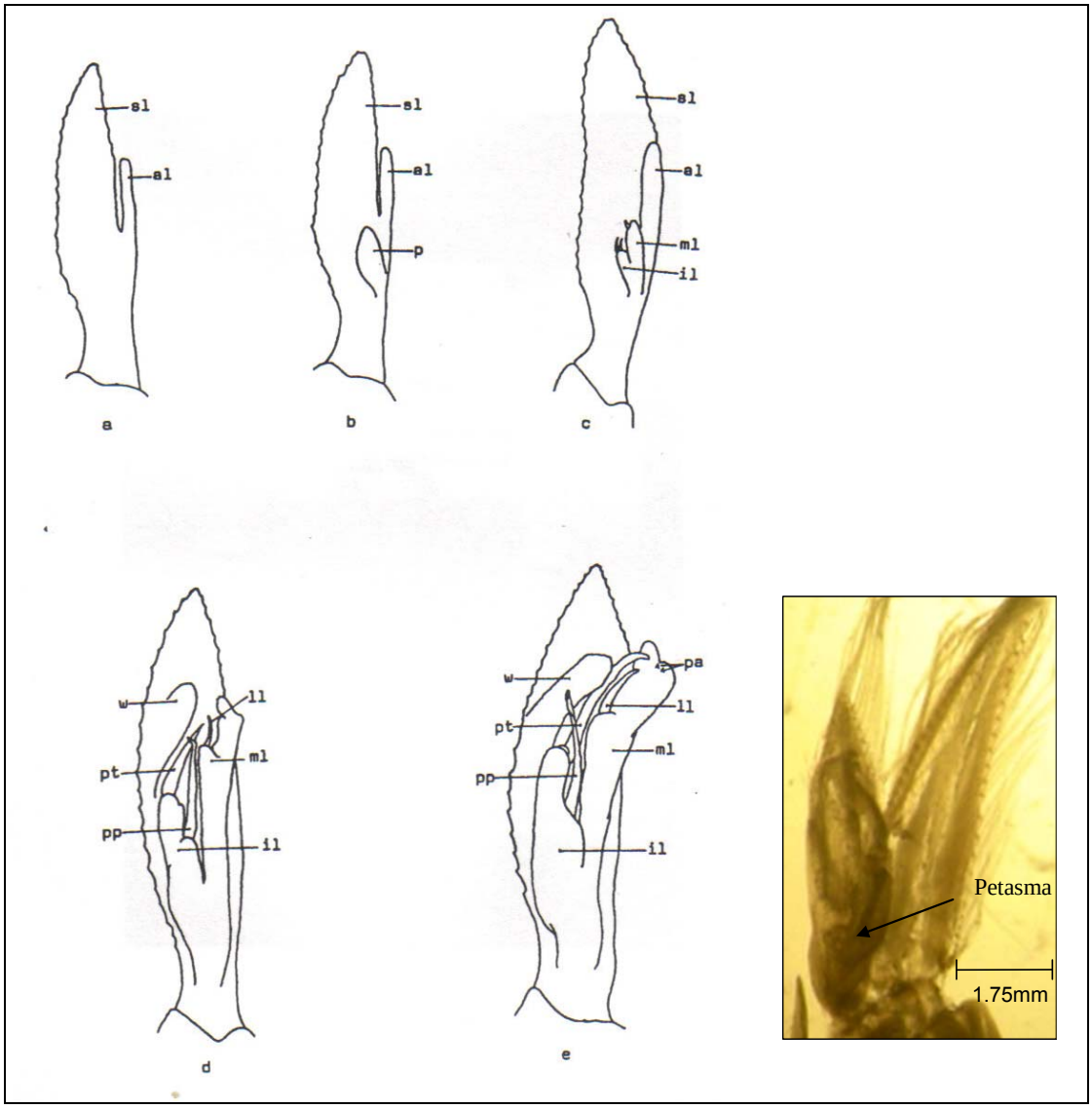
Yoshida, T. (2009). 'Environmental Influences on Reproduction in Antarctic Krill, *Euphausia superba.*' PhD Thesis, University of Tasmania, Australia.

Yoshida, T., Virtue, P., Kawaguchi, S. and Nichols, P.D. (in press). Factors determining the hatching success of Antarctic krill *Euphausia superba* embryo: lipid and fatty acid composition. *Journal of Experimental Marine Biology and Ecology*.

Yoshida, T., Kawaguchi, S., Meyer, B., Virtue, P., Penschow, J. Nash, G. (2009). Structural changes in the digestive glands of larval Antarctic krill (*Euphausia superba*) during starvation. *Polar Biology*. **32**, 503-507.

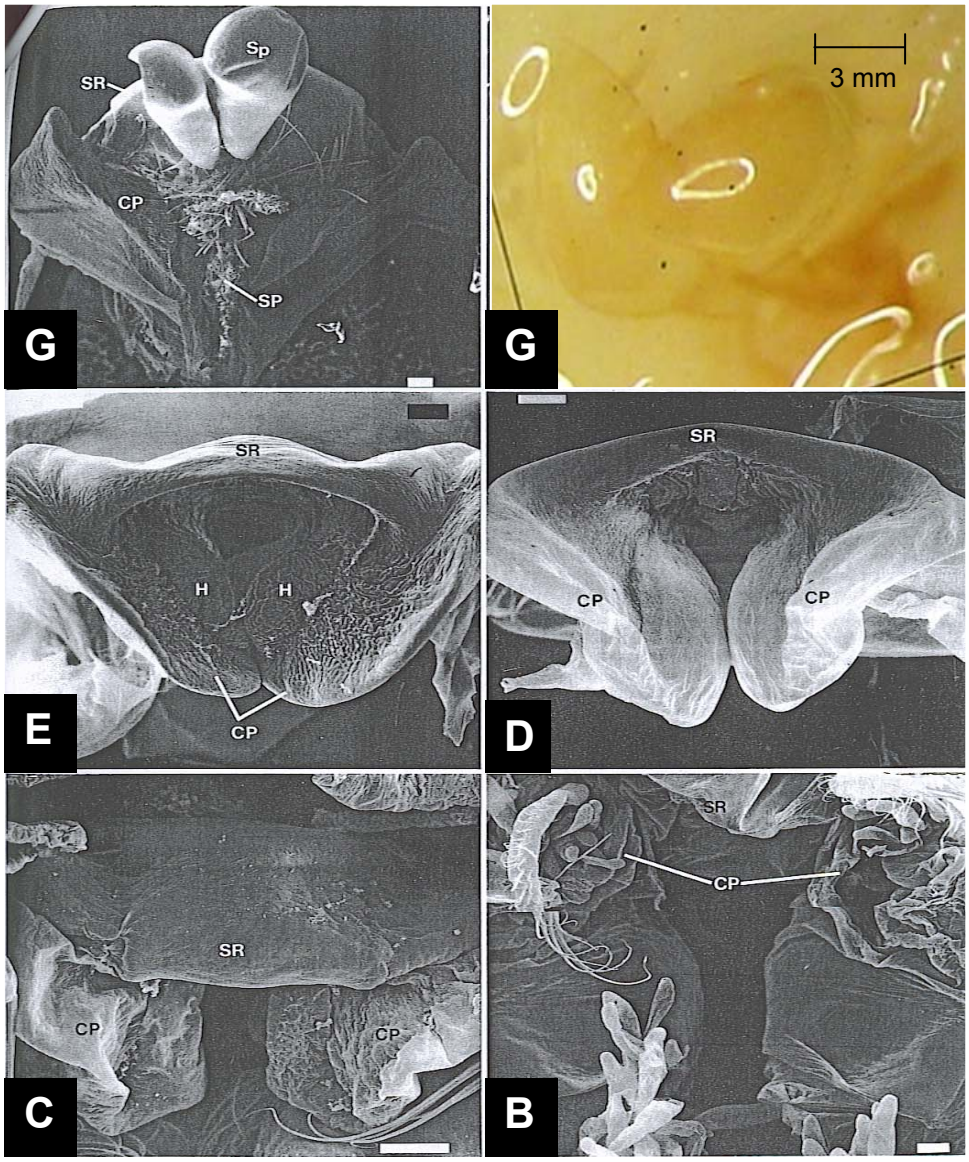
-- Appendix A --

The development of the petasma, which is the male's reproductive organ: **a)** The undifferentiated endopod of a juvenile, **b)** petasma of a 2MA male, **c)** petasma of a 2MB male, **d)** petasma of a 2MC male, **e)** fully developed petasma of a 3M adult male. There is also a photograph of a fully developed petasma of a 3M adult male. Auxiliary lobe (al); inner lobe (il); lateral lobe (ll); middle lobe (ml); petasma (p); additional process (pa); proximal process (pp); terminal process (pt); setigerous lobe (si); wing (w). Source of the petasma development diagram: Bargmann (1937).



-- Appendix A (*Continued*) --

Scanning electron micrographs of each of the female thelycum stages (B-G), as well as one photograph of a fully developed thelycum with empty spermatophores attached (stage G). Spermatophores (Sp), sperm plug (SP), sternal plate ridge (SR), coxal plates (CP), holdfasts (H). Scale bars = 100 µm, except where indicated otherwise. Source of the scanning electron micrographs: Thomas and Ikeda (1987).



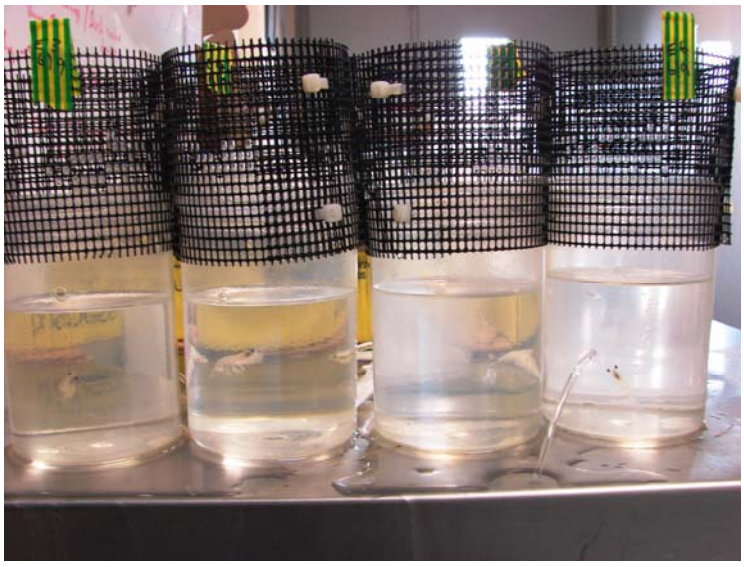
-- Appendix A (*Continued*) --

Definitions of male and female krill maturity stages and their scores used throughout this thesis. After Bargmann (1945), modified by Makarov and Denys (1980) and Thomas and Ikeda (1987).

Maturity Stage	Description	Maturity Score
Juvenile		
1	Secondary sexual characteristics, petasma or thelycum, are not visible	1
Male		
2MA	Petasma is a single and undivided lobe	2
2MB	Petasma is two-lobed, but there is no wing	3
2MC	Petasma has a wing present	4
3M	Petasma is fully developed. Sexually mature	5
Female		
2FB	Thelycum is not well developed. Two small coxal outgrowths at each end of the sternal band	2
2FC	Thelycum half-developed. Coxal part larger than sternal part, but not heavily chitinized	3
2FD	Thelycum similar to adult shape, except it is smaller and not as well chitinized. There is some pigmentation	4
2FE or 3FA	Thelycum large, visible, firm, well developed and chitinized, and brightly pigmented. Sexually mature, but no male spermatophores attached	5
2FG or 3FB-E	Thelycum large, visible, firm, well developed and chitinized, and brightly pigmented. Sexually mature, with male spermatophores attached	5

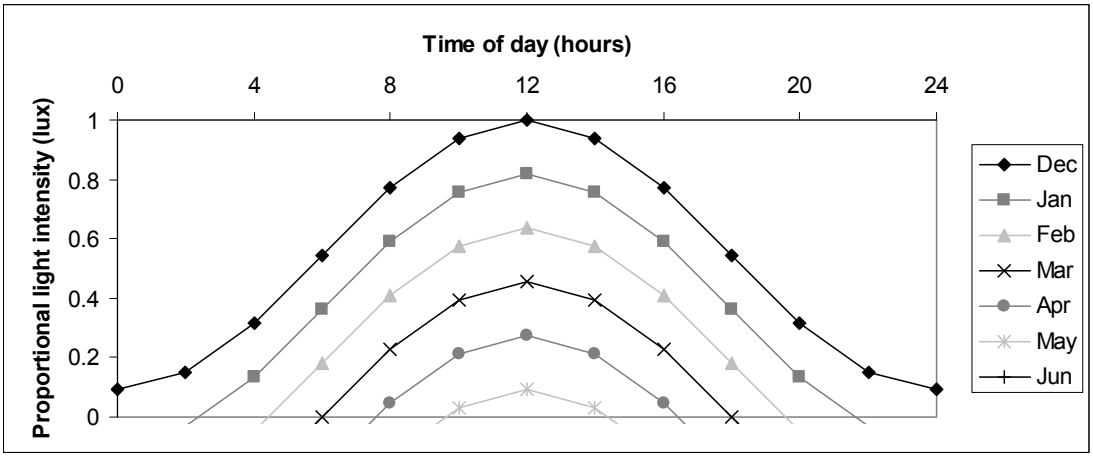
-- Appendix B --

Photographs of the five 600-L translucent acrylic tanks used to maintain the stock population of krill under the experimental conditions, as well as the 750mL jars that held individual krill. The outer ring of each tank consisted of 14 individual jars of krill.



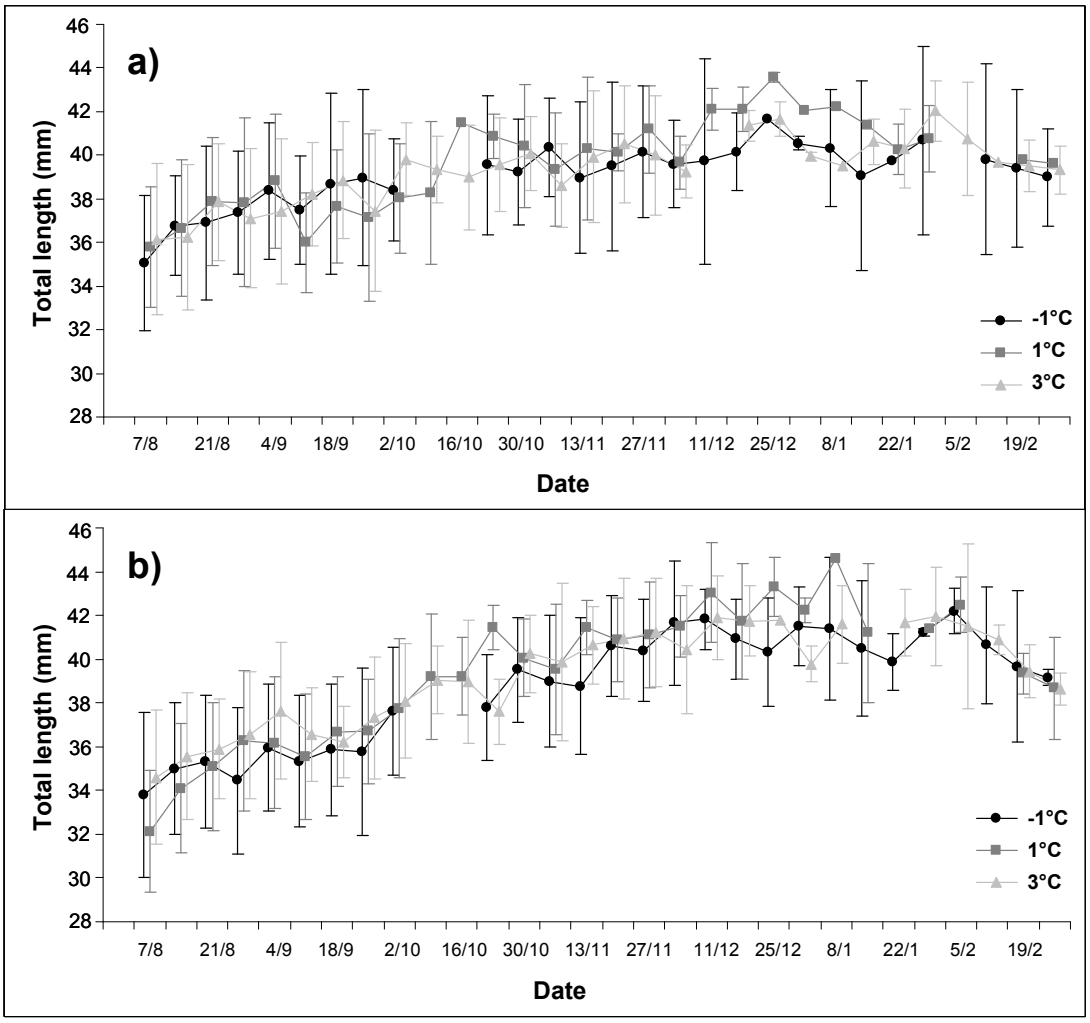
-- Appendix C --

A diagram representing the 24 hour light regimes in the experimental tanks throughout the year, simulating approximately what occurs naturally in the Southern Ocean (66°S at 30m depth). 0.1 represents 10 lux and 1 represents 100 lux. The remaining months not shown in this diagram mimic the months that are already shown **i.e** Jul is the same as May, Aug is the same as Apr, Sept and Mar, Oct and Feb, and Nov and Jan.



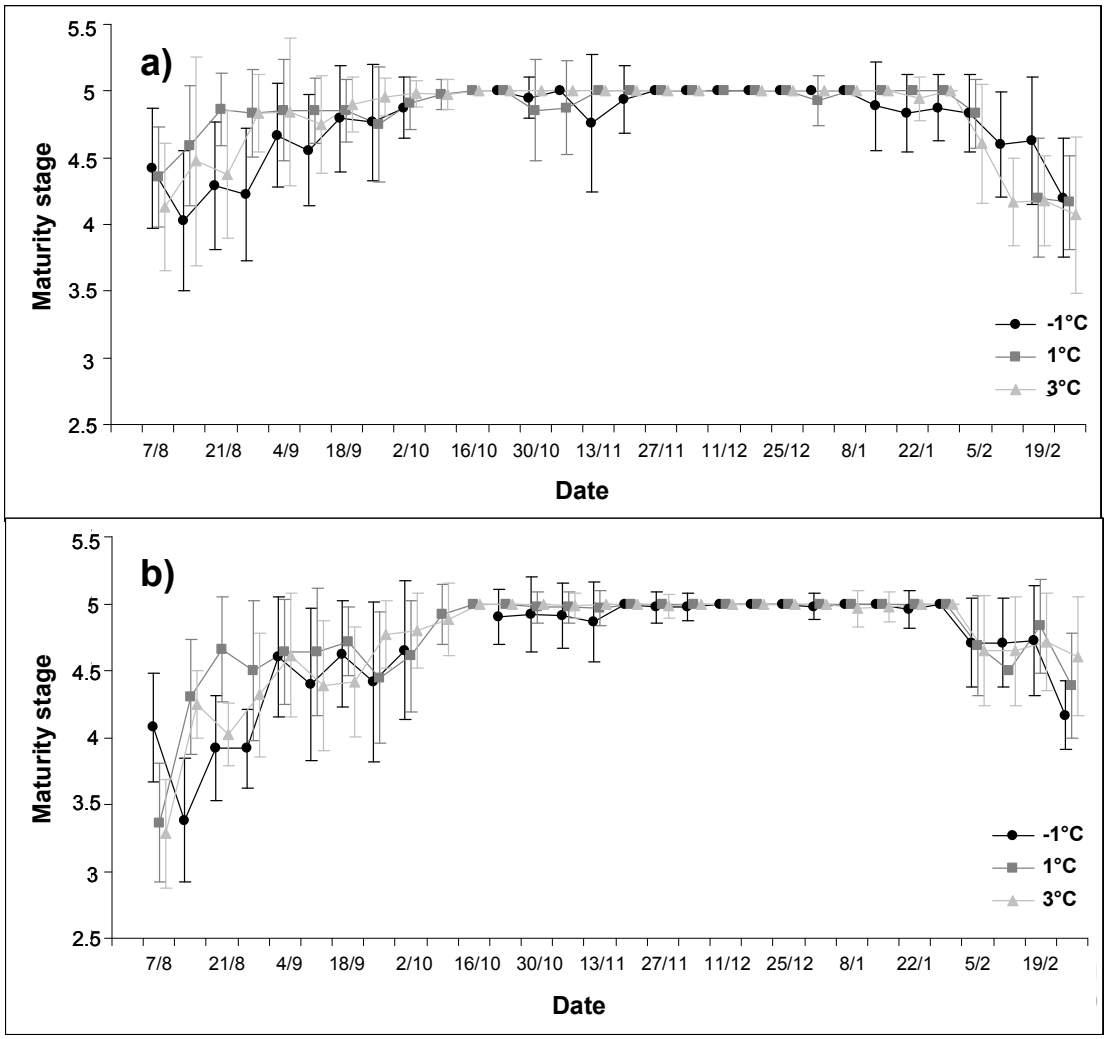
-- Appendix D --

Growth trends for **a)** males and **b)** females for the stock population of krill in the 600-L tanks within the main growth period (August-February). This is unpublished data, and was simply measured to compare the trends with the growth rates of individually maintained krill (750mL jars).



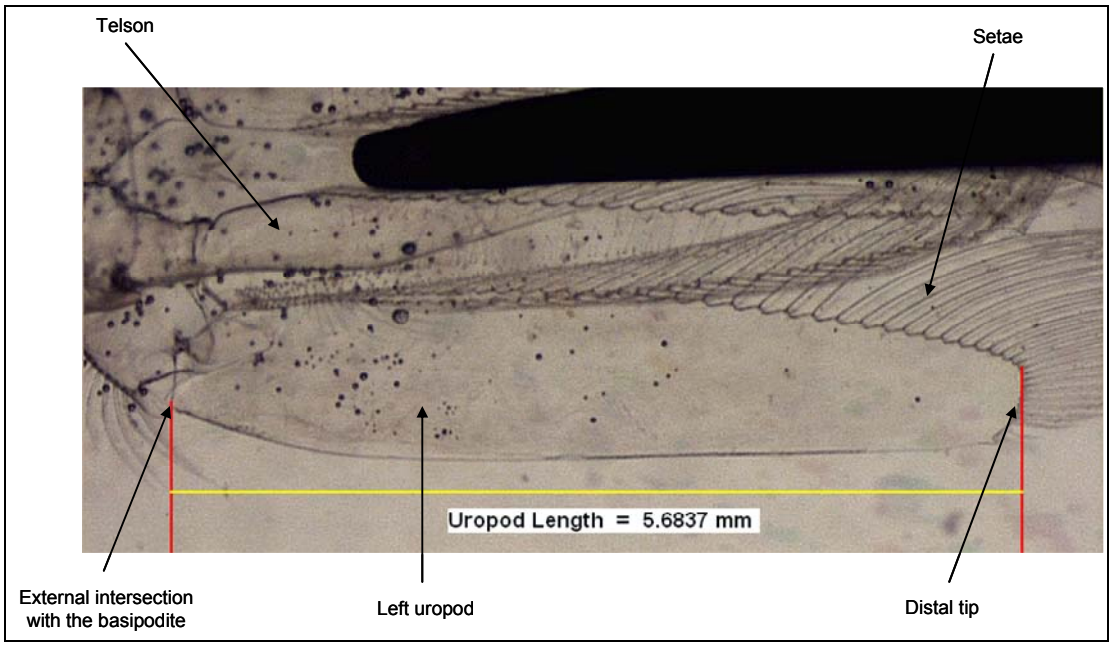
-- Appendix D (*Continued*) --

Maturity trends for **a)** males and **b)** females for the stock population of krill in the 600-L tanks within the main growth period (August-February). This is unpublished data, and was simply measured to compare the trends with the maturity cycle of individually maintained krill (750mL jars).



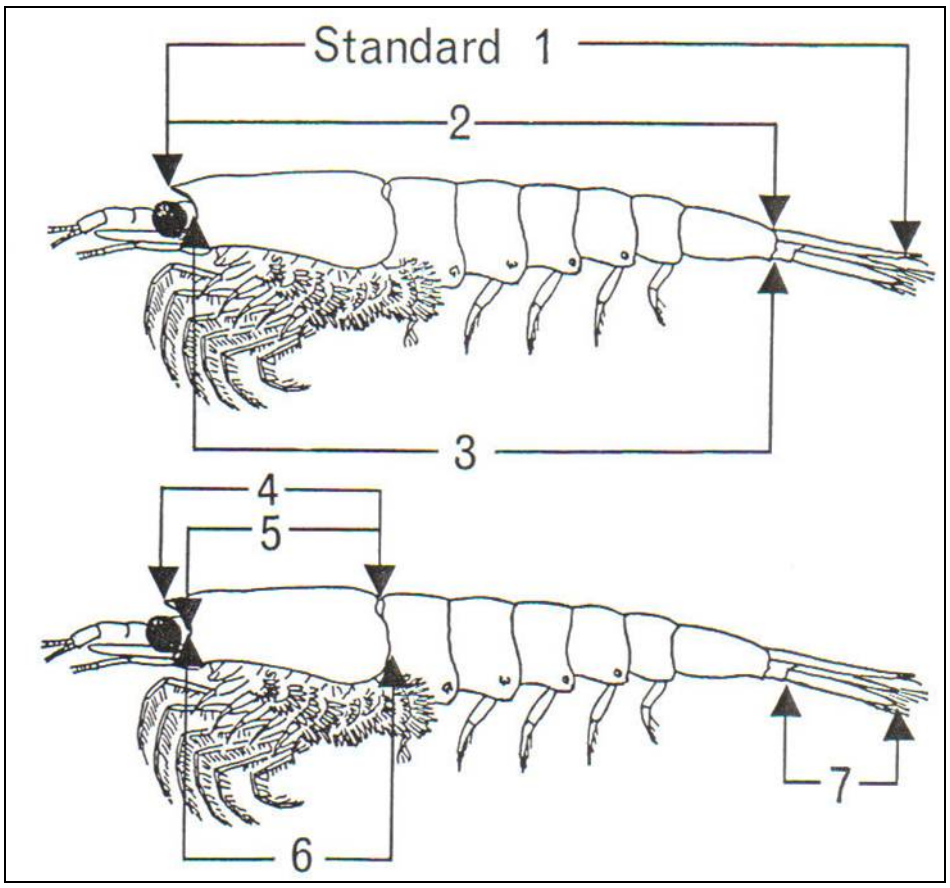
-- Appendix E --

A photograph showing the left uropod of a moulted exoskeleton. The uropod exopodite is measured from the distal tip to its external intersection with the basipodite, using image analysis software. The measurement is made parallel to the axis of the uropod rather than from point to point.



-- Appendix F --

A diagram showing the various body length measurements of krill. Source: Mauchline (1980). Standard length 1 was calculated from the measured moulted uropod length (UL) using the following equation from Hollander (2001): TL = (moulted UL – 0.1051)/0.1407



-- Appendix G --

Photographs of the 100-L white polyethylene tanks used enclosed within black lightproof plastic containers with a sliding door at the front to create a separate light compartment.

-- Appendix H --

Food quality: lipid (percent of DM) and fatty acid composition (percent of total FA) of the diet fed to krill. (LA: linoleic acid, EPA: eicosapentaenoic acid, AA: arachidonic acid, DHA: docosahexaenoic acid, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids). Source: Yoshida (2009).

Sample	*P. tricornutum*	Instant algae			Minced clam
		T. weissflogii	*Pavlova*	*Isochrysis*	
Total lipid (%DM)	22.6	10.9	29.4	18.6	7.2
Fatty acids					
14:0	6.5	9.1	17.4	12.9	1.4
16:0	14.2	22.5	13.0	14.4	16.9
18:0	0.8	1.5	0.5	1.0	7.4
16:1(n-7)c	27.4	20.5	11.3	7.9	3.7
18:1(n-9)c	8.6	1.1	1.1	15.0	2.4
18:1(n-7)c	1.0	0.6	0.3	1.4	4.5
20:1(n-9)c	0.0	0.3	0.7	0.0	1.2
22:1(n-11)c	0.0	0.0	0.0	0.0	0.2
C16PUFA	5.8	6.9	2.1	1.2	0.0
18:4(n-3)	2.9	1.0	7.9	5.8	1.3
18:2(n-6) (LA)	4.5	2.5	1.8	11.8	0.7
20:5(n-3) (EPA)	16.6	20.4	21.1	2.1	15.2
20:4(n-6) (AA)	0.0	0.7	1.2	0.2	2.2
22:5(n-6)	0.0	0.0	7.0	1.8	0.6
22:5(n-3)	0.3	0.0	0.0	0.0	2.5
22:6(n-3) (DHA)	2.1	5.6	5.5	11.5	18.9
Other	9.1	7.4	9.0	13.0	20.9
Total (n-3)	25.0	27.8	35.8	26.5	38.5
Total (n-6)	5.8	3.4	11.5	16.7	6.5
Sum SFA	23.5	38.1	31.4	30.1	28.8
Sum MUFA	39.0	23.4	18.5	25.4	21.2
Sum PUFA	36.6	38.1	49.4	44.4	50.0
Ratio (n-3)/(n-6)	4.3	8.1	3.1	1.6	5.9
Ratio DHA/EPA	0.1	0.3	0.3	5.5	1.2