CARL VON OSSIETZKY UNIVERSITÄT OLDENBURG

DISSERTATION

Seasonal, Physiological and Genetic Functions in Antarctic Krill, *Euphausia superba*, at Different Latitudes in the Southern Ocean

Jahreszeitliche, physiologische und genetische Funktionen im antarktischen Krill, *Euphausia superba*, an verschiedenen Breitengraden im Südpolarmeer

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Dedicated to my family

SUMMARY

Antarctic krill, *Euphausia superba*, is a key organism in the Southern Ocean food web with a circumpolar distribution over a large latitudinal range. Increasing commercial interest in this species and environmental changes due to global warming may pose a threat to Antarctic krill and consequently the Southern Ocean ecosystem, especially with regard to mismatches in biological timing. So far, we still lack a profound understanding of the seasonal timing system of Antarctic krill that is required for the correct prediction of Antarctic krill's response to future climate scenarios. Light regime and the endogenous timing system of Antarctic krill are known to play important roles in the regulation of its seasonal cycles of growth, maturity, metabolic activity and gene expression. However, it is not yet clear, how extreme changes in latitudinal light regime affect the flexible seasonal physiology and behaviour of Antarctic krill in its different latitudinal habitats and which molecular pathways drive the seasonal timing of these processes.

This dissertation aims to investigate the effect of different latitudinal light regimes on the seasonal cycle of Antarctic krill. It comprised two main parts: a) a field study with the goal to characterize seasonal processes on the molecular level in Antarctic krill in different latitudinal regions (Publication 1), and b) a two-year laboratory study under constant food and temperature conditions that tested the effect of the latitudinal light regimes 52°S, and 66°S, and constant darkness on the seasonal cycle of Antarctic krill (Publication 2 & 3).

Publication 1 investigated the seasonal and regional differences in gene expression between summer and winter krill from three different latitudinal regions: South Georgia (52°S), South Orkneys/Bransfield Strait (60°S-63°S), and Lazarev Sea (62°S-66°S). In the higher-latitudinal regions South Orkneys/Bransfield Strait and Lazarev Sea, we found pronounced seasonal differences in the expression of genes related to different metabolic and regulatory processes, reproduction, and development suggesting that Antarctic krill entered a state of metabolic depression and regressed development in winter. The differences in seasonal gene expression were less distinct in the low-latitude region South Georgia, most likely due to the low-latitudinal light regime and

the enhanced food availability in this ice-free region. Using an RNAseq approach, we also identified various seasonal target genes that may have putative functions in the regulation of metabolism, reproduction, development and seasonal timing in Antarctic krill.

Publication 2 analysed the effect of the latitudinal light treatments 52°S and 66°S, as well as constant darkness on the seasonal cycles of growth, feeding, lipid content, and maturity of Antarctic krill during our long-term laboratory experiment. We found that light regime affected the seasonality of these processes in Antarctic krill. The seasonal patterns of growth, feeding, and maturity persisted under constant darkness indicating the involvement of an endogenous timing system. We further demonstrated the photoperiodic plasticity of the maturity cycle in Antarctic krill showing that the critical photoperiod for maturity was longer under the high-latitudinal light regime 66°S with respect to the low-latitudinal light regime 52°S.

Publication 3 examined the seasonal expression patterns of selected metabolic and regulatory genes under the simulated light regimes 52°S and 66°S, and constant darkness during the same laboratory experiment. The simulated light regimes stimulated seasonally rhythmic expression patterns of genes involved in carbohydrate, energy, and lipid metabolism, lipid transport, translation, the biosynthesis of signalling compounds and the circadian clock. Seasonally rhythmic gene expression was most distinct under the high-latitudinal light regime 66°S with respect to 52°S, with differences in gene expression patterns detected in late summer, autumn and late winter which suggests that Antarctic krill flexibly adjusts its seasonal gene expression to different latitudinal light regimes. The study indicates that genes related to the circadian clock, prostaglandin and methyl farnesoate biosynthesis, the activation of neuropeptides, and receptor signalling may play a role in the regulation of seasonal processes in Antarctic krill.

In conclusion, this dissertation highlights the effect of latitudinal light regime on Antarctic krill and the potential molecular mechanisms that may control its phenology. Our results suggest that the seasonal timing system of Antarctic krill is highly plastic, thereby promoting regional acclimatization to different latitudinal light regimes. We further discuss that the internal 'photoperiodic-controlled' seasonal cycles in Antarctic

krill may be adjusted by other environmental cues like temperature and food supply in the field. These findings are highly relevant for future modelling approaches related to Antarctic krill. Furthermore, the identified seasonal target genes and processes form a basis for future functional studies of the seasonal timing system in Antarctic krill.

ZUSAMMENFASSUNG

Antarktischer Krill, *Euphausia superba*, ist ein Schlüsselorganismus im Nahrungsnetz des Südpolarmeers und kommt zirkumpolar über einen großen Bereich von Breitengraden vor. Das wachsende kommerzielle Interesse an dieser Art und die Umweltveränderungen durch globale Erwärmung stellen eine Bedrohung für den antarktischen Krill und folglich für das Ökosystem des Südpolarmeers dar, speziell im Hinblick auf Diskrepanzen in der zeitlichen Abstimmung von biologischen Prozessen ('Mismatch-Hypothese'). Noch fehlt uns ein tiefgreifendes Verständnis von dem saisonalen Zeitgebungssystem des antarktischen Krills, welches nötig ist um die Reaktion des antarktischen Krill auf zukünftige Klimaszenarien korrekt vorherzusagen. Es ist bereits bekannt, dass das Lichtregime und das endogene Zeitgebungssystem des antarktischen Krills ein wichtige Rolle bei der Regulierung der saisonalen Zyklen von Wachstum, sexueller Reife, metabolischer Aktivität und Genexpression spielt. Hingegen ist es unklar, wie extreme Änderungen des Lichtregimes an verschiedenen Breitengraden die flexible saisonale Physiologie und das Verhalten des antarktischen Krills in seinen verschiedenen Habitaten beeinflussen und welche molekularen Signalwege die saisonale zeitliche Abstimmung dieser Prozesse steuern.

Diese Dissertation hat das Ziel den Effekt von variablen Lichtregimen verschiedener geografischer Breite auf den saisonalen Zyklus des antarktischem Krills zu erforschen. Sie beinhaltet zwei Hauptteile: a) eine Feldstudie mit dem Ziel die saisonalen Prozesse in antarktischem Krill auf einem molekularen Level zu charakterisieren (Publikation 1), und b) ein zweijähriges Laborexperiment unter konstanten Futter- und Temperaturbedingungen welches den Effekt von den Lichtregimen an den Breitengraden 52°S und 66°S , und konstanter Dunkelheit auf den saisonalen Zyklus des antarktischen Krills testete (Publikation 2 & 3).

Publikation 1 untersuchte die saisonalen und regionalen Unterschiede in der Genexpression zwischen Sommer- und Winterkrill aus drei verschiedenen Regionen mit unterschiedlicher Lage entlang der Breitengrade: Südgeorgien (54°S), Süd-Orkney-Inseln/Bransfield-Straße (60°S-63°S), und Lazarew-See (62°S-66°S). In den Regionen an höheren Breitengraden Süd-Orkney-Inseln und Lazarew-See haben wir ausgeprägte

saisonale Unterschiede in der Expression von Genen gefunden, die Funktionen in unterschiedlichen metabolischen und regulatorischen Prozessen sowie der Reproduktion und der Entwicklung haben. Diese Ergebnisse deuteten darauf hin, dass der antarktische Krill im Winter in ein Ruhestadium mit gedämpftem Metabolismus und rückläufiger Entwicklung eingetreten war. Die Unterschiede in der saisonalen Genexpression waren in der Region Südgeorgien weniger ausgeprägt, was vermutlich durch das Lichtregime an dem niedrigeren Breitengrad und die erhöhte Futterverfügbarkeit in dieser eisfreien Region bedingt ist. Mittels der RNAseq Methode konnten wir diverse saisonale Zielgene identifizieren, welche mutmaßliche Funktionen in der Regulation des Metabolismus, der Reproduktion, Entwicklung und saisonalen Zeitgebung haben.

Publikation 2 analysierte den Effekt der simulierten Lichtregime an den Breitengraden 52°S und 66°S sowie konstanter Dunkelheit auf die saisonalen Zyklen von Wachstum, Ernährung, Lipidgehalt und sexueller Reife in antarktischem Krill während unseres Langzeit-Laborexperiments. Wir fanden heraus, dass das Lichtregime die Saisonalität dieser Prozesse im antarktischen Krill beeinflusst. Die saisonalen Muster des Wachstums, der Ernährung und der sexuellen Reife blieben auch unter konstanter Dunkelheit bestehen, was auf einen endogenen Zeitgebungsmechanismus hinweist. Zudem haben wir die photoperiodische Plastizität des sexuellen Reifezyklus von antarktischem Krill dargelegt, indem wir gezeigt haben, dass die kritische Photoperiode für sexuelle Reife länger unter dem Lichtregime des höheren Breitengrades 66°S war als unter dem Lichtregime des niedrigeren Breitengrades 52°S.

Publikation 3 untersuchte die saisonalen Expressionsmuster von metabolischen und regulatorischen Genen unter Einfluss der simulierten Lichtregime 52°S, 66°S und konstanter Dunkelheit während des gleichen Laborexperiments. Die simulierten Lichtregime stimulierten saisonal rhythmische Expressionsmuster von Genen, welche in den Kohlenstoff-, Energie- und Lipidmetabolismus, den Lipidtransport, die Translation, die Biosynthese von Signalstoffen und die zirkadiane Uhr involviert sind. Die saisonal rhythmische Genexpression war, im Vergleich zum Lichtregime 52°S, am meisten unter dem simulierten Lichtregime von dem hohen Breitengrad 66°S ausgeprägt und es zeigten sich Unterschiede in den Genexpressionsmustern im späten

Sommer, Herbst und späten Winter. Diese Beobachtung deutet darauf hin, dass antarktischer Krill seine saisonalen Genexpressionsmuster flexibel an die verschiedenen Lichtregime an unterschiedlichen Breitgraden anpassen kann. Die Studie lässt außerdem erkennen, dass Gene eine Rolle für die Regulierung von saisonalen Prozessen in antarktischem Krill spielen könnten, die im Bezug zur zirkadianen Uhr, der Biosynthese von Prostaglandinen und Methylfarnesoat und der Aktivierung von Neuropeptiden stehen.

Schlussfolgernd hebt diese Dissertation hervor, welchen Effekt verschiedene Lichtregime, die sich je nach geografischer Breite unterscheiden, auf antarktischen Krill haben und welche potentiellen molekularen Mechanismen seine Phänologie steuern. Unsere Forschungsergebnisse weisen darauf hin, dass das saisonale Zeitgebungssystem von antarktischem Krill zu hohem Maße formbar ist und damit die regionale Akklimatisierung an die Lichtbedingungen an verschiedenen Breitengraden unterstützt. Weiterhin erörtern wir, dass die internen photoperiodisch-kontrollierten saisonalen Zyklen des antarktischen Krills durch andere Umweltfaktoren wie Temperatur und Futterverfügbarkeit angepasst werden könnten. Diese Erkenntnisse sind hochrelevant für zukünftige Modellierungsansätze bezogen auf den antarktischen Krill. Darüber hinaus stellen die identifizierten saisonalen Zielgene eine Grundlage für zukünftige funktionelle Studien des saisonalen Zeitgebungssystems im antarktischen Krill dar.

Contents

List of Abbreviations

AIC	**A**kaike's **i**nformation **c**riterion
AUC	**A**rea **U**nder the **C**urve
CCAMLR	**C**ommission for the **C**onservation of **A**ntarctic **M**arine **L**iving **R**esources
CHH	**C**rustacean **H**yperglycemic **H**ormone
DD	Constant Darkness
DEGs	**D**ifferentially **E**xpressed **G**enes
FDR	**F**alse **D**iscovery **R**ate
GAMM	**G**eneralized **A**dditive **M**ixed **M**odel
GAM	**G**eneralized **A**dditive **M**odel
GLMM	**G**eneralized **L**inear **M**ixed **M**odel
GO	**G**ene **O**ntology
IKMT	**I**saacs-**K**idd **M**idwater **T**rawl
ORF	**O**pen **R**eading **F**rame
PCA	**P**rinciple **C**omponent **A**nalysis
RMT	**R**ectangular **M**idwater **T**rawl
ROC	**R**eceiver **O**perating **C**haracteristic
TMM	**T**rimmed **M**ean of **M**-values normalization method
TRAP	**T**ranslocon-**A**ssociated **P**rotein
sPLS-DA	**S**parse **P**artial **L**east **S**quares **D**iscriminant **A**nalysis

1. General Introduction

In this section, I would like to introduce the scientific background of this dissertation which studied seasonal, physiological and genetic functions in Antarctic krill, *Euphausia superba*, under different latitudinal light regimes. First, I will explain the importance of *E. superba* in the Southern Ocean ecosystem, the impact of fisheries, as well as the effects of climate change and potential phenological mismatches on this species. Afterwards, I will summarize findings from field studies that show that Antarctic krill has evolved pronounced seasonal cycles of physiology and behaviour to adapt to the highly variable environment in the Southern Ocean. Then, I will integrate information about the seasonal timing system in Antarctic krill including the role of environmental cues, the potential mechanisms of the endogenous timing system and the neuroendocrine control. In a final step, I will explain the gaps of knowledge, the research objectives of this dissertation, and the methodological approach.

1.1 *Euphausia superba*, a key organism in the Southern Ocean

With an estimated circumpolar biomass of 379 Mt (Atkinson, 1998), Antarctic krill (*Euphausia superba*) belongs to one of the most abundant organisms on Earth. Antarctic krill is a key organism in the Southern Ocean food web linking primary production to higher trophic levels such as whales, penguins, birds and seals (Fig. 1.1). *Euphausia superba* is distributed over a large latitudinal range from approximately 50°S to more than 70°S (Hill et al., 2013). These different latitudinal habitats are characterized by extreme seasonal and regional fluctuations of photoperiod, primary production and sea ice extent (Quetin and Ross, 1991). Since Antarctic krill is able to travel great distances within one year, either by active migration (Siegel, 1988) or passive transport within

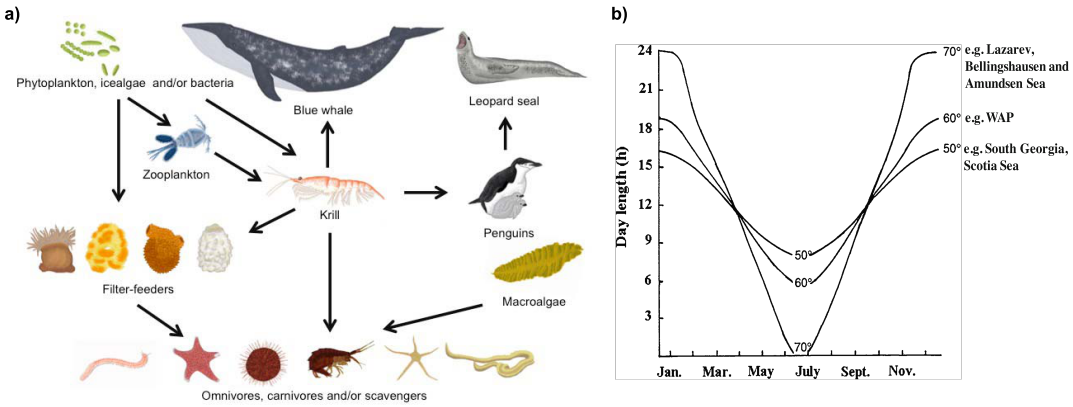

FIGURE 1.1: a) Simplified version of the Antarctic food web by Balañá (2013) licenced under CC BY-NC-ND 3.0 ES; b) Latitudinal light regimes in different regions of the Southern Ocean by Meyer (2012) licenced under CC BY 4.0.

ocean currents (Thorpe et al., 2007), it seems to be highly flexible in adjusting it's phenology to both the high annual and regional variability of environmental factors in the different latitudinal habitats of the Southern Ocean.

Commercial interest in Antarctic krill and fishery are growing considering its high biomass, improved harvesting techniques and the increasing demand for newly developed krill products (Nicol et al., 2012). Antarctic krill is considered of high nutritional value when used as 'krill meal' in aquaculture (Yoshitomi et al., 2007) and for the production of dietary supplements and pharmaceutical products because of their suggested beneficial properties for human health (Tou et al., 2008). Krill fishery in the Southern Ocean is currently managed by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR). However, sustainable fishery's management is largely dependent on our current understanding of the Southern Ocean ecosystem and the correct prediction of krill abundances under changing environmental conditions in the future.

Climate change may pose another threat to Antarctic krill. It has been predicted that global warming and associated changes in chlorophyll concentration will reduce the favourable growth habitat of Antarctic krill, and thereby its biomass in the Southern Ocean (Hill et al., 2013). In the Southwest Atlantic Sector, it has been reported that Antarctic krill densities have declined associated with a southward shift of *E. superba*'s distribution and an increase in salp densities in that region (Atkinson et al.,

2019; Atkinson et al., 2004). This shift has been explained by changes in sea ice extent (Atkinson et al., 2004) and anomalies of the Southern Annular Mode (Atkinson et al., 2019). Changes in Antarctic krill biomass have been linked to the survival of upper-level predators such as penguins which indicates that climate change may lead to profound changes in the Southern food web (Trivelpiece et al., 2011).

Climate change effects in the Southern Ocean are especially relevant under the match/mismatch hypothesis that describes how the seasonal timing of a predator and the availability of its prey may affect recruitment, reproduction or survival of the predator (Durant et al., 2007). In particular, Antarctic krill may be influenced by temporal and spatial changes in phytoplankton distribution that do not match its seasonal requirements for recruitment or reproductive processes. However, these effects are difficult to predict, because the mechanisms and the flexibility of Antarctic krill's seasonal timing system are poorly understood.

1.2 Pronounced Seasonal Cycles of *E. superba* in the Field

Pronounced seasonal variations of growth, feeding, metabolic activity (Meyer et al., 2010), lipid turnover (Ericson et al., 2018; Hellessey et al., 2018), reproduction (Siegel, 2012) and gene expression (Seear et al., 2012) have been observed in Antarctic krill in the field (Fig. 1.2). Antarctic krill has evolved different overwintering strategies to cope with the conditions of near-constant darkness and low food availability in some regions of the Southern Ocean (Meyer, 2012). The by far most important strategy is the reduction of physiological functions consistent with a state of quiescence observed in Antarctic krill. Respiration is severely reduced during the winter season and may drop down to 30% of the summer rates (Meyer et al., 2010; Quetin and Ross, 1991). Moreover, a significantly lower activity of the metabolic key enzymes citrate synthase (Cullen et al., 2003; Meyer et al., 2002) and malate dehydrogenase (Meyer et al., 2010; Pape et al., 2008) was found in autumn and winter. In the course of winter-metabolic depression, low feeding activity has been observed, with indications that Antarctic krill can switch to alternative food sources like zooplankton or ice-algae (Atkinson et al., 2002; Meyer et al., 2010). As a consequence, growth rates of adult krill are extremely reduced during winter (Meyer et al., 2010), even shrinkage has been reported

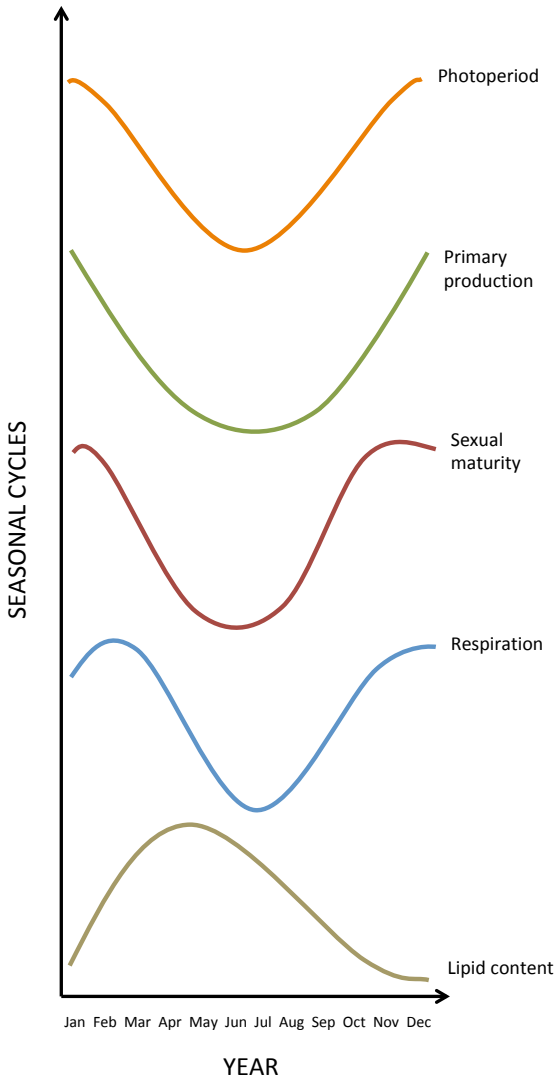

FIGURE 1.2: Schematic representation of seasonal cycles of photoperiod and primary production in the Southern Ocean, and sexual maturity, respiration and lipid content of Antarctic krill following information from Meyer (2012), Arrigo et al. (2008), Kawaguchi et al. (2007), and Hellessey et al. (2018)

(Quetin and Ross, 1991). The seasonal accumulation of lipid stores and their utilisation during winter promotes survival of adult krill during periods of low food availability (Falk-Petersen et al., 2000; Hagen, 2000; Hagen et al., 2001; Meyer et al., 2010; Quetin and Ross, 1991). Moreover, Antarctic krill shows a pronounced seasonal cycle of maturity which is characterized by the regression of its external sexual traits towards winter and the sexual re-maturation towards spring (Kawaguchi et al., 2007). Gene expression analysis of seasonal Antarctic krill samples near the Antarctic Peninsula revealed an upregulation of genes related to feeding and digestion, respiration, motor activity, immunity and vitellogenesis in summer krill with respect to winter (Seear et al., 2012).

Antarctic krill is able to synchronize its seasonal life cycle to local photoperiod and food supply in the different latitudinal habitats of the Southern Ocean. Regional differences in the timing of reproduction (Spiridonov, 1995), growth (Kawaguchi et al., 2006), feeding activity and lipid storage (Schmidt et al., 2014), and gene expression (Seear et al., 2012) have been observed in the field. Spiridonov (1995) investigated the spawning season of Antarctic krill in different regions of the Southern Ocean and discussed that the reproductive timing is largely dependent on the variation of seasonal sea ice cover and the timing of phytoplankton blooms.

Kawaguchi et al. (2006) reported differences in the growth period of Antarctic krill examining the Southwest Atlantic Sector and the Indian Ocean sector. The authors suggested that the earlier timing of the phytoplankton bloom at lower latitudes might advance the growth period of Antarctic krill. Differences in the overwintering behaviour of Antarctic krill were observed by Schmidt et al. (2014) and Seear et al. (2012) in different latitudinal habitats of the Southern Ocean. Antarctic krill from the low-latitude region South Georgia had higher feeding activities and lower lipid stores during winter compared to the high-latitude region Lazarev Sea where Antarctic krill experienced near-constant darkness and the strongest limitation in food supply (Schmidt et al., 2014). Similar differences were found on the gene expression level, where winter Antarctic krill from South Georgia showed higher gene activities related to feeding, digestion and immunity with respect to krill from the Antarctic Peninsula (Seear et al., 2012).

1.3 Seasonal timing mechanisms in *E. superba*

The variable light, food, and temperature regimes may play a role in the regulation of *E. superba*'s seasonal cycles in the different latitudinal habitats of the Southern Ocean (Fig. 1.3). Controlled laboratory experiments were conducted to unravel the specific effects of these parameters on the different seasonal processes in Antarctic krill. Higher water temperatures and high food supply were found to increase the growth rates of Antarctic krill (Brown et al., 2010; Buchholz, 1991), whereas seasonal changes of photoperiod modulated a seasonal growth pattern (Piccolin et al., 2018b). Kawaguchi et al. (2007) found that favourable feeding conditions accelerate the sexual maturation process of Antarctic krill. The initiation of sexual maturation and spawning were also observed when exposing Antarctic krill to long photoperiods (Hirano et al., 2003; Teschke et al., 2008). These long photoperiodic conditions also triggered an enhanced lipid catabolism that was suggested to be necessary for the maturation process (Teschke et al., 2008). During long-term laboratory experiments of constant food supply, different simulated light regimes flexibly adjusted the seasonal cycle of maturity in Antarctic krill (Brown et al., 2011). The metabolic activity of Antarctic krill was observed to be higher under long photoperiods similar to summer and autumn conditions compared to the simulated winter condition 'constant darkness' (Teschke et al., 2007), while the same study indicated that Antarctic krill might not be able to respond to high food concentrations under constant darkness. Although food supply and temperature have the potential to affect the respiration rates of Antarctic krill, these factors do not change the general seasonal pattern of metabolic activity (Brown et al., 2013). Recently, Piccolin et al. (2018b) confirmed in long-term experiments that a simulated annual light regime could trigger the seasonal cycle of metabolic activity in Antarctic krill. These seasonal photoperiodic effects on krill's metabolic cycle were also found on gene expression level (Piccolin et al., 2018b; Seear et al., 2009).

These studies reveal that light regime and seasonal changes in photoperiod are major cues that entrain the seasonal rhythms of growth, maturity, metabolic activity and gene expression in Antarctic krill. It has been suggested that these seasonal rhythms are controlled by an endogenous timing system with photoperiod as timing

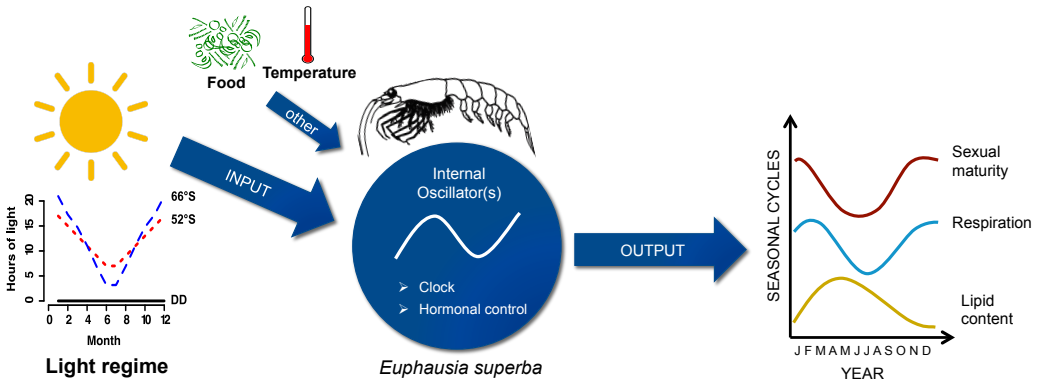

FIGURE 1.3: Schematic representation of the seasonal timing system of Antarctic krill.

cue (*Zeitgeber*) (Brown et al., 2011; Brown et al., 2013; Piccolin et al., 2018a). An endogenous timing system (circannual clock) is characterized by the observation that seasonally rhythmic patterns persist, even if the actual *Zeitgeber* is not present (Visser et al., 2010). Such evidence was found in Antarctic krill during long-term laboratory experiments where seasonal patterns of maturity, growth and metabolic activity were observed under constant darkness (Brown et al., 2011; Piccolin et al., 2018b).

In general, the molecular mechanisms of seasonal timing systems are poorly understood, but there are indications that the circadian (daily) clock may play role for photoperiodic time measurement and consequently the timing of seasonal life cycle events (Helm et al., 2013). In eukaryotes, the circadian clock functions as an approximately 24-h oscillator via interlaced transcriptional/posttranslational feedback loops that are synchronized by an environmental factor such as photoperiod (Mackey, 2007). In insects, circadian clock genes were found to play an important role for the seasonal photoperiodic timing of diapause (review by Goto (2013), Meuti and Denlinger (2013), and Meuti et al. (2015), with exceptions (e.g. Emerson et al. (2009)). The investigation of latitudinal clines of photoperiodism showed that insect populations generally showed higher critical photoperiods for the initiation of diapause at higher latitudes, and latitudinal adaptation of their photoperiodic response has been linked to clock gene polymorphisms (Hut et al., 2013). Photoperiodic plasticity may also be based on the differential expression of clock genes (Hodkova et al., 2003) depending on season

and latitude. Moreover, it is speculated, if non-coding RNA or epigenetic modifications play a role in the regulation of circannual rhythms (Helm and Stevenson, 2014).

In Antarctic krill, molecular studies have analysed the functioning of its visual perception system and its circadian clock (Biscontin et al., 2016; Biscontin et al., 2017). Different opsin genes were identified in Antarctic krill that are important for the visual perception of light of different wavelengths (Biscontin et al., 2016). The circadian clock machinery of Antarctic krill was characterized by Biscontin et al. (2017) who found that the core clock resembled both insect's and mammalian circadian clock systems with a light mediated degradation mechanism that suggested light as the main *Zeitgeber*. Controlled laboratory experiments revealed that the daily oscillations of clock genes in Antarctic krill varied under variable seasonal conditions of photoperiod and became arrhythmic under mid-winter and mid-summer conditions (Piccolin et al., 2018b). Antarctic krill's circadian clock has not only been linked to the timing of daily rhythms of metabolic activity (De Pittà et al., 2013; Piccolin et al., 2018b; Teschke et al., 2011) and diel vertical migration (Gaten et al., 2008), but it is also suggested to play a role for the seasonal day length measurement and the regulation of seasonal rhythms in Antarctic krill based on the observation of seasonal patterns of clock gene expression in Antarctic krill (Piccolin et al., 2018b).

In crustaceans, circadian pacemakers (clocks) are located in the nervous system, in particular in retina of the eye, the eyestalk, the brain and the caudal photoreceptor (Arechiga and Rodriguez-Sosa, 2002; Rodríguez-Sosa et al., 2008) and may be linked to neuroendocrine control of seasonal rhythms. Seasonal life cycle events in decapods are mediated by various neuropeptides and signalling molecules that originate from the X-organ-sinus gland system of the eyestalk ganglia, brain, the thoracic ganglia, the Y-organ and the mandibular organ (Nagaraju, 2011). Important hormones comprise the 'CHH-superfamily' including the crustacean hyperglycaemic hormone, the moult-inhibiting hormone, the gonad/vitellogenesis-inhibiting hormone and the mandibular organ-inhibiting hormone that have multiple functions in carbohydrate and lipid metabolism, reproduction, moulting, osmoregulation and the regulation of methyl farnesoate synthesis from the mandibular organ (review by Webster et al. (2012)). Other

hormones that control moulting and reproduction in crustaceans include methyl farnesoate (Reddy et al., 2004), ecdysteroids and 'vertebrate-type' steroids (Lafont and Mathieu, 2007), and prostaglandins (review by Nagaraju (2011)).

In Antarctic krill, studies on the neuroendocrine control of seasonal processes are rare (Buchholz, 1991; Pape et al., 2008; Seear et al., 2012; Toullec et al., 2013). Buchholz (1991) studied changes in hemolymph titre of ecdysone-equivalents in the different moult stages of Antarctic krill. Pape et al. (2008) were not able to detect melatonin in Antarctic krill and therefore rejected the hypothesis that it played a role in regulating the seasonal metabolic cycle of Antarctic krill (Teschke et al., 2007). In the field, seasonal gene expression patterns of the neuropeptide neuroparsin and insulin-like peptides have been discussed in relation to the seasonal reproductive physiology of Antarctic krill (Seear et al., 2012). In the ice krill, *Euphausia crystallorophias*, various neuropeptide hormones were identified including members of the CHH superfamily (Toullec et al., 2013). The recently developed transcriptome database may provide a source for *E. superba*-specific target sequences of neuropeptides (Sales et al., 2017).

1.4 Research objectives

The current environmental changes in the Southern Ocean and the increasing commercial interest in Antarctic krill emphasise the need to better understand the adaptability of *E. superba* in different latitudinal regions of the Southern Ocean, especially under the aspect of potential mismatches in biological timing. It has not yet been investigated, if different latitudinal light regimes regulate the flexible seasonal physiology and behaviour of Antarctic krill in its diverse latitudinal habitats. In general, the seasonal timing system of Antarctic krill is not well understood including the potential involvement of the circadian clock and the neuroendocrine control of seasonal rhythms in Antarctic krill. Laboratory experiments that observe the seasonal cycle of Antarctic krill under controlled photoperiodic conditions over a period of multiple years and simulating different latitudinal light regimes are still lacking.

This dissertation aimed to understand the role of different latitudinal light regimes on the seasonal cycle of Antarctic krill focussing on (a) the molecular characterization of seasonal rhythms in different latitudinal habitats in the field (Publication 1), and

(b) the investigation of seasonal rhythms under a two-year photoperiodic-controlled laboratory experiment with constant food supply (Publication 2 & 3). The following research objectives were addressed in the three different chapters of the dissertation:

1. Investigation of seasonal and regional differences in gene expression of summer and winter Antarctic krill from three different latitudinal regions: South Georgia (54°S), South Orkneys/Bransfield Strait (60°S-63°S) and Lazarev Sea (62°S-66°S) (Publication 1)

2. Analysis of seasonal cycles of growth, feeding, lipid metabolism and maturity of Antarctic krill under the simulated light regimes 52°S, 66°S, and constant darkness (Publication 2)

3. Characterization of seasonal expression patterns of genes involved in different metabolic processes, seasonal timing, reproduction, feeding and development under the simulated light regimes 52°S, 66°S, and constant darkness (Publication 3)

A range of different methods were implemented to investigate the effect of latitudinal light regime on Antarctic krill. An RNAseq approach was used to characterize the seasonal and latitudinal gene expression differences in Antarctic krill in the field and to identify suitable seasonal target genes with focus on genes with potential regulatory functions in the seasonal cycle of Antarctic krill. For the first time, a two-year laboratory experiment was conducted that simulated different latitudinal light regimes and constant food supply. Antarctic krill from the laboratory experiments was investigated using morphometric and lipid content analysis, and gene expression data from custom designed TaqMan cards.

The findings of this dissertation improve our understanding of the effect of different latitudinal light regimes on seasonal cycles in Antarctic krill and their underlying molecular mechanisms.

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2. Publication I

Seasonal gene expression profiling of Antarctic krill in three different latitudinal regions

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2.1 Abstract

The key organism Antarctic krill, *Euphausia superba*, has evolved seasonal rhythms of physiology and behaviour to survive under the extreme photoperiodic conditions in the Southern Ocean. However, the molecular mechanisms generating these rhythms remain far from understood. The aim of this study was to investigate seasonal and regional differences in gene expression in three different latitudinal regions with variable photoperiodic conditions (South Georgia, South Orkneys/Bransfield Strait, Lazarev Sea) and to identify genes with potential regulatory roles in the seasonal life cycle of

Antarctic krill. The RNAseq data were analysed (a) for seasonal differences between summer and winter field samples from each region, and (b) for regional differences within each season. In general, we found an upregulation of gene expression in summer krill in all regions with respect to winter. However, seasonal differences in gene expression were less pronounced in Antarctic krill from South Georgia where most genes related to metabolic, biological and regulatory processes were not found to be differentially expressed between summer and winter krill. We also identified genes with putative regulatory roles, for instance genes related to hormone metabolism and signalling, reproductive and developmental processes. Our results suggest that Antarctic krill entered a state of metabolic depression and regressed development (so called winter quiescence) in South Orkneys/Bransfield Strait and Lazarev Sea region in winter. The winter quiescence seems to be less pronounced in the South Georgia region, most likely due to the milder seasonal conditions, the less extreme light regime in this low-latitude region, and hence food availability. These findings including the proposed target genes provide a basis for future laboratory studies of the molecular mechanisms of seasonal rhythms in Antarctic krill.

2.2 Introduction

Seasonal rhythms of physiology and behaviour are essential for the survival of marine organisms inhabiting regions with extreme seasonal changes of photoperiod (day length) like the Southern Ocean. Antarctic krill, *Euphausia superba*, holds a pivotal position in the Southern Ocean food web where it is a major link between primary production and higher trophic levels. It has been proposed that Antarctic krill may serve as a polar model organism to study the effects of climate change in the polar ecosystem of the Southern Ocean (Meyer, 2010). For that purpose, we want to understand the mechanisms of its seasonal life cycle including its flexibility under changing environmental conditions.

In the field, pronounced seasonal differences have been found in the Antarctic krill's body composition, metabolic activity, feeding, growth (Meyer et al., 2010) and maturity (Siegel, 2012). Survival in periods of near-constant darkness and low food availability is accomplished by different overwintering strategies. These include the

accumulation of lipid reserves during summer and the reduction of metabolic activity, feeding activity and growth (Meyer, 2012) and sexual regression during winter.

Only few studies have investigated regional differences in the life cycle of krill such as the timing of reproduction (Spiridonov, 1995) and growth (Kawaguchi et al., 2006). Overwintering strategies seem to vary according to latitudinal habitat as krill near South Georgia was observed to have lower lipid stores and higher feeding activity in winter compared to higher latitudinal regions (Schmidt et al., 2014). Seasonal and regional differences in gene expression were found, for the first time, by Seear et al. (2012) who investigated seasonal effects near the Antarctic Peninsula (60°S) and spatial differences in winter comparing the Antarctic Peninsula and South Georgia (54°S) region. This study concluded that genes involved in feeding and digestion, respiration, motor activity, immunity and vitellogenesis were upregulated in krill sampled in the Peninsula region during summer with respect to winter. The regional comparison of winter krill revealed an upregulation of genes related to feeding and digestion and immunity at South Georgia compared to the Peninsula region.

The seasonal cycle of Antarctic krill is influenced by different environmental factors such as light regime, food availability and/or temperature that may contribute to krill's flexible behaviour in different latitudinal regions. Controlled lab experiments have demonstrated the effect of temperature and food supply on krill growth (Buchholz, 1991) and maturity (Kawaguchi et al., 2007). Photoperiod has been shown to affect feeding and metabolic activity (Teschke et al., 2007), growth (Brown et al., 2013), maturity (Brown et al., 2011) and gene expression (Seear et al., 2009) in Antarctic krill under laboratory conditions. Based on the photoperiodic studies, it has been suggested that seasonal rhythms in Antarctic krill are governed by an endogenous timing system with photoperiod as *Zeitgeber*. Recently, it has been confirmed in a two-year lab experiment that krill's seasonal rhythms seem to be affected by different latitudinal light regimes (Höring et al., 2018).

Studies on the molecular mechanisms of the endogenous timing system in Antarctic krill have mostly focused on daily rhythms, the circadian clock and the photoperception system in *E. superba* (Biscontin et al., 2016; Biscontin et al., 2017; De Pittà

et al., 2013; Piccolin et al., 2018a). Biscontin et al. (2016) identified the opsin repertoire of Antarctic krill which may contribute to the perception of daily and seasonal changes in irradiance and spectral composition in the Southern Ocean. Antarctic krill possesses an ancestral circadian clock machinery with both insect- and vertebrate like features and a light mediated entraining mechanism (Biscontin et al., 2017). It has been suggested that krill's circadian clock does not only control daily rhythms in *E. superba*, but may also be involved in the timing of seasonal life cycle events (Piccolin et al., 2018b).

The flexibility of krill's seasonal cycle in different latitudinal regions and the underlying molecular mechanisms are still poorly understood. Current knowledge on the seasonal behaviour of Antarctic krill in the field is based on single observations and the analysis of few regions, whereas data from the winter season is generally less frequent. Even though extensive transcriptome (Meyer et al., 2015; Sales et al., 2017), we still lack a comprehensive understanding of the molecular pathways that contribute to the regulation of seasonal rhythms in Antarctic krill in different latitudinal regions of the Southern Ocean.

This paper aims to investigate seasonal and regional differences in gene expression in Antarctic krill in three different latitudinal regions of the Southern Ocean: South Georgia (54°S), South Orkneys/Bransfield Strait (60°S-63°S) and Lazarev Sea (62°S-66°S). An RNA-seq approach is used to test for (1) seasonal differences in gene expression between summer and winter krill from each region, and (2) regional differences in gene expression between the three different regional krill samples from each season. The RNA-seq data is analysed with the goal to identify seasonal target genes with putative regulatory functions in the seasonal life cycle of Antarctic krill.

2.3 Methods

2.3.1 Sample collection and experimental design

Antarctic krill samples (*Euphausia superba*) were obtained from five different expeditions and from a Norwegian fishing vessel (Table 2.1, Fig. 2.1. Sampling was carried out with a Rectangular Midwater Trawl (RMT8+1 for expeditions ANT23-2, ANT23-6

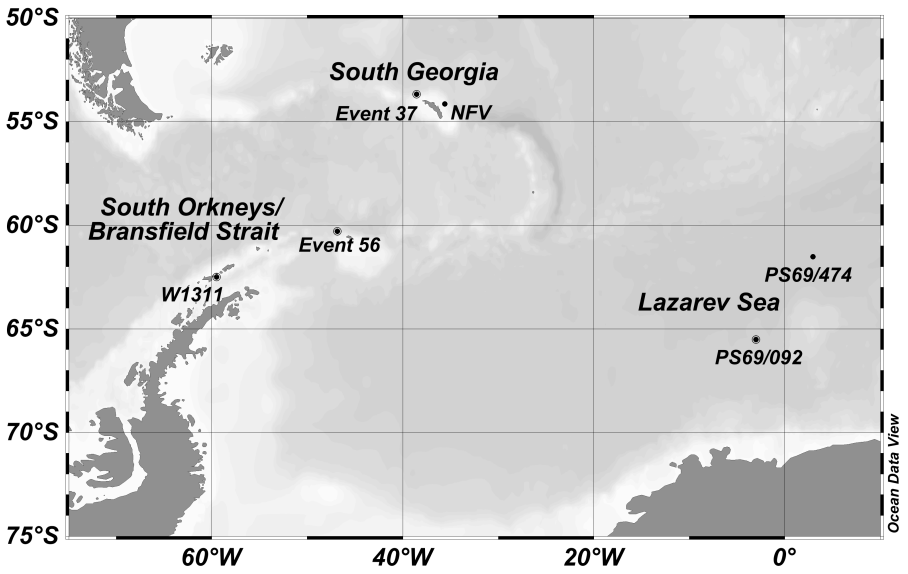

FIGURE 2.1: Station map indicating station numbers (NFV - Norwegian fishing vessel) and the three studied regions

and JR15004, RMT8 for expedition JR260B), an Isaacs-Kidd Midwater Trawl (IKMT, expedition AMLR14) and a continuous pumping system (Norwegian fishing vessel). Snap-frozen Antarctic krill samples stored at $-80\,^{\circ}$C were transferred to the Alfred-Wegener-Institute, Bremerhaven, for molecular analysis.

The Antarctic krill originated from three different latitudinal regions: a) Lazarev Sea (62°S-66°S), b) South Orkneys/Bransfield Strait (60°S-63°S), and c) South Georgia (54°S), including summer and winter samples for each region. By visual inspection of the outer sexual organs, male petasma and female thelycum, adult males and females were identified. In total, 36 individuals were chosen for further analysis, with 6-7 individuals for each regional and seasonal sample including 3-4 females and 3 males (except the South Georgia winter sample, where solely males were available, and 5 males were analysed; see Table 2.1 for full sampling scheme).

2.3.2 RNA extraction, library preparation and Illumina sequencing

RNA extraction was performed from frozen krill heads with the RNeasy Midi Kit (QIAGEN, Hilden, Germany). Frozen krill heads were cut on dry ice and transferred to

TABLE 2.1: Sampling data including cruise, region, station, sampling date and local time (h:min), season, latitude, longitude, sampling gear, number of individuals with sex, and sample code of the analysed individuals

Cruise	Region	Station	Date/Time	Season	Latitude	Longitude	Gear	Sex
ANT23-2	Lazarev Sea	PS69/092	23. Dec 05	summer	-65.51	-3.03	RMT 8+1	3 ♂
			01:07					4 ♀
ANT23-6	Lazarev Sea	PS69/474	27. Jun 06	winter	-61.52	2.93	RMT 8+1	3 ♂
			02:21					3 ♀
JR15004	South Orkneys	Event 56	02. Feb 16	summer	-60.30	-46.85	RMT8+1	3 ♂
			04:14					3 ♀
AMLR14	Bransfield Strait	W1311	26. Aug 14	winter	-62.50	-59.50	IKMT	3 ♂
			00:40					3 ♀
JR260B	South Georgia	Event 37	04. Jan 12	summer	-53.68	-38.56	RMT 8	3 ♂
			01:37					3 ♀
Norwegian fishing vessel	South Georgia		18. Jul 15	winter	-54.15	-35.60	continuous pumping system	5 ♂
			07:00					

1.5 ml RLT lysis buffer in tissue homogenizing Precellys® tubes (CKMix Tissue Homogenizing Kit, Bertin corp., Rockville, MD, USA). Homogenization was carried out at 4 °C in a Precellys® homogenizer with the Cryolys® cooling system (Bertin corp.) with two runs for 15 s at 5000 rpm and 10 s break. Further steps of RNA extraction were carried out according to the manufacturer's protocol of the RNeasy Midi Kit. The quality and quantity of the RNA was inspected using the NanoDrop™2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and the Agilent 2100 Bioanalyzer system (Agilent technologies, Santa Clara, CA, USA).

RNA samples were sent for sequencing to IGA Technology Services (Udine, Italy). cDNA libraries were performed with 1 µg to 2 µg RNA by using the TruSeq Stranded mRNA Sample Prep kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The poly-A mRNA was fragmented 3 minutes at 94 °C. 1X Agencourt AMPure XP beads (Agencourt Bioscience Cooperation, Beckman Coulter, Beverly, MA, USA) were used for every purification step. The RNA samples and final cDNA libraries were quantified with the Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and quality tested by Agilent 2100 Bioanalyzer Nano assay. For cluster generation on the flow cell, libraries were processed with cBot (Illumina, San Diego, CA,

USA) following manufacturer's instructions. Sequencing was carried out on paired-end mode (2 × 100 bp) on HiSeq2500 (Illumina) with a targeted sequencing depth of about 80 million reads per sample. Raw data were processed with the software `CASAVA v1.8.2` (Illumina) for both format conversion and de-multiplexing.

2.3.3 Quality control and analysis of RNA-seq data

The programme `BBDuk` from `BBMap package v36.38` (Bushnell, 2016) was used for the removal of adapter sequences and quality trimming of reads (set parameters: ktrim=r, k=23, mink=11, hdist=1, tpe tbo, qtrim=r, trimq=10, minlen=36). The quality of the trimmed reads was checked with the programme `FastQC v0.11.5` (Andrews, 2017). Since the `FastQC` reports indicated the presence of reads encoding ribosomal RNA, these reads were removed using the software `SortMeRNA v2.1` (Kopylova et al., 2012). Transcript abundance in each sample was estimated by aligning the processed paired-end reads to the *E. superba* reference transcriptome (Meyer et al., 2015) using the software `Trinity v2.4.0` (Grabherr et al., 2011) with the abundance estimation method `RSEM v1.2.26` (Li and Dewey, 2011) and the alignment tool `Bowtie2 v2.2.5` (Langmead and Salzberg, 2012). As reference, we chose the transcriptome by Meyer et al. (2015) instead of the recently developed KrillDB transcriptome (Sales et al., 2017), because preliminary alignment tests yielded approximately 10% higher alignment rates to the transcriptome by Meyer et al. (2015). Both transcript expression matrix of non-normalized counts and matrix of TMM-normalized expression values were calculated. Mapping rates to the reference transcriptome had a mean ± SD of 69.22 ± 3.37%. Differential gene expression was analysed with `edgeR` (Robinson et al., 2010). Significant differentially expressed genes (DEGs) were identified using a false discovery rate (FDR) cutoff value of 0.001 and a minimum absolute $\log_2$ fold change ($\log_2$FC) of 2. Pairwise comparisons included seasonal comparisons within each region and regional comparisons in both summer and winter between regions (Fig. 2.2). The PtR script was used to do a principal component analysis (PCA) of all differentially expressed genes. Both PC 1 and PC 2 were correlated to season accounting for overall 46.89% variation in the dataset (supplementary Figure S1), but did not show correlation to region, sex or sample processing.

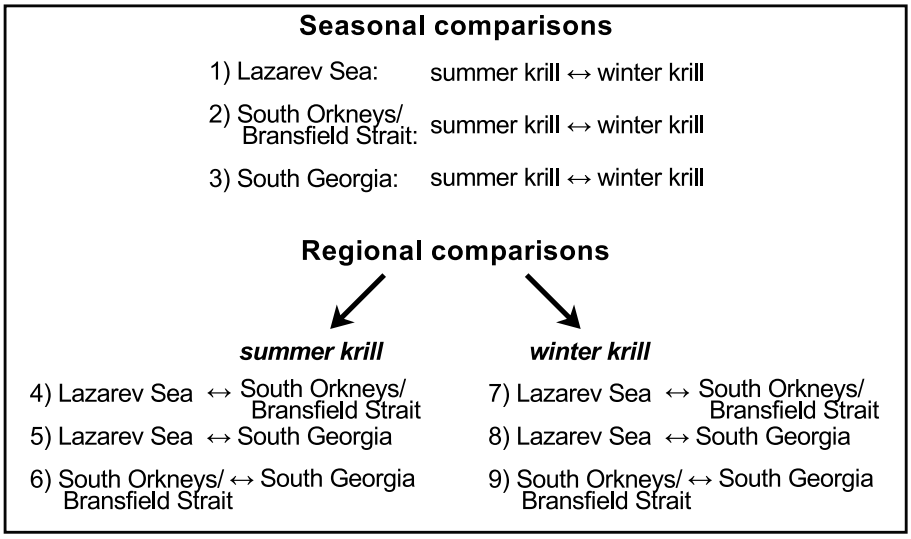

FIGURE 2.2: Pairwise comparisons tested for differentially expressed genes subdivided into seasonal comparisons between summer and winter in three different regions (1-3) and regional comparisons of summer krill (4-6) and winter krill (7-9).

To annotate the DEGs, local blastx searches against the protein UniProt databases Swiss-Prot and UniRef90 (Boutet et al., 2007) with a cutoff E value of 10^{-9} were performed using `BLAST+ v.2.5.0` (Camacho et al., 2009). From the 1929 DEGs, 693 genes could be annotated resulting in an annotation rate of 35.93%. Additional annotation information were retrieved from the UniProt website (`https://www.uniprot.org/`). To aim for a crustacean-specific annotation and functional characterization, we chose to do a manual categorization of the annotated genes rather than focussing on the enrichment of gene ontology (GO) terms. Thereby, we were also able to improve the functional characterization of DEGs for the regional and seasonal comparisons where only few DEGs were found (Table 2.2). Using the information from the UniProt website and crustacean-specific literature, if available, the annotated genes were inspected and sorted manually into categories (supplementary Table S1). For selected genes of interest, the annotation was reviewed performing blastx searches against NR using the web interface on `https://blast.ncbi.nlm.nih.gov/Blast.cgi` (Johnson et al., 2008). For contigs HACF01031034, HACF01033533, HACF01010344 and HACF01005894, improved annotation results were added to the annotation table (supplementary Table S1). For Fig. 2.3 and supplementary Figure S2 , category normalization was carried

TABLE 2.2: Number of differentially expressed genes (DEGs) identified for each tested pairwise comparison in edgeR including the number of upregulated DEGs in each sample

Test	Sample A	Sample B	Total no. of DEGs	Upregulated in sample A	Upregulated in Sample B
1	Lazarev Sea, summer	Lazarev Sea, winter	698	611	87
2	South Orkneys, summer	Bransfield Strait, winter	1121	1054	67
3	South Georgia, summer	South Georgia, winter	295	251	44
4	Lazarev Sea, summer	South Orkneys, summer	234	143	91
5	Lazarev Sea, summer	South Georgia, summer	132	69	63
6	South Orkneys, summer	South Georgia, summer	82	64	18
7	Lazarev Sea, winter	Bransfield Strait, winter	73	37	36
8	Lazarev Sea, winter	South Georgia, winter	174	24	150
9	Bransfield Strait, winter	South Georgia, winter	106	73	33

out by dividing the DEG counts per category for upregulated genes per sample by the size of the respective category (total count of DEGs within the same category). Normalized categories are shown in normalized units ranging from 0 to 1 indicating a higher importance of categories with increasing values towards 1. Top categories were defined for values higher or equal to 0.2.

2.3.4 Data archiving

Raw sequences and the transcript expression matrix of non-normalized counts have been deposited in the ArrayExpress database at `EMBL-EBI` (`www.ebi.ac.uk/arrayexpress`) under accession number `E-MTAB-7467`.

2.4 Results

2.4.1 Seasonal comparisons of gene expression in three different latitudinal regions

Highest differential gene expression was found in the seasonal pairwise comparisons in the three regions with 295 to 1121 DEGs which were mostly upregulated in summer krill (Table 2.2). Most DEGs were found in the winter-summer krill comparison of the South Orkneys/Bransfield Strait region, followed by Lazarev Sea and South Georgia. Krill from the South Georgia region had the lowest seasonal differences in gene expression compared to the other regions.

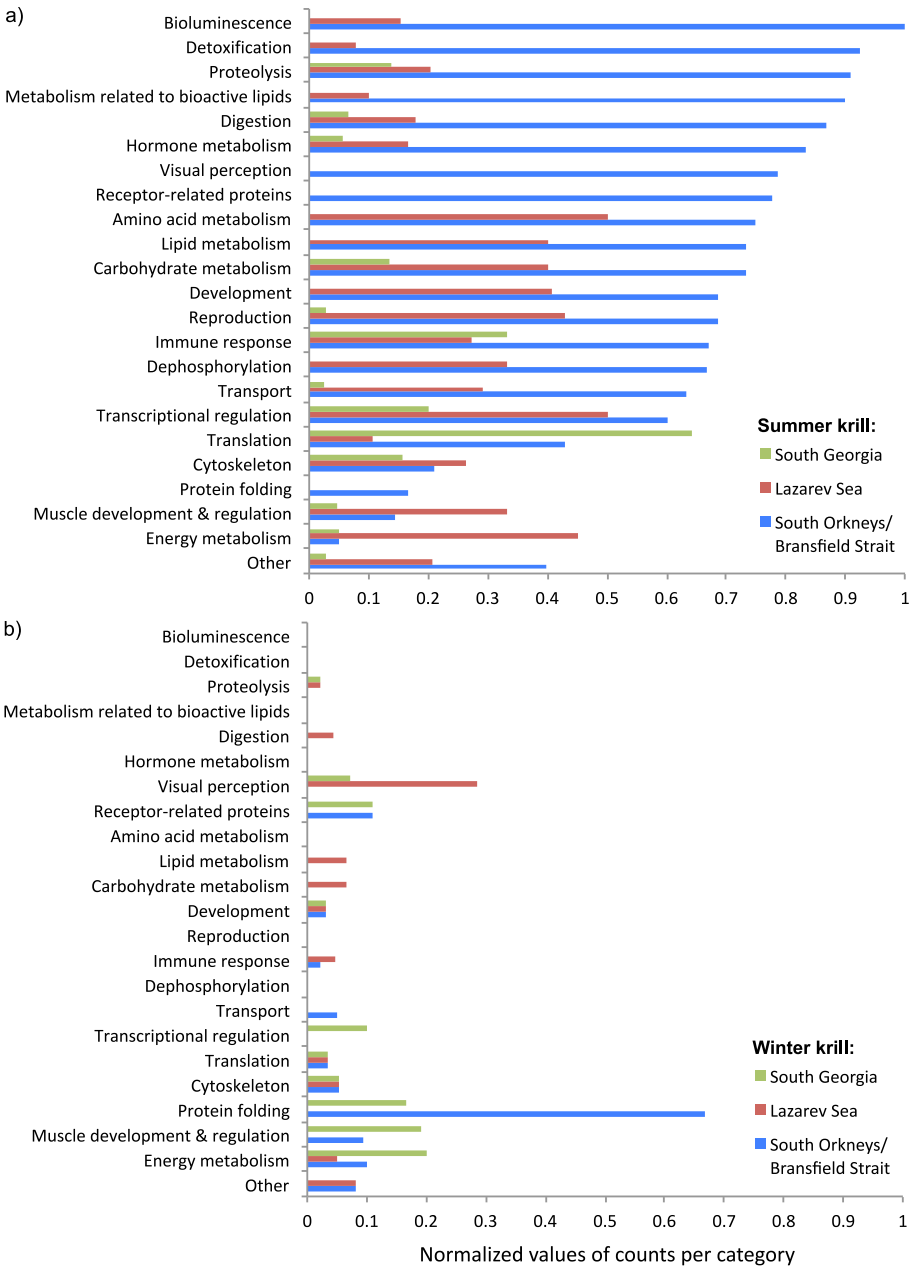

FIGURE 2.3: Bar plots showing the functional categories found in the three seasonal comparisons with a) normalized category values of up-regulated differentially expressed genes in summer krill from each region, and b) normalized category values of upregulated differentially expressed genes in winter krill from each region.

The highest functional variety of DEGs was found to be upregulated in summer krill from the South Orkneys/Bransfield Strait region (Fig. 2.3a). The 19 top categories comprised bioluminescence (1), detoxification (0.92), proteolysis (0.91), metabolism related to bioactive lipids (0.9), digestion (0.87), hormone metabolism (0.83), visual perception (0.79), receptor-related proteins (0.78), amino acid metabolism (0.75), carbohydrate and lipid metabolism (0.74 each), development and reproduction (0.69 each), immune response and dephosphorylation (0.67 each), transport (0.63), transcriptional regulation (0.6), translation (0.43) and cytoskeleton (0.21). Thereof, the first 8 categories were particularly distinct for South Orkneys/Bransfield Strait summer krill with respect to the other two studied regions.

For Lazarev Sea summer krill, 13 top categories were found: amino acid metabolism and transcriptional regulation (0.5 each), energy metabolism (0.45), reproduction (0.43), development (0.41), carbohydrate and lipid metabolism (0.4 each), muscle development & regulation and dephosphorylation (0.33 each), transport (0.29), immune response (0.27), cytoskeleton (0.26) and proteolysis (0.2). Compared to summer krill from the other two studied regions, the categories energy metabolism and muscle development & regulation had highest values in Lazarev Sea summer krill.

For South Georgia summer krill, only three top categories were identified: translation (0.64), immune response (0.33) and transcriptional regulation (0.2). Compared to the other two regions, the category translation was most pronounced in South Georgia summer krill. Most genes related to other metabolic, regulatory and biological processes were not differentially expressed in South Georgia krill.

Compared to summer krill, only few DEGs were found to be upregulated in winter krill from the three regions (Fig. 2.3b). Only one top category was found for winter krill from each studied region: protein folding (0.67) for South Orkneys/Bransfield Strait, visual perception (0.29) for Lazarev Sea, and energy metabolism (0.2) for South Georgia.

2.4.2 Detailed analysis of categories including genes with putative seasonal regulatory functions

With the aim to look for target genes with potential seasonal regulatory functions in Antarctic krill, we chose the following 7 categories for further investigation: metabolism related to bioactive lipids, hormone metabolism, visual perception, receptor-related proteins, development, reproduction, dephosphorylation and transcriptional regulation. The analysis was not restricted to the samples where these categories were enriched, but all identified DEGs within these categories were inspected. Selected members of these categories are shown in Table 2.3.

TABLE 2.3: Selected members of differentially expressed protein-coding genes of the seasonal comparisons within the categories metabolism related to bioactive lipids, hormone metabolism, visual perception, receptor-related proteins, development, reproduction, dephosphorylation and transcriptional regulation

Category	Protein	Related Swissprot / UniRef / NR ID	Putative function	Contig ID	Upregulated in
Metabolism related to bioactive lipids	Sphingomyelin phosphodiesterase	Q0VD19	sphingolipid metabolism, biosynthesis of ceramide	HACF01033273	South Orkneys, summer
	Putative glucosylceramidase 4	Q9UB00	sphingolipid metabolism, biosynthesis of ceramide	HACF01006732	South Orkneys, summer
	Neutral ceramidase	Q29C43	sphingolipid metabolism, biosynthesis of sphingosine	HACF01032702	South Orkneys, summer
Hormone metabolism	Dehydrogenase/reductase SDR family member 11	Q71R50	estrogen biosynthesis	HACF01041540	South Orkneys, summer
	Lathosterol oxidase	Q9EQS5	steroid metabolism	HACF01002294	South Orkneys, summer
	Cytochrome P450 3A21	O18993	steroid metabolism	HACF01007387	South Orkneys, summer
	Type I iodothyronine deiodinase	P24389	thyroxine metabolism	HACF01008075	South Orkneys, summer

Table 2.3 continued from previous page

Category	Protein	Related Swiss-sprot/UniRef/NR ID	Putative function	Contig ID	Upregulated in
	Beta,beta-carotene 9',10'-oxygenase	Q99NF1	retinoic acid biosynthesis	HACF01008355	South Orkneys, summer
	Aldehyde dehydrogenase family 8 member A1	Q9H2A2	retinoic acid biosynthesis	HACF01003376	Lazarev Sea, summer
	Tyrosine decarboxylase	Q95ZS2	octopamine biosynthesis	HACF01001558	Lazarev Sea, summer
	Neprilysin-1	W4VS99	breakdown of neuropeptides	HACF01002648	South Orkneys, summer
	Neuroendrocrine convertase 1	OAD61081.1	cleavage of precursors of bioactive peptides	HACF01005894	South Orkneys, summer
Visual perception	Arrestin homolog	P55274	signal transduction	HACF01056943	South Orkneys, summer
	Arrestin, lateral eye	P51484	signal transduction	HACF01005913	Lazarev Sea, winter
	Carotenoid isomerooxygenase	Q9VFS2	visual pigment biogenesis, photoreceptor development	HACF01003645	South Orkneys, summer
Receptor-related proteins	Adiponectin receptor protein	Q9VCY8	regulation of insulin secretion, glucose and lipid metabolism	HACF01002955	South Orkneys, summer
	Prolow-density lipoprotein receptor-related protein 1	Q07954	multiple functions in development, cellular lipid homeostasis, signalling and neurotransmission	HACF01040030	South Orkneys, summer
	Leucine-rich repeat-containing G-protein coupled receptor 4	A2ARI4	activator of Wnt signalling pathway, development, regulation of circadian rhythms of plasma lipids	HACF01008235	South Orkneys, summer

Table 2.3 continued from previous page

Category	Protein	Related Swiss-sprot/UniRef/NR ID	Putative function	Contig ID	Upregulated in
	Integrin beta-1-A	P12606	formation of receptor complexes with wide array of ligands	HACF01056144	South Orkneys, summer
	Translocon-associated protein subunit gamma	Q9DCF9	regulation of retention of ER resident proteins	HACF01039823	Bransfield Strait, winter
	Guanine nucleotide-binding protein subunit beta-2-like 1	O42248	recruitment, assembly and regulation of various signalling molecules	HACF01000518	South Georgia, winter
Development	Blastula protease 10	P42674	cell differentiation	HACF01031830	South Orkneys, Lazarev Sea, summer
	Protein SpAN	P98068	cell differentiation	HACF01012692	Lazarev Sea, winter
	Carbohydrate sulfotransferase 11	Q9NPF2	regulation of cell proliferation	HACF01012160	South Orkneys, Lazarev Sea, summer
	Fibrocystin-L	Q80ZA4	regulation of cell proliferation	HACF01005119	South Orkneys, Lazarev Sea, summer
	Glycoprotein 3-alpha-L-fucosyltransferase A	Q9VUL9	nervous system development	HACF01046985	South Orkneys, summer
	Neurotrophin 1	B7TB45	nervous system development	HACF01037993	Lazarev Sea, summer
	Laccase-2 isoform X4	XP_025270057.1	cuticle tanning	HACF01010344	South Orkneys, Lazarev Sea, summer
	Crustacyanin-A2 subunit	P80007	coloration	HACF01009046	Bransfield Strait, winter
	Krueppel homolog 1	P08155	metamorphosis	HACF01005245	South Georgia, winter

Table 2.3 continued from previous page

Category	Protein	Related Swissprot/UniRef/NR ID	Putative function	Contig ID	Upregulated in
Reproduction	Vitellogenin	Q6RG02	lipid transport, oogenesis	HACF01038168	South Orkneys, Lazarev Sea, summer
	Vitellogenin 2	UniRef90_V9XZL5	lipid transport, oogenesis	HACF01038626	South Orkneys, South Georgia, summer
	Hematopoietic prostaglandin D synthase	O73888	prostaglandin biosynthesis	HACF01038076	South Orkneys, Lazarev Sea, summer
	Prostamide/prostaglandin F synthase	Q8TBF2	prostaglandin biosynthesis	HACF01001735	South Orkneys, summer
	Carbonyl reductase [NADPH] 1	P48758	prostaglandin biosynthesis	HACF01007962	South Orkneys, summer
	Juvenile hormone esterase-like protein 1	ALT10383.1	potential role in degradation of methyl farnesoate	HACF01031034	South Orkneys, summer
	Juvenile hormone esterase-like carboxylesterase 1	APO14259.1	potential role in degradation of methyl farnesoate	HACF01033533	Lazarev Sea, summer
Dephosphorylation	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	P48463	modulation of phosphorylase B kinase casein kinase 2, mitogen-stimulated S6 kinase, and MAP-2 kinase	HACF01002873	Lazarev Sea, summer
Transcriptional regulation	CREB-binding protein	P45481	transcriptional regulation	HACF01024140	Lazarev Sea, summer

Genes within the category metabolism related to bioactive lipids were enriched in the South Orkneys/Bransfield Strait summer krill. Some of these genes were also found in Lazarev Sea summer krill. These genes had predicted functions in sphingolipid metabolism, such as the biosynthesis of ceramide and sphingosine, and in the biosynthesis of phosphatidylcholine.

The category hormone metabolism was found to be enriched in the South Orkneys/Bransfield strait summer krill. However, few genes within this category were also upregulated in summer krill from the other two regions. Genes within category hormone metabolism had predicted functions in steroid metabolism, thyroxine metabolism, retinoic acid biosynthesis, octopamine biosynthesis, taurine biosynthesis and the breakdown of bioactive peptides.

Genes within the category visual perception were enriched and upregulated in the South Orkneys/Bransfield Strait summer krill and in Lazarev Sea winter krill. This category comprised genes related to signal transduction, visual pigment biogenesis and eye development.

The category receptor-related proteins was enriched in the South Orkneys/Bransfield Strait summer krill. Two genes of this category were also found to be upregulated in the South Orkneys/Bransfield Strait and South Georgia winter krill. These genes coded for different receptor-related proteins with predicted functions in the formation of receptor complexes and various signalling pathways and regulatory processes, such as the Wnt and insulin signalling pathway.

The category development was found to be enriched in South Orkneys/Bransfield Strait and Lazarev Sea summer krill. Three genes of this category were upregulated in the winter krill of the three studied regions. The genes had predicted functions in cell differentiation and proliferation, nervous system development, pigmentation, metamorphosis and the regulation of other development processes.

The category reproduction was enriched in South Orkneys/Bransfield Strait and Lazarev Sea summer krill. A few DEGs within this category were also upregulated in South Georgia summer krill. This category included DEGs with putative functions as the lipid transporter vitellogenin and in the metabolism of reproduction-related hormones, in particular prostaglandin biosynthesis and juvenile hormone esterase-like carboxyesterases.

The category dephosphorylation contained only three DEGs which coded for putative phosphatases. The category was enriched in South Orkneys/Bransfield Strait and Lazarev Sea summer krill.

The category transcriptional regulation was found to be enriched in summer krill

of the three studied regions. One gene within this category was identified in South Georgia winter krill. These DEGs coded for proteins with putative RNA binding activity and regulatory functions in transcription.

2.4.3 Regional comparisons of gene expression within each season (summer and winter)

Less genes were differentially expressed in the regional pairwise comparisons in summer (82 to 234 DEGs) and winter (73 to 174 DEGs) (Table 2.2). A plot showing the results of the regional comparisons can be found in the supplementary material (Fig. S2).

Comparing the three regional summer samples from South Orkneys/Bransfield Strait, Lazarev Sea and South Georgia, least DEGs were found within the comparison South Orkneys/Bransfield Strait vs. South Georgia krill. Only few upregulated DEGs from the South Orkneys/Bransfield Strait latitudinal region could be annotated compared to the other regions in summer. In the South Orkneys/Bransfield Strait summer krill the category visual perception was enriched with respect to Lazarev Sea and South Georgia summer krill (0.21 each). Lazarev Sea summer krill showed two enriched top categories with respect to South Orkneys/Bransfield Strait summer krill only: energy metabolism (0.25) and reproduction (0.26) comprising vitellogenin-like genes only. South Georgia summer krill had two top categories with respect to Lazarev Sea summer krill only: cytoskeleton (0.32) and translation (0.68).

Comparing the three regional winter samples, only few DEGs could be annotated. The category carbohydrate metabolism (0.2) was found to be enriched for the South Georgia winter krill with respect to Lazarev Sea winter krill only.

2.5 Discussion

2.5.1 Generalisations and methodological discussion

This study investigated seasonal and regional differences in gene expression in Antarctic krill in three different latitudinal regions of the Southern Ocean (Lazarev Sea: 62°S-66°S, South Orkneys/Bransfield Strait: 60°S-63°S, and South Georgia: 54°S). Seasonal

differences between summer and winter krill were generally found to be more pronounced than regional differences in summer or winter. Most differentially expressed genes were found to be upregulated in summer krill indicating that Antarctic krill entered a less active state during winter in all studied regions. However, these seasonal differences in gene expression seemed to be less distinct in the low-latitude region South Georgia.

Differences in the seasonal gene expression pattern between the three tested regions may reflect an adaptive behaviour of Antarctic krill to the environmental conditions that krill is exposed to in the different habitats. The highest variety of functionally enriched genes was found in the South Orkneys/Bransfield Strait region which indicates that summer krill was in a highly active condition in this region with respect to winter. In particular, Antarctic summer krill from South Orkneys/Bransfield Strait were characterized by the upregulation of genes related to bioluminescence, detoxification, metabolism related to bioactive lipids, digestion, hormone metabolism, visual perception and receptor-related proteins. The upregulation of amino acid, lipid and carbohydrate metabolism and the biological categories reproduction and development in both South Orkneys/Bransfield Strait and Lazarev Sea summer krill with respect to winter supports the assumption that krill enters a state of metabolic depression and regressed development during winter in these regions (so called winter quiescence). However, genes related to energy metabolism were found to be upregulated in Lazarev Sea summer krill only. This may point to stronger seasonal differences in energy metabolism in the high-latitude region Lazarev Sea. In contrast, most genes related to metabolic, biological and regulatory processes were not found to be differentially expressed in the comparison of summer and winter krill from South Georgia which may be a response to the less extreme winter conditions in this low-latitude region (Meyer et al., 2017).

We also identified a variety of candidate genes with likely roles in the metabolism related to bioactive lipids, hormone metabolism, visual perception, as receptor-related proteins, in development, reproduction, dephosphorylation and transcriptional regulation. The selected genes may serve as target genes for future studies of seasonal rhythms in Antarctic krill.

This paper partly confirms results from a microarray study by Seear et al. (2012) who found an upregulation of genes involved in feeding and digestion, respiration, motor activity, immunity and vitellogenesis in Antarctic krill near the Antarctic Peninsula (60°S) in summer. By contrast, the present paper investigated as novel aspect the seasonal gene expression profiles of Antarctic krill from three different latitudinal regions in the Atlantic sector of the Southern Ocean. In particular, it adds seasonal gene expression data for summer and winter krill from the high-latitude region Lazarev Sea and the low-latitude region South Georgia. Moreover, the present study used a different molecular approach, RNA-seq, focussing on putative regulatory processes of seasonal rhythms in Antarctic krill and the identification of potential seasonal target genes. In contrast to the study by Seear et al. (2012), we did not find strong differences in the direct regional comparisons of the summer or winter krill samples, such as the upregulation of genes related to digestion and immunity in the South Georgia region in winter (Seear et al., 2012). Moreover, we observed a more diverse pattern of gene expression related to energy metabolism and respiration in the three studied regions.

These differences may have been caused by different methodological constraints of our RNAseq study. A limited number of Antarctic krill samples were available for sequencing. The sequenced Antarctic krill originated from the field and were sampled under highly variable conditions. Social cues, feeding condition, migratory behaviour of krill, and abiotic factors that cannot be controlled may have affected the gene expression profile detected in the samples. Moreover, differences in sampling time and station coordinates, variable sampling techniques on the different vessels and unknown parameters such as age or moulting stage of the studied individuals may have introduced variation in the dataset. To partly compensate for this variation, we used high replicate numbers for differential gene expression analysis in this RNAseq study.

In this study, the generally low level of upregulated genes in winter krill cannot directly explain the winter behaviour of Antarctic krill in some regions. For instance, the Bransfield Strait is a food-rich overwintering ground for Antarctic krill where large vertical migrations have been observed (Bernard et al., 2018; Reiss et al., 2017). Hence, gene expression needs to be activated in winter to allow for this behaviour. However,

our data analysis focussed on significant differences in seasonal gene expression. It mainly revealed genes that are important for Antarctic krill in its more active summer condition, where gene expression is apparently much larger with respect to winter. But the expression of genes below the detection level of our statistical methods may still allow for winter feeding and migration behaviour.

2.5.2 Potential influences on Antarctic krill's seasonal and regional gene expression

Our seasonal gene expression results from South Orkneys/Bransfield Strait and Lazarev Sea agree with observations in Antarctic krill from the field: a reduced metabolic activity and regressed development in winter and an enhanced metabolism, development, reproductive activity and gene expression in summer (Meyer et al., 2010; Siegel, 2012). In contrast, metabolic depression in winter and enhanced expression of reproduction- and development related genes in summer were not observed in the South Georgia krill. Yet, generally higher gene expression was also found in the South Georgia summer krill related to other processes such as translation, immune response and transcriptional regulation. These differences in seasonal gene expression discovered in krill from the South Georgia region may reflect the flexible overwintering behaviour of Antarctic krill found in this region (Schmidt et al., 2014).

Variable factors such as water temperature, reproductive timing, food availability and light regime may have influenced the regional differences observed in seasonal gene expression in Antarctic krill in this study.

However, regional differences in seasonal water temperature cannot explain why the least seasonal differences in gene expression were found in the South Georgia region. Largest seasonal differences in water temperature are observed around South Georgia, where temperature may rise above 4°C in summer and remains around 0°C in winter (Whitehouse et al., 1996). In contrast, seasonal water temperatures are more stable in the Lazarev Sea and close to the Antarctic Peninsula ranging between -1.8°C and -0.1°C (Meyer et al. (2010), and station data), but krill from these regions showed the largest differences in seasonal gene expression in our study.

The variable reproductive timing of Antarctic krill according to region and sea ice conditions (Spiridonov, 1995) may explain why in our study less seasonal differences in gene expression were observed in the Lazarev Sea compared to South Orkneys/Bransfield Strait. Summer krill samples from Lazarev Sea were obtained in the end of December, when sea ice was melting and the phytoplankton bloom just started to develop (Meyer et al., 2010). Thus, the krill from the Lazarev Sea was probably only in preparation of the spawning season. On the contrary, the South Orkneys/Bransfield Strait krill was caught in an ice-free region in the beginning of February, where krill was in the middle of the spawning period and probably in a more active condition than in Lazarev Sea. The high metabolic demand of the South Orkneys/Bransfield Strait summer krill is reflected by the high proportion of detoxification genes found in this region.

Annual feeding conditions may have especially affected the different behaviour of Antarctic krill in the lower-latitudinal region South Georgia, where krill is exposed to less extreme winter conditions compared to the other two regions. South Georgia is ice-free throughout the year and prolonged periods of phytoplankton blooms occur in this area. In winter, Antarctic krill from South Georgia was observed to be feeding on phytoplankton and seabed detritus, and contained lower lipid stores compared to Antarctic krill from Bransfield Strait and Lazarev Sea (Schmidt et al., 2014). Therefore, seasonal differences in gene expression may be less pronounced in that area.

There are indications that light regime and an endogenous timing system may play a major role in controlling the flexible behaviour and life cycle of Antarctic krill in different latitudinal regions of the Southern Ocean. Recently, a two-year lab experiment has shown that the latitudinal light regime (photoperiod, the day length) affected seasonal cycles of growth, maturity, feeding and lipid content of Antarctic krill (Höring et al., 2018). Seasonal patterns of growth, feeding and maturity were also observed under constant darkness which indicated the presence of an endogenous timing system that was most likely entrained by light regime prior to the experiments. Critical photoperiods for female maturity were found to be higher under the simulated high-latitude light regime which pointed to a flexible seasonal timing system in Antarctic krill under different latitudinal photoperiods. Piccolin et al. (2018b) demonstrated the

effect of photoperiod on the seasonal cycle of growth, enzyme activity and oxygen consumption in Antarctic krill. The authors linked the results to the seasonal expression of circadian clock genes and suggested their involvement in the seasonal timing mechanism in Antarctic krill.

These findings reveal that photoperiod is an important *Zeitgeber* for Antarctic krill that seems to entrain its seasonal timing system under the variable photoperiodic conditions in the Southern Ocean. The less pronounced seasonal cycle of Antarctic krill observed around South Georgia may therefore be partly controlled by the less extreme seasonal light conditions in that region and krill's endogenous clock. The photoperiodic seasonal timing system may be complemented by other factors such as food supply as explained above.

2.5.3 Target genes and their putative functions in Antarctic krill

We identified regulatory genes with multiple functions that may play an important role in the control of seasonal physiology and behaviour in Antarctic krill. These target genes were selected from annotated genes with putative regulatory functions that were differentially expressed between summer and winter krill in this study. The functional roles of these genes still need to be validated in Antarctic krill in future laboratory experiments. Moreover, controlled laboratory experiments may be conducted to test if these genes are rhythmically expressed under different light regimes and if they are effectively involved in the regulation of seasonal life cycle events in Antarctic krill. Thus, our study establishes a basis for future laboratory studies to further elucidate the molecular mechanisms of seasonal rhythms in Antarctic krill. In the following, we will describe the potential functional roles of our proposed seasonal target genes.

We identified several genes that are involved in the metabolism of bioactive lipids. The biosynthesis pathway of sphingolipids such as ceramide and sphingosine play a key role in the regulation of these bioactive compounds. Bioactive lipids have been shown to mediate stress-related responses and processes such as cell proliferation and differentiation, apoptosis and inflammation (Hannun and Obeid, 2008). Ceramide is also involved in the induction of protein dephosphorylation by activating Ser-Thr

phosphatases such as PP2A (Chalfant et al., 2004), potentially affecting insulin signalling and metabolism (Hannun and Obeid, 2008). The metabolism of bioactive lipids may therefore be involved in the regulation of growth, metabolism and immune response in Antarctic krill.

Target genes with putative functions in the metabolism of different hormones may play a role in the regulation of hormone levels in Antarctic krill. These included for example genes with functions in steroid, thyroxine, retinoic acid and octopamine metabolism and the breakdown of bioactive peptides. In crustaceans, ecdysteroids and vertebrate-type steroids mediate the regulation of moulting and reproduction (Lafont and Mathieu, 2007). Thyroxine and retinoic acid might have similar functions as the insect juvenile hormone, such as the regulation of development and reproduction (Laufer and Biggers, 2001). In the European hamster, the thyroid hormone metabolism has been associated with the seasonal timing of reproduction (Sáenz de Miera et al., 2014) and it remains to be clarified if thyroxine possesses a similar function in Antarctic krill. Octopamine affects heart beat and behaviour in lobsters (Battelle and Kravitz, 1978; Kravitz, 1988). We also identified two genes involved in the breakdown of bioactive peptides: neprilysin-1 which was found to inactivate the circadian neurotransmitter pigment dispersing factor (Isaac et al., 2007); and neuroendocrine convertase 1, a prohormone processing enzyme that was found to play a role in the reproduction processes in abalone (Zhou and Cai, 2010).

For visual perception, we identified arrestin, which is an important component of the visual transduction system (Montell, 2012), and carotenoid isomerooxygenase, a key enzyme for the biogenesis of visual pigments (Voolstra et al., 2010).

Receptor-related proteins play an important role for signal transduction in the nervous system and may have regulatory roles in various seasonal processes in Antarctic krill such as growth and metabolism. These candidate genes include the adiponectin receptor which is known to regulate insulin secretion, glucose and lipid metabolism in Drosophila (Kwak et al., 2013), and supports the maintenance of skeletal muscle fiber in crustaceans (Kim et al., 2016). The prolow-density lipoprotein receptor-related

protein 1 may have multiple functions in Antarctic krill such as in development, cellular lipid homeostasis, endocytosis and the regulation of signalling pathways (Franchini and Montagnana, 2011). The leucine-rich repeat-containing G-protein coupled receptor 4 may be involved in the Wnt/β-catenin signaling pathway and development (Carmon et al., 2011) and the regulation of circadian rhythms of plasma lipids (Wang et al., 2014). Integrins form cell-surface-adhesion receptors with functions for instance in development, immune response and signalling (Harburger and Calderwood, 2009). The translocon-associated protein (TRAP) subunit gamma has been suggested to contribute to cellular homeostasis during stress responses such as glucose deprivation (Yamaguchi et al., 2011), and may therefore play a similar role in the winter quiescence of Antarctic krill. The Guanine nucleotide-binding protein subunit beta-2-like 1(alias RACK1) has been found be involved in developmental processes (Vani et al., 1997), maturation (Ron and Mochly-Rosen, 1994), and immune response in crustaceans (Jia et al., 2016).

As candidate gene for the future investigations of reproductive processes in Antarctic krill, we propose the lipid transport molecule vitellogenin. It is an essential component in the process of egg maturation (Krishnan et al., 2008), but may also be required for other processes with high energy demand such as growth and moulting. The hormone-like prostaglandins have been related to the regulation of ovarian maturation in crustaceans (Wimuttisuk et al., 2013). We also identified genes closely related to juvenile hormone esterase-like carboxylesterases which may potentially degrade and thereby inactivate methyl farnesoate in crustaceans (Lee et al., 2011). Methyl farnesoate was found to promote both reproductive maturation and moulting in crustaceans (Reddy et al., 2004).

We propose target genes that may affect various developmental and growth-related processes during the seasonal cycle of Antarctic krill. These genes coded amongst others for the blastula protease 10 (alias SpAN), which has been functionally described during sea urchin embryogenesis (Lepage et al., 1992), and may also play a role for cell differentiation in Antarctic krill. Carbohydrate sulfotransferase 11 (alias chondroitin 4-sulfotransferase 1) has been linked to the Wnt signalling pathway affecting developmental processes such as cell proliferation (Nadanaka et al., 2008). From structural

similarities, fibrocystin-L (gene PKHD1) is proposed to play a role for cell proliferation, adhesion and repulsion (Onuchic et al., 2002). Potential candidate genes for nervous system development comprise for instance glycoprotein 3-alpha-L-fucosyltransferase A which catalyzes the glycosylation of neural-specific proteins (Yamamoto-Hino et al., 2010) and neurotrophin 1, a secreted protein with regulatory functions in the nervous system (Zhu et al., 2008). Laccase 2 is a phenoloxidase gene that has been related to cuticle tanning in insects (Arakane et al., 2005) and may have a similar function in Antarctic krill, but may also be involved in immune response in crustaceans (Clark and Greenwood, 2016). We also found crustacyanin-A2 subunit which is known to generate the colouration of the lobster shell (Cianci et al., 2002). Krüppel homolog 1 seems to be linked to juvenile hormone during metamorphosis in Drosophila (Minakuchi et al., 2008), but may also regulate development in an independent pathway in crustaceans (Miyakawa et al., 2018). The role of krüppel may be versatile as effects have also been observed on vitellogenin expression in the fat body and ovarian maturation and growth in insects (Song et al., 2014) and on fat metabolism in nematods (Zhang et al., 2009).

Dephosphorylation by phosphatases represents another important step in the post-translational regulation of proteins. We found for instance the serine/threonine-protein phosphatase 2A (PP2A) which may contribute to a variety of processes including ovarian maturation (Zhao et al., 2017), visual perception (Wang et al., 2008) and circadian timing (Pegoraro and Tauber, 2011).

On the transcriptional level, we found for example a gene coding for the CREB-binding protein, which is a transcriptional coactivator affecting circadian behavioural activity (Maurer et al., 2016), postembryonic development (Roy et al., 2017) and eye development (Kumar et al., 2004) in insects and may therefore have similar functions in Antarctic krill.

2.5.4 Application to future studies of seasonal rhythms

This study provides further understanding of the gene expression profiles behind the flexible seasonal behaviour of Antarctic krill in different latitudinal regions of the

Southern Ocean. It further discusses the potential environmental factors that may affect the observed regional differences in seasonal gene expression. Our data suggests that a number of genes related to sphingolipid metabolism, hormone metabolism, visual perception, receptor-related proteins, reproduction, development, dephosphorylation and transcriptional regulation may have regulatory functions in krill's seasonal physiology. This study provides a basis for future laboratory studies where the effect of different environmental factors such as light regime or food supply on the expression of these seasonal candidate genes may be tested.

Genes related to insulin and the juvenile hormone like signalling pathway in crustaceans may be of special interest. Insulin signalling (Sim and Denlinger, 2013) and the absence of juvenile hormone has been related to reproductive diapause and associated metabolic processes in insects (Liu et al., 2017) and may have a similar role in Antarctic krill for the preparation of winter quiescence.

Even though seasonal differences in clock gene expression could not be detected in this study, genes involved in the circadian clock and downstream pathways may still be appropriate for the investigation of seasonal rhythms in Antarctic krill. Clock genes were found to affect photoperiodic diapause in insects (Ikeno et al., 2010) and a potential seasonal role has also been suggested for Antarctic krill (Piccolin et al., 2018b). However, a seasonal timing system independent of the circadian clock may also exist (Bradshaw et al., 2006) and other levels of seasonal control may comprise non-coding RNAs or epigenetic modifications (Helm and Stevenson, 2014).

2.6 Conclusion

This study examined seasonal and regional differences in gene expression in Antarctic krill from three latitudinal regions of the Southern Ocean (Lazarev Sea, South Orkneys/Bransfield Strait, South Georgia) with the additional goal to identify target genes with putative regulatory functions in the seasonal cycle of Antarctic krill. The studied regions were characterized by different latitudinal light regimes with more extreme annual changes of photoperiod and therefore more severe winter conditions experienced by Antarctic krill in higher latitudinal regions such as Lazarev Sea. We found a downregulation of most differentially expressed genes in the winter samples

indicating that Antarctic krill entered a less active state in winter. However, seasonal differences in gene expression seemed to be less pronounced in Antarctic krill from the South Georgia region compared to the South Orkneys/Bransfield Strait and Lazarev Sea region. In the South Georgia krill, the seasonally differential expression of most genes related to metabolic, biological and regulatory processes was missing. This may be explained by a less pronounced seasonal cycle of Antarctic krill in this low-latitude region that is characterized by less extreme light conditions, milder winters with no sea ice coverage and enhanced food availability. We propose that seasonal gene expression may be partly governed by a photoperiodic timing system that may influence the flexible behaviour and physiology of Antarctic krill in different latitudinal regions of the Southern Ocean. Moreover, we identified target genes with potential regulatory roles in the seasonal cycle of Antarctic krill including processes of growth, reproduction and metabolism. These genes are functionally linked to different regulatory pathways such as hormone and sphingolipid metabolism, and juvenile hormone like and insulin signalling pathways and may serve as starting point for understanding the molecular mechanisms of seasonal rhythms in Antarctic krill.

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3. Publication 2

Light regime affects the seasonal cycle of Antarctic krill (*Euphausia superba*): impacts on growth, feeding, lipid metabolism, and maturity

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3.1 Abstract

Light regime is an important *Zeitgeber* for Antarctic krill (*Euphausia superba*), which seems to entrain an endogenous timing system that synchronizes its life cycle to the extreme light conditions in the Southern Ocean. To understand the flexibility of Antarctic krill's seasonal cycle, we investigated its physiological and behavioural responses to

different light regimes and if an endogenous timing system was involved in the regulation of these seasonal processes. We analysed growth, feeding, lipid content, and maturity in a 2-year laboratory experiment simulating the latitudinal light regimes at 52°S and 66°S and constant darkness under constant food level. Our results showed that light regime affected seasonal cycles of growth, feeding, lipid metabolism, and maturity in Antarctic krill. Seasonal patterns of growth, feeding, and maturity persisted under constant darkness, indicating the presence of an endogenous timing system. The maturity cycle showed differences in critical photoperiods according to the simulated latitudinal light regime. This suggests a flexible endogenous timing mechanism in Antarctic krill, which may determine its response to future environmental changes.

3.2 Introduction

Concerns are growing about the impact of global warming on the Antarctic marine ecosystem. The observed changes in sea-ice extent and zooplankton distribution may lead to trophic mismatches and thereby profound changes in the Southern Ocean food web (Atkinson et al., 2004; Steinberg et al., 2015). To be able to predict future changes, we need to better understand the adaptive potential of polar key organisms such as the Antarctic krill (*Euphausia superba*) (Meyer, 2010).

Antarctic krill's success in the Southern Ocean likely originates from its ability to synchronize its life cycle to local photoperiod and food supply. It has evolved seasonal patterns of growth, lipid turnover, metabolic activity (Meyer et al., 2010), and maturation (Kawaguchi et al., 2007) that bring an evolutionary advantage to survive in an environment with strong seasonal fluctuations of sea-ice extent, photoperiod, and primary production. These seasonal patterns seem to vary according to latitudinal region, as it has been observed that Antarctic krill near South Georgia (54°S) had lower lipid stores and higher feeding activities in winter compared with regions at higher latitudes where near-constant darkness during winter limits food supply (Schmidt et al., 2014). However, the mechanisms shaping these seasonal rhythms remain poorly understood.

Photoperiod seems to play a major role in the modulation of the seasonal rhythms of Antarctic krill. Laboratory experiments revealed that photoperiod affected seasonal patterns of growth (Brown et al., 2010), maturity (Hirano et al., 2003; Teschke et al., 2008; Brown et al., 2011), feeding, and metabolic activity (Teschke et al., 2007). It is not yet clear if light regime also promotes acclimatization to the varying seasonal conditions in different latitudinal habitats of Antarctic krill.

An endogenous timing system may be involved in the regulation of seasonal rhythms in Antarctic krill. Seasonal patterns of maturity were observed to persist under constant darkness (Brown et al., 2011), indicating an endogenous timing system that maintained the rhythm even if the *Zeitgeber* (environmental cue) was absent (= concept of a biological clock). Recent studies suggest that Antarctic krill possesses a circadian clock that regulates its daily metabolic output rhythms and is entrained by photoperiod (Mazzotta et al., 2010; Teschke et al., 2011). However, it is unknown if the circadian clock is also involved in the timing of seasonal events in Antarctic krill.

This study aims to investigate the effect of different light regimes on growth, feeding, lipid metabolism, and maturity in Antarctic krill, as well as the involvement of an endogenous timing system in the modulation of seasonal rhythms. We analyse a unique data set from multiyear laboratory experiments simulating different latitudinal light regimes (52°S, 66°S, constant darkness) and constant food supply over 2 years. We will test (i) if light regime stimulates seasonal patterns of growth, feeding, lipid metabolism, and maturity; (ii) if different latitudinal light regimes cause different seasonal patterns; and (iii) if seasonal patterns persist under constant darkness indicating an endogenous timing system.

3.3 Materials and Methods

3.3.1 Antarctic krill collection and maintenance prior to the experiments

Antarctic krill were caught with a rectangular mid-water trawl (RMT 8) on 12 February, 2013 (66°47′S, 65°8′E) during the voyage V3 12/13 of RSV Aurora australis and on 15 January, 2015 (65°31′S, 141°23°E) during voyage V2 14/15. The sampling methods are described in detail by King et al. (2003). The sampled Antarctic krill arrived at

the Australian Antarctic Division aquarium in Hobart on 22 February, 2013 and on 25 January, 2015, respectively. For acclimation and for keeping of Antarctic krill until the start of the experiments, they were transferred to 800 L tanks (temperature 0.5 °C) that simulated the natural light regime at 66°S. A detailed description of the Antarctic krill aquarium facility and the simulated light regime can be found in Kawaguchi et al. (2010).

3.3.2 Photoperiodic-controlled laboratory experiments

Long-term laboratory experiments were conducted over a period of 2 years starting in January 2015. Three different light regimes were tested, simulating (1) natural light conditions at 52°S, (2) natural light conditions at 66°S, and (3) constant darkness (DD) (Figs. 3.1a, 3.1b). For each treatment, 250 Antarctic krill were transferred from the 800 L acclimation tanks to a 250 l experimental tank connected to a recirculating chilled seawater system with a constant water temperature of 0.5 °C. For the initial experimental set-up, Antarctic krill collected in 2013 were used (tanks A, B, E, F).

However, due to increased mortality in tank A (treatment DD), an additional tank for treatment DD (tank K) was set up in the beginning of March 2015 using freshly caught Antarctic krill collected in 2015. The three different light conditions were simulated within black lightproof plastic containers, one for each experimental tank, using twin fluorescent tubes (Osram L18W/640 Cool White) with a marine blue gel filter (Marine Blue 131; ARRI Australia Pty. Ltd.). Light adjustment under treatments 52°S and 66°S was carried out using a PC-controlled timer and dimming system (`winDIM version 4.0e`; EEE, Portugal) with a maximum light intensity of 100 lx (photon flux = 1.3 µmol m^{-1} s^{-2}) during midday in January (corresponds to 1% light penetration at 30 m depth). According to the light regime, photoperiod and light-intensity profiles were adjusted at the beginning of each month for each treatment. The simulated light-intensity profiles for each treatment and month can be found in the supplementary material of the publication that is available online on the journal webpage.

The food level was held constant to remove that effect from our experiments because we solely wanted to identify the effect that light regime had on the seasonal cycle of Antarctic krill. Antarctic krill were fed daily between the hours of 08:30 and 09:30

FIGURE 3.1: Long-term lab experiments at the Australian Antarctic Division: a) Experimental set-up and tested hypotheses, b) Simulated light regimes 55°S and 66°S.

and the water flow in the tanks was turned off for approximately 2 h to ensure feeding. The food comprised three live laboratory-cultured algae (final concentrations were 1.5×10^4 cells mL^{-1} of *Phaeodactylum tricornutum* Bohlin, 1897, 2×10^4 cells mL^{-1} of *Geminigera cryophila* (D.L. Taylor and C.C. Lee) D.R.A. Hill, 1991, 2.2×10^4 cells mL^{-1} of *Pyramimonas gelidicola* McFadden, Moestrup and Wetherbee, 1982), three types of commercial algal paste (1×10^4 cells mL^{-1} of *Thalassiosira weissflogii* (Grunow) G. Fryxell and Hasle, 1977 "TW 1200TM", 5.1×10^4 cells mL^{-1} of *Isochrysis* Parke, 1949 "Iso 1800TM", 4.8×10^4 cells mL^{-1} of *Pavlova* Butcher, 1952 "Pavlova 1800TM"; Reed Mariculture, USA), and two types of prawn hatchery feeds (0.5 g of FRiPPAK FRESH

TABLE 3.1: Sampling scheme of the long-term experiment. Carapace length, digestive gland length and maturity score from these krill (*E. superba*) were used for analysis of growth, feeding and maturity in this study. For lipid content analysis a reduced dataset was analysed. Month of the experiment is abbreviated as M.

Treatment	Tank	M 2-6	M 7-13	M 14-17	M 18-19	M 20-21	M 22	M 23	M 24
52°S	E	10†	6†	6†	6†		8		
52°S	F	10	6	6	6		6	8	
66°S	B	10†	6*†	6†	6†		6	8	
DD	A	10†	6†	6†					
DD	K			6	6†	6	6	10	16

Given numbers represent sampled individuals per month (n = 617).
*: in July15 (month 7) one additional krill was sampled
†: in April 15, July 15, October 15, January 16, April 16 and July 16
(months 4, 7, 10, 13, 16, 19) lipid content of 6 krill per month was analysed

#1CAR, 0.5 g of FRiPPAK FRESH #2CD; INVE, Thailand). Antarctic krill under treatment DD were fed in dim red light. Moults and dead Antarctic krill were removed regularly from the tanks.

Antarctic krill sampling of 6–10 individuals per tank and month was carried out in the middle of each month during midday starting in February 2015 (for treatment DD in dim red light). Due to different rates of mortality in the tanks, the sampling scheme had to be adjusted during the course of the experiment (Table 3.1) to assure sampling over the whole experimental period. Due to the problem with increased mortality under treatment DD mentioned above, we decided to sample tanks A and K sequentially to ensure the completion of the experiment over the 2-year period.

Live Antarctic krill was inspected under a stereomicroscope and the sex was determined. Pictures of the carapace and the sexual organs (female thelycum and male petasma) were taken with a Leica DFC 400 camera system (Leica Microsystems, Germany). Carapace length (tip of the rostrum to posterior notch) and digestive gland length (longest axis through carapace) were determined from the pictures within the Leica `DFC Camera` software `version 7.7.1` (Leica Microsystems, Switzerland).

After visual inspection, the sampled Antarctic krill was immediately frozen in liquid nitrogen. Frozen samples were stored at $-80\,^{\circ}\mathrm{C}$.

The first inspection of the sex ratio within the experimental tanks revealed that females dominated, with proportions of 71%– 85% per tank.

3.3.3 Growth analysis

Carapace length was used as a proxy for growth in the experiments. Antarctic krill were sampled randomly from each experimental tank; thus, a general trend observed in the carapace length data are assumed to display the general trend of growth.

The data analysis was performed in `RStudio version 1.0.136` (RStudio Team, 2016). Before the modelling process, a Pearson's product moment correlation was conducted to determine a potential difference in growth pattern between male and female Antarctic krill; thus, the need for separate models for each sex. Due to the strong correlation ($r = 0.82$, $p < 0.001$) between males and females, based on the mean carapace length for each sex across all treatments, data from both sexes were combined ($n = 617$). To investigate the long-term trend (variable "time") and the seasonal variability (variable "month") of Antarctic krill growth for each "treatment" (light regime), a generalized additive mixed model (GAMM) with a Gaussian distribution was used. An additive model was chosen over a linear one to resolve the nonlinear relationship of the response and explanatory variables. The GAMM takes the structure as specified by Hastie and Tibshirani (1987) and was fitted using the `gamm` function in the `mgcv` package (Wood, 2007). Random effects for "tank" were included in the model to account for potential dependencies between individuals from the same tank. Prior to the modelling process, temporal autocorrelation was examined using the `acf` function in `R`. Time series are often subject to latitudinal dependencies between data points and not accounting for the autocorrelation can result in biased estimates of model parameters (Panigada et al., 2008). As autocorrelation was neither detected, nor evident in residual analysis during model validation, no temporal autocorrelation term was included in the final model.

Smoothed terms were fitted as regression splines (variable "time"), apart for the variable "month", which was modelled using cyclic cubic regression splines, setting knots manually between 1 (January) and 12 (December) to account for the circular nature of this term. Differences in temporal pattern between the three light regimes (52°S, 66°S, DD) were implemented using the `by`-argument of the `gamm` function, which allows for the creation of separate smoothers for each level of the treatment factor

(light regime) over the temporal variables "month" and "time". Hence, separate parameter estimates for the temporal variables are obtained for each treatment level. To avoid overfitting, the smooth function of the variable "month" was manually restricted to k = 5. Model selection was conducted using manual stepwise-backward selection based on Akaike's information criterion (AIC) (Akaike, 1981). If the addition of a term led to an AIC decrease of >2 per degree of freedom, or an increase of the adjusted R^2, or if the term was significant, then the term was included in the model. Model fit was examined by residual analysis.

3.3.4 Feeding Analysis

The feeding index (%) was calculated as digestive gland length $\times$ (carapace length)$^{-1}$ $\times$ 100. Data of males and females were combined because of the strong correlation of monthly mean values (Pearson's product moment correlation, r = 0.95, p < 0.001). To investigate a temporal pattern in the feeding index of Antarctic krill for each treatment, a GAMM was employed as described above (section Growth analysis). The smooth function of the variable "time" was manually restricted to k = 6.

3.3.5 Lipid content analysis

Every 3 months from April 2015 to July 2016, six replicate samples from each treatment were tested for their lipid content. Lipids were extracted from the carapace, which was separated from the frozen samples with a scalpel on dry ice prior to extraction. Lipid extraction was performed with dichloromethane:methanol (2:1, v:v) according to the method described by Hagen (2000). Lipid content was determined gravimetrically and was calculated in percentage of dry mass. One data point (sample code "Jan16_E04") was removed due to the negative value of lipid content that indicated incorrect measurement for that individual.

Lipid content differed between male and female Antarctic krill (Pearson's product moment correlation of pooled monthly mean values, r = 0.26, p = 0.62); therefore, statistical analysis was performed separately for each sex. Data for males were not sufficient for robust modelling and only females were considered for this analysis (n = 83). Only one tank for each time point and treatment was available, therefore a

mixed model to resolve a potential tank effect could not be employed. For treatment DD, five samples were available from a second tank, but these were not sufficient for the inclusion of a random effect. Therefore, a generalized additive model (GAM) was employed to examine the temporal pattern of female Antarctic krill lipid content, following the protocol described in section Growth analysis. The smooth function of the variable "time" was manually restricted to k = 6. Because the variable "month" was not significant, it was excluded from the final model.

3.3.6 Maturity Analysis

The maturity stage of the sampled Antarctic krill was assessed by analysing pictures of the external sexual organs according to Makarov and Denys (1980) and Thomas and Nash (1987). A maturity score was assigned using the method of Brown et al. (2010) and Brown et al. (2011). Due to the ordinal characteristic of the maturity scores, Pearson correlation of monthly mean values could not be performed with the data set. Therefore, we visually inspected the relationship between maturity score and hours of light in males and females. Seasonal maturity scores differed between male and female Antarctic krill (Fig. 3.2); therefore, statistical analysis was performed on females only (n = 493), as there were not sufficient data to allow for modelling males separately. To investigate the temporal pattern of maturity of female Antarctic krill for each treatment, a GAMM was employed as described in section Growth analysis. Because model residuals were autocorrelated, an auto-regressive correlation structure of the order 1 was added, which improved model fit and resolved the dependencies between residuals. Maturity scores are represented as whole numbers and take values between 3 and 5. Therefore, the GAMM was initially modelled using a Poisson distribution with a logarithmic link function between predictor and response. Due to overdispersion, a negative binomial GAMM had to be used. The smooth function of the variable "time" was manually restricted to k = 6.

To examine differences in the critical photoperiod between latitudinal light regimes 52°S and 66°S, a logistic regression was used. As only full maturity was investigated, maturity scores <5 were set to zero and full maturity (score = 5) was set to one in all

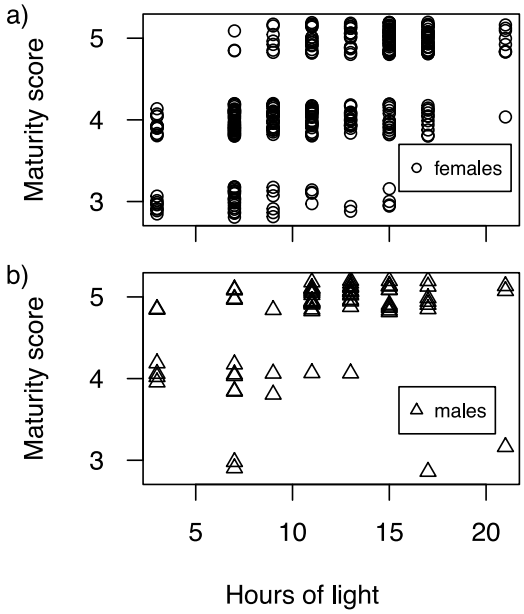

FIGURE 3.2: Relationship between maturity score and hours of light in a) females and b) males.

samples, resulting in a data set of zeros and ones. The relationship between full maturity of female Antarctic krill and photoperiod was modelled with a binomial generalized linear mixed model (GLMM) with a logit function between predictor and response and an interaction term for factor "treatment" and continuous variable "hours of light". The model was fitted using the `glmer` function from the `lme4` library. To account for dependencies between individuals from the same tank, random effects for "tank" were included in the model. Model fit was assessed by constructing a receiver operating characteristic (ROC) curve using the `pROC` package in R, where the area under the curve (AUC) indicates the goodness of fit (Boyce et al., 2002). Values below 0.7 are considered poor and 1.0 represents a perfect fit (Cumming, 2000). The critical photoperiod (= photoperiod, when the probability to be fully mature is 50%) was predicted from the 95% confidence intervals.

3.3.7 Data archiving

Processed data have been uploaded to the database PANGAEA and can be accessed under `https://doi.pangaea.de/10.1594/PANGAEA.885889`.

3.4 Results

3.4.1 Growth Analysis

Carapace length ranged from 8.1 to 19.02 mm with a mean (±SD) of 11.71 mm (±1.61 mm) across the whole data set. The GAMM (model M1; Table 3.2) revealed significant seasonal and interannual patterns in growth, which were similar across all treatments (Figs. 3.3a, 3.3b). Shrinkage was observed in the beginning of the experiments. A significant seasonal variability with shrinkage towards austral winter (June to August) and growth towards austral summer (December to February) was observed under treatments 52°S and DD (not significant under treatment 66°S).

3.4.2 Feeding

The feeding index data ranged from 25.15% to 66.09% with a mean (±SD) of 42.00% (±6.58%).

The GAMM revealed significant changes in the feeding index over time (model M2; Table 2). We observed an increase of the feeding index throughout the experimental period in all treatments and a final stagnation in treatments 52°S and DD (Figs. 3.4a, 3.4b). The seasonal trend differed between treatments. In treatment 52°S, the feeding index strongly increased during the autumn period (March to May) with a subsequent decrease and stabilization during the rest of the year. The seasonal trend in treatment 66°S was very weak and will therefore not be described further. In treatment DD, the feeding index increased over a longer period (March to July) and decreased during the rest of the year.

3.4.3 Lipids

The lipid content data of males and females ranged from 2.53% to 57.75% with a mean (±SD) of 17.04% (±9.12%). The GAM considering female lipid content data only (model M3; Table 2) revealed significant differences in temporal variability of lipid content between the experimental treatments (Fig. 3.5). Even though the variable "month" was not significant, a resembling seasonal pattern was observed in the interannual trend under treatment 66°S with an increase towards austral winter and

TABLE 3.2: Model results, showing model statistics for parametric coefficients (estimates, standard errors (SD), z- or t-values and p-values), a measure of explained variance of the model (Deviance or Adjusted R^2 (Adj. R^2)) and non-parametric terms where applicable (estimated degrees of freedom (edf), F-statistic and p-values).

Intercept	**Estimate**	**SD**	***t*-value**	***p*-value**	**Adj. R^2**	
M1	11.67	0.12	101	< 0.001	0.39	
	Treatment 52°S		Treatment 66°S		Treatment DD	
Variable	**Time**	**Month**	**Time**	**Month**	**Time**	**Month**
Smooth (edf)	2.84	1.74	3.56	0.39	3.32	1.8
F-value	32.8	1.95	20.65	0.15	20.31	2.46
p-value	<0.001	0.003	<0.001	0.13	<0.001	<0.001
Intercept	**Estimate**	**SD**	***t*-value**	***p*-value**	**Adj. R^2**	
M2	42.08	0.22	189.8	< 0.001	0.64	
Variable	**Time**	**Month**	**Time**	**Month**	**Time**	**Month**
Smooth (edf)	2.87	5.09	2.55	1.47	3.13	2.79
F-value	41.42	4.2	92.84	0.39	64.52	1.4
p-value	<0.001	<0.001	<0.001	0.041	<0.001	<0.001
Intercept	**Estimate**	**SD**	***t*-value**	***p*-value**	**Deviance**	
M3	17.21	0.77	22.33	< 0.001	50.9%	
Variable	**Time**		**Time**		**Time**	
Smooth (edf)	3.58		3.82		1.0	
F-value	1.16		14.97		0.05	
p-value	0.3		<0.001		0.82	
Intercept	**Estimate**	**SD**	***t*-value**	***p*-value**	**Adj. R^2**	
M4	1.43	0.01	134.9	< 0.001	0.45	
Variable	**Time**	**Month**	**Time**	**Month**	**Time**	**Month**
Smooth (edf)	1.0	4.14	1.88	4.07	3.33	2.88
F-value	6.1	15.0	5.84	7.7	4.58	1.19
p-value	0.014	<0.001	0.002	<0.001	0.04	<0.001
M5	**Estimate**	**SD**	***z*-value**	***p*-value**	**AUC**	
Intercept	-6.09	0.78	-7.86	< 0.001	0.77	
Hours of Light	0.49	0.06	7.86	< 0.001		
Treatment	1.22	1.25	0.98	0.4		
Interaction: Light * Latitude	-0.16	0.09	-1.73	0.084		

Significant p-values of explanatory variables are in bold.
M1: GAMM for carapace length over time for each treatment with random effects for tank
M2: GAMM for feeding index over time for each treatment and random effects for tank
M3: GAM for lipid content of females over time for each treatment
M4: Negative binomial GAMM for female maturity over time for each treatment with random effects for tank and AR1-correlation structure
M5: Binomial GLMM for full maturity of females in relation to hours of light with interaction term for treatment (52°S and 66°S) and random effects for tank effect, AUC = 'Area under the curve' from ROC-curve analysis serves as an indication of model fit

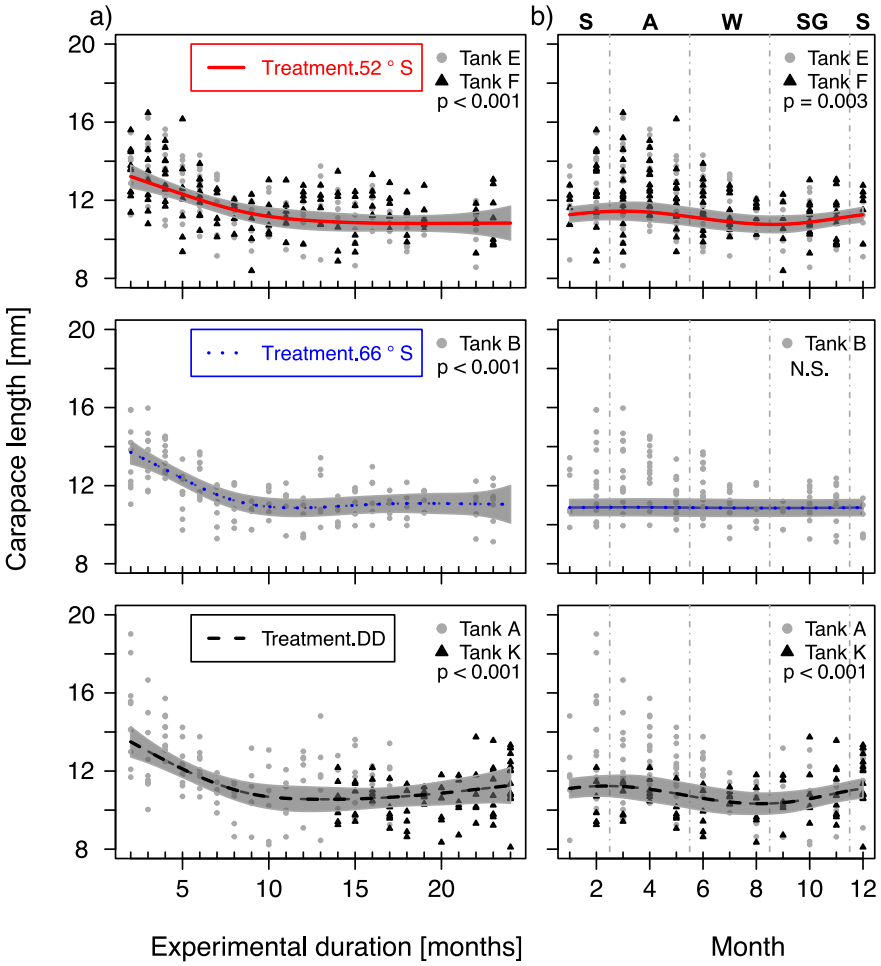

FIGURE 3.3: Estimated smooth terms of the GAMM for carapace length within treatments 52°S, 66°S and DD with a) explanatory variable time (thin plate regression spline smooth term) showing the general trend over the whole experimental period and b) explanatory variable month (cyclic smooth term) representing the seasonal trend over the months of the year. The smoothers (lines) are displayed with 95% confidence intervals (shading), the raw data points for experimental tanks (shapes) and the p-value. The seasonal periods are indicated by vertical dashed lines and the following abbreviations: SG - spring, S - summer, A - autumn, W - winter.

a decrease towards austral summer. The increase of lipid content during the second winter was much stronger than the first winter. No significant patterns were found for treatments 52°S and DD.

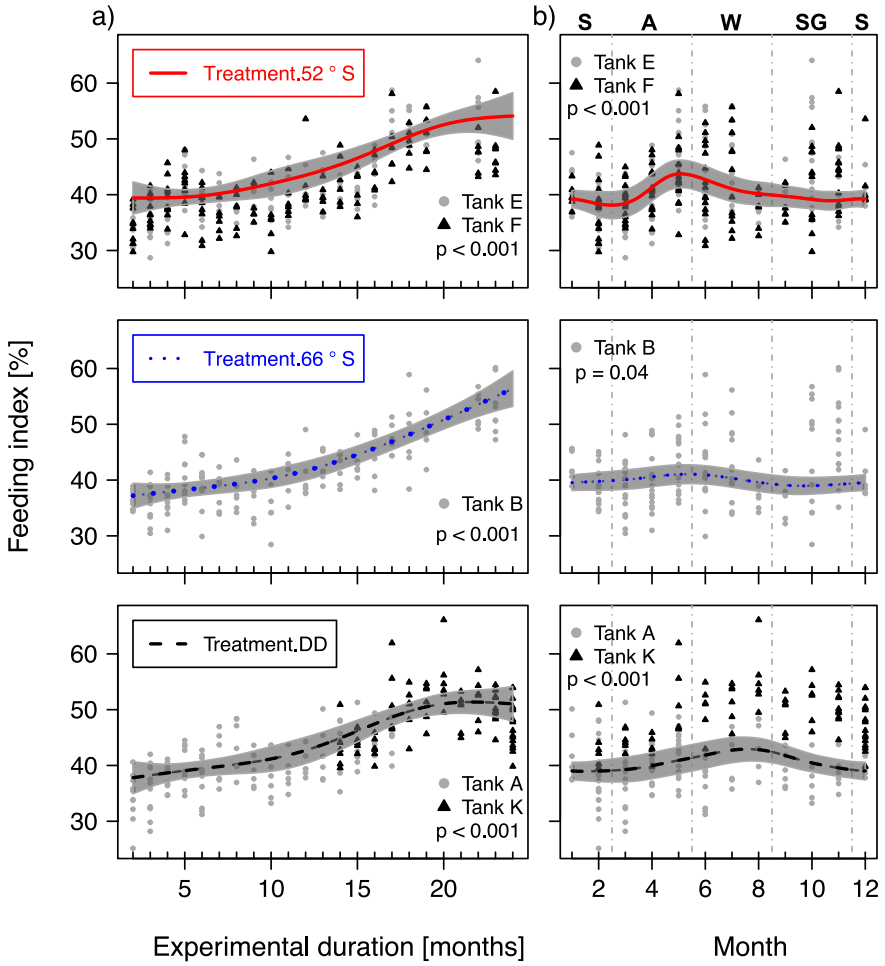

FIGURE 3.4: Estimated smooth terms of the GAMM for feeding index within treatments 52°S, 66°S and DD with a) explanatory variable time (thin plate regression spline smooth term) showing the general trend over the whole experimental period and b) explanatory variable month (cyclic smooth term) representing the seasonal trend over the months of the year. The smoothers (lines) are displayed with 95% confidence intervals (shading), the raw data points for experimental tanks (shapes) and the p-value. The seasonal periods are indicated by vertical dashed lines and the following abbreviations: SG - spring, S - summer, A - autumn, W - winter.

3.4.4 Maturity

Implementing the negative binomial GAMM for female maturity (model M4; Table 2), we found a significant seasonal cycle of maturity under treatments 52°S, 66°S, and DD with sexual regression towards austral winter and sexual re-maturation towards austral spring and summer (Figs. 3.6a, 3.6b). Significant interannual patterns differed

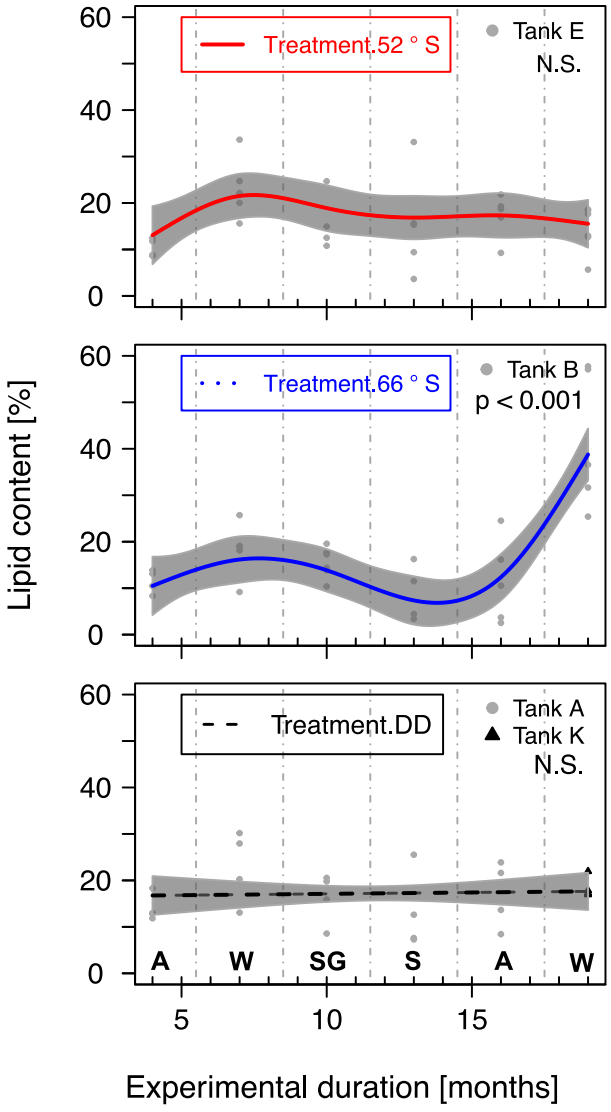

FIGURE 3.5: Estimated smooth terms of the GAM for lipid content in females within treatments 52°S, 66°S and DD. The explanatory variable time (thin plate regression spline smooth term) is showing the general trend over the whole experimental period. The smoothers (lines) are displayed with 95% confidence intervals (shading), the raw data points for experimental tanks (shapes) and the p-value. The seasonal periods are indicated by vertical dashed lines and the following abbreviations: SG - spring, S - summer, A - autumn, W - winter.

between treatments. In treatments 52°S and 66°S, a slight decrease of maturity over the whole study period was observed. The interannual pattern in treatment DD showed that sexual regression was only completed during the first winter of the experiments.

The binomial GLMM (model M5; Table 2) suggests that the variable "hours of light" significantly affects female maturity in treatments 52°S and 66°S. The interaction term between "hours of light" and "treatment" was marginally not significant. When investigating the critical photoperiod at the probability of 50%, differences between the treatments were found (Fig. 3.7). For treatment 52°S, the critical photoperiod was estimated as 12.5 h of light with 95% confidence intervals (11.86, 13.22). For treatment 66°S, an estimate of 14.76 h of light with 95% confidence intervals (13.3, 16.3) was found.

3.5 Discussion

We present findings from the first 2-year laboratory experiments investigating the effect of light regime and the biological clock on the seasonal cycle of Antarctic krill.

The observed seasonal cycles of growth, feeding, lipid metabolism, and maturity under the simulated latitudinal light regimes suggest that light regime is an essential *Zeitgeber* for Antarctic krill. The occurrence of a pronounced lipid cycle under treatment 66°S and the observed differences in critical photoperiods for the maturation cycle indicate that Antarctic krill may respond flexibly to different latitudinal light regimes. This may represent an adaptive mechanism to the extreme light regimes in the Southern Ocean and ensure survival of Antarctic krill in different latitudinal habitats, especially during winter. Moreover, seasonal patterns of growth, feeding, and maturity persisted under constant darkness indicating the presence of an endogenous timing system modulating these rhythms. High food supply does not suppress endogenously driven seasonal rhythms of growth, feeding, lipid metabolism, and maturity.

The following considerations should be taken into account when interpreting the findings of this study. Due to limits in space and costs for the long-term laboratory experiments and variable mortality rates in the tanks, we had to adjust the experimental set-up and sampling scheme accordingly. This led to a sampling design with

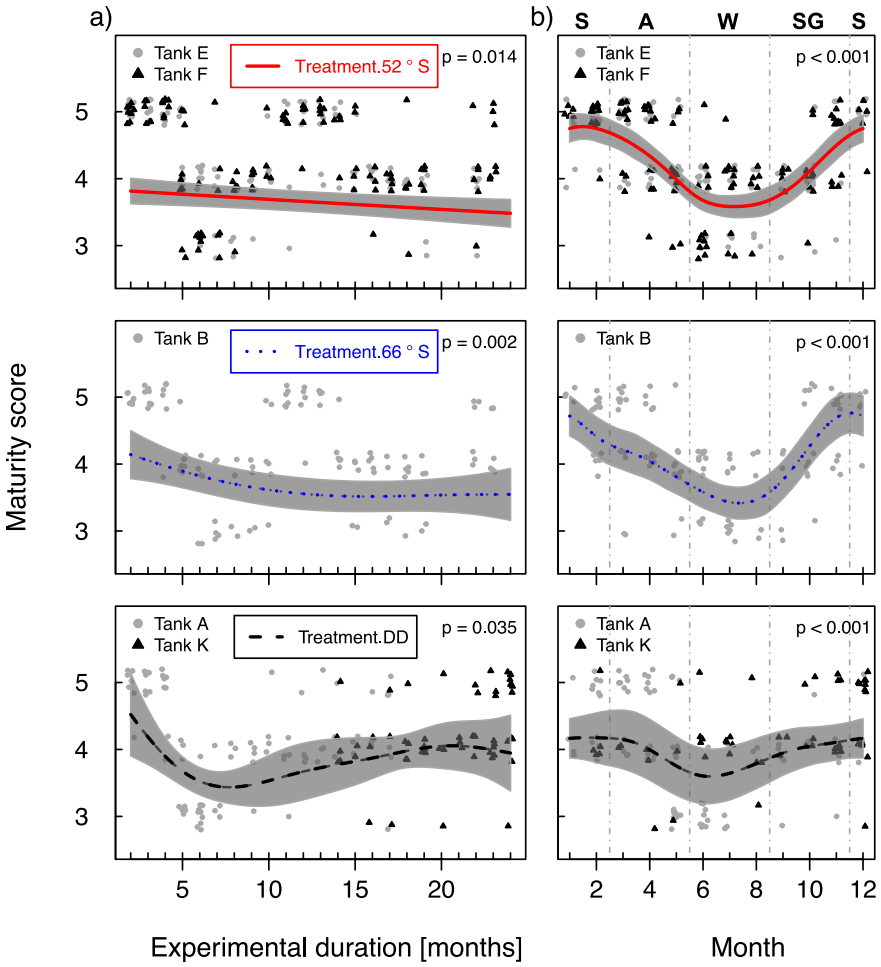

FIGURE 3.6: Estimated smooth terms of negative binomial GAMM for female maturity within treatments 52°, 66°S and DD with a) explanatory variable time (thin plate regression spline smooth term) showing the general trend over the whole experimental period and b) explanatory variable month (cyclic smooth term) representing the seasonal trend over the months of the year. The smoothers (lines) are displayed with 95% confidence intervals (shading), the jittered raw data points for experimental tanks (shapes) and the p-value. The seasonal periods are indicated by vertical dashed lines and the following abbreviations: SG - spring, S - summer, A - autumn, W - winter.

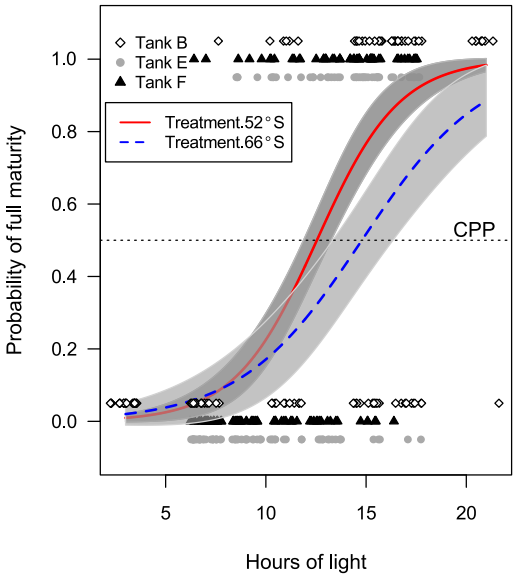

FIGURE 3.7: Results from the logistic regressions for 52°S and 66°S: Estimated trends of the binomial GLMM (lines) are shown with 95% confidence intervals (shading) and jittered raw data points for experimental tanks (shapes). The horizontal line indicates the 50% probability level for the critical photoperiod (CPP).

replication in experimental units over the full study period for treatment 52°S only. Carapace length, digestive gland length, and maturity data from treatment 66°S and partly treatment DD, as well as the lipid content data set, may be regarded as pseudoreplicated (Colegrave and Ruxton, 2018) because the replication in experimental units over the full study period is incomplete. We have included the random effect "experimental tank" in our models, where appropriate, during statistical analysis of the data to account for a potential tank effect as far as possible. How- ever, we cannot exclude that differences in tank and replicate number may have influenced the results of our tests.

To interpret the response of Antarctic krill to constant darkness over the full 2-year period, we combined data from two different cohorts of Antarctic krill. The "new" cohort was acclimated to the laboratory conditions for 1 year, before sampling started. Preliminary analysis revealed similar trends in both cohorts under constant darkness, which supports our assumption that both cohorts responded similarly to the treatment.

Moreover, we decided to solely analyse a reduced data set for lipid content because frozen Antarctic krill samples from the 2-year experiments are very valuable and can be used for multiple analyses. The reduced data set is adequate to display the pronounced seasonal lipid cycle under the high latitudinal light regime, but it may be insufficient to test for weaker patterns in the other treatments. Since potential differences in the male pattern were indicated and the number of males was too low to conduct a separate analysis, we decided to analyse females only for lipid content and maturity.

Moreover, we presume that the observations made in the first few months of the experiment represent a general period of acclimation to the experimental conditions. It may explain the strong shrinkage, suppressed lipid accumulation, and a general similarity of the data under all treatments in the beginning of the experiments.

Our observation of a seasonal cycle of growth confirms findings by Brown et al. (2010) that suggest growth is influenced by light regime, independently of food supply. For the first time, we show that Antarctic krill's growth cycle is endogenous and persists under constant darkness. The observed shrinkage in autumn and winter in this study may be partly related to the maturity cycle. Females have been observed to shrink during sexual regression (Thomas and Nash, 1987) and Tarling et al. (2016) suggested that it might be explained by morphometric changes due to the contraction of the ovaries. On the other hand, the shrinkage may reflect an overwintering mechanism (Quetin and Ross, 1991). This is supported by our observation of significant seasonal shrinkage under constant darkness where we did not find a pronounced maturity cycle over the 2-year period.

The seasonal increase of feeding in autumn, which was observed under treatment 52°S, may represent an inherent strategy to be able to accumulate enough lipid stores for winter (Hagen et al., 2001; Meyer et al., 2010). These results partly agree with the short-term study by Teschke et al. (2007) who observed higher clearance rates under autumn and summer light conditions compared with constant darkness, suggesting enhanced feeding activity under light conditions of prolonged day length. The comparability of both studies may be limited because we solely used a morphometric index

as a measure of feeding activity. The feeding index may be biased by the strong shrinkage that occurred in the beginning of our experiments, which could have masked a suppressed feeding activity in the first months. In our long-term study, the seasonal feeding trend under treatment DD resembled the other treatments with a shift of peak feeding activity towards winter that may indicate an endogenous control of seasonal feeding activity in Antarctic krill. The general increase of feeding index during the experiments suggests that Antarctic krill is able to make use of food supply throughout the whole experimental period. This observation may also indicate a flexible feeding behaviour of Antarctic krill (Atkinson et al., 2002) that has also been observed in the field in winter (Quetin and Ross, 1991; Huntley, 1994; Schmidt et al., 2014).

In our study, we observed a seasonal pattern of lipid content under treatment 66°S that may be stimulated by the high latitudinal light regime. It resembles the lipid cycle observed in the field with highest values of lipid content in autumn and lowest values in early spring (Hagen et al., 2001; Meyer et al., 2010). This is the first study that shows the possible influence of light regime on the lipid cycle in Antarctic krill. The accumulation of lipid reserves may be adjusted according to the latitudinal light regime, which may explain the differences observed in the field with higher lipid stores found in regions at higher latitudes (Schmidt et al., 2014). We also observed a match of the period of lipid depletion and re-maturation, which supports the assumption that lipid stores may be used for the maturation process (Teschke et al., 2008).

The effect of light regime on the maturity cycle (Hirano et al., 2003; Teschke et al., 2008; Brown et al., 2011) is confirmed by our study. The endogenous cycle of maturity under constant darkness has been observed in short-term experiments before (Thomas and Nash, 1987; Kawaguchi et al., 2007; Brown et al., 2011). We show that this pattern does not persist during the second year under constant darkness and suggest that the *Zeitgeber* photoperiod is required for the entrainment of the maturity cycle over longer periods. Results from former experiments (Hirano et al., 2003; Brown et al., 2011) indicate that Antarctic krill's maturity cycle may be entrained by the timing of two contrasting photoperiods (peak and trough light regimes).

To study potential differences in the physiological response of Antarctic krill to different latitudinal light regimes, we used the critical photoperiod (defines the day

length when 50% of the population shift from one state to another, here maturity) as an indicator to determine the time of the year that is a turning point in the seasonal cycle. However, using critical photoperiod, we cannot give rise to any conclusion regarding the mechanism of entrainment of these rhythms. We observed that the critical photoperiod for maturity differed between latitudinal light regimes, being longer under the high latitudinal light regime. An increase of critical photoperiod with latitude has also been found in insects in relation to diapause (Bradshaw and Holzapfel, 2007; Tyukmaeva et al., 2011; Hut et al., 2013). Organisms with longer critical photoperiods have an adaptive advantage under the extreme seasonal changes of photoperiod at higher latitudes where they have to prepare early enough to ensure survival during winter. Specifically, a higher critical photoperiod for maturity implies that Antarctic krill is able to undertake the critical stage of sexual regression and re-maturation during the time of the year when photoperiods are longer compared with regions at lower latitudes. In regions with extreme changes of photoperiod and severe winter conditions, this adaptive mechanism may ensure that Antarctic krill prepares early enough for winter and keeps up energy-saving mechanisms long enough.

Antarctic krill's flexibility in adjusting its photoperiodic response to a wide range of latitudinal light regimes may be advantageous under future climate change, as a southward migration trend of Antarctic krill to higher latitudes at the western Antarctic Peninsula has been reported (Ross et al., 2014). Still, changes in sea-ice dynamics, such as the timing of sea-ice formation or melt, may lead to mismatches in the timing of critical life-cycle events (Clarke et al., 2007). For instance, an earlier phytoplankton bloom associated with earlier sea-ice melt may influence the survival and reproductive success of Antarctic krill. Therefore, its potential to adapt to future environmental changes may also depend on its genetic flexibility in adjusting its photoperiodic response and the timing of critical life-cycle events (Bradshaw and Holzapfel, 2007).

Our findings support the assumption of a circannual timing system synchronized by light regime in Antarctic krill (Meyer, 2012). The modulation of seasonal rhythms of growth, feeding, lipid metabolism, and maturity happen independently of constant

food supply, indicating an inherent mechanism in Antarctic krill that regulates the timing of these processes according to the light regime. Photoperiod may play a significant role in the initiation of neuroendocrine cascades (on–off mechanism) in Antarctic krill, as it has been found to be the primary signal initiating diapause, migration, or reproduction in other arthropods (Bradshaw and Holzapfel, 2007). It remains to be clarified if the photoperiodic time measurement inducing seasonal events in Antarctic krill is related to the circadian clock (Hut et al., 2013; Meuti et al., 2015) or represents an independent circannual timing system. Using light regime as a seasonal *Zeitgeber* makes ecologically sense because it is a more reliable cue than food availability. The intensity of the initiated seasonal physiological processes may be regulated in the field by the interaction with other factors such as food or temperature. High food quality and quantity were found to advance growth (Ross et al., 2000; Atkinson et al., 2006) and maturation (Quetin and Ross, 2001) in Antarctic krill. We propose that this effect is restricted to specific seasonal periods that are determined by the response of Antarctic krill's endogenous timing system to the exposed latitudinal light regime.

This study has high relevance for future modelling approaches of Antarctic krill densities in the Southern Ocean, especially under the aspect of climate change. Recent Antarctic krill models have focussed on intraspecific food competition (Ryabov et al., 2017) or have been conducted on a conceptual basis (Groeneveld et al., 2015). The incorporation of light regime into dynamic models may significantly improve the predictability of growth, energy budget, and reproduction in Antarctic krill. Recently, a coupled energetics and moult-cycle model has been developed for Antarctic krill that considered resource allocation based on the seasonal cycles of growth and maturity (Constable and Kawaguchi, 2017). Further research on the phenology and biological clock of Antarctic krill will help to better understand its adaptive potential to environmental changes.

3.6 Conclusion

This study aimed to investigate the impact of light regime on Antarctic krill's phenology and the role of its endogenous timing system. Our observations suggest that light regime affects seasonal cycles of growth, feeding, lipid metabolism, and maturity

under constantly high food supply. Antarctic krill possesses an endogenous timing system that maintains seasonal rhythms under constant darkness and is most likely entrained by light regime. Varying critical photoperiods under different latitudinal light regimes indicate that this timing system is flexible, allowing Antarctic krill to adjust its physiological and behavioural responses to the extreme light conditions in the Southern Ocean.

3.7 Acknowledgements

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4. Publication 3

The effect of latitudinal light regime on seasonal gene expression in Antarctic krill (*Euphausia superba*)

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4.1 Abstract

Antarctic krill, *Euphausia superba*, flexibly adapts its seasonal life cycle to the highly variable conditions in the different latitudinal habitats of the Southern Ocean. This study investigated how different latitudinal light regimes affected seasonal gene expression in Antarctic krill. We analysed the expression of various metabolic and regulatory genes in a controlled long-term laboratory experiment under constant food

supply simulating the latitudinal light regimes 52°S, 66°S, and constant darkness as reference. We found that latitudinal light regime induced seasonally rhythmic patterns of gene expression that were more distinct under the high-latitude light regime 66°S and were mostly disrupted under constant darkness. The simulated light regimes clearly affected the expression patterns of genes related to carbohydrate, energy, and lipid metabolism, lipid transport, translation, the biosynthesis of signalling molecules as well as to the circadian clock. These results suggest that Antarctic krill is able to adjust its seasonal gene expression patterns and associated metabolic and regulatory processes to different latitudinal light regimes. We propose that genes related to the circadian clock, as well as prostaglandin, methyl farnesoate, neuropeptide biosynthesis and receptor signalling may play a role in the regulation of seasonal rhythms in Antarctic krill.

4.2 Introduction

Antarctic krill, *Euphausia superba*, holds a pivotal position in the Southern Ocean food web forming a major link between primary production and higher trophic levels such as fish, penguins, seals and whales. In the Atlantic sector of the Southern Ocean, krill distribution extends to a remarkably broad latitudinal range from 51°S to 70°S where it is exposed to strong seasonality with extreme shifts in photoperiod, primary productivity and sea ice extent (Quetin and Ross, 1991).

Our knowledge on the molecular mechanisms that underlie Antarctic krill's flexible behaviour in different latitudinal regions still remains fragmentary. A better understanding of the regulatory and metabolic processes that govern seasonal rhythms in Antarctic krill may improve the explanatory power of Antarctic krill models. This is especially relevant under the recently observed changes in sea ice extent and macrozooplankton distribution in the South West Atlantic Sector (Atkinson et al., 2004; Atkinson et al., 2019; Steinberg et al., 2015) that point to fundamental changes in the Southern Ocean food web.

In the field, Antarctic krill shows pronounced seasonal rhythms of growth, lipid metabolism, metabolic activity and maturity (Kawaguchi et al., 2007; Meyer et al., 2010). Survival during periods of near-constant darkness is accomplished by different

overwintering strategies such as metabolic depression, regressed development and growth, and the utilisation of body lipid stores (Meyer, 2012). Regional differences in the timing of reproduction (Spiridonov, 1995), growth (Kawaguchi et al., 2006), gene expression (Seear et al., 2012),, winter lipid storage and feeding activity (Schmidt et al., 2014) have also been observed in Antarctic krill.

Interestingly, winter krill from the low latitude region around South Georgia (54°S) have been found to differ the most, in terms of feeding activity, body lipid storage and gene expression from higher latitude krill (Schmidt et al., 2014, and Höring et al., in prep. (Publication 1)). At South Georgia, Antarctic krill was found to have higher feeding activities and lower lipid stores in winter (Schmidt et al., 2014; Seear et al., 2012). Moreover, seasonal differences in gene expression between summer and winter were less pronounced in Antarctic krill from South Georgia region indicating that Antarctic krill does not enter a distinct period of rest in this region in winter (Höring et al., in prep.; Publication 1). The flexible seasonal behaviour of Antarctic krill at South Georgia may be an adaptation to the low-latitude light regime which enhances food availability in that region year-round.

Recently, a long-term laboratory study has shown that Antarctic krill possesses a flexible endogenous timing system, likely entrained by the prevailing light regime (Höring et al., 2018). It seems to affect krill's seasonal response, controlling multiple seasonal processes such as growth, feeding, lipid metabolism, maturity, oxygen consumption and metabolic activity (Höring et al., 2018; Piccolin et al., 2018b).

It has been suggested that Antarctic krill's circadian clock may play a role in the timing of seasonal life cycle events (Piccolin et al., 2018b). The circadian clock system has been functionally characterized in Antarctic krill and includes a light mediated entraining mechanism (Biscontin et al., 2017). It may not only be important for the synchronisation of daily rhythms of metabolic activity, gene expression (De Pittà et al., 2013; Teschke et al., 2011), and diel vertical migration (Gaten et al., 2008), but also for the measurement of seasonal day length. Light conditions in early autumn may be critical for seasonal entrainment in Antarctic krill as an upregulation of genes related to the circadian clock and circadian-related opsins were observed in photoperiodic controlled laboratory experiments during this time of the year (Piccolin et al., 2018b).

This study complements the results from a recent two-year lab experiment, analysing the effect of different latitudinal light regimes on the seasonal cycle of Antarctic krill (Höring et al., 2018). Höring et al. (2018) focussed on morphometric and lipid content data, whereas data on gene expression and metabolic pathways were still missing. How different latitudinal light regimes affect the molecular processes during the seasonal cycle of Antarctic krill is still not clear.

The goal of this study was to characterize molecular pathways that contribute to different seasonal responses of Antarctic krill under the simulated latitudinal light regimes 52°S and 66°S, as well as constant darkness (DD), under constant food supply. A set of genes known to be involved in seasonal timing, reproduction, feeding, development, lipid, energy and carbohydrate metabolism was analysed for (i) seasonal rhythmicity of gene expression under the treatments 52°S, 66°S and DD over one year, and (ii) differences in gene expression patterns between the three treatments in seven months within a one year seasonal light cycle (April, June, August, October, December, February and April the next year).

4.3 Materials & Methods

4.3.1 Long-term lab experiments at the Australian Antarctic Division

To test the effect of different light regimes on Antarctic krill, long-term lab experiments were performed under constant food supply at the Australian Antarctic Division aquarium in Hobart, Australia, over a period of two years (January 2015 to December 2016). Three different light regimes were simulated: 1) natural light regime at 52°S, 2) natural light regime at 66°S, and 3) constant darkness (DD). The detailed experimental conditions including details on krill sampling, experimental set-up, the simulated light regimes and food supply can be found in an earlier study investigating morphometric and lipid content data from the same experiment (Höring et al., 2018). Frozen krill samples from the experiments were sent to the Alfred-Wegener-Institute, Bremerhaven, for molecular analysis and stored at $-80\,^{\circ}$C. Overall 126 individuals from three tanks/treatments (52°S, 66°S, DD) were analysed including seven

TABLE 4.1: Sampling scheme showing the number of investigated Antarctic krill samples per treatment and month.

Tank/Treatment	Apr15	Jun15	Aug15	Oct15	Dec15	Feb15	Apr16
Tank A - DD	6	6	6	6	6	6	6
Tank B - 66°S	6	6	6	6	6	6	6
Tank E - 52°S	6	6	6	6	6	6	6

time points per treatment (April 2015 to April 2016) with six biological replicates per time point (Table 4.1).

4.3.2 Sample processing and preparation of RNA and cDNA

Krill heads were cut on dry ice and transferred to RNA*later*™-ICE (Thermo Fisher Scientific, Waltham, MA, USA) according to manual instructions. The heads were dissected using the stereomicroscope Leica M125 C (Wetzlar, Germany) and a cooling element, adjusted to 4 °C by a Minichiller (Huber, Offenburg, Germany). Loose parts such as thoracopods, the pigmented part of the eyes, and the front part of the antennas were removed from the krill heads. The homogenization of the dissected krill heads was carried out in Precellys® tubes (CKMix Tissue Homogenizing Kit, Bertin corp., Rockville, MD, USA) containing 600 µL TRIzol™Reagent per tube and sample (Thermo Fisher Scientific) using the Precellys® homogenisator with the Cryolys® cooling system (Bertin corp.) for 2*15 s at 5000 rpm at 4 °C. After 5 min of incubation at room temperature, a phase separation step with chloroform was carried out according to the TRIzol protocol and 200 µL of aqueous phase per sample were transferred to a new tube. RNA was then purified with the Direct-zol™RNA MiniPrep Plus kit (Zymo Research Europe, Freiburg, Germany) including an on-column DNase I treatment step, following manual instructions. RNA was eluted in 50 µL of RNase-free water and stored at −80 °C. Quality and quantity of the RNA were validated using a NanoDrop™2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific) and the Agilent 2100 Bioanalyzer system (Agilent technologies, Santa Clara, CA, USA). cDNA was synthesized in 50 µL reaction volume with final concentrations of 4 µg RNA, 100U RevertAid™H Minus Reverse Transcriptase and Reaction Buffer (Thermo Fisher Scientific), 40U RiboLock RNase Inhibitor (Thermo Fisher Scientific), 0.4 mM Deoxynucleotide (dNTP) Solution Mix (New England BioLabs, Ipswich, MA, USA) and 100μM

pentadecamers (Eurofins Genomics, Ebersberg, Germany). cDNA synthesis included -RT and non-template controls. The following temperature profile was used for cDNA synthesis: pre-primer extension (10 min at 25 °C), DNA polymerization (50 min at 37 °C) and enzyme deactivation (15 min at 70 °C).

4.3.3 TaqMan card design and measurement

For gene expression analysis, Custom TaqMan™Gene Expression Array Cards format 32 (Thermo Fisher Scientific) were designed. Genes of interest were chosen from previous studies on Antarctic krill (Piccolin et al., 2018b, and Höring et al., in prep. (Publication 1)) and from the Krill Transcriptome Database (Sales et al., 2017). For some genes of interest, the primer/probe design was adopted from the study by Piccolin et al. (2018b). For the other genes, where the primer/probe design was not yet available, annotation was reviewed by blastn and blastx searches on the web interface of NCBI (`https://blast.ncbi.nlm.nih.gov/Blast.cgi`; Johnson et al. (2008)). ORFfinder (`https://www.ncbi.nlm.nih.gov/orffinder/`) was used to identify the open reading frame (ORF) of the sequences that conformed with the reading frame and the identified protein domains from the blastx searches. If applicable, the alignment of seasonal RNAseq data for each sequence (Höring et al., in prep.; Publication 1) was then used to find an amplicon within the ORF with the least single nucleotide polymorphisms using the programme Tablet (Milne et al., 2010). The segments of interest were cut in the programme `ApE v.2.0.53c` (`http://jorgensen.biology.utah.edu/wayned/ape/`). No repeats were found for these parts of the genes (`http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker`). The sequences were then submitted to the Custom TaqMan® Assay Design tool for automatic primer and probe design for each gene (Thermo Fisher Scientific). The sequences of primers and probes used in this study are summarized in supplementary Table B.1.

Gene expression analysis via TaqMan® Cards was conducted on a ViiA™7 Real-Time PCR System (Thermo Fisher Scientific) according to manual instructions. 400 ng cDNA per sample and TaqMan® Gene Expression Master Mix (Thermo Fisher Scientific) with a final loading volume of 100 µL per sample were used. Dilution curves were performed for the analysis of primer efficiency.

4.3.4 Gene expression analysis

Data quality control and normalisation were carried out using the software `qbase+ 3.2` (Biogazelle, Zwijnaarde, Belgium). The median Cq value was calculated for each data point from three technical replicates. The preliminary geNorm analysis (Vandesompele et al., 2002) implemented in `qbase+` suggested two housekeeping genes for data normalisation: *RNA polymerase I-specific transcription initiation factor RRN3 isoform 1 (rrn3)* and *ubiquitin carboxyl-terminal hydrolase 46 (usp46)*. These genes have already been found to show stable gene expression in Antarctic krill in previous photoperiodic controlled laboratory experiments (Piccolin et al., 2018b, Biscontin et al., in prep.). The stability of the two reference genes was validated for the final dataset by calculating the reference target stability in `qbase+` (*rrn3*: M=0.285 & CV=0.1; *usp46*: M=0.285 & CV=0.098). Gene expression data were then normalised using the method by Hellemans et al. (2007), a modified version of the delta-delta-Ct method that incorporates PCR efficiency correction for each gene, multiple reference gene normalisation as well as proper error propagation, implemented in `qbase`. The normalised expression values of the target genes with standard error will be archived upon publication of the manuscript.

Statistical analysis and visualisation of gene expression results were performed in `RStudio version 1.1.456` (RStudio Team, 2016). The heatmap for Fig. 4.1 was generated in the `R` package `gplots` (Warnes et al., 2016) using the median values per time point and treatment. To test for rhythmic seasonal expression patterns of the analysed genes, the `R` package `rain` (Thaben and Westermark, 2014) was used.

To identify differences in gene expression patterns between light treatments/conditions (52°, 66°S and DD), sparse partial least squares discriminant analysis (sPLS-DA) was used (`R` package `mixOmics` (Rohart et al., 2017)). sPLS-DA analysis was conducted for each month separately (7 subsets). The subsets included krill of both sexes that were analysed together, because a preliminary Principal Component Analysis (PCA) revealed that major parts of the variation in the subsets were not related to sex. sPLS-DA fitted a classifier multivariate model for each subset while assigning the samples into the three light treatments (52°, 66°S and DD). In addition, this method allowed variable selection including the optimal number of components and features (genes)

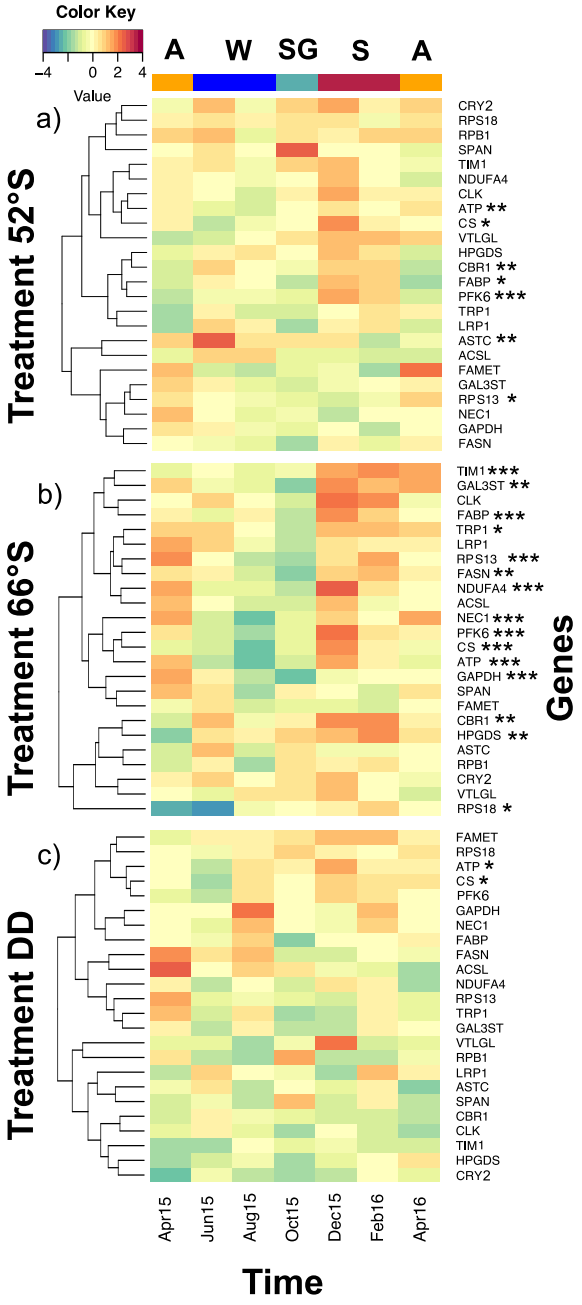

FIGURE 4.1: Heatmap showing the median values of expression for each gene and sampled time point per treatment: a) simulated light regime at 52°S, b) simulated light regime at 66°S, and c) constant darkness (DD). Significantly rhythmic genes per treatment were identified with the RAIN algorithm and are indicated with asterisks (* p< 0.05, ** p<0.01, *** p<0.001). The sampling seasons are abbreviated as follows: A - autumn, W - winter, SG -spring, and S - summer.

TABLE 4.2: Parameters for the sPLS-DA models for each subset (month) including the chosen number of components; the number of selected genes and the overall error rate for component 1 (C1), component 2 (C2), and component 3 (C3).

subset	components	genes C1	genes C2	genes C3	overall error rate C1 (max.dist)	overall error rate C2 (max.dist)	overall error rate C3 (max.dist)
Apr15	1(2)*	24	24		0.75	0.80	
Jun15	1(2)*	23	5		0.52	0.57	
Aug15	1(2)*	5	18		0.49	0.66	
Oct15	2	24	1		0.64	0.57	
Dec15	2	24	1		0.45	0.37	
Feb16	1(2)*	8	24		0.56	0.59	
Apr16	3	23	1	12	0.68	0.69	0.65

leading to the lowest overall error rates of the final model. If the parameter tuning of the sPLS-DA (5-fold cross-validation, repeated 100 times) indicated an optimal number of components = 1, we considered using a model with two components for visualisation purposes. Performance of the final models was assessed by the overall error rates for each component and the stability of the selected genes. The parameters chosen for the final models can be found in Table 4.2. Sample plots, loading plots and clustered image maps from the `R` package `mixOmics` were used for the visualisation of the sPLS-DA results.

4.4 Results

4.4.1 Analysis of seasonal rhythmicity of gene expression

Using `RAIN`, significant rhythmic patterns of gene expression were detected in Antarctic krill over a period of 12 months in the three treatments simulating the low-latitude light regime 52°S, the high latitude light regime 66°S and constant darkness (DD) (Fig. 4.1, Table 4.3).

Under treatment 52°S, seven genes had significant seasonal rhythmicity with functions in glycolysis (*phosphofructokinase-6* alias *pfk6*), citric acid cycle (*citrate synthase* alias *cs*), respiratory chain (*ATP synthase subunit gamma* alias *atp*), prostaglandin biosynthesis (*carbonyl reductase 1* alias *cbr1*), lipid transport (*fatty acid binding protein* alias *fabp*), translation (*ribosomal protein S13* alias *rps13*), and feeding (*allatostatin C* alias *astc*).

TABLE 4.3: RAIN results sorted after the functional process of the studied genes including the p-value for each gene and treatment. P-values that indicated significant seasonally rhythmic gene expression patterns over a period of 12 months are displayed in bold.

Process	Gene	Treatment 66°S p-value	Treatment 52°S p-value	Treatment DD p-value
Glycolysis	*pfk6*	**<0.001**	**<0.001**	0.389
	gapdh	**<0.001**	0.095	0.907
Citric acid cycle	*cs*	**<0.001**	**0.018**	**0.039**
Respiratory chain	*atp*	**<0.001**	**0.004**	**0.019**
	ndufa4	**<0.001**	0.089	0.086
Lipid metabolism	*acsl*	0.649	0.757	0.541
	fasn	**0.004**	0.883	0.22
	gal3st	**0.007**	0.276	0.923
Lipid transport	*fabp*	**<0.001**	**0.011**	0.407
	vtlgl	0.174	0.063	0.652
Prostaglandin biosynthesis	*hpgds*	**0.006**	0.076	0.806
	cbr1	**0.009**	**0.005**	0.227
Activation of neuropeptides	*nec1*	**<0.001**	0.537	0.881
Biosynthesis of methyl farnesoate	*famet*	0.704	0.414	0.236
Circadian clock	*tim1*	**<0.001**	0.696	0.668
	clk	0.194	0.588	0.838
	cry2	0.872	0.687	0.814
Translation	*rps18*	**0.031**	0.826	0.256
	rps13	**<0.001**	**0.011**	0.113
Transcription	*rpb1*	0.887	0.832	0.833
Feeding	*trp1*	**0.015**	0.576	0.631
	astc	0.922	**0.005**	0.897
Development	*span*	0.221	0.505	0.78
Multifunctional cell-surface receptor	*lrp1*	0.058	0.725	0.576

Under treatment 66°S, we found the highest number of genes displaying significant rhythmicity throughout the year (15). These genes are involved in glycolysis (*pfk6* & *glyceraldehyde-3-phosphate dehydrogenase* alias *gapdh*), citric acid cycle (*cs*), respiratory chain (*atp* & *cytochrome c oxidase subunit NDUFA4* alias *ndufa4*), lipid transport and metabolism (*fabp* & *fatty acid synthase* alias *fasn* & *galactosylceramide sulfotransferase* alias *gal3st*), prostaglandin biosynthesis (*cbr1* & *hematopoietic prostaglandin D synthase* alias *hpgds*), activation of neuropeptides (*neuroendocrine convertase 1* alias *nec1*), circadian clock (*timeless1* alias *tim1*), translation (*rps13* and *ribosomal protein S18* alias *rps18*), and feeding (*anionic trypsin 1* alias *trp1*).

Under treatment DD, only two genes showed significant seasonal rhythmicity, which were involved in energy metabolism (*atp*) and citric acid cycle (*cs*).

We did not find significant rhythmicity in any of the treatments for the following genes: *long-chain-fatty-acid-CoA ligase (acsl), vitellogenin-like gene (vtlgl), farnesoic acid O-methyltransferase (famet), clock (clk), cryptochrome 2 (cry2), RNA polymerase II largest subunit RPB1 (rpb1), span-like gene (span),* and *low-density lipoprotein receptor-related protein 1 (lrp1)* (for function see Table 4.3).

4.4.2 Analysis of monthly gene expression signatures using sPLS-DA

We used sPLS-DA to exploratively assess monthly differences in gene expression of Antarctic krill in response to the different light treatments (52°S, 66°S, and DD) for the first time. Gene expression data from each month were examined separately within the following subsets: Apr15 (autumn), Jun15 (early winter), Aug15 (late winter), Oct15 (spring), Dec15 (early summer), Feb16 (late summer), and Apr16 (second autumn). Even though we were not able to fully separate the treatments in most of the subsets (Fig. 4.2 & Fig. 4.3) (likely due to the low number of replicates per month/treatment), this type of analysis provided first insights into general gene expression patterns, putatively characterizing the response to the three different light treatments throughout the year. The gene sets selected by sPLS-DA for each monthly subset contributed to the separation of the treatments (Fig. 4.2-4.3, details for each subset are outlined below). They were analysed in terms of loadings for each gene per component, which can be interpreted as a positive correlation of the gene

expression pattern with the corresponding treatment of the sample plots (i.e. "up-/downregulated"; Fig. 4.2-4.3), irrespective of positive or negative loading values. The sPLS-DA results for each subset are summarized in Tables 4.4 to 4.10 including the selected genes for each component of interest, their respective functions, and their loadings.

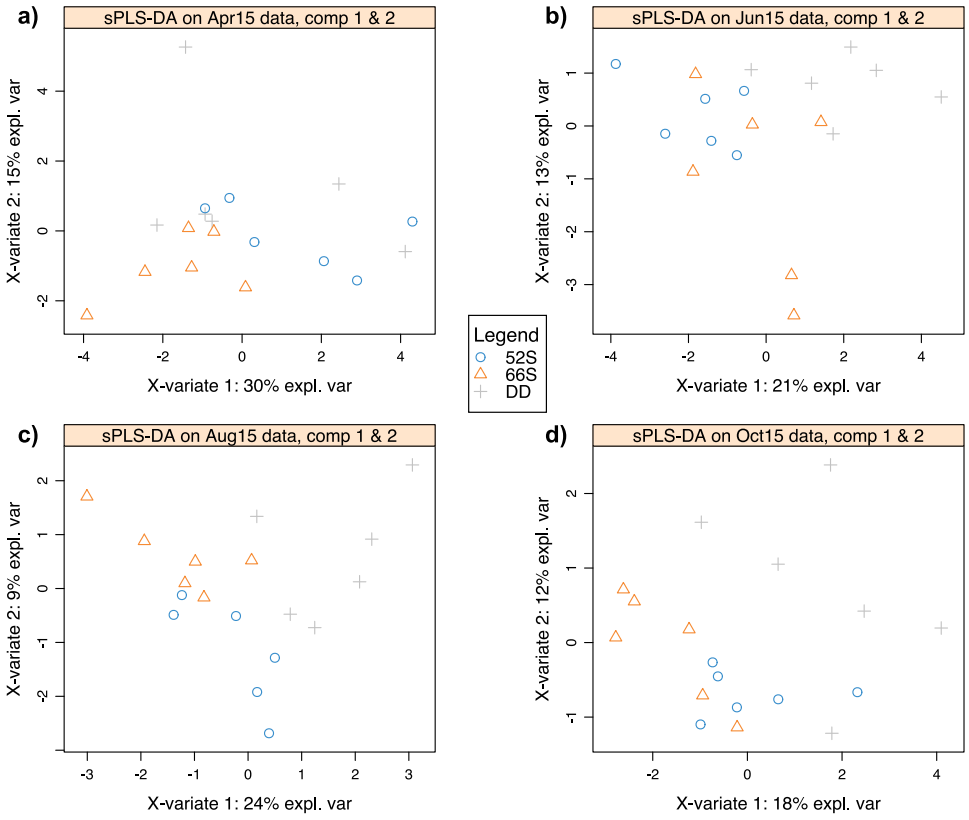

FIGURE 4.2: Sample plots for component 1 and 2 from sPLSDA analysis of each subset: a) Apr15 (autumn) b) Jun15 (early winter), c) Aug15 (late winter), and d) Oct15 (spring)

For the Apr15 subset (autumn), we focussed on the sPLS-DA results of component 1, as component 2 did not improve the discrimination of the three treatments (Fig. 4.2a). Component 1 discriminated between individual krill from the two light treatments (66°S and 52°S). Krill from treatment DD displayed a high variability in gene expression with similarities to krill samples of both light treatments (66°S and 52°S) and no distinct pattern. The detected gene signature of component 1 that contributed to the separation of treatment 66°S and 52°S can be found in Table 4.4.

TABLE 4.4: sPLS-DA results for the Apr15 subset (autumn). Genes analysed in the course of this study are sorted according to their respective functional process. Genes selected by sPLS-DA are indicated by loading values. Negative and positive loadings are given for component 1 (C1). Within this monthly subset, gene expression patterns mostly contributed to a separation of treatment 66°S (negative loadings) and 52° (positive loadings)

Process	**Gene**	**Loadings on C1**	
		negative	positive
Glycolysis	*pfk6*	-0.30	
	gapdh	-0.26	
Citric acid cycle	*cs*		0.25
Respiratory chain	*atp*	-0.25	
	ndufa4	-0.21	
Lipid metabolism	*acsl*	-0.13	
	fasn	-0.16	
	gal3st	-0.09	
Lipid transport	*fabp*	-0.20	
	vtlgl	-0.42	
Prostglandin biosynthesis	*hpgds*		0.19
	cbr1		0.09
Activation of neuropeptides	*nec1*	-0.15	
Biosynthesis of methyl farnesoate	*famet*		0.09
Circadian clock	*tim1*		0.16
	clk		0.07
	cry2		0.10
Translation	*rps18*		0.15
	rps13	-0.21	
Transcription	*rpb1*		0.07
Feeding	*trp1*	-0.36	
	astc		0.18
Development	*span*	-0.10	
Multifunctional cell-surface receptor	*lrp1*	-0.20	

TABLE 4.5: sPLS-DA results for the Jun15 subset (early winter). Genes analysed in the course of this study are sorted according to their respective functional process. Genes selected by sPLS-DA are indicated by loading values. Negative and positive loadings are given for component 1 (C1). Within this monthly subset, gene expression patterns mostly contributed to a separation of the light treatments 52°S and 66°S (negative loadings) and DD (positive loadings).

Process	**Gene**	**Jun15 Loadings on C1**	
		negative	**positive**
Glycolysis	*pfk6*	-0.10	
	gapdh	-0.08	
Citric acid cycle	*cs*		
Respiratory chain	*atp*		0.05
	ndufa4	-0.35	
Lipid metabolism	*acsl*	-0.14	
	fasn		0.15
	gal3st	-0.18	
Lipid transport	*fabp*		0.04
	vtlgl		0.25
Prostglandin biosynthesis	*hpgds*	-0.18	
	cbr1	-0.20	
Activation of neuropeptides	*nec1*	-0.07	
Biosynthesis of methyl farnesoate	*famet*		0.18
Circadian clock	*tim1*	-0.39	
	clk	-0.13	
	cry2	-0.24	
Translation	*rps18*		0.07
	rps13	-0.30	
Transcription	*rpb1*	-0.24	
Feeding	*trp1*	-0.19	
	astc	-0.31	
Development	*span*	-0.30	
Multifunctional cell-surface receptor	*lrp1*	-0.07	

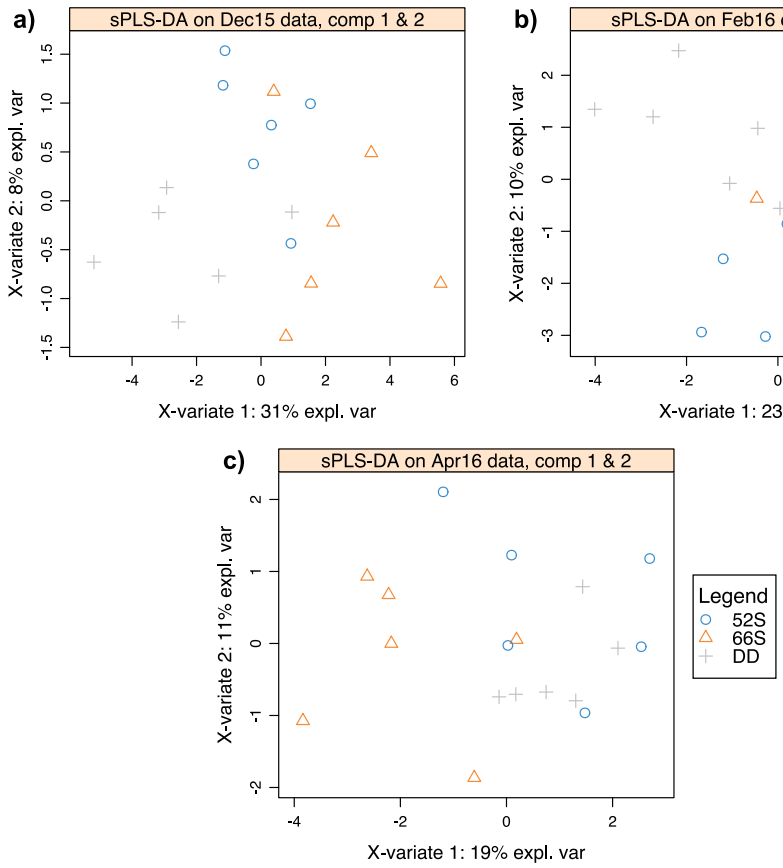

FIGURE 4.3: Sample plots for component 1 and 2 from sPLSDA analysis of each subset: a) Dec15 (early summer), b) Feb16 (late summer), and c) Apr16 (second autumn)

For the Jun15 subset (early winter), component 1 mostly contributed to the separation of individual krill from the light treatments 52°S/66°S and treatment DD, with no further discrimination between the latitudinal light treatments 52°S and 66°S, and a less distinct pattern in individual krill from treatment 66°S (Fig. 4.2b). Component 2 of the Jun15 model did not improve the discrimination between the treatments. The gene expression patterns of component 1 that characterized the separation between the treatments 52°S/66°S and DD are summarized in Table 4.5.

For the Aug15 subset (late winter), both component 1 and component 2 contributed to the discrimination of the treatments (Fig. 4.2). Component 1 distinguished between individual krill from the light treatments 66°S/52°S and treatment DD, with a less distinct pattern for individual krill from treatment 52°S. The second component contributed to the distinction between krill from latitudinal light treatments 66°S and

TABLE 4.6: sPLS-DA results for the Aug15 subset (late winter). Genes analysed in the course of this study are sorted according to their respective functional process. Genes selected by sPLS-DA are indicated by loading values. Negative and positive loadings are given for component 1 (C1) and component 2 (C2). Within this monthly subset, gene expression patterns of C1 mostly contributed to a separation of the light treatments 66°S and 52°S (negative loadings) and DD (positive loadings), whereas the gene signature of C2 mostly contributed to the separation of light treatments 52°S (negative loadings) and 66°S (positive loadings).

Process	**Gene**	**Aug15 Loadings on C1**		**Aug15 Loadings on C2**	
		negative	positive	negative	positive
Glycolysis	*pfk6*			-0.06	
	gapdh		0.58		
Citric acid cycle	*cs*		0.51		
Respiratory chain	*atp*		0.62		0.05
	ndufa4			-0.04	
Lipid metabolism	*acsl*				
	fasn				
	gal3st		<0.01		0.17
Lipid transport	*fabp*				0.23
	vtlgl				0.03
Prostglandin biosynthesis	*hpgds*				0.16
	cbr1			-0.20	
Activation of neuropeptides	*nec1*		0.17	-0.31	
Biosynthesis of methyl farnesoate	*famet*				0.23
Circadian clock	*tim1*				
	clk				0.24
	cry2			-0.07	
Translation	*rps18*			-0.18	
	rps13			-0.37	
Transcription	*rpb1*			-0.30	
Feeding	*trp1*				0.36
	astc			-0.22	
Development	*span*			-0.44	
Multifunctional cell-surface receptor	*lrp1*				

TABLE 4.7: sPLS-DA results for the Oct15 subset (spring). Genes analysed in the course of this study are sorted according to their respective functional process. Genes selected by sPLS-DA are indicated by loading values. Negative and positive loadings are given for component 1 (C1) and component 2 (C2). Within this monthly subset, gene expression patterns of both C1 and C2 mostly contributed to a separation of the light treatments 66°S and 52°S (negative loadings) and DD (positive loadings).

Process	Gene	Oct15 Loadings on C1		Oct15 Loadings on C2	
		negative	positive	negative	positive
Glycolysis	*pfk6*		<0.01		
	gapdh		0.23		
Citric acid cycle	*cs*		0.23		
Respiratory chain	*atp*		0.21		
	ndufa4		0.12		
Lipid metabolism	*acsl*		0.13		
	fasn		0.27		
	gal3st		0.04		
Lipid transport	*fabp*	-0.05			
	vtlgl	-0.04			
Prostglandin biosynthesis	*hpgds*	-0.38			
	cbr1	-0.28			
Activation of neuropeptides	*nec1*		0.21		
Biosynthesis of methyl farnesoate	*famet*		0.34		
Circadian clock	*tim1*		0.17		
	clk	-0.23		-1.00	
	cry2	-0.08			
Translation	*rps18*		0.16		
	rps13		0.28		
Transcription	*rpb1*		0.11		
Feeding	*trp1*	-0.10			
	astc		0.14		
Development	*span*		0.09		
Multifunctional cell-surface receptor	*lrp1*		0.34		

52°S, whereas the pattern in krill from treatment DD was less distinct. The gene signatures for components 1 and 2 and respective functional information for these genes can be found in Table 4.6.

For the Oct15 subset (spring), both component 1 and 2 discriminated between individual krill from the latitudinal light treatments 66°S/52°S and treatment DD (Fig. 4.2d). For the first component the pattern was less distinct for individual krill from treatment 52°S, whereas for the second component the pattern was less clear for individual krill from treatment 66°S. The selected gene sets that characterized the separation between treatments 66°S/52°S and DD in 'spring' are shown in Table 4.7.

TABLE 4.8: sPLS-DA results for the Dec15 subset (early summer). Genes analysed in the course of this study are sorted according to their respective functional process. Genes selected by sPLS-DA are indicated by loading values. Negative and positive loadings are given for component 1 (C1) and component 2 (C2). Within this monthly subset, gene expression patterns of C1 mostly contributed to a separation of treatment DD (negative loadings) and the light treatments 66°S and 52°S (positive loadings), whereas the gene signature of C2 mostly contributed to the separation of light treatments 66°S/DD (negative loadings) and 52°S (positive loadings).

Process	**Gene**	**Dec15 Loadings on C1**		**Dec15 Loadings on C2**	
		negative	**positive**	**negative**	**positive**
Glycolysis	*pfk6*		0.19		
	gapdh		0.09		
Citric acid cycle	*cs*		0.18		
Respiratory chain	*atp*		0.10		
	ndufa4		0.32		
Lipid metabolism	*acsl*		0.13		
	fasn		0.11		
	gal3st		0.33		
Lipid transport	*fabp*		0.29		
	vtlgl		0.13		
Prostglandin biosynthesis	*hpgds*		0.17		
	cbr1		0.36		
Activation of neuropeptides	*nec1*		0.14	-1.00	
Biosynthesis of methyl farnesoate	*famet*	-0.24			
Circadian clock	*tim1*		0.24		
	clk		0.17		
	cry2		0.17		
Translation	*rps18*		0.01		
	rps13		0.21		
Transcription	*rpb1*		0.20		
Feeding	*trp1*		0.22		
	astc	-0.01			
Development	*span*		0.14		
Multifunctional cell-surface receptor	*lrp1*		0.25		

For the Dec15 subset (early summer), component 1 discriminated between individual krill from treatment DD and the light treatments 66°S/52°S, with a less distinct pattern for krill from treatment 52°S (Fig. 4.3a). Component 2 contributed to the separation of individual krill from treatment DD/66°S and treatment 52°S. The respective gene signatures can be found in Table 4.8.

For the Feb16 subset (late summer), component 1 contributed to the separation of individual krill from treatment DD and the light treatments 66°S/52°S, with a less distinct pattern for krill from treatment 52°S (Fig. 4.3b). The second component discriminated between krill from treatment 52°S and treatments DD/66°S. The selected

TABLE 4.9: sPLS-DA results for the Feb16 subset (late summer). Genes analysed in the course of this study are sorted according to their respective functional process. Genes selected by sPLS-DA are indicated by loading values. Negative and positive loadings are given for component 1 (C1) and component 2 (C2). Within this monthly subset, gene expression patterns of C1 mostly contributed to a separation of treatment DD (negative loadings) and of the light treatments 66°S and 52°S (positive loadings), whereas the gene signature of C2 mostly contributed to the separation of light treatments 52°S (negative loadings) and DD/66°S (positive loadings).

Process	**Gene**	**Feb16 Loadings on C1**		**Feb16 Loadings on C2**	
		negative	**positive**	**negative**	**positive**
Glycolysis	*pfk6*			-0.24	
	gapdh	-0.34			0.18
Citric acid cycle	*cs*				0.34
Respiratory chain	*atp*				0.27
	ndufa4				0.09
Lipid metabolism	*acsl*		0.01		0.10
	fasn				0.19
	gal3st				0.14
Lipid transport	*fabp*		0.25	-0.14	
	vtlgl			-0.24	
Prostglandin biosynthesis	*hpgds*		0.29		0.21
	cbr1		0.58	-0.13	
Activation of neuropeptides	*nec1*				0.09
Biosynthesis of methyl farnesoate	*famet*				0.44
Circadian clock	*tim1*		0.43		0.19
	clk		0.43		0.10
	cry2			-0.02	
Translation	*rps18*				0.24
	rps13				0.30
Transcription	*rpb1*			-0.07	
Feeding	*trp1*		0.16	-0.09	
	astc				0.20
Development	*span*				0.12
Multifunctional cell-surface receptor	*lrp1*				0.18

gene sets for component 1 and 2 are shown in Table 4.9.

For the Apr16 subset (second autumn), we focussed on component 1, because the sPLS-DA results for component 2 did not improve the discrimination of the treatments (Fig. 4.3c). The first component discriminated between individual krill from treatment 66°S and treatments 52°S/DD. The gene signature of component 1 that contributes to this separation is summarized in Table 4.10.

Distinct differences in the gene expression patterns between krill from the latitudinal light treatments 52°S and 66°S were found in the period of late summer (see loadings for component 2 of the Feb16 subset in Table 4.9) and autumn (see loadings

TABLE 4.10: sPLS-DA results for the Apr16 subset (second autumn). Genes analysed in the course of this study are sorted according to their respective functional process. Genes selected by sPLS-DA are indicated by loading values. Negative and positive loadings are given for component 1 (C1). Within this monthly subset, gene expression patterns of C1 mostly contributed to a separation of light treatments 66°S (negative loadings) and 52°S/DD (positive loadings).

Process	**Gene**	**Apr16 Loadings on C1**	
		negative	**positive**
Glycolysis	*pfk6*	-0.14	
	gapdh	-0.11	
Citric acid cycle	*cs*		0.13
Respiratory chain	*atp*		0.30
	ndufa4	-0.14	
Lipid metabolism	*acsl*	-0.19	
	fasn	-0.09	
	gal3st	-0.22	
Lipid transport	*fabp*	-0.10	
	vtlgl	-0.14	
Prostglandin biosynthesis	*hpgds*	-0.24	
	cbr1	-0.40	
Activation of neuropeptides	*nec1*	-0.28	
Biosynthesis of methyl farnesoate	*famet*		0.19
Circadian clock	*tim1*	-0.36	
	clk	-0.06	
	cry2		
Translation	*rps18*		0.35
	rps13		0.13
Transcription	*rpb1*	-0.04	
Feeding	*trp1*	-0.21	
	astc	-0.12	
Development	*span*	-0.20	
Multifunctional cell-surface receptor	*lrp1*	-0.13	

for component 1 of Apr15 subset in Table 4.4 & component 1 of Apr16 subset in Table 4.10). In late summer (Feb16 subset, Table 4.9), the positive loadings of component 2 of the sPLS-DA results indicated an upregulation of genes related to translation and the upregulation of metabolic genes with functions in the citric acid cycle, the respiratory chain, and lipid metabolism in individual krill from treatment 66°S and DD with respect to krill from treatment 52°S. For the first and second autumn, we found similar expression patterns of metabolism-related genes when comparing krill from the latitudinal light treatments 66°S and 52°S. In the first autumn (Apr15 subset, Table 4.4), the negative loadings of component 1 of the selected gene set showed an upregulation of metabolic genes related to glycolysis, respiratory chain, lipid metabolism, and an upregulation of genes related to lipid transport in krill from treatment 66°S with respect to krill from treatment 52°S. In the second autumn (Apr16 subset, Table 4.10), the negative loadings of component 1 of the detected gene signature indicated an upregulation of genes related to glycolysis, lipid metabolism, and lipid transport in krill from treatment 66°S with respect to treatments 52°S/DD. In the late summer and autumn subsets (Feb16, Apr15, Apr16), the upregulation of genes related to general metabolism was also accompanied by the upregulation of the development-related gene *span* and two genes with putative regulatory roles (*nec1*, *lrp1*), and for the autumn subsets only the upregulation of the feeding-related gene *trp1*.

In early winter (Jun15 subset) and spring (Oct15 subset), krill from the latitudinal light treatments 66°S and 52° were not discriminated by sPLS-DA analysis, and only minor differences were detected in early summer (Dec15 subset, Table 4.8). However, in late winter (Aug15 subset, Table 4.9), the negative loadings of component 2 indicated an upregulation of genes related to the regulatory processes (*nec1*), development (*span*), and translation and transcription in krill from treatment 52°S with respect to treatment 66°S. Moreover, the positive loadings of the same component showed an upregulation of genes related to lipid transport in krill from treatment 66°S with respect to treatment 52°S.

Under treatment DD, we observed an upregulation of genes related to metabolic and potential regulatory processes during winter and spring. In early winter (Jun15 subset, Table 4.5), the selected genes with positive loadings indicated an upregulation

of the lipid metabolic gene *fasn*, and of genes related to lipid transport and the biosynthesis of methyl farnesoate (*famet*) in krill from treatment DD with respect to the light treatments 52°S/66°S. In late winter (Aug15 subset, Table 4.6), the positive loadings of component 1 showed an upregulation of metabolic genes related to glycolysis, citric acid cycle and the respiratory chain, and the regulatory gene *nec1* in individual krill of treatment DD with respect to the light treatments 52°S/66°S. In spring (Oct15 subset, Table 4.7), the positive loadings of component 1 indicated an upregulation of genes related to glycolysis, citric acid cycle, respiratory chain, translation and transcription, and of genes with putative regulatory functions (*nec1*, *famet*, *lrp1*) in krill from treatment DD with respect to treatments 66°S/52°S.

The sPLS-DA results indicate that krill from treatment DD showed a downregulation of most genes during summer with respect to the light treatments. In early summer (Dec15 subset, Table 4.8), the identified gene signature of component 1 points to an upregulation of the putative regulatory gene *famet* and the down-regulation of the majority of the other selected genes (amongst others metabolic genes) in krill from treatment DD with respect to krill from the light treatments 66°S/52°S. In late summer (Feb16 subset, Table 4.9), component 1 solely indicated an upregulation of the glycolysis-related gene *gapdh* in krill from treatment DD (with respect to the light treatments 66°S/52°S), whereas the other selected genes e.g. related to lipid metabolism, lipid transport were down-regulated.

For clock gene expression and prostaglandin metabolism, we observed similar expression patterns with an upregulation in the light treatments 52°S/66°S and a down-regulation under constant darkness. This was especially evident from the sPLS-DA analysis of the subsets Jun15 (early winter, Table 4.5), Oct15 (spring, Table 4.6), Dec15 (early summer, Table 4.8), and Feb16 (late summer, Table 4.9) that all revealed a discrimination between krill from the light treatments 52°S/66°S and constant darkness. The detected gene signatures of these subsets suggested a mostly complete upregulation of genes related to the circadian clock and prostaglandin metabolism in krill from the light treatments 52°S/66°S with respect to treatment DD. A 'co-upregulation' of clock genes and prostaglandin metabolic genes was also indicated in autumn, with an upregulation of these genes in individual krill from treatment 52°S with respect

to treatment 66°S during the first autumn (Apr15 subset, Table 4.4), and a upregulation of these genes in individual krill from 66°S with respect to treatment 52°S/DD in the second autumn (Apr16 subset, Table 4.10). In late winter (Aug15 subset, Table 4.6), genes related to circadian clock and prostaglandin metabolism were not selected for the first component which contributed to the separation of krill from the light treatments 66°S/52°S and treatment DD, and for the second component, these genes showed partly upregulation in both individual krill from treatment 52°S and 66°S.

We did not find fully conclusive seasonal expression patterns for the feeding indicator genes *astc* and *trp1* for most of the sPLS-DA results. For instance for the late winter subset (Aug15, Table 4.6), component 2 indicated the upregulation of the gene *astc* (putative feeding inhibitor) and the downregulation of *trp1* (putative digestive enzyme) in krill from treatment 52°S with respect to treatment 66°S (where we would expect the opposite). Therefore, we did not focus on these genes for our main conclusions.

4.5 Discussion

This study analysed the seasonal gene expression patterns of Antarctic krill in a long-term laboratory experiment at simulated low-latitude light regime 52°S, at high-latitude light regime 66°S and at constant darkness over two years. It complemented the earlier study by Höring et al. (2018) that analysed morphometric and lipid content data from the same experiment.

The simulated light regimes 52°S and 66°S seem to induce seasonally rhythmic gene expression patterns that are more pronounced under the high latitude light regime (66°S). The rhythmically expressed genes have functional roles in the processes of glycolysis, citric acid cycle, respiratory chain, lipid metabolism, lipid transport, prostaglandin biosynthesis, the activation of neuropeptides, the circadian clock, translation, and feeding. The predominant loss of rhythmic gene expression under constant darkness further suggests that photoperiodic cues are required to maintain the seasonal gene expression patterns of Antarctic krill over longer periods.

The analysiss of monthly gene expression patterns of our laboratory experiment further indicates that different latitudinal light regimes may trigger flexible gene expression patterns in Antarctic krill. In late summer and autumn, we found an upregulation of genes related to different metabolic processes, lipid metabolism and transport in Antarctic krill kept under the high-latitude light regime 66°S with respect to krill from the low-latitude light regime, which may be an adaptive mechanism to facilitate enhanced lipid accumulation at higher latitudes in this period of the year. Antarctic krill that is affected by a low latitude light regime may also prepare earlier for spring, which is suggested by our observation of an upregulation of genes related to the activation of neuropeptides, development, translation and transcription in krill kept under the low-latitude light regime 52°S with respect to krill kept under the high-latitude light regime 66°S in late winter.

Under constant darkness, Antarctic krill does not seem to enter a strong state of metabolic depression, based on the observation of the upregulation of genes related to different metabolic and regulatory processes with respect to the light treatments during winter and spring. The observed downregulation of most genes during summer in Antarctic krill kept under constant darkness may be explained by the missing *Zeitgeber* that may have caused an arrhythmic condition in these krill.

The prostaglandin metabolism related genes *hpgds* and *crb1* as well as the putative regulatory genes *famet nec1*, and *lrp1* may have important roles in the regulation of the seasonal reproductive cycle and metabolism of Antarctic krill. Constant darkness seems to lead to a disruption of the prostaglandin metabolism and an upregulation of *famet*, *nec1* and *lrp1* during winter and spring which coincide with the observation of a less pronounced reproductive cycle under constant darkness (Höring et al., 2018) and the enhanced expression of metabolic genes in winter. Moreover, a link between clock gene expression and the expression of genes related to prostaglandin biosynthesis may be suggested by the finding of similar expression patterns in these genes. Both simulated latitudinal light regimes seem to lead to an upregulation of genes related to the circadian clock and prostaglandin biosynthesis, whereas constant darkness seems to trigger a downregulation of these processes.

The genes *rps13, rps18, rpb1, usp46* and *rrn3* were originally tested as housekeeper

candidates, because they proved to have a stable expression under variable photoperiods and seasonal conditions in previous studies (Häfker et al., 2018; Piccolin et al., 2018a; Piccolin et al., 2018b; Shi et al., 2016, and Biscontin et al., in prep). Based on geNorm analysis and the observation of partly seasonally rhythmic expression patterns, we cannot confirm the stability of *rps13*, *rps18* and *rpb1* in photoperiodic-controlled long-term laboratory experiments with Antarctic krill. Our data rather suggests that *usp46* and *rrn3* are suitable reference genes when studying gene expression of Antarctic krill under different simulated light regimes over longer periods.

The observed expression patterns of the feeding related genes *astc* and *trp1* may only partly explain the seasonal pattern of feeding that was derived from a morphometric index measured in the same experiment (Höring et al., 2018). Höring et al. (2018) observed a steady increase in feeding index with a certain seasonality showing a peak of feeding in late autumn/winter under the three different light treatments (52°S, 66°S and DD). The gene *trp1* coding for a digestive enzyme shows a significant rhythmic pattern under the high latitude light regime 66°S (but not significant for treatment 52°S and DD) in this study. The lower expression of *trp1* during winter and spring and its higher expression during summer and autumn may indicate a seasonal cycle of digestive activity that may be mediated by the photoperiod-dependent feeding activity (Teschke et al., 2007) and thereby leads to a peak in feeding index in late autumn (Höring et al., 2018). However, trypsin may also be involved in the degradation of cuticle during the moult cycle of Antarctic krill (Seear et al., 2010).

The potential feeding inhibitor *astc* showed a more irregular pattern in our study where a significant rhythmic pattern was solely observed under treatment 52°S. For instance, the high expression values of *astc* under the low latitude light regime 52°S with respect to 66°S in late winter cannot be explained by the simulated light conditions, because one would rather expect a higher feeding inhibition in winter under the high latitude light regime. This may likely be explained by the multiple functional roles of *astc* which may have introduced additional variation in our dataset. In crustaceans, C-AST like peptides were found to have modulatory roles in feeding and locomotion (Wilson and Christie, 2010) and cardioactive activity (Dickinson et al., 2009). In insects, allatostatins have been shown to inhibit the production of juvenile hormone, an

important regulator of growth, development and reproduction (Weaver and Audsley, 2009).

The gene expression results under the latitudinal light treatment 66°S and constant darkness are generally comparable to the study by Piccolin et al. (2018b) who investigated the seasonal metabolic cycle of Antarctic krill in a 1-year laboratory experiment. However, the metabolic genes *cs*, *pfk6* and *atp* had their lowest gene expression values in late winter (August) in the current study, whereas Piccolin et al. (2018b) has already found an increase of expression of these genes under treatment 66°S in the same month. Moreover, we did not find a peak expression of the clock gene *cry2* in February, but the other two clock genes *tim1* and *clock* revealed similar patterns as in the study by Piccolin et al. (2018b). These differences may be caused by the slight differences in the simulation conditions of the latitudinal light regime 66°S as well as the different sampling conditions of Antarctic krill in both studies. Piccolin et al. (2018b) collected the Antarctic krill over a 24h period (instead of sampling at one time point of the day) and used much higher replicate numbers for each sampling month than in the current study.

The incomplete discrimination between the simulated latitudinal light regimes 52°S, 66°S and constant darkness in this study may on the one hand represent a genetic or phenotypic variability in the population of Antarctic krill that was used for the photoperiodic long-term experiments. On the other hand, the multivariate statistical approach sPLS-DA used in this study was originally developed for high throughput biological datasets (Lê Cao et al., 2011).

Thus, our dataset might be too small, the gene expression patterns between the treatments too similar, or it may not contain the genes that are most relevant to discriminate between the treatments. Due to the limited number of Antarctic krill samples available from the long-term experiment, we decided to analyse krill of mixed sex, size and moult stages, which may have introduced further variability in the dataset. Moreover, higher replicate numbers ($\leq$10 krill samples per month and treatment) may further improve the gene expression results in future long-term experiments with Antarctic krill.

When comparing the results from this long-term experiment to observations in

Antarctic krill in the field, we are able to draw conclusions regarding the possible control mechanisms of the different seasonal processes in Antarctic krill.

Most of our results show strong similarities to observations from the field which indicate that the seasonal cycle of Antarctic krill and the underlying gene expression patterns seem to be mainly regulated by light regime. Under the photoperiodic controlled laboratory conditions applied here, an upregulation of genes with various metabolic and regulatory functions was found during summer. This observation agrees with findings for Antarctic krill in the field that were sampled in different latitudinal regions in the Southwest Atlantic sector of the Southern Ocean (Seear et al., 2012, and Höring et al., in prep. (Publication 1)). Moreover, the repressed gene expression levels suggest that Antarctic krill enters a state of winter quiescence under the photoperiodic controlled laboratory conditions and constant food supply. Therefore, we propose that the observed winter quiescence in Antarctic krill in the field (Meyer et al., 2010) may be mainly controlled by light regime.

The field study by Höring et al. (in prep.; Publication 1) formed the basis for the selection of several genes in this study that were found to be upregulated in Antarctic krill from the field during summer. These genes included the hormone metabolism related genes *hpgds*, *cbr1* and *nec1*, the lipid transport related genes *vtlgl* and *fabp*, the lipid metabolism related genes *fasn*, *acsl* and *gal3st*, the feeding related gene *trp1*, the development related gene *span*, the energy metabolism related gene *ndufa4*, the carbohydrate metabolism related gene *gapdh*, and the receptor related gene *lrp1*. Most of these genes showed significantly seasonal patterns of gene expression under the simulated light regimes in this study, which suggests that their seasonal expression patterns may also be regulated by light regime in the field.

For the first time, we show that Antarctic krill is able to adjust its gene expression to different latitudinal light regimes in the Southern Ocean. In late summer and autumn, we found an upregulation of genes mostly involved in carbohydrate and energy metabolism, lipid metabolism and lipid transport under the simulated light regime 66°S. The lipid metabolic gene *fasn* plays a role in fatty acid synthesis for storage (Chirala and Wakil, 2004), whereas the gene *acsl* codes for an enzyme that activates long-chain fatty acids for both synthesis and degradation of lipids (Mashek et

al., 2007). The upregulation of these processes in late summer and autumn under the simulated light regime 66°S (with respect to 52°S) may be explained by an enhanced lipid accumulation in Antarctic krill in this period that is required under the high-latitude light regime in preparation for winter. This is also found in the field where higher lipid stores were observed in the Lazarev Sea region (light conditions similar to light regime 66°S) with respect to the low-latitude region South Georgia where prolonged photoperiods and no sea ice cover occur during winter (light conditions similar to light regime 52°S) (Schmidt et al., 2014). These results support the assumption that the seasonal lipid metabolic cycle of krill is regulated by latitudinal light regime (Höring et al., 2018).

Another indication for the variable effect of different latitudinal light regimes on Antarctic krill is the observation of the more pronounced seasonal cycle of gene expression that was found under the high latitude light regime 66°S in this study indicated by the highest number of significantly rhythmic genes compared to the other treatments. Moreover, we found an upregulation of genes related to the activation of neuropeptides, development, translation and transcription under the simulated light regime 52°S (with respect to 66°S) in late winter, which may indicate that the low-latitude light regime triggers an earlier preparation for spring in these krill, and consequently a shorter/or less pronounced winter quiescence at lower latitudes. Similar effects have already been observed in the field by Höring et al. (in prep., Publication 1) who found less seasonal differences in gene expression between summer and winter krill from the low-latitude region South Georgia compared with regions at higher latitudes.

However, some observations from the field cannot be confirmed in this study. In the low-latitude region South Georgia, a higher feeding activity and an enhanced expression of feeding related genes was observed with respect to regions at higher latitudes in winter (Schmidt et al., 2014; Seear et al., 2012). Although we found a seasonal pattern of feeding in our photoperiodic controlled long-term experiment, we were not able to detect a higher feeding activity under the low-latitude light regime 52°S in winter. These findings may support the idea that the seasonal feeding behaviour of Antarctic krill may be regionally adjusted in the field by other environmental cues

than light regime such as regional food supply (Schmidt et al., 2014). Regional feeding conditions may also explain the complex gene expression patterns that were observed in Antarctic krill in different latitudinal regions by Höring et al. (in prep., Publication 1).

Moreover, this study gives insight into different signalling pathways that seem to be involved in the regulation of the seasonal cycle of Antarctic krill. This study suggests that seasonal clock gene expression is regulated by light regime. We found partly seasonal rhythmicity of clock gene expression and a general upregulation of clock genes under the simulated light regimes 52°S and 66°S throughout the entire study period, whereas clock gene expression was disrupted in Antarctic krill under constant darkness due to the missing *Zeitgeber*. Piccolin et al. (2018b) proposed that seasonal differences in clock gene expression may be linked to the regulation of seasonal life cycle events in Antarctic krill. Clock genes may play a role in the seasonal time measurement in Antarctic krill, because a similar regulation mechanism was found to control diapause in insects (Meuti et al., 2015). However, the functional link between clock gene expression and the seasonal output pathways still needs to be validated in knock-out experiments with Antarctic krill.

Another level of regulation includes prostaglandins which are physiologically active lipid compounds that may control the reproductive cycle of Antarctic krill. Prostaglandins regulate the ovarian development (Wimuttisuk et al., 2013), gonad maturation and moulting in crustaceans (Nagaraju, 2011). For the prostaglandin biosynthesis related genes *hpgds* and *cbr1* (Wimuttisuk et al., 2013), we found a similar pattern as in the clock genes with a disruption of the seasonal rhythmic expression patterns of these genes in Antarctic krill under constant darkness. Based on the observation that Antarctic krill's reproductive cycle was weakened under constant darkness (Höring et al., 2018), we suggest that prostaglandins are mainly involved in the regulation of the seasonal cycle of reproduction in Antarctic krill. Moreover, seasonal patterns of prostaglandin biosynthesis are regulated by light regime, and possibly linked to clock gene expression.

We further propose that the gene *famet* may play a role in the regulation of reproduction, moulting and the initiation of winter quiescence in Antarctic krill. The

gene *famet* codes for an enzyme that catalyses the final step of the synthesis of methyl farnesoate, a precursor of the insect juvenile hormone III (Gunawardene et al., 2002). Methyl farnesoate stimulates moulting and reproductive development in crustaceans (Reddy et al., 2004). In insects, the suppression of the related molecule juvenile hormone plays a role in the initiation of diapause (Liu et al., 2017). Under constant darkness, we detected an upregulation of *famet* in early winter, spring, and early summer. This may be explained by the inhibitory mechanism that controls the secretion of methyl farnesoate from the mandibular organ which is controlled by the eyestalk neuropeptide mandibular organ-inhibiting hormone in crustaceans (Swetha et al., 2011). Eye stalk ablation leads to increased levels in methyl farnesoate in the mandibular organ and hemolymph (Tsukimura and Borst, 1992). This may explain our observation of increased *famet* levels and the disruption of Antarctic krill's reproductive cycle under constant darkness. Constant darkness probably interrupts the inhibitory mechanism that controls the secretion from the mandibular organ which leads to higher synthesis levels and secretion of methyl farnesoate and therefore advanced maturation stages of Antarctic krill.

This study also indicates that enzymes related to the family of subtilisin-like proprotein convertases may play a role in the regulation of seasonal hormone levels in Antarctic krill. These enzymes are known to activate precursors of hormones and neuropeptides via proteolysis (Zhou et al., 1999). They comprise the prohormone convertase 1 (gene *nec1*), which was found to be involved in the reproduction process of the abalone (Zhou and Cai, 2010). In crustaceans, prohormone convertase 2 like genes were found to be expressed in the neuroendocrine cells of the eyestalk (Toullec et al., 2002) and to bind the crustacean hyperglygemic hormone (Tangprasittipap et al., 2012). Therefore, they could be involved in the activation of many neuropeptides that are produced in the neuroendocrine cells of the eyestalk containing the X-organ-sinus gland complex in crustaceans (Tangprasittipap et al., 2012; Toullec et al., 2002). These neuropeptides include the crustacean hyperglemic hormone, the gonad (vitellogenic) inbiting factor, and the molt inhibiting hormone which have various functions in the regulation of glucose level, reproduction and growth in crustaceans (Nagaraju, 2011).

In our study, the prohormone convertase related gene *nec1* showed a significant seasonal pattern of expression under the simulated light regime 66°S with a downregulation during the winter months. In contrast, *nec1* was found to be upregulated during late winter and spring in Antarctic krill under constant darkness. These observations suggest that the gene *nec1* is influenced by light regime and that its seasonal pattern is disrupted under constant darkness. This may subsequently lead to a disturbance of the hormonal control mechanisms of seasonal processes in Antarctic krill such as reproduction or metabolism.

Moreover, the cell-surface receptor related gene *lrp1* may be involved in the regulation of metabolic processes in Antarctic krill. We observed an upregulation of *lrp1* (and *nec1*) in krill under the latitudinal light regime 66°S in late summer and autumn, and under constant darkness in spring, which was always accompanied with an upregulation of different metabolic genes. The low-density lipoprotein receptor-related protein 1 (gene *lpr1*) may have multiple functions, for instance in cellular lipid homeostasis and the regulation of signalling pathways (Franchini and Montagnana, 2011).

This study provides further evidence for the effect of different latitudinal light regimes on the seasonal cycle of Antarctic krill and may therefore be relevant for future modelling studies of *E. superba* in the Southern Ocean (Höring et al., 2018). Our findings point to a highly flexible seasonal timing system in Antarctic krill that is highly advantageous under the extreme latitudinal light regimes in the Southern Ocean, especially under the observed southward shift of Antarctic krill in the Southwest Atlantic Sector due to effects by global warming (Atkinson et al., 2019). However, more research is needed to understand if its seasonal timing system is flexible enough to respond to climate-induced changes in the timing of phytoplankton blooms that may lead to 'mismatches' in the energy requirements of Antarctic krill (Durant et al., 2007).

Moreover, we give novel insights in the molecular mechanisms that govern the seasonal cycle of Antarctic krill under the influence of different latitudinal light regimes. These findings form a basis for future functional studies of the seasonal timing system in Antarctic krill, by applying e.g. the gene silencing technique RNA interference (Hannon, 2002). If the genome sequence of *E. superba* becomes available and stable breeding conditions are established under laboratory conditions in the future,

the genome editing technique CRISPR/Cas9 (Sander and Joung, 2014) may also be considered for further functional analyses of genes related to the seasonal cycle of Antarctic krill.

4.6 Conclusion

We analysed the expression patterns of multiple genes with metabolic and regulatory functions in Antarctic krill, kept in a controlled two-year laboratory experiment under simulated latitudinal light regimes of 52°S and 66°S and under constant darkness. On gene expression level, we show that Antarctic krill responds differently to latitudinal light regimes with a stronger seasonality observed under the more extreme high-latitude light regime 66°S. On the contrary, constant darkness seems to disrupt the seasonal expression patterns of different metabolic and regulatory genes, eventually affecting Antarctic krill's seasonal cycle of physiology and development over longer periods. These results suggest that latitudinal light regime is an important *Zeitgeber* for Antarctic krill and may be the prominent environmental factor contributing to Antarctic krill's flexible seasonal response in the different latitudinal habitats of the Southern Ocean. Moreover, our findings reveal that the circadian clock genes, the physiologically active compounds prostaglandins and methyl farnesoate as well as the neuropeptide-processing enzyme prohormone convertase 1 and the low-density lipoprotein receptor-related protein 1 may contribute to the regulation of seasonal processes in Antarctic krill such as reproduction, growth and metabolism.

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5. General Discussion

This dissertation gives new insights in the flexible seasonal timing system of Antarctic krill at different latitudes in the Southern Ocean and in the molecular mechanisms underlying the seasonal cycles of Antarctic krill. First, I will discuss how different environmental factors influence seasonal processes in Antarctic krill with special focus on the effect of latitudinal light regime. Then, I will move on to the endogenous timing of Antarctic krill referring to potential mechanisms of photoperiodic entrainment, the molecular basis of seasonal time measurement and photoperiodic plasticity. Subsequently, I will give insights in regulatory pathways that may control seasonal processes in Antarctic krill focussing on genes related to the visual transduction system, the circadian clock, insulin and juvenile hormone-like signalling, and the metabolism of signalling compounds in Antarctic krill.

5.1 The effect of latitudinal light regime on the seasonal cycle of *E. superba*

From field studies, we know that Antarctic krill is able to synchronize its seasonal physiological functions to the extreme seasonal changes of photoperiod, food regime and sea ice conditions in the Southern Ocean by having evolved different overwintering strategies (Ericson et al., 2018; Hellessey et al., 2018; Meyer et al., 2010; Seear et al., 2012; Siegel, 2012). However given the large circumpolar distributional range of Antarctic krill, it is still a matter of discussion which environmental factors shape the seasonal patterns of physiology and behaviour in the different latitudinal habitats of Antarctic krill in the field (Kawaguchi et al., 2006; Schmidt et al., 2014; Seear et al., 2012; Spiridonov, 1995). Variable environmental conditions in the distributional range

of Antarctic krill include local differences of ambient water temperature, food availability and latitudinal light regime. The potential involvement of these environmental factors in the regional adaptation and the regulation of seasonal cycles in Antarctic krill will be discussed in detail in the following paragraphs.

Ambient water temperature may influence local differences in Antarctic krill growth, maturity and metabolic activity, but is most likely not the main environmental cue triggering Antarctic krill's seasonal cycle of growth, maturity and metabolism. Higher water temperatures may increase the metabolic activity of Antarctic krill (Segawa et al., 1979) and its growth rates by shortening the intermoult period (Brown et al., 2010; Buchholz, 1991). Different temperature regimes also led to differences in the timing of the shortening of the intermoult period during the maturity cycle of Antarctic krill (Kawaguchi et al., 2007). Seasonal differences in the intermoult period of Antarctic krill were often linked to the seasonal cycle of water temperature (Kawaguchi et al., 2006; Tarling et al., 2006). However, long-term laboratory experiments under constant food supply and temperature rather suggest that the general seasonal patterns of growth, maturity and metabolic activity are mediated by light regime and an endogenous timing system in Antarctic krill (Brown et al., 2010; Brown et al., 2011; Brown et al., 2013). Results from this dissertation (Publication 1) further suggest that temperature is not the main factor shaping the seasonal differences in gene expression between summer and winter krill in its different latitudinal habitats, because least differences were observed in the low-latitude region South Georgia where seasonal temperature differences are the strongest (Whitehouse et al., 1996).

Seasonal and regional differences in food quantity and quality may affect the seasonal physiological and behavioural processes in Antarctic krill. The variable timing of phytoplankton blooms in the different latitudinal habitats of Antarctic krill has been discussed in relation to regional differences in the reproductive timing of Antarctic krill (Spiridonov, 1995) and the timing of its main growth period (Kawaguchi et al., 2006). Indeed, it has been shown in laboratory studies that high food supply (Buchholz, 1991) can accelerate growth and maturation in Antarctic krill (Kawaguchi et al., 2007). In the field, differences in food availability have also been associated with the observed regional variation of feeding behaviour in Antarctic krill in winter (Schmidt

et al., 2014). We explained the comparatively low seasonal differences in gene expression in the low-latitude region South Georgia by the milder seasonal conditions including effects of the low-latitude light regime (16 h light in mid-summer and even 9 h light in mid-winter, see Figure 1b), no sea ice cover in winter and consequently the generally higher food availability in that region (Publication 1).

Indeed, results from our controlled laboratory study suggest that feeding and digestion in Antarctic krill might be controlled by a combination of light regime and food supply (Publication 2 & 3). We found that light regime generally affected the seasonal pattern of feeding index and the expression of feeding-related genes in Antarctic krill indicating a slightly reduced feeding activity during winter. However, the field observations of extremely low feeding activity of Antarctic krill during winter (Meyer et al., 2010) and local differences of winter feeding behaviour (Schmidt et al., 2014) were not confirmed under the simulation of different latitudinal light regimes and constant food supply in the laboratory. This indicates that the regional feeding behaviour of Antarctic krill is probably largely dependent on regional food availability.

This dissertation shows that light regime, and in particular changes in latitudinal light regime, play a major role for the synchronization of Antarctic krill's seasonal cycles to the variable seasonal conditions in its different latitudinal habitats of the Southern Ocean. For the first time, a controlled two-year laboratory experiment was conducted that simulated the latitudinal light regimes 52°S and 66°S, and constant darkness under stable food supply and temperature conditions (Publication 2 & 3). This two-year laboratory experiment partly confirms results from other shorter laboratory studies showing that light regime is the major cue that entrains seasonal cycles of maturity (Brown et al., 2011), growth, metabolic activity and gene expression in Antarctic krill (Piccolin et al., 2018). Under controlled laboratory conditions, light regime stimulates the different overwintering mechanisms (an their recovery towards summer) of Antarctic krill that are also observed in the field: including the regression of the outer sexual organs (Siegel, 2012), the stagnation of growth or shrinkage (Meyer et al., 2010; Quetin and Ross, 1991), metabolic depression and reduced gene expression during winter (Meyer et al., 2010; Seear et al., 2012), and the seasonal accumulation of lipid stores (Meyer et al., 2010). In particular, publication 2 revealed a seasonal cycle

of sexual maturity and growth under both simulated latitudinal light regimes over a two-year period, and gave novel insights in the effect of latitudinal light regime on the seasonal cycle of feeding, lipid content and the critical photoperiod for maturity in Antarctic krill. Publication 3 showed that different latitudinal light regimes could flexibly affect the seasonal expression patterns of different metabolic and regulatory genes in Antarctic krill.

The following observations support our hypothesis that latitudinal light regime affects the seasonal cycle of Antarctic krill (Publications 2 & 3).

Results from publication 2 & 3 suggest that the seasonal cycle of lipid metabolism is regulated and adjusted according to the latitudinal light regime. Under the simulated high-latitude light regime 66°S, we observed a pronounced seasonal cycle of lipid content as well as an enhanced expression of different metabolic and lipid-transport related genes in late summer and autumn, possibly related to increased lipid accumulation during this period. This may be a potential adaptation mechanism of Antarctic krill that is regulated by the high-latitude light regime leading to larger lipid stores that are required to survive in regions at higher latitudes, e.g. Lazarev Sea, that are characterized by near-constant darkness and extremely low food availability during winter. Therefore, differences in latitudinal light regime may partly explain why winter krill from the low-latitude region South Georgia was found to have lower lipid stores than Antarctic krill from the higher latitudinal regions Bransfield Strait and Lazarev Sea (Schmidt et al., 2014).

On gene expression level, the effect of latitudinal light regime on Antarctic krill further manifested. We observed the occurrence of seasonally rhythmic gene expression under the simulated high latitudinal light regime 66°S, whereas the seasonally rhythmic patterns in gene expression seemed to be weaker under the low-latitude light regime 52°S (Publication 3). In late winter, we also found an upregulation of genes related to the activation of neuropeptides, development, translation and transcription in Antarctic krill kept under the simulated low-latitude light regime 52°S which may point to a shorter/or less distinct state of winter quiescence in these krill. Similar observations were made in Antarctic krill from the field where the least seasonal differences in gene expression were observed in the low-latitude region South Georgia

(54°S) with respect to the higher latitudinal regions South Orkneys/Bransfield Strait and Lazarev Sea (60°S-66°S) (Publication 1). Therefore, we suggest that latitudinal light regime is an important cue for Antarctic krill to be able to regionally adjust its seasonal patterns of gene expression in the field.

To which extent the seasonal cycles of metabolism, growth, maturity, lipid utilisation and feeding in Antarctic krill are directly affected by the latitudinal light regime or indirectly modulated by the interaction with other internal regulatory processes, it is not yet clear. This comprises the open question if seasonal changes in metabolic activity may affect feeding activity in Antarctic krill, or the other way around (Teschke et al., 2007). The seasonal maturity cycle of Antarctic krill also seems to partly interact with seasonal growth patterns (Tarling et al., 2016; Thomas and Nash, 1987) and the seasonal utilisation of lipid stores (Teschke et al., 2008). Moreover, it is known that seasonal processes in Antarctic krill are regulated by an endogenous timing system that will be discussed in the following section.

5.2 Endogenous timing of seasonal processes in *E. superba*

This dissertation complements findings from earlier studies revealing that seasonal cycles of maturity, growth and metabolic activity are regulated by an endogenous timing system in Antarctic that is likely synchronized by light regime (Brown et al., 2011; Brown et al., 2013; Piccolin et al., 2018). During our controlled two-year laboratory experiment, we show that seasonal patterns of growth, feeding and maturity persist in Antarctic krill exposed to constant darkness (Publication 2) which is an indication for the presence of a biological (or internal) clock controlling these seasonal processes (Visser et al., 2010). Circannual clocks control the internal timing of seasonal life cycle events and they are typically entrained by photoperiodic cues (Helm et al., 2013). This is also likely the case for Antarctic krill, because light regime has been found to be the major cue for the stimulation of circannual rhythms in Antarctic krill (discussed in detail above). Antarctic krill's susceptibility to other environmental factors such as temperature, food supply or social cues may be restricted to particular periods of the underlying clock-controlled rhythms in Antarctic krill, a mechanism also known in

other animals (Visser et al., 2010). Interestingly, social cues have been found to influence the seasonal behaviour and reproduction of birds (Helm et al., 2006). A similar mechanism of social interaction may also play a role for the adjustment of seasonal processes in the different latitudinal habitats of Antarctic krill, such as the synchronization of the spawning season.

Considering the huge latitudinal range of Antarctic krill and the corresponding differences in seasonal day length, Antarctic krill must have evolved a highly flexible clock machinery to ensure survival under these extreme light conditions.

In publication 2, we used the concept of critical photoperiod to emphasize Antarctic krill's flexibility in photoperiodic timing under different latitudinal light regimes. Interestingly, the critical photoperiod for maturity in Antarctic krill was found to be higher under the high-latitude light regime 66°S with respect to the low-latitude regime 52°. The increasing trend in critical photoperiod with latitude in Antarctic krill seems to be an adaptation to the more extreme light conditions at higher latitudes where Antarctic krill need to enter the state of sexual regression and re-maturation at comparatively longer photoperiods than at lower latitudes. Similar adaptations have been observed in insects for diapause initiation that showed a similar increasing trend of critical photoperiod with latitude (Bradshaw and Holzapfel, 2007; Hut et al., 2013; Tyukmaeva et al., 2011)

Which specific light cues are required to synchronize Antarctic krill's seasonal timing system in different latitudinal regions is not yet clear. A concept of photoperiodic entrainment has been suggested for the seasonal cycles of maturity and metabolic activity in Antarctic krill (Brown et al., 2011; Piccolin et al., 2018). From observations under photoperiodic controlled laboratory experiments, these authors suggest that the timing of sexual regression and the reduction of metabolic activity towards winter are dependent on a decrease in day length, whereas sexual re-maturation and metabolic recovery towards summer are initiated by the endogenous timing system of Antarctic krill independent of light cues. A similar mechanism could have triggered the endogenous cycle of maturity, and the more robust endogenous rhythms of growth and feeding in Antarctic krill under constant darkness of our two-year laboratory experiment (Publication 2). However, the study by Hirano et al. (2003) also indicates that

maturation and spawning in Antarctic krill may be triggered by a dark period followed by a period of long photoperiods. Similar photoperiodic cues are required in some bird species for the initiation of the breeding season (Helm et al., 2013). These findings suggest that the seasonal re-maturation of Antarctic krill may be controlled by a combination of endogenous cues that may mostly trigger the re-maturation of the outer sexual traits and additional light cues that may induce vitellogenesis and spawning in Antarctic krill.

The molecular mechanisms that control the seasonal timing of physiological and behavioural responses are still poorly understood in animals (Helm et al., 2013). Conceptionally, the photoperiodic signal is perceived by an internal photoperiodic timer that regulates gene expression and neuroendocrine signalling, thereby initiating complex physiological, reproductive and developmental processes (Bradshaw and Holzapfel, 2007; Visser et al., 2010).

A recent study suggests that the circadian clock may be involved in the regulation of the seasonal cycles of Antarctic krill (Piccolin et al., 2018). Under photoperiodic-controlled laboratory experiments, Piccolin et al. (2018) found a seasonal expression pattern of the clock genes *clk*, *cry2*, and *tim1* which was mostly similar to our observations made in publication 3. There is evidence from RNAi experiments that the circadian clock as a functional entity may control the photoperiodic initiation of reproductive diapause in insects (reviewed by Meuti and Denlinger (2013) and Meuti et al. (2015)). A similar mechanism may be present in Antarctic krill, given that the circadian clock-work in Antarctic krill is functionally characterized and comprises a photoperiod-induced entraining mechanism (Biscontin et al., 2017). However, the functional involvement of the circadian clock in the timing of seasonal cycles of Antarctic krill still has to be proven in knock-out experiments.

On the other hand, Antarctic krill may have evolved a seasonal timing mechanism that is independent of the circadian timing system, such as in the pitcher-plant mosquito (Emerson et al., 2009). Emerson et al. (2009) also states that this does not exclude the possibility that individual clock genes are involved in the seasonal time measurement. Other potential seasonal timing mechanisms include the regulation by non-coding RNAs and epigenetic modification ((Helm and Stevenson, 2014). Both

processes have been studied in hibernating mammals and were associated with the regulation of various processes such as lipid metabolism and the timing of reproductive development (Lang-Ouellette et al., 2014; Stevenson and Lincoln, 2017; Stevenson and Prendergast, 2013).

Which molecular factors promote the seasonal and regional photoperiodic plasticity in Antarctic krill are unknown. Clock gene polymorphisms have been linked to the photoperiodic plasticity of seasonal timing in insects, birds and fish (Caprioli et al., 2012; Hut et al., 2013; O'Malley et al., 2010). Genetic variation, such as clock gene polymorphisms, may also play a role for latitudinal adaptation of the seasonal response of Antarctic krill in the field. However in the same population of Antarctic krill, different seasonal gene expression patterns were detected during our simulation of the latitudinal light regimes 66°S and 52°S (Publication 3). These findings likely suggest a general plasticity of the seasonal timing system of Antarctic krill independent of genetic variation. The visual plasticity within the extreme light conditions of the Southern Ocean may be linked to the flexible 'light' adjustment of components within the visual perception system of Antarctic krill similar to the adaptive mechanisms explained for the circadian visual system in arthropods by Mazzotta and Costa (2016). The discovery of the versatile opsin photopigments of Antarctic krill (Biscontin et al., 2016) and the recent observation of seasonal expression patterns of circadian-related opsins under a simulated light regime (Piccolin et al., 2018) further support the assumption that the visual perception system may play a major role in the regulation of the flexible phenology of Antarctic krill.

5.3 Insights into the regulatory pathways mediating the seasonality in *E. superba*

To identify regulatory processes that are potentially involved in the seasonal timing and latitudinal adaptation of Antarctic krill, we used an RNAseq approach analysing regional and seasonal differences in gene expression of summer and winter krill from three different latitudinal regions: South Georgia (54°S), South Orkneys/Bransfield Strait (60°S-63°S) and Lazarev Sea (62°S -66°S) (Publication 1). Thereby, we were not

TABLE 5.1: List of seasonally expressed genes with functional process that were found to be significantly rhythmic over a period of 12 months under the light regime 66°S using RAIN (Publication 3).

Gene	Functional process
phosphofructokinase-6	glycolysis
glyceraldehyde-3-phosphate dehydrogenase	glycolysis
citrate synthase	citric acid cycle
ATP synthase subunit gamma	respiratory chain
cytochrome c oxidase subunit NDUFA4	respiratory chain
carbonyl reductase 1	prostaglandin biosynthesis
hematopoietic prostaglandin D synthase	prostaglandin biosynthesis
neuroendocrine convertase 1	activation of neuropeptides
fatty acid binding protein	lipid transport
fatty acid synthase	fatty acid synthesis
galactosylceramide sulfotransferase	sulfation of membrane glycolipids
ribosomal protein S13	protein synthesis
ribosomal protein S18	protein synthesis
anionic trypsin 1	digestion of peptides
timeless1	circadian clock

only able to characterize seasonal gene expression in Antarctic krill under variable environmental conditions, but also to propose various target genes that are possibly involved in the regulation of seasonal processes in Antarctic krill. These target genes may be a starting point to elucidate the molecular mechanisms of the seasonal timing system in Antarctic krill. In publication 3, we chose a few of these target genes, in combination with other genes of interest, to clarify the effect of latitudinal light regime on the expression of these genes. We showed that light regime affected genes with functions in glycolysis and the citric acid cycle, the respiratory chain, lipid metabolism and transport, prostaglandin biosynthesis, the activation of neuropeptides, translation, the digestion of peptides and the circadian clock (Table 5.1).

RNAseq target gene selection was carried out from differentially expressed genes between summer and winter krill including the following gene categories: metabolism related to bioactive lipids, hormone metabolism, visual perception, receptor-related proteins, development, reproduction, dephosphorylation and transcriptional regulation (Publication 1).

In the following, I will focus on the most relevant target genes and regulatory processes that are connected to a) visual perception, b) the circadian clock, c) the insulin signalling and the juvenile-hormone like pathway, and d) the metabolism of other signalling compounds and hormones. If appropriate, they will be discussed in relation to findings from our controlled laboratory experiments (Publication 3).

With respect to the visual perception system of Antarctic krill, we identified genes coding for the signal transduction-related protein arrestin and the enzyme carotenoid isomerooxygenase (gene = *ninaB*) (Publication 1). The carotenoid isomerooxygenase catalyses the biogenesis of photopigments (visual opsins) (Voolstra et al., 2010). In *Drosophila*, The absorption of light by visual pigments causes a conformational change that activates G-proteins and thereby initiates the phototransduction cascade (Mazzotta and Costa, 2016). Arrestins are involved in the termination of the phototransduction cascade by inhibiting the interaction between visual pigments and G-proteins (Montell, 2012). A daily bimodal gene expression pattern of arrestin has already been identified in Antarctic krill in the field (De Pittà et al., 2013). Therefore, these genes may be interesting candidates for the investigation of seasonal and regional differences in visual transduction system in Antarctic krill.

Seasonal photoperiodic cues may be transmitted to the circadian clock system that may also play a role for the timing of seasonal processes in Antarctic krill (as discussed above). In the field, we found pronounced seasonal differences in the expression of genes that have known connections to the circadian clock and therefore we suggest that these genes may additionally have seasonal regulatory roles in Antarctic krill (Publication 1).

We identified a target gene coding for the endopeptidase neprilysin-1 (Publication 1). Neprilysin-like peptidases are involved in in the breakdown of neuropeptides, and have been found to terminate synaptic signalling of the circadian neurotransmitter pigment dispersing factor (PDF) (Isaac et al., 2007). In insects, it has been proposed that PDF-neuroactive neurons play a role for the transmission of photoperiodic signals that initiate the diapause in insects (Hamanaka et al., 2005; Ikeno et al., 2014). Moreover, neprilysins have important functions in the regulation of the reproductive processes of *Drosophila* (Sitnik et al., 2014).

Various genes that coded for receptor-related proteins were found (Publicaton 1). These candidate genes included the leucine-rich repeat-containing G-protein coupled receptor 4 (protein LGR4) that has been associated with the circadian regulation of plasma lipids in mice (Wang et al., 2014). In addition, LGR4 plays a role in the regulation of Wnt/β-catenin signalling (Carmon et al., 2011) that has known functions in

various developmental processes in arthropods (Murat et al., 2010). Therefore, LGR4 may be an interesting target to study its possible role in the seasonal developmental processes and lipid metabolism of Antarctic krill.

Another potential target gene may be the serine/threonine-protein phosphatase 2A (PP2A) (Publication 1) that is an important post-translational regulator of the circadian clock system (Pegoraro and Tauber, 2011). Interestingly, PP2A is also involved in the regulation of visual transduction in *Drosophila* (Wang et al., 2008) and the ovarian maturation in crustaceans (Zhao et al., 2017).

We also identified a gene coding for the CREB-binding protein (Publication 1) which is a transcriptional co-regulator of the circadian clock and independently affects circadian locomotor activity in *Drosophila* (Maurer et al., 2016). The CREB-binding protein is also involved in the transcriptional regulation of various developmental processes in *Drosophila*, and in particular the juvenile hormone signalling pathway (Roy et al., 2017).

In insects, insulin and juvenile hormone signalling have been found to stimulate reproductive development, growth and metabolism, whereas the interruption of these pathways leads to diapause formation that is characterized by lipid accumulation, reproductive arrest and supressed metabolism (Flatt et al., 2005; Hahn and Denlinger, 2011; Liu et al., 2017; Schiesari et al., 2016; Sim and Denlinger, 2013). We suggest that similar regulatory pathways may be important for the regulation of seasonal cycles of reproduction, growth and metabolism in Antarctic krill, especially with respect to its pronounced overwintering mechanisms. A gene coding for an insulin-like peptide has been identified in Antarctic krill in the field by Seear et al. (2012) who discussed its function with respect to the reproductive physiology of Antarctic krill in summer. We add several target genes that are related to insulin signalling and have juvenile-hormone like functions in Antarctic krill (Publication 1).

Regarding insulin signalling, we identified a gene coding for the adiponectin receptor (Publication 1). In *Drosophila*, the adiponectin receptor is involved in the regulation of insulin secretion and respectively controls glucose and lipid metabolism (Kwak et al., 2013), whereas in crustaceans it has been found to play a role in the maintenance of skeletal muscles (Kim et al., 2016).

The transcriptional regulator Krüppel homolog 1 may be another target gene with potential similarities to the juvenile hormone signalling pathway (Publication 1). In insects, Krüppel homolog 1 acts as a mediator of the juvenile hormone signal, thereby affecting vitellogenesis and oocyte maturation (Song et al., 2014) and development (Minakuchi et al., 2008).

Moreover, we propose that genes related to the metabolism of methyl farnesoate may be involved in the seasonal regulation of moulting and reproduction in Antarctic krill (Publication 1 & 3). Methyl farnesoate is a precursor of the insect juvenile hormone III and it is considered to be the 'crustacean juvenile hormone' (Homola and Chang, 1997). Methyl farnesoate has known functions in the stimulation of moulting and reproduction in crustaceans (Reddy et al., 2004). In publication 1, we identified target genes coding for juvenile hormone esterase-like carboxylesterases which are potentially involved in the inactivation of methyl farnesoate (Lee et al., 2011). Under our three simulated light regimes (Publication 3), we also investigated the gene expression profile of the farnesoic acid O-methyltransferase (gene famet) which catalyses the biosynthesis of methyl farnesoate in crustaceans (Gunawardene et al., 2002). We found an upregulation of *famet* under constant darkness in early winter, spring and early summer, probably due to generally higher levels of methyl farnesoate secretion in the absence of light cues (Publication 3). We suggest that constant darkness disrupts the photoperiodic-controlled inhibitory mechanism that controls methyl farnesoate secretion in crustaceans (Swetha et al., 2011; Tsukimura and Borst, 1992). The consequently elevated levels of methyl farnesoate secretion may be partly responsible for the dampening of the seasonal maturity cycle of Antarctic krill under constant darkness (Höring et al., 2018).

Last but not least, we identified several other target genes that code for enzymes involved in the metabolism of hormones and bioactive compounds, such as steroid, octopamine and thyroxine metabolism, and may have regulatory roles in seasonal processes in Antarctic krill (Publication 1). Ecdysteroids and vertebrate-type steroids have known functions in the regulation of moulting and reproduction in crustaceans (Lafont and Mathieu, 2007). The aminergic neurotransmitter octopamine has been found to stimulate heart beat and behaviour in lobsters (Battelle and Kravitz, 1978;

Kravitz, 1988), whereas it inhibits dormancy in *Drosophila* (Andreatta et al., 2018) and may have similar effect on Antarctic krill in its 'active' seasons. In vertebrates, thyroid hormone (thyroxine as precursor) and its metabolism are important for the seasonal timing of reproduction (Nishiwaki-Ohkawa and Yoshimura, 2016; Sáenz de Miera et al., 2014). In insects however, the functional relevance and mechanism of thyroid hormone signalling is not clear (Flatt et al., 2006).

Interestingly, our selected target genes also included the *prohormone convertase 1 (nec1)* coding for a prohormone processing enzyme, and *hematopoietic prostaglandin D synthase (hpgds)* and *carbonyl reductase 1 (cbr1)* that are involved in the biosynthesis of prostaglandins (Publication 1). For the first time, these genes were investigated during our long-term laboratory study and showed significant seasonal rhythmicity under the simulated light regime 66°S. These results suggest the photoperiodic control of their seasonal expression patterns with a possible link to clock gene expression and a de-synchronization of these seasonal patterns under constant darkness (Publication 3).

Prohormone convertases that catalyse the activation of hormones and neuropeptides (Zhou et al., 1999) may play an important role in the seasonal regulation of growth, reproduction and metabolism in Antarctic krill. Prohormone convertases have been found in the neuroendocrine tissue of the crustacean eyestalk including the X-organ-sinus gland complex (Tangprasittipap et al., 2012; Toullec et al., 2002) that is the major production site for many neuropeptides such as the crustacean hyperglycemic hormone (CHH) (Nagaraju, 2011). From our laboratory observations (Publication2), we may suggest that a photoperiodic-controlled signalling pathway in the eyestalk regulates the seasonal expression profile of *nec1* in Antarctic krill which in turn may affect the activation of various hormones that have known regulatory roles in reproduction, growth and metabolism (Nagaraju, 2011).

Prostaglandins are physiologically active lipid compounds that are found in various crustacean tissues including the X-organ-sinus gland, the brain and the ovary (Nagaraju, 2011). In crustaceans, prostaglandins play a major role in ovarian development (Wimuttisuk et al., 2013) and may have similar effects on the reproductive physiology of Antarctic krill. Our laboratory results (Publication 3) give new insights

into the the transcriptional regulation of the biosynthesis pathway of prostaglandins that is mediated by seasonal light cues in Antarctic krill.

5.4 Conclusions and outlook

This dissertation gave new insights in the effect of different latitudinal light regimes on the seasonal cycle of Antarctic krill and the potential internal regulatory mechanisms of its seasonal timing system. In publication 1, we explored the seasonally differential gene expression patterns in Antarctic krill in different latitudinal regions in the Southern Ocean and found that krill from the low-latitude region South Georgia showed the least seasonal differences, probably due to Antarctic krill's flexible adaptation to the low-latitude light regime and enhanced food availability in this region. Moreover, we detected several target genes that may be the basis for future studies of the internal mechanisms governing the seasonal cycles in Antarctic krill. To further elucidate the role of latitudinal light regime and of the endogenous timing system on the phenology in Antarctic krill, we conducted the first two-year laboratory experiment under constant food and temperature conditions simulating the latitudinal light regimes 52°S, 66°S, and constant darkness (Publication 2 & 3).

Publication 2 highlighted that the simulated latitudinal light regimes affected the seasonal cycles of growth, feeding, lipid metabolism and maturity in Antarctic krill with evidence that an endogenous timing system was controlling these seasonal processes (except for seasonal cycle of lipid content). We emphasized the photoperiodic flexibility of Antarctic krill by showing that the critical photoperiod for maturity is higher under the high-latitude light regime 66°S with respect to the low-latitude light regime 52°S. Antarctic krill's flexible response to different latitudinal light regimes was further manifested on gene expression level that was investigated in publication 3. These results also suggested the involvement of genes related to the circadian clock, the activation of neuropeptides, and the metabolism of methyl farnesoate and prostaglandins in the light-dependent regulation of seasonal processes in Antarctic krill.

Our findings point to a highly flexible seasonal timing system that enables Antarctic krill to adjust its seasonal photoperiodic response to extreme differences in latitudinal light regime. In the field, the underlying 'clock-controlled' seasonal cycles of physiology seem to be adjusted by other environmental factors such as temperature and food supply which further promotes the regional acclimatization of Antarctic krill.

It is not yet clear if Antarctic krill is able to adjust its phenology to anthropogenic environmental changes in the Southern Ocean. Its flexible timing system may be an advantage under the observed southward shift of Antarctic krill in the Southwest Atlantic Sector (Atkinson et al., 2019), because Antarctic krill is already well adapted to the extreme light regimes at higher latitudes. However, it is still unknown if Antarctic krill will be able to adapt in time to climate change-related alterations in sea-ice dynamics and the timing of phytoplankton blooms. Further research is required to better understand Antarctic krill's flexibility in responding to variable food conditions in different seasons in conjunction with its photoperiodic controlled seasonal timing system. The findings of this dissertation are highly relevant for future modelling approaches that may help to predict how Antarctic krill will respond to climate change. The prediction of growth, energy budget and reproduction may be significantly improved by incorporating the factor latitudinal light regime as the main driver for underlying seasonal cycles of growth, feeding, lipid metabolism and maturity in Antarctic krill. In addition, the effect of regionally variable factors such as temperature and food availability may be added during particular periods of the seasonal cycle.

We are just in the beginning of understanding the molecular mechanisms that control seasonal physiological cycles in Antarctic krill. Antarctic krill may be an interesting target organism to understand the flexibility of seasonal timing systems. Future laboratory experiments may test the combined effects of different environmental factors on seasonal processes in Antarctic krill, such as light regime, temperature and food supply. The selected target genes from our seasonal RNAseq study (Publication 1) may be a starting point for further molecular investigations such as testing the influence of light regime on their seasonal expression patterns. An important step towards the functional understanding of seasonal timing in Antarctic krill may be the establishment of gene silencing techniques such as RNAi that could for instance solve the

question if circadian clock genes are involved in the seasonal regulatory processes in Antarctic krill. Further studies should also focus on the effects of endocrine signalling pathways in Antarctic krill which are not well understood and are an important link for the regulation of seasonal processes in Antarctic krill.

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Erklärung des Autors

Erklärung gemäß §12 (2) b) der Promotionsordnung der Fakultät V (Mathematik & Naturwissenschaften) der Carl von Ossietzky Universität Oldenburg (Datum 05.09.2014)

Ich erkläre hiermit:

- dass die vorliegende Dissertation selbständig verfasst wurde und alle genutzten Quellen angegeben wurden.
- dass Teile der Dissertation bereits veröffentlich wurden. Der Status zur Veröffentlichung der Publikationen I, II, und III (Sektionen 2, 3, und 4) angegeben.
- dass die Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen Hochschule vorgelegt wurde.
- dass der akademische Grad Dr. rer. nat. angestrebt wird.
- dass die Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg befolgt wurden.
- dass beim Verfassen der Dissertation keinerlei kommerzielle Beratungsdienste in Anspruch genommen wurden.

Flavia Höring

A. Supplementary Information Publication I

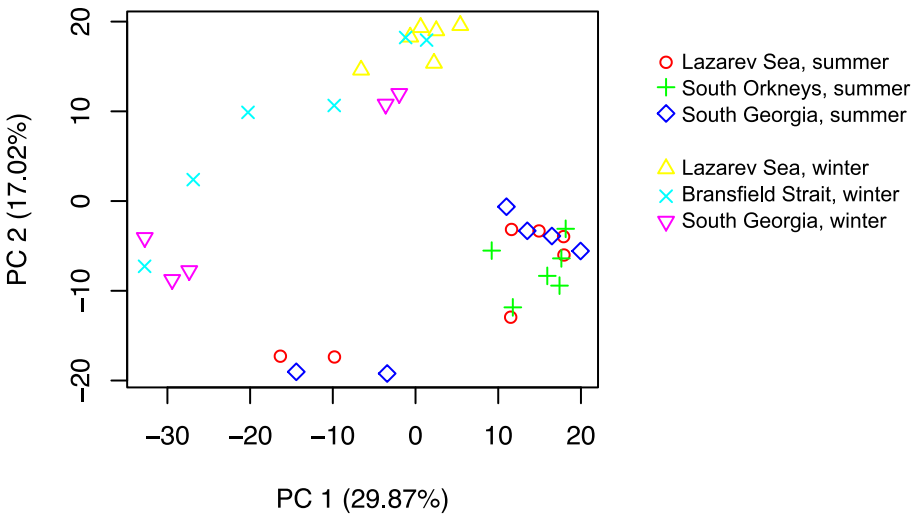

FIGURE A.1: Sample plot of the principal component analysis for all differentially expressed genes. The samples are colored according to region & season.

TABLE A.1: Annotation results including query/contig ID, subject ID (Swissprot, UniRef, or NR ID), % identity, alignment length, e-value, bit score and manually assigned functional category.

Query/Contig ID	Subject ID	% Id.	Alignment length	E-value	Bit score	Category
HACF01000044	P02707	32,773	119	1,09E-12	68,2	immune response
HACF01000131	UniRef90_E0VF74	46,914	81	9,70E-11	72,4	other
HACF01000142	P42674	35,641	390	4,47E-58	209	development
HACF01000143	P42674	35,897	390	6,09E-59	212	development
HACF01000178	P00762	42,029	138	2,41E-34	84,7	digestion
HACF01000205	P81384	52	100	4,78E-28	110	other
HACF01000296	UniRef90_E9FRH4	29,798	198	2,40E-16	85,9	
HACF01000337	UniRef90_E9FSZ1	67,742	31	1,14E-13	55,1	other
HACF01000348	P69526	32,258	155	5,50E-31	74,3	proteolysis
HACF01000389	Q26365	72,575	299	2,51E-142	414	transport
HACF01000397	B3EWR1	43,548	124	2,54E-24	101	immune response
HACF01000398	B3EWR1	43,548	124	1,31E-24	101	immune response
HACF01000408	Q9VMU4	53,933	89	1,71E-28	103	translation
HACF01000458	UniRef90_Q9NDS2	37,398	123	9,10E-11	67	other

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01000482	UniRef90_Q7YUE2	61,429	70	2,08E-18	87	other
HACF01000502	P35786	47,549	204	5,97E-41	148	other
HACF01000518	O42248	84,553	123	1,41E-70	223	receptor-related protein
HACF01000575	Q26636	42,405	158	2,00E-61	125	immune response
HACF01000629	UniRef90_I6VVL1	40,667	150	1,03E-26	118	immune response
HACF01000635	P18700	93,333	255	4,58E-174	514	cytoskeleton
HACF01000682	Q99538	37,176	347	3,29E-62	169	proteolysis
HACF01000685	O70244	32,231	121	4,24E-20	72	transport
HACF01000774	P27591	44,231	104	3,65E-15	77,4	other
HACF01000879	Q3ZCJ8	47,939	461	7,21E-146	431	proteolysis
HACF01000883	UniRef90_UPI0006B0AA51	48,78	287	9,07E-90	283	
HACF01000914	Q8N0N3	50	246	8,57E-106	239	immune response
HACF01000937	UniRef90_UPI0006B10B0C	36,228	403	2,04E-70	237	
HACF01000968	Q5RB02	48,424	349	1,47E-116	354	proteolysis
HACF01000970	Q0IIS3	37,226	274	5,66E-57	194	lipid metabolism
HACF01000988	Q9GV46	25,472	212	5,35E-15	61,2	bioluminescence
HACF01000996	UniRef90_Q9NDT7	57,895	57	4,15E-15	78,2	other
HACF01001048	P82120	38,71	93	3,08E-10	60,5	other
HACF01001088	Q01177	26,457	223	6,60E-11	69,3	immune response
HACF01001110	A5PJF6	40,187	214	3,51E-53	156	hormone_metabolism
HACF01001112	Q3MI05	42,704	466	7,07E-124	373	immune response
HACF01001113	Q3MI05	34,973	366	9,65E-59	200	immune response
HACF01001228	P00765	39,004	241	3,21E-50	169	digestion
HACF01001243	Q9XY07	44,011	359	8,68E-97	300	energy metabolism
HACF01001275	Q95M17	39,896	386	2,02E-88	288	other
HACF01001290	Q7RTY7	31,818	396	9,30E-42	164	immune response
HACF01001309	Q16822	65,327	597	0	850	carbohydrate metabolism
HACF01001341	Q9I7U4	28,679	265	1,80E-25	108	muscle development and regulation
HACF01001348	UniRef90_Q009U0	36,17	141	3,36E-23	103	immune response
HACF01001407	O46559	33,053	357	2,27E-38	152	digestion
HACF01001416	Q26636	42,395	309	1,51E-78	249	immune_response
HACF01001422	Q93Z80	32,821	195	1,18E-20	97,8	transport
HACF01001433	UniRef90_C1BV96	35,252	139	1,50E-12	72	other
HACF01001438	UniRef90_UPI000359830C	34,361	227	1,28E-36	144	
HACF01001481	Q8BFW4	29,747	158	1,31E-10	67,8	immune response
HACF01001491	UniRef90_E9FRH4	34,225	187	6,57E-22	103	
HACF01001502	P51523	43,396	106	7,91E-18	84,3	transcriptional regulation
HACF01001558	Q95ZS2	56	150	3,46E-55	194	hormone metabolism
HACF01001652	Q9W5U2	43,683	467	7,46E-116	388	other
HACF01001653	Q9QZU7	37,195	164	2,09E-52	110	lipid metabolism
HACF01001681	Q9Y5Y6	34,091	352	3,64E-41	164	proteolysis
HACF01001689	UniRef90_A0A067RDJ7	51,402	428	1,84E-143	429	other
HACF01001735	Q8TBF2	38,75	160	2,90E-34	129	reproduction
HACF01001785	UniRef90_V3ZXQ2	52,564	78	1,77E-33	90,1	other
HACF01001792	O94813	29,448	163	3,21E-10	66,2	development
HACF01001798	P05946	65,445	191	3,84E-87	262	muscle development and regulation
HACF01001800	UniRef90_E9HZE2	77,193	57	6,34E-21	95,5	
HACF01001802	Q9D8B1	38,384	99	1,45E-24	70,1	lipid metabolism
HACF01001857	UniRef90_A0A067R334	48,276	58	2,65E-10	64,7	energy metabolism
HACF01001907	Q9TU53	32,759	116	3,73E-11	66,2	transport

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01001916	UniRef90_T1W366	73,418	79	3,59E-33	125	immune response
HACF01001926	Q7Z5L0	35,429	175	4,45E-24	98,6	immune response
HACF01001941	UniRef90_Q9NDT7	57,377	61	2,64E-16	80,9	other
HACF01001955	P81589	58,333	48	1,27E-10	58,2	other
HACF01002031	P98068	30,481	187	1,17E-18	88,6	development
HACF01002097	P19096	43,768	1067	0	752	lipid metabolism
HACF01002278	O46581	40,909	132	4,16E-25	104	energy metabolism
HACF01002294	Q9EQS5	31,707	164	5,86E-13	73,2	hormone metabolism
HACF01002310	Q09225	27,147	361	3,94E-32	104	transport
HACF01002423	Q923L3	41,964	112	1,52E-17	85,9	other
HACF01002433	O60760	40,488	205	1,03E-45	155	reproduction
HACF01002609	Q80ZA4	34,264	394	1,28E-150	241	development
HACF01002648	W4VS99	31,638	708	1,12E-117	382	hormone metabolism
HACF01002764	P12107	40,755	265	1,86E-55	209	development
HACF01002793	P15684	33,962	212	4,49E-55	126	proteolysis
HACF01002797	Q9W092	43,467	375	5,21E-101	318	other
HACF01002826	P35443	49,015	406	7,95E-112	341	immune response
HACF01002831	UniRef90_C3YC49	39,779	362	3,93E-72	250	
HACF01002861	Q9U572	38,298	141	1,57E-22	100	immune response
HACF01002862	B7Z031	28,896	308	1,67E-38	151	other
HACF01002867	UniRef90_H2FEH6	89,583	48	8,45E-16	89,4	energy metabolism
HACF01002869	Q62867	39,493	276	1,13E-51	181	proteolysis
HACF01002873	P48463	58,962	212	3,34E-94	272	dephosphorylation
HACF01002882	Q5RB02	39,661	295	2,41E-106	230	proteolysis
HACF01002892	Q9U572	26,266	316	3,45E-18	92	immune response
HACF01002893	Q92820	35,484	124	2,84E-34	77,8	proteolysis
HACF01002955	Q9VCY8	53,629	248	1,11E-81	266	receptor-related protein
HACF01003025	P53634	49,096	387	3,19E-130	385	proteolysis
HACF01003047	UniRef90_E9HFT6	52,273	88	4,72E-39	99,8	
HACF01003087	Q91WG0	35,583	326	3,97E-54	192	reproduction
HACF01003114	Q9FR44	32,246	276	1,70E-37	144	metabolism related to bioactive lipids
HACF01003148	Q0IH73	36	175	9,68E-26	112	hormone metabolism
HACF01003158	UniRef90_Q5XLK0	52,5	80	3,19E-19	89,7	other
HACF01003219	Q9U8W8	48	200	1,42E-55	186	immune response
HACF01003240	Q6DKE1	78	100	1,83E-52	171	energy metabolism
HACF01003283	P28493	38,996	259	1,42E-41	147	immune response
HACF01003306	Q09225	30,952	210	8,79E-18	77,4	transport
HACF01003339	UniRef90_UPI0005D062D1	23,511	319	9,11E-17	92	
HACF01003365	Q9R257	33,333	186	1,91E-22	95,1	other
HACF01003371	Q9XSK2	32,597	181	3,28E-23	98,6	other
HACF01003376	Q9H2A2	64,179	67	1,26E-21	100	hormone metabolism
HACF01003386	UniRef90_C3YPK9	36,275	204	1,62E-25	114	
HACF01003435	UniRef90 A0A0B4NFP6	35,762	151	1,97E-16	88,2	immune response
HACF01003499	UniRef90_E9FRH4	27,933	179	1,41E-12	73,9	
HACF01003564	UniRef90 C7B731	59,615	52	5,64E-14	63,2	immune response
HACF01003628	UniRef90_T1W366	72,84	81	1,24E-32	127	immune response
HACF01003645	Q9VFS2	42,202	109	1,11E-18	87,8	visual perception
HACF01003666	P23593	41,27	63	1,46E-12	48,1	transport
HACF01003677	Q9XZ71	54,198	262	5,66E-41	151	muscle development and regulation
HACF01003684	UniRef90_T1W2K0	52,941	85	1,06E-16	81,3	immune response
HACF01003714	Q9GV46	36,154	130	1,47E-16	67,8	bioluminescence
HACF01003779	P41366	36,735	98	3,71E-18	57,8	immune response

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01003833	P52760	64,646	99	9,59E-41	138	transcriptional regulation
HACF01003865	UniRef90_A0A087TU70	40,566	106	1,98E-13	75,9	lipid metabolism
HACF01003892	P0C1J5	67,308	52	8,85E-15	73,9	protein folding
HACF01004004	C0KJQ4	54,902	51	1,86E-13	67	immune response
HACF01004340	UniRef90_Q75PH3	60,606	66	1,09E-18	83,2	other
HACF01005022	Q23551	28,713	202	5,20E-28	82,4	muscle development and regulation
HACF01005119	Q80ZA4	47,222	468	0	439	development
HACF01005155	Q9U572	24,554	505	1,18E-32	141	immune response
HACF01005189	Q91437	71,713	502	0	732	other
HACF01005245	P08155	73,099	171	1,22E-82	280	development
HACF01005314	Q7PT10	78,419	329	0	518	protein folding
HACF01005441	Q64459	31,429	490	1,83E-76	259	hormone metabolism
HACF01005577	Q9U572	33,426	359	7,05E-46	180	immune response
HACF01005621	Q8IW92	40,476	630	2,46E-133	409	carbohydrate metabolism
HACF01005645	P31335	70,137	586	0	759	other
HACF01005690	B8DKP2	44,074	270	6,87E-74	242	other
HACF01005824	P09242	46,545	492	8,76E-122	373	dephosphorylation
HACF01005866	UniRef90_V4ALG6	32,589	224	8,82E-25	112	
HACF01005894	P30432	58,252	103	3,47E-69	115	hormone metabolism
v.s.	OAD61081.1	57,692	104	1,51E-82	132	
HACF01005913	P51484	36	350	1,86E-60	209	visual perception
HACF01005952	Q54P79	41,042	307	8,01E-70	237	other
HACF01005953	P05661	60	150	2,64E-132	172	muscle development and regulation
HACF01005964	P32138	45	380	4,39E-114	337	lipid metabolism
HACF01005998	Q72HI4	35,714	112	4,85E-10	63,2	other
HACF01006135	Q9GV46	28,013	307	5,39E-28	118	bioluminescence
HACF01006153	Q8BML2	31,548	168	3,74E-13	75,9	other
HACF01006235	O60218	57,627	295	1,27E-120	359	carbohydrate metabolism
HACF01006264	Q3MI05	46,667	360	3,14E-101	315	immune response
HACF01006310	P26221	48,913	460	8,81E-107	341	digestion
HACF01006390	O97951	39,109	202	2,17E-49	162	detoxification
HACF01006433	P36178	52,805	303	9,22E-98	298	digestion
HACF01006448	P82198	32,075	318	8,46E-37	147	development
HACF01006479	UniRef90_E9H6P2	27,897	233	2,34E-31	115	
HACF01006492	UniRef90 Q9VQR0	23,276	348	4,50E-12	77	
HACF01006527	UniRef90_T1K075	31,373	102	1,30E-12	45,4	
HACF01006530	Q8C8R3	36,62	71	6,06E-18	52,4	other
HACF01006612	UniRef90_R4PL90	72,021	193	4,31E-89	284	other
HACF01006655	UniRef90_E4XN23	31,414	191	4,78E-11	73,9	
HACF01006728	Q9U572	22,5	400	1,25E-12	73,9	immune response
HACF01006732	Q9UB00	47,418	213	1,22E-59	206	metabolism related to bioactive lipids
HACF01006808	Q64737	52,61	249	2,06E-108	242	other
HACF01006972	P86704	83,916	143	2,34E-110	224	muscle development and regulation
HACF01006975	Q6RG02	39,85	399	1,12E-87	294	reproduction
HACF01006980	Q9FKS8	26,087	345	1,54E-15	81,6	transport
HACF01007012	P35575	31,389	360	2,29E-59	200	carbohydrate metabolism
HACF01007056	Q9NRA2	43,678	348	8,99E-95	296	transport
HACF01007095	Q29IL2	40,8	125	2,52E-17	87,4	muscle development and regulation
HACF01007177	Q6GPQ3	29,596	223	1,10E-25	82,8	transport
HACF01007217	UniRef90_B7UCF9	34,932	146	8,89E-22	80,9	immune response
HACF01007227	Q9U1G6	31,776	107	1,51E-12	67,8	transport
HACF01007273	P22648	30,476	105	2,71E-18	62	development
HACF01007277	Q5M8Z0	54,545	187	1,00E-107	207	amino acid metabolism

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01007287	Q0VD19	41,558	231	4,14E-59	186	metabolism related to bioactive lipids
HACF01007295	Q9GV46	30,198	202	3,36E-21	86,7	bioluminescence
HACF01007379	Q27256	38,889	306	1,75E-53	191	transport
HACF01007383	UniRef90_UPI00037076B2	36,813	182	1,00E-21	105	
HACF01007387	O18993	37,589	141	7,00E-44	114	hormone metabolism
HACF01007513	Q9GV46	31,045	335	5,15E-38	142	bioluminescence
HACF01007526	P25782	53,918	319	7,13E-118	347	proteolysis
HACF01007528	Q01484	28	400	2,71E-25	111	other
HACF01007597	P55112	30,12	249	1,37E-21	97,1	digestion
HACF01007617	Q92820	43,667	300	7,64E-70	224	proteolysis
HACF01007686	UniRef90_D2CGA8	29,06	117	1,87E-13	55,8	other
HACF01007769	Q9GV46	31,325	249	7,59E-33	110	bioluminescence
HACF01007802	P70079	48,583	247	5,38E-67	219	energy metabolism
HACF01007869	A1DNL0	55,455	330	1,14E-127	379	digestion
HACF01007872	Q6NSJ0	41,036	251	3,24E-49	178	muscle development and regulation
HACF01007908	A8E657	46,479	213	2,55E-55	197	amino acid metabolism
HACF01007937	Q9QZ25	24,756	307	9,48E-15	78,6	other
HACF01007938	UniRef90_UPI0006B108DE	27,976	336	1,11E-18	93,6	
HACF01007960	Q6RG02	29,396	364	2,74E-36	142	reproduction
HACF01007962	P48758	48,837	129	6,38E-58	125	reproduction
HACF01007990	UniRef90_Q5XLK0	51,19	84	1,24E-19	90,9	other
HACF01008041	UniRef90_UPI0003F07610	13,736	182	3,37E-16	89	
HACF01008047	P49010	53,333	225	4,52E-80	254	other
HACF01008075	P24389	40,86	186	2,64E-48	166	hormone metabolism
HACF01008166	UniRef90_T1W366	72,84	81	7,97E-33	127	immune response
HACF01008178	Q8NCI6	32,52	123	4,43E-29	58,5	carbohydrate metabolism
HACF01008235	A2ARI4	29,389	262	2,67E-16	83,2	receptor-related protein
HACF01008300	UniRef90_D3PHC6	38,514	148	7,18E-17	86,7	visual perception
HACF01008330	Q9V4N3	48,077	104	1,14E-24	100	hormone metabolism
HACF01008355	Q99NF1	42,06	233	4,71E-53	184	hormone metabolism
HACF01008485	Q9I3S3	65,844	243	1,98E-110	326	amino acid metabolism
HACF01008557	Q09225	29,371	143	3,78E-20	62	transport
HACF01008564	O13395	34,127	126	1,73E-16	83,6	other
HACF01008573	UniRef90_D3PIA3	28,761	226	4,76E-10	67,4	visual perception
HACF01008625	O75521	47,17	265	5,77E-72	230	lipid metabolism
HACF01008757	P52033	46,875	96	1,21E-26	83,2	detoxification
HACF01008786	Q941X2	39,231	130	2,39E-20	94,4	development
HACF01008896	UniRef90_E9FRH4	32,086	187	5,57E-18	89,4	
HACF01008904	O43897	37,419	155	1,49E-20	95,1	development
HACF01008909	P51910	32,065	184	1,26E-20	90,1	transport
HACF01008929	Q9P2U7	41,071	112	3,34E-30	89,7	other
HACF01008981	UniRef90_A0A0B1PMD7	30,114	176	5,10E-15	80,9	other
HACF01009046	P80007	41,358	162	2,02E-31	118	development
HACF01009136	P22102	55,372	242	7,47E-77	255	other
HACF01009304	UniRef90_T1W366	72,5	80	1,68E-31	122	immune response
HACF01009310	Q0VD19	47,253	91	3,63E-37	94,4	metabolism related to bioactive lipids
HACF01009457	UniRef90_A0A087UDE2	32,292	192	4,17E-23	106	
HACF01009592	UniRef90_A0A099KQ44	32,5	240	1,48E-24	111	
HACF01009640	Q9U8W8	43,498	223	1,77E-52	175	immune response

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01009651	O97972	28,495	186	1,67E-14	74,7	detoxification
HACF01009668	UniRef90_E9FRH4	31,016	187	6,79E-16	82,8	
HACF01009672	Q9N0V4	61,29	155	1,09E-62	199	detoxification
HACF01009748	Q8N6F8	31,126	151	1,37E-17	82,8	other
HACF01009920	Q86IC9	47,418	213	1,55E-63	201	other
HACF01009951	P51910	35,152	165	2,87E-26	104	transport
HACF01009964	UniRef90_C3ZK66	43,878	98	5,94E-20	90,9	
HACF01009977	Q7Z5L0	31,361	169	1,11E-14	73,9	immune response
HACF01010090	P32320	54,478	134	2,46E-47	157	other
HACF01010228	Q7SXP0	48,585	212	1,03E-58	188	transport
HACF01010244	Q7Z5L0	37,079	89	3,60E-14	53,1	immune response
HACF01010276	P51910	30,769	169	2,33E-14	72,4	transport
HACF01010285	Q5YLG1	40,851	235	2,99E-44	160	digestion
HACF01010344	Q5N7B4	40,476	126	5,53E-19	89	development
v.s.	XP_025270057.1	41,615	161	5,03E-32	133	
HACF01010500	Q5M7F4	53,275	229	4,83E-81	249	lipid metabolism
HACF01010521	UniRef90_D3PIA3	31,797	217	1,27E-20	95,1	visual perception
HACF01010578	P61916	30,469	128	7,02E-13	67,4	transport
HACF01010709	P81429	38,272	162	3,25E-21	90,9	reproduction
HACF01010717	P15396	32,287	223	5,23E-24	103	other
HACF01010723	UniRef90_D3PIA3	30,89	191	1,50E-17	86,7	visual perception
HACF01010749	Q9FR44	38,889	72	4,50E-15	60,5	metabolism related to bioactive lipids
HACF01010815	Q04519	29,586	169	2,95E-17	83,6	metabolism related to bioactive lipids
HACF01010818	UniRef90_E9HPA2	48,408	157	4,18E-50	169	
HACF01010840	UniRef90_E9FRT8	30	200	5,11E-14	76,3	
HACF01010865	Q95RA9	29,5	200	1,69E-26	105	immune response
HACF01010891	P14140	90,043	231	1,86E-146	419	cytoskeleton
HACF01010928	Q0Q028	35,252	139	2,94E-12	65,9	immune response
HACF01010941	UniRef90_A0A0K2V6Y8	32,044	181	4,61E-15	79,7	visual perception
HACF01011005	UniRef90_R7UGW9	30,597	134	4,92E-10	67,4	
HACF01011328	Q9Y600	54,639	97	2,44E-27	111	hormone metabolism
HACF01011568	Q91437	63,768	207	1,36E-89	291	other
HACF01011596	Q9GV46	27,5	200	1,31E-10	63,2	bioluminescence
HACF01011614	Q6UWM9	46,377	138	7,25E-39	143	detoxification
HACF01011665	Q9PUH5	41,667	108	1,45E-20	87	lipid metabolism
HACF01011744	O15992	38,144	97	2,26E-23	73,2	energy metabolism
HACF01011747	P35033	40,659	182	1,40E-43	149	digestion
HACF01011931	P10039	38,931	131	5,21E-22	96,7	development
HACF01011946	O07610	40,278	72	3,01E-10	62,4	lipid metabolism
HACF01012154	UniRef90_T1W366	78,571	56	6,15E-22	88,6	immune response
HACF01012160	Q9NPF2	31,148	122	8,33E-15	64,3	development
HACF01012398	P21213	39,634	164	1,70E-27	112	amino acid metabolism
HACF01012427	Q95M12	49,451	91	3,92E-24	101	proteolysis
HACF01012551	P28287	93,627	204	2,46E-144	412	cytoskeleton
HACF01012653	P35035	37,755	196	1,10E-32	121	digestion
HACF01012692	P98068	33,871	186	4,24E-19	87,8	development
HACF01012730	P70079	54,598	174	2,02E-55	184	energy metabolism
HACF01013033	P45521	87,5	152	5,04E-100	295	cytoskeleton
HACF01013192	Q24621	45,652	138	1,89E-37	130	muscle development and regulation
HACF01013353	P10982	75	128	2,34E-64	197	cytoskeleton
HACF01013403	Q90474	69,412	85	9,79E-49	134	protein folding
HACF01013684	UniRef90_E9FRH4	34,94	166	3,44E-20	92,4	
HACF01013778	UniRef90_T1W366	72,84	81	1,88E-33	125	immune response

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01013806	P60204	46,875	128	7,42E-31	112	other
HACF01013819	P97353	43,75	112	2,54E-19	87	other
HACF01013910	Q5SXG7	42,647	68	1,43E-10	60,8	immune response
HACF01013976	P45521	86,047	86	2,93E-49	165	cytoskeleton
HACF01014327	UniRef90_T1W366	57,778	45	1,30E-10	64,3	immune response
HACF01014343	Q7Z5L0	37,615	109	5,82E-14	64,7	immune response
HACF01014543	P26221	50	100	4,45E-24	101	digestion
HACF01014557	UniRef90_B7SP41	32,432	111	5,27E-11	67	proteolysis
HACF01014633	UniRef90_UPI0005EFB468	32,353	136	4,04E-15	77	
HACF01014684	UniRef90_D2DSQ3	76,596	47	5,71E-13	73,9	transcriptional regulation
HACF01014698	P81589	56,25	48	6,36E-10	55,5	other
HACF01014726	Q26065	89,831	177	3,15E-117	339	cytoskeleton
HACF01014736	UniRef90_Q7Q112	40,223	179	4,52E-24	106	
HACF01015196	P0C1I7	74,59	122	7,45E-52	166	protein folding
HACF01015402	UniRef90_Q9NDT7	51,282	78	5,29E-17	81,3	other
HACF01015708	Q9N290	29,839	124	1,83E-10	61,2	other
HACF01015743	P46784	39,08	87	1,55E-15	71,2	translation
HACF01015970	UniRef90 E9G8U0	30,882	136	3,50E-11	67,8	
HACF01016440	UniRef90_Q9NDT7	60	65	9,03E-20	88,2	other
HACF01016483	P0DJ56	44,526	137	1,44E-26	102	translation
HACF01016689	UniRef90_G5CJW5	80,46	87	4,57E-46	158	immune response
HACF01016714	UniRef90_V9P9Q9	41,353	133	7,77E-28	113	immune response
HACF01016741	P05756	58,94	151	6,75E-51	162	translation
HACF01017324	P45887	90	150	2,95E-91	273	cytoskeleton
HACF01017718	UniRef90_E9HJT2	53,448	58	1,68E-11	69,7	other
HACF01018018	Q4KMC4	65,385	156	1,22E-69	225	other
HACF01018022	Q9NRB3	36,667	120	9,12E-12	64,7	development
HACF01018027	Q9JII6	49,254	134	3,06E-36	130	carbohydrate metabolism
HACF01018229	P48159	57,554	139	2,14E-52	166	translation
HACF01018379	UniRef90_I4DMP6	41,667	48	3,71E-11	48,5	
HACF01018445	Q6RG02	36,975	119	3,01E-23	98,2	reproduction
HACF01018820	P62268	81	100	3,01E-56	175	translation
HACF01018943	Q3UP87	50,794	63	1,19E-14	72	immune response
HACF01019157	Q7SXK5	67,361	144	4,21E-66	209	amino acid metabolism
HACF01019315	Q9FY66	48,551	138	3,06E-38	130	translation
HACF01019429	P59229	57,037	135	3,25E-20	84,3	other
HACF01019457	Q9TWG0	48,276	58	8,17E-14	64,7	other
HACF01020870	P49215	64,655	116	2,68E-48	155	translation
HACF01021096	Q7XYA7	33,333	102	1,05E-13	66,6	translation
HACF01021243	P0DJ57	36,283	113	2,68E-16	73,6	translation
HACF01021442	UniRef90_D3PK53	31,193	109	7,95E-10	63,9	other
HACF01021473	UniRef90_Q7YUE2	70,968	62	2,15E-22	92,4	other
HACF01021690	Q9U1G6	38,824	85	5,77E-14	67	transport
HACF01022194	Q15661	60	65	7,51E-19	82,8	proteolysis
HACF01022240	Q00328	45,802	131	3,86E-32	120	digestion
HACF01022797	P0DJ19	50,806	124	1,95E-38	129	translation
HACF01022903	Q08699	61,616	99	1,44E-30	110	translation
HACF01023063	P69149	75	92	1,81E-46	133	transcriptional regulation
HACF01023486	P22643	51,667	60	6,21E-12	64,3	bioluminescence
HACF01023524	UniRef90_Q75PH3	68,333	60	4,58E-20	85,9	other
HACF01023630	P97435	36,364	132	1,10E-18	84	digestion
HACF01023639	UniRef90_A0A0A9YC52	46,512	86	1,68E-14	77	

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01023804	Q9SS17	54,082	98	2,53E-15	70,5	translation
HACF01023907	Q9FLF0	52,857	70	1,10E-26	79,3	translation
HACF01024029	Q8BN82	41,129	124	5,65E-26	103	transport
HACF01024140	P45481	34,314	102	4,58E-13	67,4	transcriptional regulation
HACF01025452	O73888	47	100	5,12E-28	104	reproduction
HACF01025536	Q9LZ41	41,346	104	2,46E-15	69,7	translation
HACF01025839	UniRef90_V3ZNV5	64,444	45	1,07E-11	68,9	carbohydrate metabolism
HACF01025863	Q90YT6	50	118	2,51E-34	118	translation
HACF01026034	Q962U1	62,727	110	1,42E-37	129	translation
HACF01026558	Q26365	79,048	105	2,83E-45	151	transport
HACF01026761	P24119	60,215	93	6,14E-34	118	translation
HACF01028145	P26225	54,955	111	3,14E-24	99	digestion
HACF01028283	P0DJ60	62,766	94	1,74E-28	104	translation
HACF01028468	UniRef90_E9FRT8	41,772	79	6,23E-10	61,6	
HACF01029006	Q94726	63,889	36	1,53E-16	53,5	cytoskeleton
HACF01029513	O16087	87,879	33	9,90E-19	59,7	protein folding
HACF01029924	Q56K03	69,048	84	4,24E-10	56,2	translation
HACF01030159	O44119	79,487	39	6,51E-11	58,9	muscle development and regulation
HACF01030412	P04254	53,704	54	5,21E-14	69,3	energy metabolism
HACF01030985	Q9VUY0	34,652	632	6,85E-120	380	hormone metabolism
HACF01031026	Q9GV46	28,649	185	1,10E-17	84,3	bioluminescence
HACF01031034	Q91WG0	32,783	424	2,34E-57	202	reproduction
v.s	ALT10383.1	43	400	1,77E-97	317	
HACF01031108	Q96PZ7	32,593	135	8,78E-11	69,3	other
HACF01031140	Q9V7S5	39,453	256	7,05E-57	197	transport
HACF01031166	P81577	43,243	74	1,90E-15	71,2	other
HACF01031213	UniRef90_Q7Q3K2	34,194	155	4,51E-10	68,2	detoxification
HACF01031233	O75897	33,436	326	8,88E-50	176	other
HACF01031346	UniRef90_C3Y2L0	52,294	218	2,38E-91	250	
HACF01031367	Q1HPS0	43,678	87	4,52E-29	86,7	muscle development and regulation
HACF01031394	Q9VCY8	49,474	95	3,73E-25	105	receptor-related protein
HACF01031530	P42860	49,515	206	2,31E-61	196	detoxification
HACF01031533	Q9UBG0	23,656	279	8,28E-10	64,7	immune response
HACF01031567	Q93113	42,64	197	9,09E-44	153	detoxification
HACF01031736	P05547	50,495	101	8,67E-27	110	muscle development and regulation
HACF01031794	Q15782	38,806	134	1,79E-17	87	other
HACF01031811	Q008X1	35,465	172	1,53E-23	98,6	immune response
HACF01031830	P42674	31,759	381	3,82E-48	177	development
HACF01031889	Q7Z5L0	34,32	169	3,35E-21	91,7	immune response
HACF01031906	P35041	29,289	239	1,99E-25	105	digestion
HACF01031956	Q4V8T0	70,796	113	9,12E-89	184	carbohydrate metabolism
HACF01031980	Q8IUS5	38,346	266	2,32E-54	189	lipid metabolism
HACF01032026	P98068	30,236	592	2,39E-52	197	development
HACF01032062	Q90YU5	92,683	123	3,91E-76	235	translation
HACF01032112	Q64425	28,571	161	3,90E-18	62,8	digestion
HACF01032129	UniRef90_A0A067R7Z4	33,835	133	4,51E-12	74,7	
HACF01032146	Q9GV46	27,134	328	3,37E-24	105	bioluminescence
HACF01032153	Q504N0	40	375	2,28E-86	273	proteolysis
HACF01032160	Q09225	28,571	217	3,89E-34	84,3	transport
HACF01032202	P05090	36,517	178	3,12E-24	100	transport
HACF01032209	P05186	47,6	250	3,69E-98	221	dephosphorylation
HACF01032282	P52556	40,642	187	4,21E-37	134	other
HACF01032283	Q6RG02	35,714	518	4,21E-106	353	reproduction

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01032289	Q6RG02	35,606	264	1,43E-49	163	reproduction
HACF01032360	P00762	44,118	238	3,33E-46	168	digestion
HACF01032388	Q0VD19	37,879	396	1,68E-82	268	metabolism related to bioactive lipids
HACF01032409	Q5RF16	44,56	193	7,75E-56	157	hormone metabolism
HACF01032424	Q9CPU4	50,746	134	6,45E-35	125	detoxification
HACF01032444	Q3SXM5	40,83	289	6,20E-68	220	hormone metabolism
HACF01032455	P22105	40,556	180	4,06E-37	141	development
HACF01032458	Q6RG02	40,394	203	3,84E-87	137	reproduction
HACF01032595	P30275	41,88	117	6,08E-20	94,7	energy metabolism
HACF01032702	Q29C43	43,404	470	3,67E-154	400	metabolism related to bioactive lipids
HACF01032709	P11247	34,576	590	2,42E-85	287	immune response
HACF01032722	P80276	58,721	172	1,19E-65	209	carbohydrate metabolism
HACF01032734	Q6RG02	38,462	104	4,27E-33	89	reproduction
HACF01032752	Q8N130	45,385	260	8,10E-77	160	transport
HACF01032755	UniRef90_UPI0006B0F3DB	56,995	193	1,05E-59	195	
HACF01032805	UniRef90_E9GB99	32,85	207	1,68E-25	120	reproduction
HACF01032919	Q6IRK9	54,377	377	1,08E-134	399	proteolysis
HACF01033004	UniRef90_E9K6N5	37,849	251	2,03E-47	173	immune response
HACF01033025	UniRef90_T1W366	71,795	78	3,18E-30	118	immune response
HACF01033068	Q6PBH5	46,341	82	6,10E-17	78,6	energy metabolism
HACF01033147	P80646	27,168	173	1,60E-14	66,2	digestion
HACF01033158	UniRef90_E9HZE2	77,193	57	2,88E-20	95,9	
HACF01033194	Q6RG02	36,683	199	1,10E-30	122	reproduction
HACF01033240	Q5BLE8	46,61	118	2,12E-32	124	other
HACF01033273	Q0VD19	47,368	266	5,32E-76	245	metabolism related to bioactive lipids
HACF01033287	UniRef90_C3YJK1	37,662	154	5,45E-22	104	
HACF01033299	UniRef90_A0A131Z7Z0	51,515	198	7,66E-123	245	
HACF01033318	Q6RG02	26,633	199	8,00E-20	79,3	reproduction
HACF01033361	Q20191	36,503	326	1,55E-47	177	proteolysis
HACF01033470	UniRef90_U3KST7	30,055	183	2,42E-19	94	immune response
HACF01033483	Q6NU46	57,466	221	3,51E-117	231	lipid metabolism
HACF01033533	UniRef90_F5A5Q6	38,571	140	1,63E-21	93,2	reproduction
v.s.	APO14259.1	35,766	137	3,16E-21	85,5	
HACF01033536	UniRef90_T1JCW7	28,077	260	8,26E-14	83,2	
HACF01033568	Q54Y41	47,368	76	5,69E-15	69,3	translation
HACF01033591	O33752	45,6	125	6,69E-27	103	
HACF01033638	Q17RR3	36,161	224	6,61E-31	124	digestion
HACF01033846	P09849	47,603	292	5,01E-109	250	carbohydrate metabolism
HACF01033977	Q92400	53,509	228	1,56E-76	245	digestion
HACF01033985	UniRef90_UPI0005EF71C6	42,574	101	7,42E-14	75,1	proteolysis
HACF01033990	Q920A5	40,235	425	1,95E-97	311	proteolysis
HACF01034026	Q6RG02	28,889	225	7,64E-49	115	reproduction
HACF01034183	Q13231	40,488	205	2,58E-47	164	other
HACF01034200	P35787	42,13	216	3,50E-41	147	other
HACF01034213	Q7Z408	41,509	106	7,66E-14	78,2	other
HACF01034315	UniRef90_UPI0003F0CB3B	41,758	91	6,04E-13	73,6	
HACF01034325	Q9DBW0	60,135	148	2,55E-70	196	hormone metabolism
HACF01034357	P00765	46,154	143	2,36E-29	111	digestion
HACF01034453	P02399	52,252	111	1,07E-14	74,3	translation
HACF01034502	UniRef90_K1QH31	36,508	126	1,12E-17	90,9	
HACF01034543	UniRef90_I2C0B2	41,912	136	2,56E-34	133	immune response

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01034547	Q1JPJ2	48,175	274	1,38E-78	253	proteolysis
HACF01034549	UniRef90_H2ZK53	41,176	119	5,90E-13	80,1	
HACF01034699	P07829	82,796	93	1,66E-58	173	cytoskeleton
HACF01034769	UniRef90_A0A182JG93	31,351	185	2,86E-14	68,9	
HACF01034805	Q9GV46	31,348	319	1,53E-44	161	bioluminescence
HACF01034824	UniRef90_E7D1Q4	81,579	38	1,88E-14	69,3	
HACF01035018	UniRef90_C3Z3F0	40,86	93	3,29E-13	73,9	
HACF01035154	UniRef90_U3LVT5	25,51	196	1,87E-10	68,6	immune response
HACF01035166	P00765	50	122	4,75E-55	118	digestion
HACF01035168	P16257	35,503	169	6,55E-25	101	transport
HACF01035233	P20693	34,127	126	2,81E-13	72,4	immune response
HACF01035277	P00765	48,182	220	8,95E-65	205	digestion
HACF01035301	O08782	51,064	94	8,82E-22	95,5	carbohydrate metabolism
HACF01035321	UniRef90_C3YLJ6	37,391	115	2,14E-10	66,6	
HACF01035373	P00765	39	200	4,54E-40	141	digestion
HACF01035384	Q8N357	53,012	166	8,01E-46	159	transport
HACF01035455	UniRef90_E9FRH4	31,765	170	2,87E-15	81,3	
HACF01035463	Q8WP19	64,035	228	6,91E-104	320	other
HACF01035512	UniRef90_J3KVJ4	65,909	88	3,68E-26	111	
HACF01035560	Q3T100	47,761	134	1,48E-31	116	detoxification
HACF01035609	O18783	48,571	70	3,09E-12	67,8	immune response
HACF01035623	Q9CPP7	48,454	97	7,21E-18	81,6	digestion
HACF01035800	P32320	52,222	90	1,43E-26	101	other
HACF01035883	Q8MKK4	28,947	304	8,78E-37	142	transport
HACF01035927	P53603	41,837	98	2,58E-24	85,5	amino acid metabolism
HACF01035995	UniRef90_W8PFL4	51,111	90	1,56E-19	95,5	other
HACF01036007	P97821	37,966	295	9,36E-69	224	proteolysis
HACF01036035	Q6RG02	22,075	453	4,30E-23	110	reproduction
HACF01036048	Q6Q151	43,503	177	1,25E-59	132	protein folding
HACF01036072	O60494	24,303	251	2,13E-10	68,6	transport
HACF01036180	Q54JL3	57,143	98	7,71E-35	107	amino acid metabolism
HACF01036187	Q5XIC0	57,534	73	1,81E-37	94,7	lipid metabolism
HACF01036424	A8Y9I2	55,14	107	1,38E-38	140	visual perception
HACF01036611	UniRef90_I6V2P8	33,333	159	2,17E-21	96,3	immune response
HACF01036683	Q2KIR8	59,016	61	3,55E-25	74,3	amino acid metabolism
HACF01036840	Q43291	53,383	133	1,55E-44	146	translation
HACF01036880	Q86IC8	43,891	221	4,91E-54	180	other
HACF01036905	P00689	56,557	122	6,36E-44	153	digestion
HACF01036912	Q9XZ71	53,892	167	9,47E-34	129	muscle development and regulation
HACF01036966	UniRef90_A0A093GNN4	36,441	118	3,09E-10	68,9	proteolysis
HACF01037090	P11169	32,589	224	1,16E-34	134	transport
HACF01037116	P00762	42,276	246	2,35E-44	160	digestion
HACF01037134	P33727	36,757	185	2,32E-35	92,8	development
HACF01037140	O70247	47,5	120	1,72E-28	112	transport
HACF01037169	O75897	31,788	302	2,23E-46	164	other
HACF01037174	Q298G6	71,942	139	4,35E-54	189	other
HACF01037226	Q8N0N3	56,593	182	9,53E-65	214	immune response
HACF01037236	P70059	45,378	238	1,92E-49	177	digestion
HACF01037328	P27708	64,803	304	5,61E-132	413	other
HACF01037421	Q0VD19	43,655	197	5,51E-40	158	metabolism related to bioactive lipids
HACF01037443	P00765	69,362	235	4,55E-114	333	digestion
HACF01037517	Q6RG02	30,127	395	3,02E-52	206	reproduction

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Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01037559	UniRef90_K9J9X8	50	70	1,34E-16	80,1	immune response
HACF01037579	UniRef90_D3PIA3	30,531	226	1,87E-16	84,3	visual perception
HACF01037596	UniRef90_K9J9X8	62,319	69	9,56E-21	89,7	immune response
HACF01037601	UniRef90_E9FRH4	28,959	221	1,37E-18	94	
HACF01037606	UniRef90_E9FRH4	30,542	203	2,35E-18	91,7	
HACF01037611	Q6RG02	18,133	375	7,27E-19	98,2	reproduction
HACF01037618	Q7RTY3	40,541	74	3,15E-11	67,4	proteolysis
HACF01037626	Q9EPH8	71,014	69	2,52E-20	97,1	transcriptional regulation
HACF01037639	Q9UBQ7	53,727	322	1,94E-108	323	other
HACF01037647	Q9GV46	30,795	302	9,12E-31	124	bioluminescence
HACF01037661	O46373	78,534	191	8,63E-95	283	transport
HACF01037683	P00765	66,809	235	6,28E-110	323	digestion
HACF01037695	P70059	44,958	238	1,97E-50	177	digestion
HACF01037696	Q6RG02	30,149	335	3,04E-51	188	reproduction
HACF01037715	P00765	69,787	235	5,93E-114	337	digestion
HACF01037721	O60494	23,346	257	2,12E-10	68,9	transport
HACF01037734	Q1HPK6	80,556	144	8,34E-71	251	translation
HACF01037740	P12785	50,735	136	1,87E-32	137	lipid metabolism
HACF01037755	Q6RG02	34,728	239	9,53E-42	159	reproduction
HACF01037774	UniRef90_E9HXY1	76,19	63	4,51E-29	94,4	
HACF01037778	P00765	70,042	237	1,51E-115	336	digestion
HACF01037791	UniRef90_UPI00021A8433	25,243	412	8,29E-15	85,9	proteolysis
HACF01037800	UniRef90_T1JCW7	27,237	257	3,71E-13	79,7	
HACF01037811	P80067	34,783	115	6,19E-15	76,3	proteolysis
HACF01037845	P16295	27,556	225	3,47E-10	64,7	immune response
HACF01037860	UniRef90_Q9NDT7	58,065	62	1,01E-16	81,6	other
HACF01037882	Q6RG02	31,832	333	1,45E-51	198	reproduction
HACF01037894	UniRef90_D3PIA3	31,156	199	2,22E-15	81,6	visual perception
HACF01037906	Q7RTY7	33,582	402	3,48E-44	176	immune response
HACF01037914	P02707	32,331	133	4,43E-13	72	immune response
HACF01037927	P80226	31,655	139	7,38E-10	58,9	transport
HACF01037993	B7TB45	26,702	191	1,62E-12	72,4	development
HACF01038003	P29289	64	150	2,58E-58	185	muscle development and regulation
HACF01038018	P83777	67,925	53	7,49E-17	82	lipid metabolism
HACF01038020	Q9W092	43,678	174	1,34E-78	182	other
HACF01038048	C0ITL3	46,154	117	9,03E-33	108	other
HACF01038062	UniRef90_T1W2K0	56,41	78	5,96E-18	84	immune response
HACF01038076	O73888	35,644	202	1,06E-38	136	reproduction
HACF01038103	UniRef90_C3ZK66	42,157	102	1,20E-17	93,6	
HACF01038111	Q920A5	40,909	132	1,43E-24	107	proteolysis
HACF01038112	Q6GQ29	51,673	269	3,00E-76	242	proteolysis
HACF01038168	Q6RG02	32,53	332	1,63E-51	199	reproduction
HACF01038169	UniRef90_Q9NDT7	45,57	79	1,05E-14	76,3	other
HACF01038180	UniRef90_H8YI21	57,627	118	2,61E-36	147	other
HACF01038219	P05661	66,197	284	3,80E-168	359	muscle development and regulation
HACF01038254	Q6GQ29	52,97	202	1,25E-63	187	proteolysis
HACF01038260	Q6RG02	45,161	93	5,12E-16	86,3	reproduction
HACF01038271	UniRef90_UPI00021A8433	26,566	399	4,20E-16	90,1	proteolysis
HACF01038298	UniRef90_Q9NDT7	56,667	60	5,12E-16	79,3	other
HACF01038300	P45521	95	40	4,03E-33	86,7	cytoskeleton
HACF01038322	P82119	47,967	123	5,15E-22	95,9	other
HACF01038355	UniRef90_I6VVL1	43,59	117	2,44E-22	103	immune response

Table A.1 continued from previous page

Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01038393	Q6RG02	40,909	110	6,17E-18	90,9	reproduction
HACF01038429	UniRef90_D3PIA3	35,821	201	1,32E-22	102	visual perception
HACF01038460	Q9TTK8	46,377	138	2,54E-30	115	energy metabolism
HACF01038467	P00740	40,909	44	8,62E-10	43,1	immune response
HACF01038502	P53471	91,176	170	6,62E-115	333	cytoskeleton
HACF01038520	P97675	30,303	132	1,21E-22	64,7	other
HACF01038531	Q6RG02	22,274	431	1,11E-25	118	reproduction
HACF01038534	UniRef90_A0A0B7BRV5	35,862	145	1,93E-10	72	
HACF01038545	P02707	30,07	143	2,26E-13	72,4	immune response
HACF01038559	B7TB45	38,889	90	1,58E-12	72,8	development
HACF01038626	UniRef90_V9XZL5	22,513	191	3,10E-22	72	reproduction
HACF01038712	P29290	73,77	61	2,17E-25	97,4	muscle development and regulation
HACF01038729	G9JJU2	59,524	168	1,50E-65	208	detoxification
HACF01038736	P69526	38,172	186	4,16E-30	103	proteolysis
HACF01038786	P07436	92,199	141	1,37E-94	284	cytoskeleton
HACF01038834	UniRef90_K9J9X8	44,304	79	3,95E-17	77,8	immune response
HACF01038858	UniRef90_C5HF66	63,333	60	1,48E-16	78,2	other
HACF01038860	UniRef90_D7F1R4	36,364	154	4,08E-19	91,7	development
HACF01038869	Q6RG02	40	105	5,75E-23	91,7	reproduction
HACF01038918	P00762	42,683	164	1,04E-30	114	digestion
HACF01038960	Q6RG02	41,818	110	3,36E-17	90,9	reproduction
HACF01038982	P82119	53,448	116	5,41E-27	108	other
HACF01038987	Q5RE33	50,685	146	7,70E-50	166	receptor-related protein
HACF01039009	UniRef90_E9FRH4	38,191	199	1,18E-29	120	
HACF01039013	UniRef90_E9FRH4	30,208	192	2,09E-15	82	
HACF01039027	UniRef90_E9FRH4	31,383	188	9,98E-14	77	
HACF01039037	P81580	52,542	118	6,38E-27	105	other
HACF01039063	UniRef90_D2DT32	97,059	34	8,75E-13	70,1	
HACF01039072	P05661	51,02	49	3,17E-16	54,7	muscle development and regulation
HACF01039091	UniRef90_Q7YUE2	65,217	69	6,69E-23	94,4	other
HACF01039126	UniRef90_C5HF66	48,101	79	1,38E-11	68,2	other
HACF01039176	Q8BIK6	35,484	93	2,24E-14	72,8	proteolysis
HACF01039326	P45521	75,41	61	1,18E-22	94,7	cytoskeleton
HACF01039334	Q9XZ71	61,111	36	1,69E-11	50,1	muscle development and regulation
HACF01039336	Q9VZ49	38,679	106	3,48E-14	72,4	transcriptional regulation
HACF01039354	UniRef90_G5CJW5	80,645	93	5,05E-54	172	immune response
HACF01039373	Q9Y5Y6	31,566	415	9,41E-48	176	proteolysis
HACF01039476	UniRef90 A0A078A2G4	84,848	66	9,81E-25	111	
HACF01039540	UniRef90_G5CJW5	74,809	131	3,26E-65	213	immune response
HACF01039544	UniRef90_Q7YUE2	65,789	76	1,71E-24	98,2	other
HACF01039549	P81589	58,333	48	1,13E-10	57,4	other
HACF01039576	UniRef90_K4D7B6	75,949	79	4,30E-27	113	
HACF01039584	P00765	48,98	98	2,54E-23	95,5	digestion
HACF01039614	P79953	26,667	270	1,81E-11	70,5	immune response
HACF01039618	Q6RG02	42,727	110	2,57E-20	98,6	reproduction
HACF01039663	UniRef90_T1W366	70,833	72	1,57E-24	104	immune response
HACF01039750	UniRef90_UPI00045761B8	41,667	72	8,83E-13	67,4	other
HACF01039823	Q9DCF9	61,538	52	2,12E-15	68,9	receptor-related protein
HACF01039944	Q27081	42,857	105	1,96E-17	82,4	immune response
HACF01039946	UniRef90_D3PIA3	31,633	196	1,65E-15	82,4	visual perception
HACF01039954	P82119	48,837	129	3,53E-19	84,7	other

Table A.1 continued from previous page

Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01039959	P00762	41,463	246	1,17E-45	160	digestion
HACF01039972	UniRef90_T1W366	71,25	80	8,27E-31	119	immune response
HACF01039973	C0ITL3	50,962	104	6,00E-26	104	other
HACF01040014	Q6RG02	57,377	61	8,23E-12	70,5	reproduction
HACF01040030	Q07954	48,214	112	5,33E-20	90,9	receptor-related protein
HACF01040056	UniRef90 T1W366	74,576	59	1,87E-20	90,1	immune response
HACF01040083	UniRef90 K9J9X8	53,846	65	1,02E-13	73,2	immune response
HACF01040100	UniRef90_C8CB63	37,984	129	5,84E-17	83,2	transport
HACF01040101	UniRef90_K9J9X8	50	80	1,52E-18	84	immune response
HACF01040107	UniRef90_Q9NDT7	56,667	60	9,01E-16	78,2	other
HACF01040211	UniRef90_A0A087TTB9	69,231	78	2,36E-24	82,8	
HACF01040284	UniRef90_Q9NDT7	57,377	61	7,87E-17	81,3	other
HACF01040745	Q5VT66	37,398	123	5,28E-21	81,6	detoxification
HACF01040973	Q24756	42,373	118	1,16E-21	93,2	muscle development and regulation
HACF01041044	P35033	40,704	199	8,53E-47	157	digestion
HACF01041111	Q5BLE8	41,706	211	3,05E-41	152	other
HACF01041289	Q9NBX4	28	150	4,43E-17	63,2	other
HACF01041437	P98068	29,327	208	4,95E-20	91,3	development
HACF01041540	Q71R50	46,667	210	1,17E-58	186	hormone metabolism
HACF01041730	P00765	53,271	107	4,49E-30	116	digestion
HACF01041865	Q9CPU4	51,493	134	8,68E-28	107	detoxification
HACF01042536	Q19QT7	59,669	181	1,60E-69	219	amino acid metabolism
HACF01042729	Q6WV16	55,102	98	3,45E-39	123	development
HACF01042789	P03951	35,664	143	2,82E-24	77,4	immune response
HACF01042801	Q94572	79,487	78	1,50E-43	145	cytoskeleton
HACF01042814	Q9Y5Z4	42,697	178	1,74E-38	134	other
HACF01042876	P14866	42,241	116	2,31E-20	90,9	transcriptional regulation
HACF01043021	P48148	70,161	124	5,12E-61	192	development
HACF01043218	Q5R9A1	39	100	4,03E-14	70,9	transport
HACF01043290	UniRef90_W6MEX3	56	75	8,22E-26	103	proteolysis
HACF01043364	UniRef90_H2LV15	25,116	215	7,93E-13	77,4	development
HACF01043400	UniRef90_M9PCJ6	32,278	158	3,57E-15	72,8	development
HACF01043466	O33752	47,541	61	3,99E-11	61,2	
HACF01043540	P43236	57,895	76	1,30E-20	90,9	proteolysis
HACF01043607	Q5NVR2	58,261	115	1,51E-33	123	carbohydrate metabolism
HACF01044065	UniRef90_E9GTQ5	37,374	99	1,49E-10	65,9	
HACF01044235	P98157	45,217	115	5,47E-23	80,5	receptor-related protein
HACF01044952	UniRef90_A0S0Q2	35,659	129	1,70E-19	92,8	carbohydrate metabolism
HACF01045106	UniRef90_Q9NDT7	58,333	60	1,96E-16	80,5	other
HACF01045479	P49746	43,373	166	3,31E-40	149	immune response
HACF01045495	O15072	57,407	54	1,92E-12	68,2	proteolysis
HACF01045639	P14642	88,542	96	7,57E-59	190	cytoskeleton
HACF01046303	O60494	33,333	165	1,71E-16	80,9	transport
HACF01046386	Q33820	81,081	37	3,73E-15	62,8	energy_metabolism
HACF01046440	P00740	39,759	83	7,72E-14	72,8	immune response
HACF01046465	P81388	58,182	55	8,33E-17	74,3	other
HACF01046950	A7YWP4	71,375	269	3,90E-136	404	amino acid metabolism
HACF01046985	Q9VUL9	37,647	170	1,11E-26	107	development
HACF01047135	UniRef90_Q9NDT7	59,322	59	7,13E-17	81,3	other
HACF01047354	Q23551	49,612	129	9,46E-32	118	muscle development and regulation
HACF01047426	P98021	75,714	70	1,18E-33	117	energy metabolism
HACF01047519	P04069	43,919	148	6,03E-38	135	proteolysis
HACF01047927	P10787	54,31	116	3,84E-33	129	energy metabolism

Table A.1 continued from previous page

Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01047995	Q9VZ49	50,588	85	2,19E-21	93,6	transcriptional regulation
HACF01048503	P50671	72,222	90	2,61E-37	133	energy metabolism
HACF01048950	Q8TGM7	64,912	57	3,06E-17	77	
HACF01048959	P29691	67,883	137	3,86E-55	190	translation
HACF01048962	Q6WV16	58,333	108	1,07E-42	120	development
HACF01049254	O15992	39,806	103	2,33E-16	77,4	energy metabolism
HACF01049487	P00765	58,947	95	2,65E-29	112	digestion
HACF01049514	Q26636	48,361	122	2,31E-28	113	immune response
HACF01049639	Q6QAP7	86,747	83	4,82E-48	147	translation
HACF01049656	P00765	51,724	58	4,81E-11	62,4	digestion
HACF01049690	P59669	86,957	46	2,88E-47	82	other
HACF01049730	P11426	78,125	32	1,85E-11	62	cytoskeleton
HACF01049815	P21845	50,602	83	2,67E-18	84,7	proteolysis
HACF01049870	UniRef90_A7YJ4	84,848	33	1,57E-10	67	energy metabolism
HACF01050177	P48775	51,316	76	1,87E-14	73,2	amino acid metabolism
HACF01050261	UniRef90_A0A0J7KQN9	29,703	101	3,21E-10	64,3	transport
HACF01050400	P10994	88,06	67	3,35E-38	128	cytoskeleton
HACF01050446	UniRef90_A0A087U712	32,667	150	1,03E-17	85,9	
HACF01050862	UniRef90_Q1PSV4	34,615	78	2,06E-12	51,2	immune response
HACF01051814	UniRef90_Q4LAX5	62,857	70	6,94E-12	67,4	translation
HACF01052181	P53445	64,045	89	3,53E-28	109	carbohydrate metabolism
HACF01052391	O15992	44,915	118	5,93E-23	84	energy metabolism
HACF01052509	Q54JL3	71,171	111	2,69E-52	177	amino acid metabolism
HACF01053521	P05661	51,095	137	1,49E-42	153	muscle development and regulation
HACF01053608	P47990	49,219	128	2,37E-31	124	other
HACF01053956	UniRef90_E9H014	33,858	127	3,00E-12	69,7	
HACF01053969	Q6UWB4	58,333	48	9,59E-11	62	proteolysis
HACF01054213	Q7KRW8	64,286	56	1,15E-17	80,9	transcriptional regulation
HACF01054214	P00765	57,377	61	1,25E-14	70,5	digestion
HACF01054864	Q25381	89,062	128	2,35E-80	242	cytoskeleton
HACF01054879	UniRef90_Q8WSX2	32,231	121	1,66E-11	71,2	immune response
HACF01055057	P49010	50	84	2,00E-19	87	other
HACF01055430	P00762	48,333	60	9,90E-16	56,2	digestion
HACF01055521	Q9JM99	38,158	76	4,23E-16	80,1	immune response
HACF01055587	Q9GV46	29,319	191	1,51E-17	82	bioluminescence
HACF01055624	UniRef90_UPI0006B09756	46,667	75	2,76E-15	75,1	proteolysis
HACF01055666	UniRef90_Q967X8	30,579	242	7,10E-15	81,6	proteolysis
HACF01055895	P81580	65,306	49	9,47E-13	66,2	other
HACF01055903	P00765	37,815	119	6,23E-19	85,1	digestion
HACF01055931	Q7JIG6	41,803	122	6,19E-16	77	proteolysis
HACF01055952	P56677	43,333	60	4,60E-13	52,4	proteolysis
HACF01056011	P41366	45,833	48	4,80E-14	53,5	immune response
HACF01056144	P12606	34,667	150	1,06E-11	51,6	receptor-related protein
HACF01056276	P35785	42,941	170	2,25E-26	104	other
HACF01056344	UniRef90_U5EED9	44,156	231	1,93E-46	158	transport
HACF01056429	P36178	58,929	56	7,93E-14	69,7	digestion
HACF01056477	UniRef90_D3PIA3	34,5	200	8,25E-20	94,4	visual perception
HACF01056496	P98021	73,171	123	3,26E-52	172	energy metabolism
HACF01056572	Q9Y5Z4	39,429	175	1,89E-33	123	other
HACF01056630	UniRef90_W6MEZ7	51,923	52	3,40E-10	63,2	transport
HACF01056943	P55274	63,636	66	1,49E-16	79,7	visual perception

Table A.1 continued from previous page

Query/Contig ID	Subject ID	% Identity	Alignment length	E-value	Bit score	Category
HACF01056957	UniRef90_A0A078A2G4	93,939	66	2,88E-31	128	
HACF01057510	Q27081	41,237	97	6,33E-15	73,6	immune response
HACF01057519	UniRef90_Q9NDT7	56,667	60	2,72E-16	79,7	other
HACF01057530	UniRef90_Q7YUE2	70,833	48	5,89E-15	72	other
HACF01057543	P56649	92,593	27	4,06E-11	59,3	carbohydrate metabolism
HACF01057802	Q8N2E2	50,588	85	2,28E-21	91,3	
HACF01058039	P27779	36,364	88	8,22E-10	56,6	other
HACF01058112	Q27081	50	68	1,17E-14	72,4	immune response
HACF01058151	Q00871	67,647	136	9,47E-60	188	digestion
HACF01058182	P43236	54,286	105	3,43E-30	114	proteolysis
HACF01058312	B4MYA4	35,976	164	5,05E-18	83,6	transport

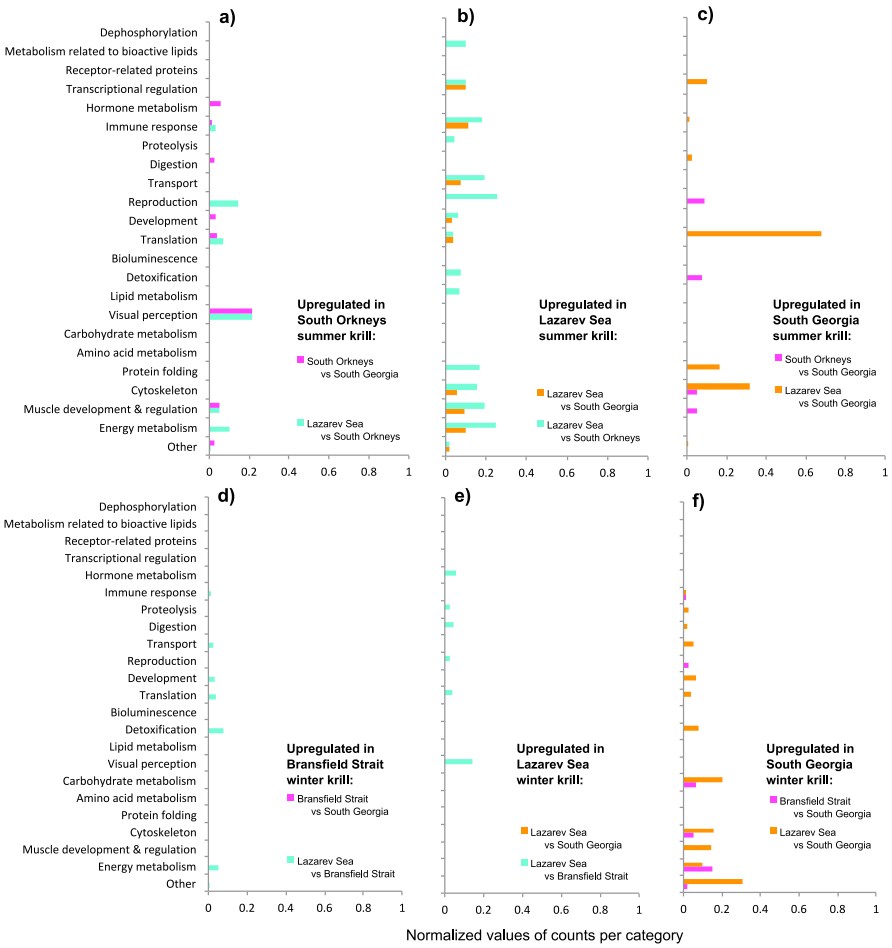

FIGURE A.2: Annotated differentially expressed genes sorted into functional categories from the regional summer (a-c) and winter comparisons (d-f). Upregulared genes are shown for each region and season: a) South Orkneys summer krill, b) Lazarev Sea summer krill, c) South Georgia summer krill, d) Bransfield Strait winter krill, e) Lazarev Sea winter krill, and f) South Georgia winter krill.

B. Supplementary Information Publication 3

TABLE B.1: Investigated genes including gene name, abbreviated gene name, TaqMan Assay ID, Sequence ID, and probe and primer sequences.

Abbreviation	Sequence ID	Probe sequence 5′-3′	Forward primer sequence 5′-3′	Reverse primer sequence 5′-3′
usp46	Piccolin et al. 2018	AAGAGCCACAGATTTT	TGGAACTGGTATTAACAGAGGACACT	ACTGCATCGTCATCAAAGAGCA
rrn3	HACF01031378	CAGGGCTTGGAACCAT	CTGCTCATGTGCAATTTATTGTCTTCT	TTCAAACTTTTCTTTTCGTAGATATTCTAGT
rpb1	ESS023330	CCGGACTGAGGATACC	AAGCCCCTTTAAAGTCTGTCAAGAG	AGTCACTGACATTCGCCGAATTT
rps18	ESS061187	CAGTCATGGCAAACAT	ACATCGATGGTAGGCGTAAAGTC	CGACGACCAACACCCTTGAT
rps13	Piccolin et al. 2018	CACACGCAGGATTTT	TGCTCAGGTTCGCTTTGTCA	CTCTGGGATATCTGGAGCAAGTC
cry2	Piccolin et al. 2018	ACTGCACCAGAAAAT	CAGTGCTCAAGAACTTCCCAACTAA	GCGTCCTATGACACATTTAGACTGT
tim1	Piccolin et al. 2018	TCGGCGTTCACTCTTC	CAAGACAAAGCGAGATGGCATTT	AGGGTTGGAAGAAGGTTTTGTGAAA
clk	Piccolin et al. 2018	TTGGCTCCAGAATCAT	GGCCTCAGTTGGTACGAGAAATG	AATTTCCATTCTATACTGTGCCTTGATGT
pfk6	Piccolin et al. 2018	ATACCGACCAAAATCA	AGTTATAACAAGAGTTAAGCAGCAGTGT	CCCTGACATGCCAAAAATGCA
cs	Piccolin et al. 2018	CAAGTCCTCAAAATTC	ATGTCTCGCTGCTGCTATCTC	TCTTCACTAGTTATTGTTGATGCCAGAA
gapdh	HACF01057543	CACGGTATGAATATCC	TCGTTTCCTGGTATGACAATGAGTT	ACCTAACCATCAACCTTCTGCATAT
ndufa4	HACF01033068	CCTCAGCCACAATCAG	GAGTTGGAATCGCACTGCTAAC	ACAGGTGAGAAGAACTTGTATTGCTT
atp	Piccolin et al. 2018	CTGCTGCCAAGTTTGC	GTCAAGAACATCCAGAAGATCACTCA	GCTTCAACTCCCTTTCAGCTCTT
vtlgl	HACF01036035	TCCACGTTCCCGTTCTT	CAGCTGAAACAACATTCAAAGCAATT	CCTGAGGTAACAATAGCACCATATTCA
fabp	HACF01040100	CTCCCAGGAGAACTTT	CCCGGCAAGTACGAATTGGA	CCATCTCTGCAGCCTTCTCA
fasn	HACF01037740	CCAGTGCTACGGCTCC	GCAGGTGAACACAGTGAAAGGT	CATGTGCAAGTCAGGACAAAGC
acsl	HACF01011946	CCTGACACTGAACATGC	ACATCAATAAATGGAGTAACCAAGAAGCA	TCATACAGTTCAGCATATTTGTAGCTCTT
gal3st	HACF01003865	CCTGCACTCAGAGCAA	TTACGGTATGGCTATCAACATGATCT	CTTGAATTTTTTAGGATGCCCAAGGT
hpgds	HACF01038076	CCCGCGCCGAACTG	ATGCCAGAATACAAGCTCATATACTTCA	TCATAGGGTATTCCTCCATAGGCAAA
cbr1	HACF01007962	CACCTATGGAGGATTTG	TCACAGAACAAGCAAGTATTGATCGA	AAGCCTGCATTGTTTACTAAGCA
famet	HACF01006689	CTCTGCGGAAAGACAC	CACAAGAACTACCGTTTCCGATCA	GGGCAGCTTTCACCTGGAA
nec1	HACF01005894	CATGTGTCCCGTCATCC	AGGTAGATGTTGTGTCCTGTTCTTG	CTAACTTCCCAGGGCCCTCTA
lrp1	HACF01044235	CCTCAGCCCAATTTG	CGGATCAGATGAAAAAGACTGCAAA	GCATTTTGAAGACGAACTGACACAT

Table B.1 continued from previous page

Abbreviation	Sequence ID	Probe sequence 5′-3′	Forward primer sequence 5′-3′	Reverse primer sequence 5′-3′
trp1	HACF01000178	CCCGACAACGTCCTCG	CTCATCATTCTCTCTCCAACCTTCA	CCACAGTACCTTTGTTTTCCAAGAG
astc	HACF01043311	CAACTGCGAGAAACAT	GATGTCCTCTGCCAGTCAACA	GCGCTGCAGATTGCCAAA
span	HACF01000142	ACCTGCTGACCCTTCC	GCTGGTCCTATATTGGCATGATCTC	GCATCCTGAGCCAACGGA