

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

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Abstract: Light regime is an important zeitgeber for Antarctic krill (*Euphausia superba* Dana, 1850), 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.

Key words: *Euphausia superba*, Antarctic krill, latitudinal light regime, endogenous timing system, critical photoperiod, reproduction.

Résumé : Le régime lumineux est un facteur environnemental important pour le krill de l'Antarctique (*Euphausia superba* Dana, 1850), qui semble permettre l'entraînement d'un système temporel endogène permettant la synchronisation de son cycle de vie aux conditions lumineuses extrêmes de l'océan Austral. Pour comprendre la souplesse du cycle saisonnier du krill antarctique, nous avons examiné ses réactions physiologique et comportementale à différents régimes lumineux et vérifié si son système temporel endogène intervenait dans la régulation de ces processus saisonniers. Nous avons analysé la croissance, l'alimentation, le contenu lipidique et la maturité dans le cadre d'une expérience en laboratoire sur deux ans qui simulait les régimes lumineux latitudinaux à 52°S et 66°S et en obscurité constante, dans des conditions d'apport de nourriture constant. Nos résultats montrent que le régime lumineux a une incidence sur les cycles saisonniers de croissance, d'alimentation, de métabolisme des lipides et de maturité chez le krill antarctique. Les motifs saisonniers de croissance, d'alimentation et de maturité persistent en obscurité constante, ce qui indique la présence d'un système temporel endogène. Le cycle de maturité présente des différences sur le plan des photopériodes critiques selon le régime lumineux latitudinal simulé. Cela indiquerait un mécanisme temporel endogène souple chez le krill antarctique qui pourrait déterminer sa réaction à des changements environnementaux futurs. [Traduit par la Rédaction]

Mots-clés : *Euphausia superba*, krill antarctique, régime lumineux latitudinal, système temporel endogène, photopériode critique, reproduction.

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 adap-

tive potential of polar key organisms such as the Antarctic krill (*Euphausia superba* Dana, 1850) (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

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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.

Materials and methods

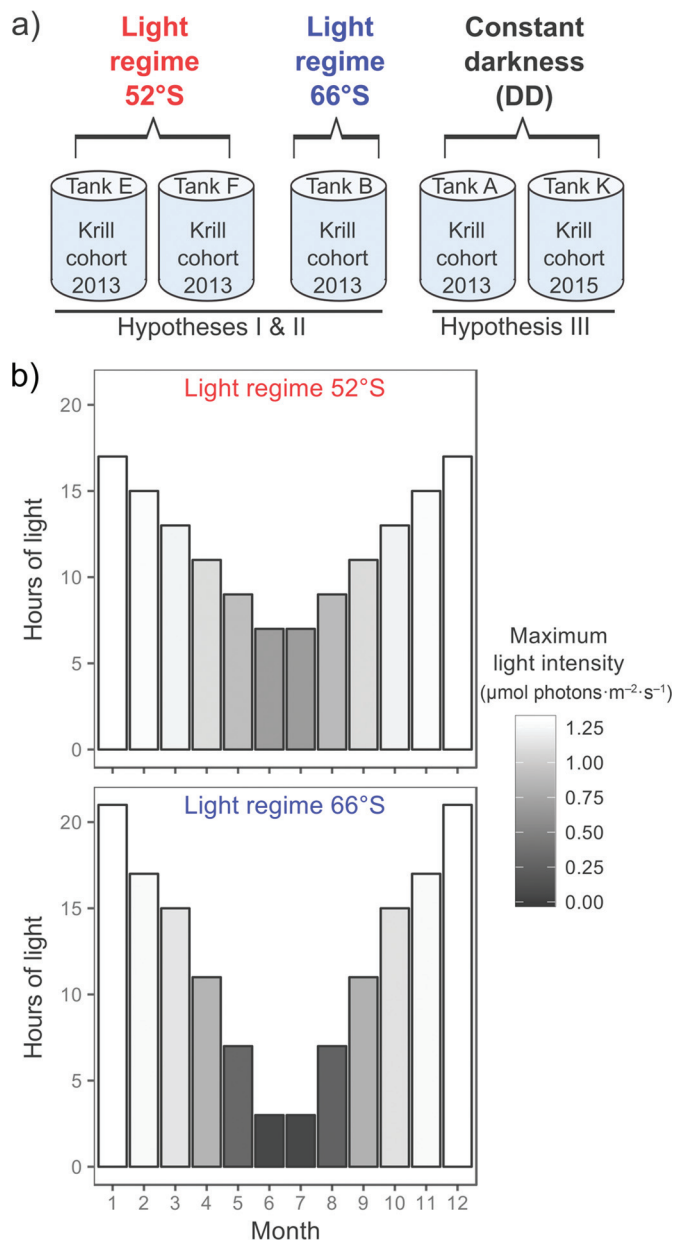
Antarctic krill collection and maintenance prior to the experiments

Antarctic krill were caught with a rectangular mid-water trawl (RMT 8) on 12 February 2013 (66°47'S, 65°08'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).

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. 1a, 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).

Fig. 1. Long-term laboratory experiments at the Australian Antarctic Division: (a) experimental set-up and tested hypotheses; (b) simulated light regimes at latitudes 52°S and 66°S. Colour version online.



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⁻²·s⁻¹) 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

Table 1. Sampling scheme of the long-term experiment.

Treatment	Tank	Month							
		2–6	7–13	14–17	18–19	20–21	22	23	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

Note: Given numbers represent sampled individuals per month ($n = 617$). Carapace length, digestive gland length, and maturity score from these Antarctic krill (*Euphausia superba*) were used for the analysis of growth, feeding, and maturity in this study. For lipid-content analysis, a reduced data set was analysed.

*In April 2015 (month 4), July 2015 (month 7), October 2015 (month 10), January 2016 (month 13), April 2016 (month 16), and July 2016 (month 19), lipid content of six Antarctic krill per month was analysed.

†In July 2015 (month 7), one additional Antarctic krill was sampled.

simulated light-intensity profiles for each treatment and month can be found in Supplementary Table S1.¹

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 0830 and 0930 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 tricoratum* 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 1200™”, 5.1×10^4 cells·mL⁻¹ of *Isochrysis* Parke, 1949 “Iso 1800™”, 4.8×10^4 cells·mL⁻¹ of *Pavlova* Butcher, 1952 “Pavlova 1800™”; Reed Mariculture, USA), and two types of prawn hatchery feeds (0.5 g of FRIPPAK FRESH #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 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.

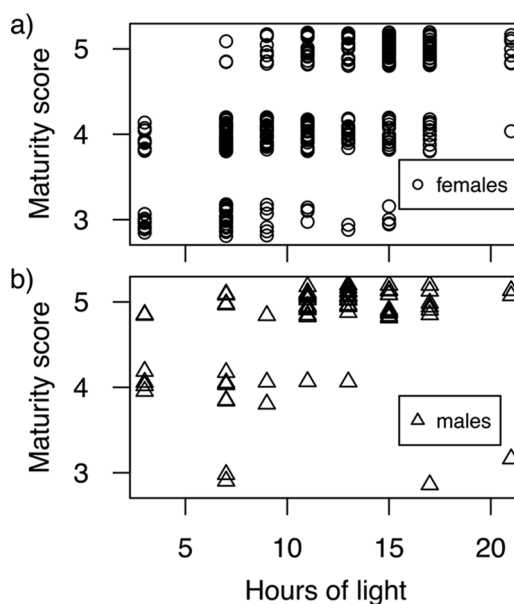
Live Antarctic krill was inspected under a stereomicroscope and the sex was determined. Pictures of the carapace and the sexual organs (female thelycum and male petasma) were taken with a Leica DFC 400 camera system (Leica Microsystems, Germany). Carapace length (tip of the rostrum to posterior notch) 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 °C.

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

Growth analysis

Carapace length was used as a proxy for growth in the experiments. Antarctic krill were sampled randomly from each experi-

Fig. 2. Relationship between maturity score and hours of light in (a) female and (b) male Antarctic krill (*Euphausia superba*).

mental 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 2006). 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

¹Supplementary table is available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2017-0353>.

Table 2. Model results showing model statistics for parametric coefficients (estimates, standard errors (SE), z or t values, and p values), a measure of explained variance of the model (deviance or adjusted R^2), and nonparametric terms where applicable (estimated degrees of freedom (edf), F statistic, and p values).

Model M1 ^a	Estimate	SE	t	p	Adjusted R^2	
Intercept	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	32.8	1.95	20.65	0.15	20.31	2.46
p	<0.001	0.003	<0.001	0.13	<0.001	<0.001
Model M2 ^b	Estimate	SE	t	p	Adjusted R^2	
Intercept	42.08	0.22	189.8	<0.001	0.64	
	Treatment 52°S		Treatment 66°S		Treatment DD	
Variable	Time	Month	Time	Month	Time	Month
Smooth (edf)	2.87	5.09	2.55	1.47	3.13	2.79
F	41.42	4.2	92.84	0.39	64.52	1.4
p	<0.001	<0.001	<0.001	0.041	<0.001	<0.001
Model M3 ^c	Estimate	SE	t	p	Deviance (%)	
Intercept	17.21	0.77	22.33	<0.001	50.9	
	Treatment 52°S		Treatment 66°S		Treatment DD	
Variable	Time		Time		Time	
Smooth (edf)	3.58		3.82		1.0	
F	1.16		14.97		0.05	
p	0.3		<0.001		0.82	
Model M4 ^d	Estimate	SE	t	p	Adjusted R^2	
Intercept	1.43	0.01	134.9	<0.001	0.45	
	Treatment 52°S		Treatment 66°S		Treatment DD	
Variable	Time	Month	Time	Month	Time	Month
Smooth (edf)	1.0	4.14	1.88	4.07	3.33	2.88
F	6.1	16.0	5.84	7.7	4.58	1.19
p	0.014	<0.001	0.002	<0.001	0.04	<0.001
Model M5 ^e	Estimate	SE	z	p	AUC	
Intercept	-6.09	0.78	-7.86	<0.001	0.77	
Hours of light	0.49	0.06	7.82	<0.001		
Treatment	1.22	1.25	0.98	0.4		
Interaction: light × latitude	-0.16	0.09	-1.73	0.084		

Note: Treatment 52°S refers to simulated light regime at latitude 52°S; treatment 66°S refers to simulated light regime at latitude 66°S; treatment DD refers to constant darkness. Significant p values of explanatory variables are set in boldface type.

^aModel M1 is a generalized additive mixed model (GAMM) for carapace length of Antarctic krill (*Euphausia superba*) over time for each treatment with random effects for tank.

^bModel M2 is a GAMM for feeding index over time for each treatment and random effects for tank.

^cModel M3 is a generalized additive model (GAM) for lipid content of females over time for each treatment.

^dModel M4 is a negative binomial GAMM for female maturity over time for each treatment with random effects for tank and autoregressive correlation structure of the order 1.

^eModel M5 is a binomial generalized linear mixed model (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, which is the area under the curve from a receiver operating characteristic (ROC) curve analysis, serves as an indication of model fit.

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.

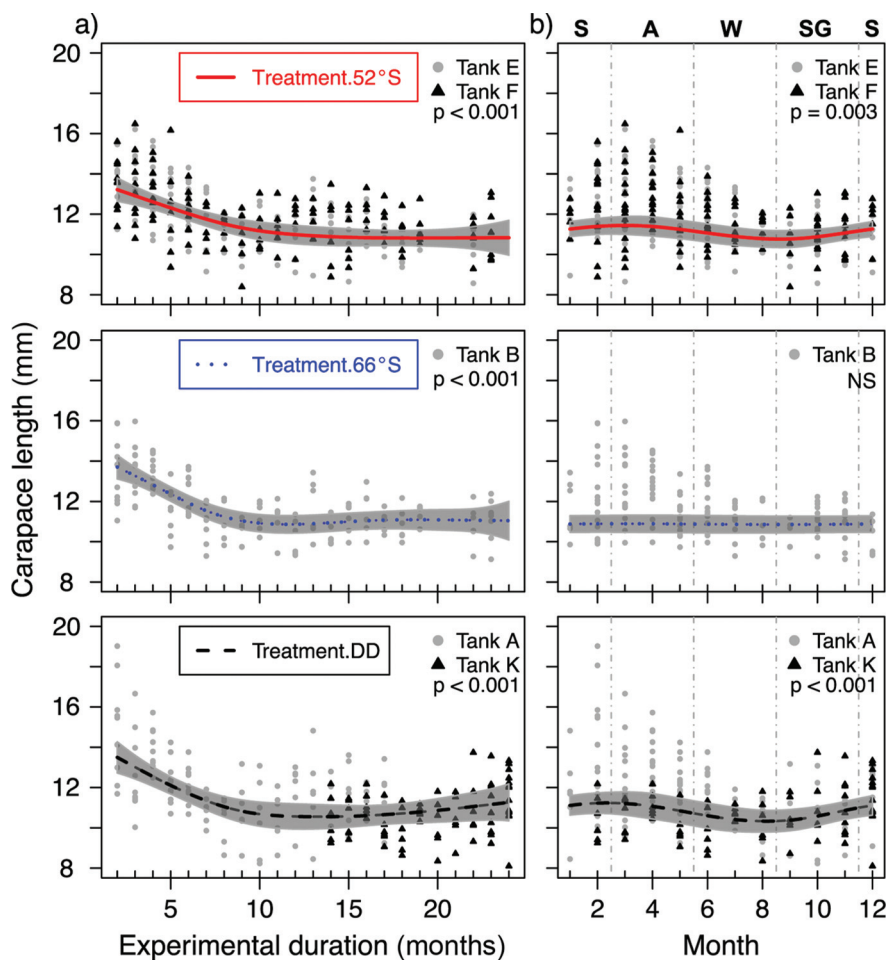
Smoothed terms were fitted as regression splines (variable “time”), apart for the variable “month”, which was modeled using cyclic cubic regression splines, setting knots manually between 1 (January) and 12 (December) to account for the circular nature of this term. Differences in temporal pattern between 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.

Feeding analysis

The feeding index (%) was calculated as digestive gland length × (carapace length)⁻¹ × 100. Data of males and females were com-

Fig. 3. Estimated smooth terms of the generalized additive mixed model for carapace length of Antarctic krill (*Euphausia superba*) within light regime treatments 52°S, 66°S, and constant darkness (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* values are also displayed; NS is not significant. In b, the seasonal periods (S, summer; A, autumn; W, winter; SG, spring) are indicated by vertical dash-dotted lines. Colour version online.



bined 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 GMM was employed as described above (section Growth analysis). The smooth function of the variable “time” was manually restricted to $k = 6$.

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.

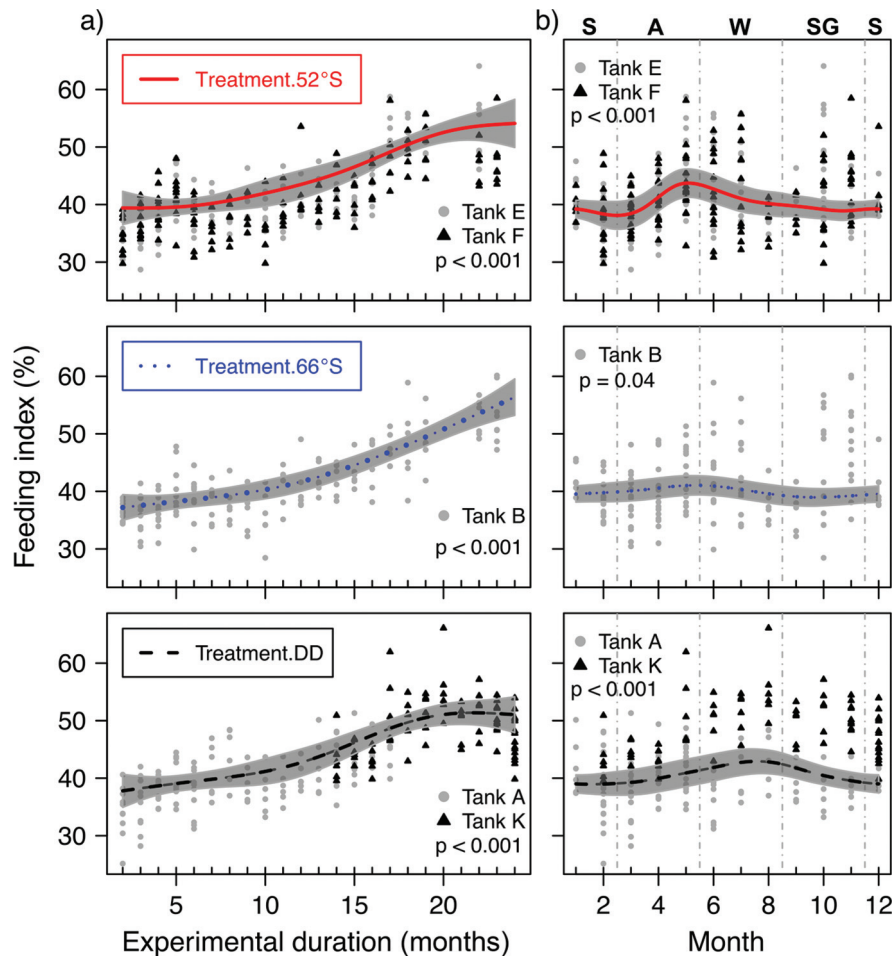
Lipid content differed between male and female Antarctic krill (Pearson's product moment correlation of pooled monthly mean values, $r = 0.26$, $p = 0.62$); therefore, statistical analysis was performed separately for each sex. Data for males were not sufficient for robust modelling and only females 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.

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 Ikeda (1987). A maturity score was assigned using the method of Brown et al. (2010, 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. 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

Fig. 4. Estimated smooth terms of the generalized additive mixed model for feeding index of Antarctic krill (*Euphausia superba*) within light regime treatments 52°S, 66°S, and constant darkness (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* values are also displayed. In b, the seasonal periods (S, summer; A, autumn; W, winter; SG, spring) are indicated by vertical dash-dotted lines. Colour version online.



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 autoregressive 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 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.

Data archiving

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

Results

Growth analysis

Carapace length ranged from 8.1 to 19.02 mm with a mean (\pm SD) of 11.71 mm (\pm 1.61 mm) across the whole data set. The GAMM (model M1; Table 2) revealed significant seasonal and interannual patterns in growth, which were similar across all treatments (Figs. 3a, 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).

Feeding

The feeding index data ranged from 25.15% to 66.09% with a mean (\pm SD) of 42.00% (\pm 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. 4a, 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.

Lipids

The lipid content data of males and females ranged from 2.53% to 57.75% with a mean (\pm SD) of 17.04% (\pm 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. 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 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.

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. 6a, 6b). Significant interannual patterns differed 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.

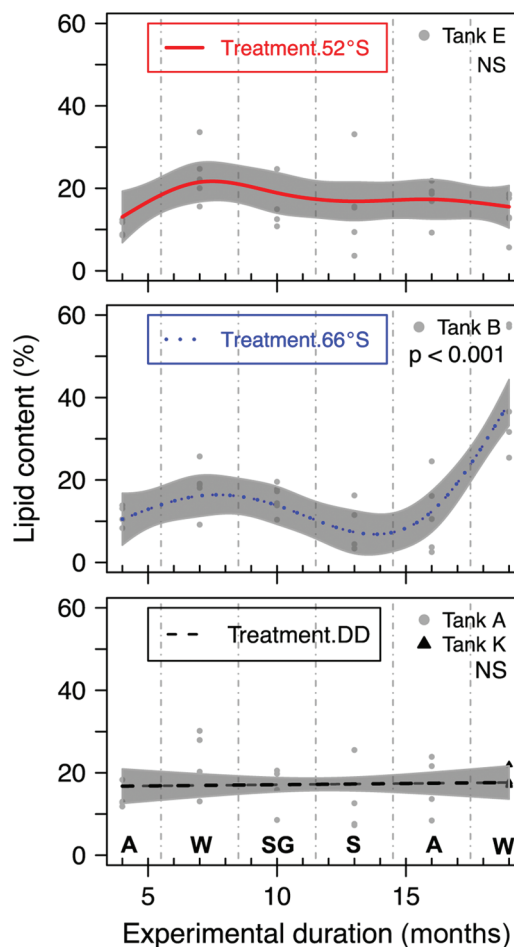
The binomial GLMM (model M5; Table 2) suggests that the variable “hours of light” significantly affects female maturity in treatments 52°S and 66°S. The interaction term between “hours of light” and “treatment” was marginally not significant. When investigating the critical photoperiod at the probability of 50%, differences between the treatments were found (Fig. 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.

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

Fig. 5. Estimated smooth terms of the generalized additive model for lipid content in female Antarctic krill (*Euphausia superba*) within light regime treatments 52°S, 66°S, and constant darkness (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 are also displayed; NS is not significant. The seasonal periods (A, autumn; W, winter; SG, spring; S, summer) are indicated by vertical dash-dotted lines. Colour version online.

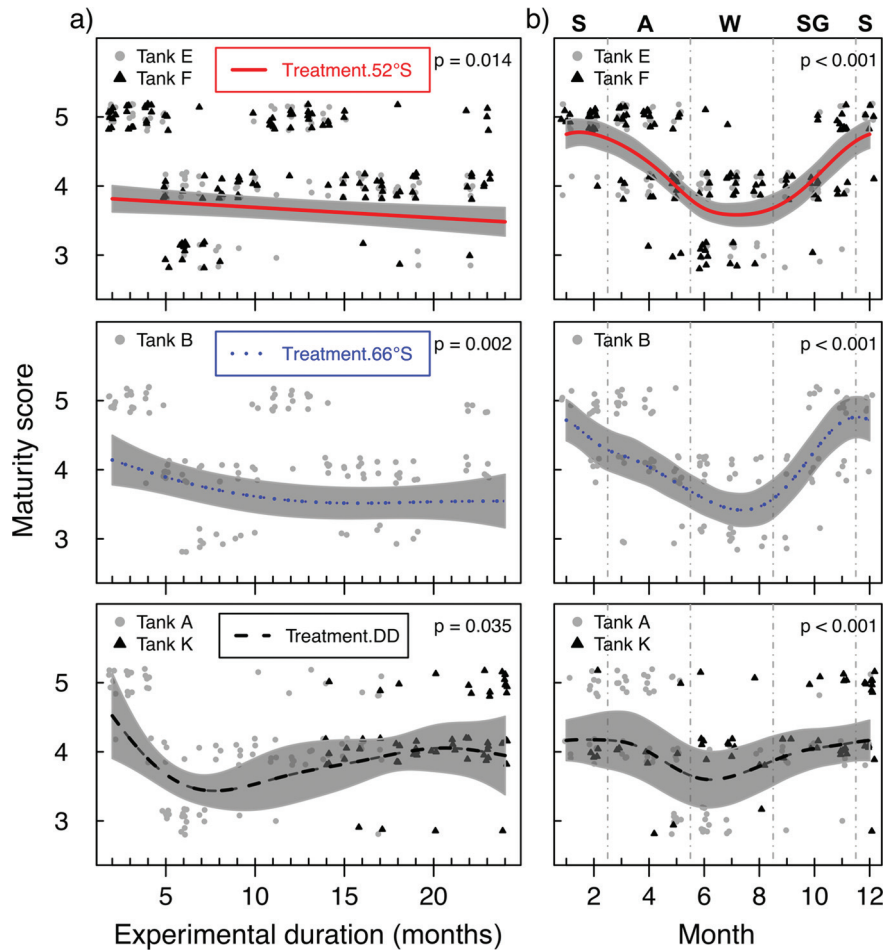


endogenously driven seasonal rhythms of growth, feeding, lipid metabolism, and maturity.

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 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. However, 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

Fig. 6. Estimated smooth terms of negative binomial generalized additive mixed model for female Antarctic krill (*Euphausia superba*) maturity within light regime treatments 52°S, 66°S, and constant darkness (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 values are also displayed. The seasonal periods (S, summer; A, autumn; W, winter; SG, spring) are indicated by vertical dash-dotted lines. Colour version online.



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.

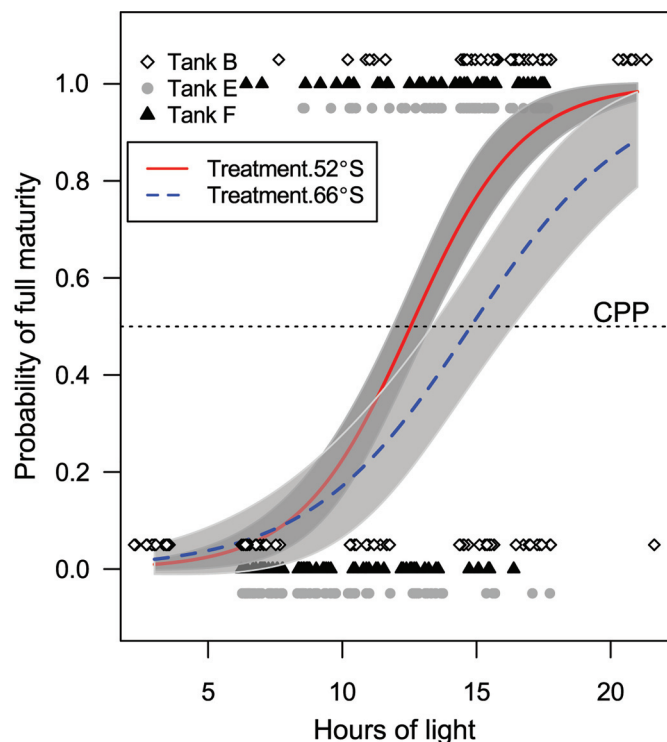
Moreover, we presume that the observations made in the first few months of the experiment represent a general period of acclimation to the experimental conditions. It may explain the strong shrinkage, suppressed lipid accumulation, and a general similarity of the data under all treatments in the beginning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

der 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 Ikeda 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

Fig. 7. Results from the logistic regressions for the latitudinal light regime treatments 52°S and 66°S. Estimated trends of the binomial generalized linear mixed model (lines) are shown with 95% confidence intervals (shading); jittered raw data points for experimental tanks (shapes) are also displayed. The horizontal dotted line indicates the 50% probability level for the critical photoperiod (CPP). Colour version online.



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 et al. 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 Ikeda 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 higher 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 higher 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 2011). 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 2018). Further research on the phenology and biological clock of Antarctic krill will help to better understand its adaptive potential to environmental changes.

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.

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