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

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ABSTRACT

The 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 differences in gene expression in three different latitudinal regions (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 RNA-seq data were analysed (a) for seasonal differences between summer and winter krill sampled from each region, and (b) for regional differences within each season. A large majority of genes showed an up-regulation in summer krill in all regions with respect to winter krill. However, seasonal differences in gene expression were less pronounced in Antarctic krill from South Georgia, most likely due to the milder seasonal conditions of the lower latitudes of this region, with a less extreme light regime and food availability between summer and winter. Our results suggest that in the South Orkneys/Bransfield Strait and Lazarev Sea region, Antarctic krill entered a state of metabolic depression and regressed development (winter quiescence) in winter. Moreover, seasonal gene expression signatures seem to be driven by a photoperiodic timing system that may adapt the flexible behaviour and physiology of Antarctic krill to the highly seasonal environment according to the latitudinal region. However, at the lower latitude South Georgia region, food availability might represent the main environmental cue influencing seasonal physiology.

1. 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) such as 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 and feeding activity as well growth (Meyer, 2011) and sexual regression during winter (Siegel, 2012; Tarling et al., 2016; Höring et al., 2018).

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: krill near South Georgia have lower

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Table 1
Sampling data, including: cruise, region, sampling date, local time (hh:min), season, latitude (Lat.), longitude (Lon.), sampling gear, number of analysed individuals with sex and maturity (mat.) stage (according to Makarov and Denys, 1981, and Kawaguchi et al., 2007), sampling depth range, average chlorophyll *a* (Chla) concentration and mean seawater surface temperature (SST) from satellite data (www.polarwatch.noaa.gov), or literature (* Reiss et al., 2017, ** Meyer et al., 2017).

Cruise	Region	Date /time	Season	Lat/Lon	Gear	Sex/Mat. stage	Depth (m)	Chla (mg m^{-3})	SST (°C)
ANT23–2	Lazarev Sea	23-Dec-2005 01:07	Summer	−65.51/−3.03	RMT 8 + 1	3 ♂/ IIIB 4 ♀/IIIG	0–50	2.50	−1.50
ANT23–6	Lazarev Sea	27-Jun-2006 02:21	Winter	−61.52/2.93	RMT 8 + 1	3 ♂/III 3 ♀/IIC	0–50	0.02	−1.80
JR15004	South Orkneys	02-Feb-2016 04:14	Summer	−60.30/ −46.85	RMT8 + 1	3 ♂/IIIB 3 ♀/IIIG	0–50	2.7	−0.05
AMLR14	Bransfield Strait	26-Aug-2014 00:40	Winter	−62.50/ −59.50	IKMT	3 ♂/III 3 ♀/IIC	0–170	0.25*	−1.66
JR260B	South Georgia	04-Jan-2012 01:37	Summer	−53.68/ −38.56	RMT 8	3 ♂/III 3 ♀/IIIG	0–50	0.29	2.30
Norwegian fishing vessel	South Georgia	18-Jul-2015 07:00	Winter	−54.15/ −35.60	Continuous pumping system	5 ♂/III	147	0.3**	0.86

lipid stores and higher feeding activity in winter compared to krill from 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 with respect to the Antarctic Peninsula and South Georgia (54°S) region. These authors concluded that genes involved in feeding and digestion, respiration, motor activity, immunity and vitellogenesis were upregulated in krill sampled in the Peninsula region during the summer with respect to winter. Whereas, 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., 2010), 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 machinery and the photoperception system in *E. superba* (Biscontin et al., 2016, 2017, 2019; 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, but may also be involved in the timing of seasonal life cycle events (Piccolin et al., 2018b). Recently, the first picture of the krill's circadian transcriptome under laboratory free-running conditions (in constant darkness, DD) as well as under a 16:8 light-dark (LD) regime was described. Interestingly, a large proportion of genes, including several clock components (*clock*, *period*, *cry2*, *vrille*, and *slimb*), showed sinusoidal expression patterns in DD, with a periodicity shorter than 24 h. Energy-storage pathways seemed to be regulated by the endogenous clock in accordance with their ecological relevance in daily energy managing and overwintering (Biscontin et al., 2019).

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 are generally less frequent. Even though extensive transcriptome studies have been conducted in *E. superba* (De Pittà et al., 2013; Meyer et al., 2015; Sales et al., 2017; Biscontin et al., 2019), 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 study investigates seasonal and regional differences in gene expression profiles in Antarctic krill sampled in three regions of the Southern Ocean at different latitudes: 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 investigate (1) seasonal transcriptional signatures of krill caught in summer and winter in these three regions, and (2) regional gene expression profiles of krill sampled at these three different latitudes during winter and summer in order to identify differentially expressed genes with regulatory functions in the seasonal life cycle of Antarctic krill.

2. Methods

2.1. Sample collection and experimental design

Antarctic krill samples (*Euphausia superba*) were obtained from five different expeditions and from a Norwegian fishing vessel (Table 1). Sampling was carried out with a Rectangular Midwater Trawl (RMT8 + 1 for expeditions ANT23–2, ANT23–6 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 °C were transferred to the Alfred-Wegener-Institute, Bremerhaven, for molecular analysis.

The Antarctic krill were caught at 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 sampling, including 3–4 females and 3 males with the exception of South Georgia where only 5 males were analysed for the winter sampling (see Table 1 for details).

2.2. RNA extraction, library preparation and Illumina sequencing

Total RNA was individually extracted from frozen krill heads with the RNeasy Midi Kit (QIAGEN, Hilden, Germany). Frozen krill heads were cut on dry ice and transferred to 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 3000 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 quantity and quality of total 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), respectively.

The RNA-Seq of each individual sample was carried out from IGA Technology Services (Udine, Italy). cDNA libraries were constructed with 1–2 μg of total 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 min at 94 °C. 1X Agencourt AMPure XP beads (Agencourt Bioscience Cooperation, Beckman Coulter, Beverly, MA, USA). Total 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 the manufacturer's instructions. Sequencing was carried out on paired-end mode (2 × 100 bp) by using HiSeq 2500 (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. 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 for 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 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 by 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). Trimmed reads showed an average mapping rate of 69.22 ± 3.37% (mean ± SD) to the reference transcriptome. TMM-normalized expression values were calculated. Differential gene expression analysis was performed with edgeR (Robinson et al., 2010). Differentially expressed genes (DEGs) were identified using a false discovery rate (FDR) cut-off value of 0.001 and a minimum absolute Log_2 fold change (log_2FC) of 2. Pairwise comparisons included seasonal comparisons within each region and regional comparisons in both summer and winter. Principal component analyses (PCA) were performed using the *PtR* script (Trinity v2.8.4).

The original annotation (Meyer et al., 2015) of all identified DEGs was updated using BLAST+ v.2.5.0 (Camacho et al., 2009) search against UniProtKB and UniRef90 databases (UniProt release 2018_11; Boutet et al., 2007) with a cut-off e-value of 10^{-9}. Additional information was retrieved from the UniProt website (https://www.uniprot.org/) including Gene Ontology (GO) terms and KEGG references. The annotation of DEGs was further manually inspected.

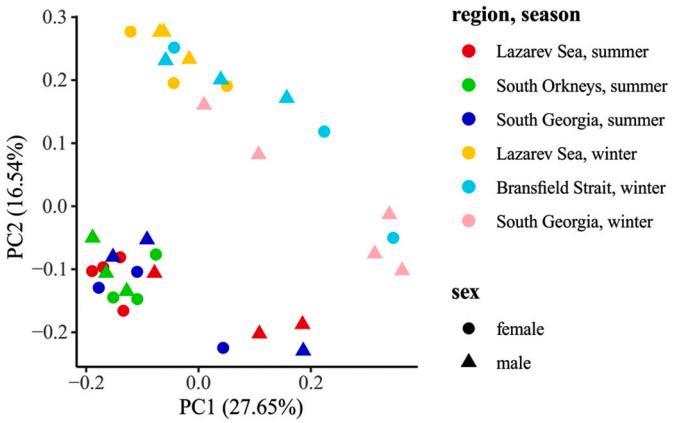

Fig. 1. Principal component analysis of normalized RNA-seq read counts (differentially expressed genes only). The colours indicate the sampling of each animal (region & sampling season), whereas the shape identifies the sex of each krill.

2.4. 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 the accession number E-MTAB-7467.

3. Results and discussion

This study investigated seasonal and regional differences in gene expression in Antarctic krill sampled in the Southern Ocean at three different latitudes (Lazarev Sea: 62°S-66°S, South Orkneys/Bransfield Strait: 60°S-63°S, and South Georgia: 54°S; Table 1).

Krill from the Lazarev Sea (LS) were sampled at the beginning of the spawning season and winter season, respectively. Despite surface water temperatures were quite similar between seasons (Table 1), the two sampling seasons were characterized by large differences in food availability in terms of chlorophyll concentration (Table 1) and photoperiod (Fig. S1). Samples from the Antarctic Peninsula (SO/BS) were sampled in the middle of the spawning season and in late winter, respectively. Bransfield Strait showed good food availability also at the time of winter sampling (Table 1). South Georgia (SG) is a year round ice-free region (Kawaguchi, 2016), where higher sea water temperatures (Table 1) and less extreme photoperiods (Fig. S1) lead to prolonged periods of phytoplankton blooms (Kawaguchi, 2016), ensuring good food supply throughout the year (Table 1). Summer and winter krill have been sampled in the middle of the spawning season and in mid-winter, respectively.

All animals showed similar maturity stages within seasons, independently of sampling region. In particular, females, that undergo a more complex sexual regression process compared to males, were in sub-adult stage in winter (IIC: thelycum half developed) and reached the fully maturity (IIIG: vitellogenesis) in summer.

Finally, all organisms have been caught at the same time of the day, in the second part of the night, at least 1 h before sunrise, to limit the number of differentially expressed genes under circadian control.

3.1. Seasonal comparisons of gene expression profiles in the three latitudinal regions

Seasonal differences between summer and winter were more pronounced than regional ones. Principal component analysis of all

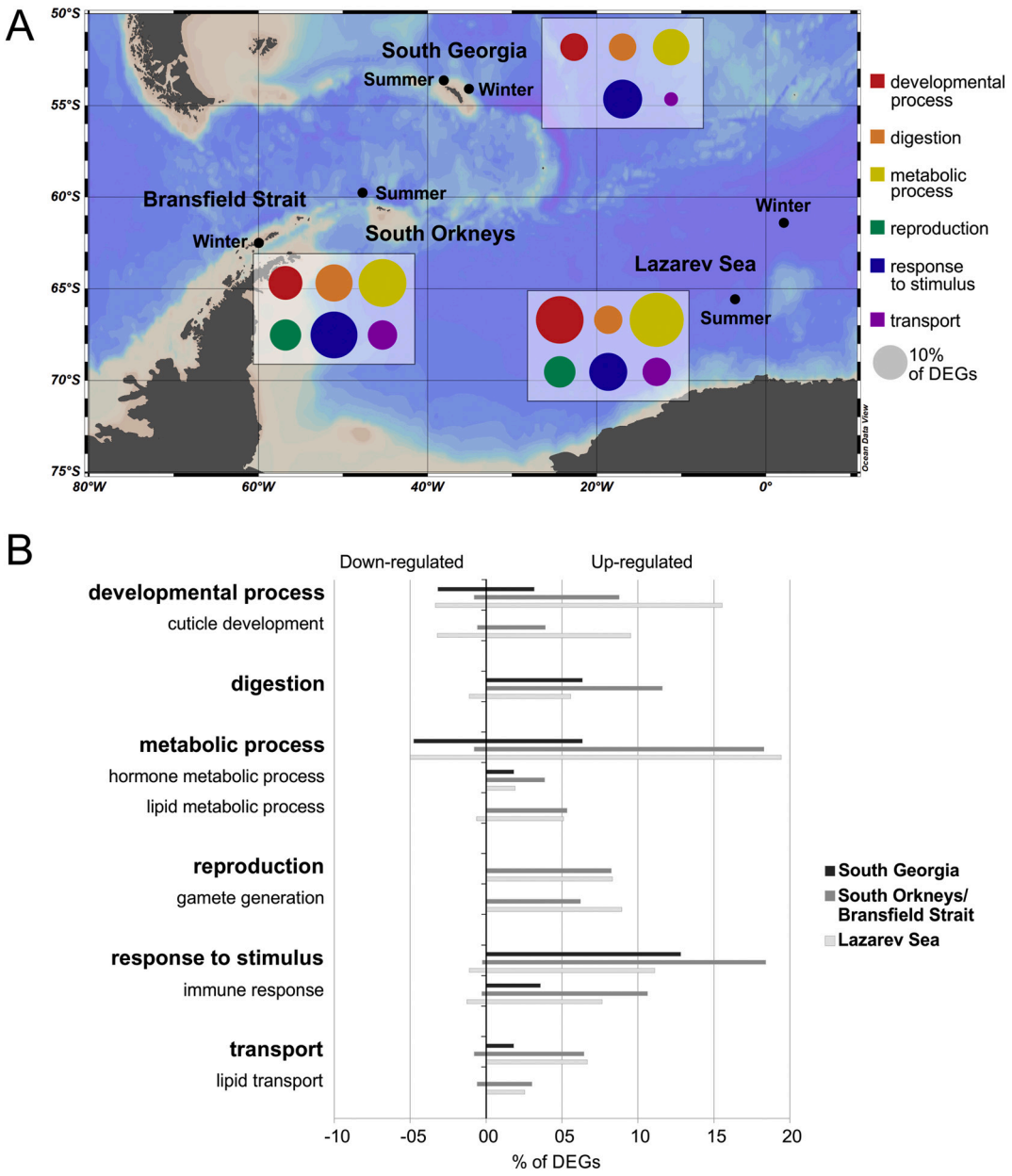

Fig. 2. Seasonal comparisons of gene expression signatures obtained at the three latitudinal regions. **A)** Map of the three sampling regions. Bubble charts show the functional enrichment analysis of differentially expressed genes found in the seasonal comparisons (summer vs. winter) at the three latitudinal regions. Six main GO terms, related to the biological processes discussed, are represented. Circle size represents the number of DEGs. **B)** Bar plots show all the GO terms discussed at the three different latitudinal regions. Up- and down-regulated genes are normalized on the total number of DEGs of each comparison. See Fig. S2 for the complete list of GO terms.

differentially expressed genes revealed that 44.2% of the total variance in the matrix of normalized read counts was mainly correlated to season (Fig. 1). No correlations were observed to latitudinal regions and krill sex, which indicated that these factors had minor effects on differential gene expression.

A total of 1654 differentially expressed genes (DEGs) were found in the seasonal pairwise comparisons performed independently in the three regions (Table 2). 1121 of these genes were found in the summer-winter comparison of krill caught in the South Orkneys/Bransfield Strait region; 698 genes were differentially expressed in krill sampled in the Lazarev Sea, and 295 genes off South Georgia. This pronounced difference in the number of seasonal DEGs between the three regions may reflect an adaptive strategy of Antarctic krill to environmental conditions in different habitats. The smaller number of DEGs found in krill sampled from South Georgia with respect to the other two regions (Table 2) may reflect the less extreme conditions in this low-latitude region, e.g. less extreme summer and winter photoperiod, greater food availability, absence of sea ice (Fig. S1; Table 1).

Most of DEGs (about 85%) were up-regulated in summer krill compared to winter krill. According to GO analysis (32% annotation; Table 2), development, digestion, metabolism, response to stimuli, and reproduction represent the most enriched biological processes (Fig. 2; see Fig. S2 for a complete list of GO terms and Table S1 and S2 for DEGs annotation). Similarly, Seear et al. (2012) found an up-regulation of genes involved in feeding, respiration, motor activity, immunity, and vitellogenesis in summer compared to winter krill caught in the Antarctic Peninsula region (60°S). Reduced metabolism, feeding, and reproductive activity, as well as development during winter might reflect the quiescent state of krill at this time of the year, when food availability is lowest, in order to maximize the chances of a successful

Table 2
Differentially expressed genes identified by pairwise comparisons between summer and winter (seasonal comparisons) in the three different regions (1–3) and regional comparisons of summer krill (4–6) and winter krill (7–9). The number of differentially expressed genes (in bold), and how many of them have been successfully annotated, are reported.

Seasonal comparisons				
			vs.	DEGs (annotated)
1	Lazarev Sea	Summer	Winter	**698** (228)
2	South Orkneys /Bransfield Strait	Summer	Winter	**1121** (495)
3	South Georgia	Summer	Winter	**295** (75)
Regional comparisons				
			vs.	DEGs (annotated)
4	Summer	Lazarev Sea	South Orkneys	**234** (92)
5		Lazarev Sea	South Georgia	**132** (59)
6		South Orkneys	South Georgia	**82** (23)
7	Winter	Lazarev Sea	Bransfield Strait	**73** (25)
8		Lazarev Sea	South Georgia	**174** (53)
9		Bransfield Strait	South Georgia	**106** (18)

overwintering.

In the comparison of seasonal gene expression profiles, only 106 differentially expressed genes were common to the three latitudinal regions (Fig. S3; only 12 annotated genes), suggesting three different seasonal adaptations according to latitudinal habitat. Furthermore, genes involved in cuticle development, lipid metabolism, lipid transport, and reproduction seemed to be differentially expressed between summer and winter only in krill sampled at the two high latitude sites (South Orkneys/Bransfield Strait, Lazarev Sea: 60–66°S), but not at the lower latitude region (South Georgia: 52°S; Fig. 2B). The seasonal regulation of these biological processes in krill from South Orkneys/Bransfield Strait and Lazarev Sea may suggest a more severe quiescent state in winter, characterized by a reduction in moulting and in processes related to the reproductive cycles. According to this hypothesis, winter krill from South Georgia showed higher feeding activities and lower lipid stores, whereas a delayed development coupled with a reduced average length has been observed in winter krill from the Lazarev Sea (Schmidt et al., 2014).

From an ecological point of view, the most unexpected result is represented by the lack of differentially expressed genes belonging to reproductive processes (Fig. 2B) in winter krill caught off South Georgia. This result contrasts with field observations that indicate an interruption of krill reproductive activity during winter in the Southern Ocean (Kawaguchi, 2016), but these observations were referred only to animals sampled at higher latitude south of South Georgia (Ross and Quetin, 2000). Moreover, stable food availability throughout the year, as observed during our sampling off South Georgia (Table 1), has been already associated with a reduced sexual regression and a faster re-maturation (Kawaguchi, 2016). From a technical point of view, the absence of females in the winter sample from South Georgia certainly reduced the number of DEGs involved in reproduction observed at this latitude but it did not explain the complete absence of DEGs involved in male reproductive physiology. So, the hypothesis of a more severe reduction in reproductive processes at higher latitudes cannot be excluded.

In order to identify which environmental factors have more influence on krill seasonal physiology, we analysed water temperature, food availability, and light regime at the different latitudes where krill were caught. The largest seasonal differences in water temperature were observed in South Georgia, where surface water temperature ranged from 0.8 °C in winter to 2.3 °C in summer (Table 1). However, this high seasonal temperature variability doesn't match with the small number of differentially expressed genes in that region (Table 2). In contrast, we found the largest seasonal differences in gene expression from krill sampled in the Lazarev Sea and Antarctic Peninsula (Table 2) where water temperature was more stable between summer and winter, ranging between −1.8 °C and − 0.05 °C (Table 1). Therefore, regional differences in seasonal water temperature are not able to explain the observed differences in gene expression profiles.

Annual feeding availability deeply affects the entire Antarctic ecosystem and krill behaviour (Schmidt et al., 2014). South Georgia is ice-free throughout the year with a rather stable food availability (Table 1). In this region, krill growth, development (Schmidt et al., 2014), and reproductive cycle (Kawaguchi, 2016) seem to be less affected during winter supporting our evidence that a low number of differentially expressed genes involved in "developmental processes" and "reproduction" was found (Fig. 2B). Less pronounced seasonal differences in gene expression in South Georgia might reflect a mild overwintering strategy likely due to a stable and secure energy supply throughout the year. Greater food availability at low latitudes possibly represents the main environmental factor determining favourable winter conditions.

At higher latitudes, feeding availability and reproductive timing are closely linked to sea ice extent (Spiridonov, 1995). Summer krill samples from the Lazarev Sea were caught in late December when sea ice was melting and when both the phytoplankton bloom and the spawning season had just begun (Meyer et al., 2010). Whereas krill from the South Orkneys/Bransfield Strait area were caught in ice-free regions in early February, the middle of the spawning season. However, these environmental and hence physiological differences in terms of spawning did not significantly affect the gene expression profiles of krill from the two high-latitude regions (Fig. 2B). The only exceptions were a higher proportion of differentially expressed genes involved in "developmental processes" and "response to stimulus" in specimens from the Lazarev Sea compare to South Orkneys/Bransfield Strait (Fig. 2B).

Day length (photoperiod) plays a major role in controlling behaviours and life cycle of Antarctic krill in different latitudinal regions of the Southern Ocean (Meyer et al., 2010; Meyer, 2011; Piccolin et al., 2018b). Höring et al. (2018) compared the seasonal effects of simulated light regimes at 52°S and 66°S in a two-year lab experiment. Different photoperiods affected seasonal cycles in growth, maturity, feeding, and lipid storage, suggesting particular overwintering strategies at different latitudes. Interestingly, seasonal cycles in lipid storage were more pronounced under the simulated 66°S light regime, supporting our results that found DEGs involved in "lipid transport" and "lipid metabolism" between summer and winter in high-latitude regions (Lazarev Sea, South Orkneys/Bransfield Strait; Fig. 2B). Moreover, the critical photoperiod, when 50% of the population shift to a full maturity stage, is higher under the high latitudinal light regime. In high latitude 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 (Höring et al., 2018). These findings might explain the lack of DEGs involved in "reproduction" in the low latitude region South Georgia between summer and winter. Interestingly, seasonal patterns of growth, feeding and maturity were also observed under constant darkness indicating the presence of an endogenous timing system, most likely entrained by light regime (Höring et al., 2018).

These results suggest that reduced food availability and extreme photoperiods likely represent the main environmental cues that influence krill's seasonal gene expression profiles and its physiology, leading to more severe overwintering strategies at high latitudinal regions.

Table 3
Set of 35 biologically relevant DEGs involved in seasonal physiology and behaviour. The ratio of expression levels between summer and winter in the three regional comparisons (LS: Lazarev Sea; SO/BS: South Orkneys/Bransfield Strait; SG: South Georgia) are reported as logFC.

Gene	LS	SO/BS	SG	GO Term	Function	Biological relevance	Reference
Peritrophin	6.0	6.6		Cuticle development	Chitin binding	Involved in keeping cuticle integrity in *T. castaneum*	Jasrapuria et al., 2010
Laccase	3.6	5.6			Cuticle tanning	May be involved in immune response in *T. castaneum*	Arakane et al., 2005
Chitin synthase		2.9			Chitin synthase activity	Gene expression under circadian control in krill	Nagasawa, 2012; Biscontin et al.,2019
chitinase 2		8.5			Chitin catabolic process	Differentially expressed during the moult cycle of *Euphausia superba*	Seear et al., 2010
Chitooligosaccharidolytic beta-N-acetylglucosaminidase	−3.6						
Calcification-associated peptide	9.7				Structural constituent of cuticle		
Carbohydrate sulfotransferase 11	2.3	2.9		Developmental growth	Regulation of cell population proliferation	Linked to the Wnt signalling pathway affecting cell proliferation in mammals	Nadanaka et al., 2008
Blastula protease 10	3.7	4.2		Multicellular organism development	Differentiation of ectodermal lineages	Involved in sea urchin embryogenesis	Lepage et al., 1992
Krueppel homolog 1			−6.2		DNA-binding transcription factor activity	Involved in *Drosophila* metamorphosis regulation and larval development	Truman, 2019
Aldehyde dehydrogenase family 8	3.2			Hormone metabolic process	Retinoic acid metabolic process	Regulation of development and reproduction in insects	Laufer and Biggers, 2001
All-trans-retinol 13,14-reductase	3.0	3.8					
Retinoid-inducible serine carboxypeptidase		4.1					
Dehydrogenase/reductase SDR family member 11		4.0			Steroid biosynthetic process	Regulation of moulting and reproduction in aquatic invertebrates	Lafont and Mathieu, 2007
Inactive hydroxysteroid dehydrogenase-like protein 1		2.8					
Lathosterol oxidase		2.6					
Short-chain dehydrogenase/reductase family 42E member 1		7.8					
MOXD1 homolog 1	5.3		8.2		Octopamine biosynthetic process	Octopamine affects heart beat and behaviour in lobsters	Kravitz, 1988
Neprilysin 1		3.9			Metalloendopeptidase activity	Was found to inactivate the circadian neurotransmitter pigment dispersing factor in *Drosophila*	Isaac et al., 2007
Type I iodothyronine deiodinase		3.2			Thyroid hormone metabolism	Associated with the seasonal timing of reproduction in European hamster	Sáenz de Miera et al., 2014
Furin-like protease 2		3.2			Regulation of glucose metabolic process	Involved in notch signalling in *Drosophila*	Kidd and Lieber, 2002
Epoxide hydrolase		4.0		Lipid metabolic process	Hydrolase activity	Catalyses juvenile hormone hydrolysis in *Drosophila*	Harshman et al., 1991
Fatty acid synthase 2	3.9	4.0			Fatty acid biosynthetic process	Contributes to diapause preparation in insects	Tan et al., 2017
Acetyl-CoA acetyltransferase		2.4			Fatty acid beta-oxidation	Involved in dopamine metabolism and cuticle tanning in insects and crustaceans	Horst and Freeman, 1993
Enoyl-CoA isomerase	4.5	2.3				Gene expression under circadian control in krill	Biscontin et al., 2019
Long-chain-fatty-acid–CoA ligase	2.2					Knockdown leads to decreased fat body and reduced reproductive capacity in insects	Alves-Bezerra et al., 2016
Vitellogenin	13.0	10.8		Gamete generation	Nutrient reservoir activity	Steroidal hormones have been implicated in the stimulation of vitellogenesis	Subramoniam, 2011
Hematopoietic prostaglandin D synthase	3.7	4.9			Prostaglandin metabolism	Involved in the control of oogenesis, spermatogenesis, and defence in marine invertebrates	Di Costanzo et al., 2019
15-hydroxyprostaglandin dehydrogenase [NAD(+)]	3.2	4.3		Mating		prostaglandin inactivation	
Carboxylic ester hydrolase	3.2	3.7			Regulation of female receptivity, post-mating	Esterase is associated with male reproductive fitness	Gilbert and Richmond, 1982
CREB-binding protein	3.5			Regulation of gene expression	Rhythmic process	Transcriptional coactivator affecting circadian behavioural activity in *Drosophila*	Maurer et al., 2016
	2.8			Protein dephosphorylation		Involved in ovarian maturation, visual perception,	

(*continued on next page*)

Table 3 (*continued*)

Gene	LS	SO/BS	SG	GO Term	Function	Biological relevance	Reference
Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform (PP2A)						and circadian timing in *Drosophila*	Zhao et al., 2017; Wang et al., 2008; Pegoraro and Tauber, 2011
Cryptochrome 1				Light perception		Light-dependent regulator of the circadian feedback loop	Biscontin et al., 2017
Arrestin	−3.7				Visual transduction	Gene expression under circadian control in krill	Biscontin et al., 2019
Adiponectin receptor protein		3.9		Receptor transactivation	Positive regulation of insulin secretion	Insulin signalling has been related to reproductive diapause in *Drosophila*	Sim and Denlinger, 2013
Leucine-rich repeat-containing G-protein coupled receptor 4		3.9			Positive regulation of Wnt signalling pathway	involved in the regulation of circadian rhythms of plasma lipids in mammals	Wang et al., 2014

3.2. Regional comparisons of gene expression profiles within each season

Very few DEGs were found in the regional pairwise comparisons in summer (from 82 to 234 DEGs) and winter (from 73 to 174 DEGs) (Tables 2) and Table S3. The small number of DEGs did not allow a statistically reliable GO analysis. Nevertheless, among the annotated regional DEGs we found the circadian clock component *cryptochrome 1* and several genes involved in phototransduction (including *arrestin*, *beta-crystallin A*, and *gamma-crystallin A*). *Cryptocrome 1* is a blue-light photoreceptor likely responsible for the light-mediated synchronization between the endogenous molecular clock and the photoperiod (Biscontin et al., 2017). The up-regulation of genes involved in phototransduction in the two high-latitude regions (Lazarev Sea, South Orkneys/Bransfield Strait) during summer supports our hypothesis about a pivotal role of photoperiod in the entrainment of the seasonal timing system of krill at high latitudes.

3.3. Putative genes involved in seasonal adaptation

Among seasonal and regional DEGs, we selected a set of 35 biologically relevant genes that have been previously described in literature to be associated with seasonal physiology and behaviours (Table 3). These genes could provide the basis for future studies to elucidate the molecular mechanisms of seasonal adaptation in Antarctic krill under different environmental factors (e.g. light regime; food supply). The strong seasonality of these selection of genes has been shown by principal component analysis performed only on their normalized read counts that is still able to separate our samples by season (Fig. S4).

Seasonal genes that are involved in development, lipid and hormone metabolic process, reproduction, visual perception, and circadian rhythms could be usefully studied. Genes involved in development and cuticle synthesis (including *chitin synthase*, *calcification-associated peptide*, and *carbohydrate sulfotransferase 11*) could be used as molecular markers to identify krill's active growth phases throughout the year. A reduction in moulting activity and reproductive processes as well as a finely orchestrated management of lipid storage participate in krill's overwintering strategy. Since these biological processes can be modulated to adapt krill to Antarctic winter (Schmidt et al., 2014); genes involved in cuticle development, reproduction (such as *vitellogenin* and *hematopoietic prostaglandin D synthase*), and lipid metabolism (including *fatty acid synthase 2* and *enoyl-CoA isomerase*) could be used to investigate the flexibility of krill's overwintering strategies (Meyer, 2011), under different environmental conditions. Several genes involved in the metabolism of different hormones including steroid (such as *lathosterol oxidase*), thyroxine (*iodothyronine deiodinase*), and retinoic acid (*all-trans-retinol 13,14-reductase*) were also found among seasonal DEGs. Interestingly, ecdysteroids, vertebrate-like steroids, thyroxine, and retinoic acid regulate several seasonal biological processes including moulting, seasonal timing of reproduction, and development in arthropods (Laufer and Biggers, 2001; Lafont and Mathieu, 2007). Since clock genes were found to affect photoperiodic diapause in insects (Ikeno et al., 2010), a potential seasonal role has also been suggested for Antarctic krill (Piccolin et al., 2018b). Genes involved in the entrainment of the circadian clock (*cryptochrome 1*, *pp2a*, *arrestin*) and in downstream pathways (*CREB-binding protein*) could provide important information about seasonal rhythms especially at high latitudes.

4. Conclusion

This study examined seasonal differences in Antarctic krill's gene expression profiles sampled from three latitudinal regions of the Southern Ocean (South Orkneys/Bransfield Strait, Lazarev Sea: 60–66°S, and South Georgia: 52°S), with emphasis on the genes involved in the seasonal cycle of krill. Most of the DEGs were found to be down regulated in winter showing that krill enter a physiological quiescent state at this time of the year. However, seasonal differences in gene expression profiles seemed to be less pronounced in Antarctic krill sampled from the South Georgia region, due to less extreme light conditions, milder winters with no sea ice coverage and hence more favourable food availability during winter. After the analysis of environmental factors particular to the three latitudinal regions (seawater temperature, sea ice conditions, food availability, photoperiod), we propose that food availability and photoperiod represent the main environmental cues causing seasonal physiological shifts in krill. Seasonal gene expression signatures may be partly affected by a photoperiodic timing system that may modulate behaviour and physiology of Antarctic krill in different latitudinal regions of the Southern Ocean. These findings provide a basis for future investigations about the molecular mechanisms of seasonal rhythms in Antarctic krill.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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