

Biological timing in Antarctic krill: Endogenous clocks and physiological rhythms at the daily and annual scale

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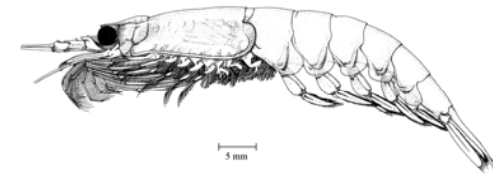


Photo: J. v. Franecker

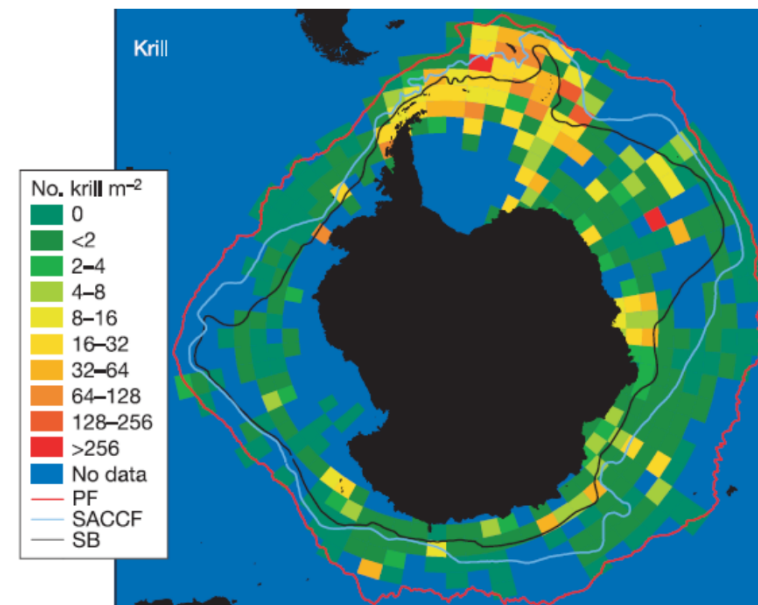
The Antarctic krill: *Euphausia superba*



- The most abundant of the world's euphausiids
- Plays a central role in the Southern Ocean ecosystem:



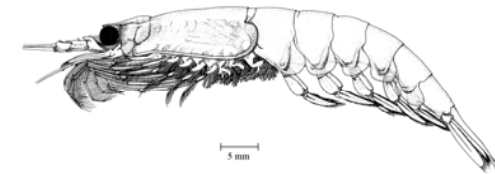
- Circumpolar distribution
- Highly abundant: **170-379 Mt**



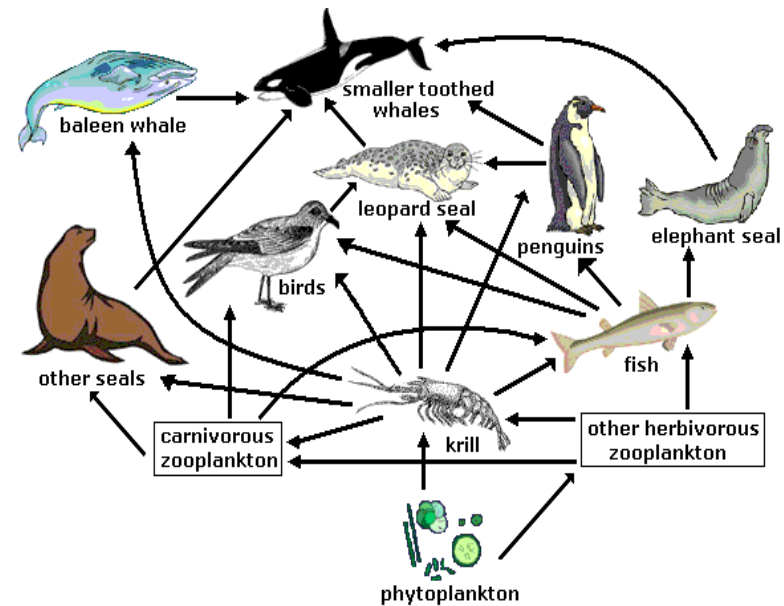
Atkinson et al. 2004

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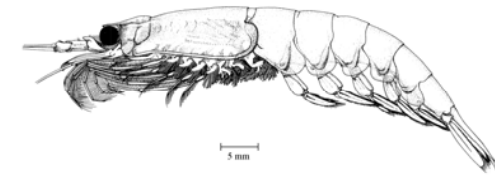
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- Important grazer & prey item
- Growing commercial interest

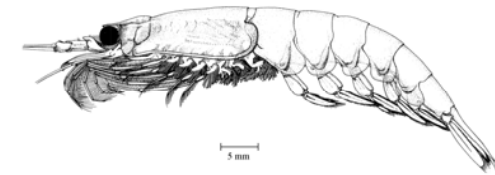


from www.supplementquanda.com

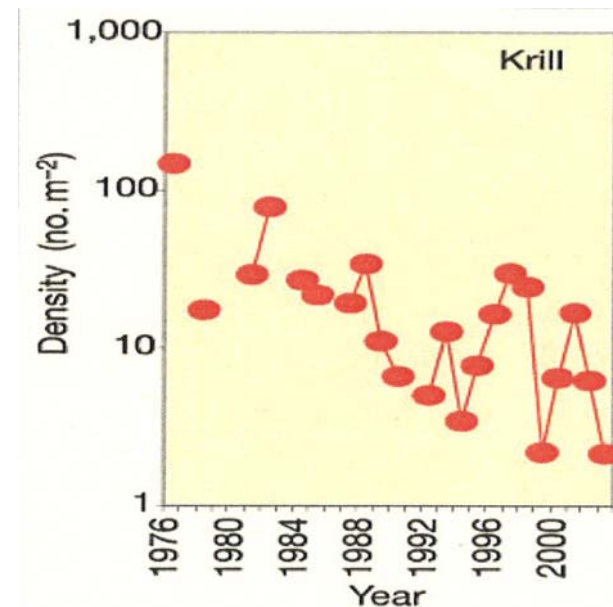
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- Circumpolar distribution
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- Important grazer & prey item
- Growing commercial interest
- Long-term decline in biomass

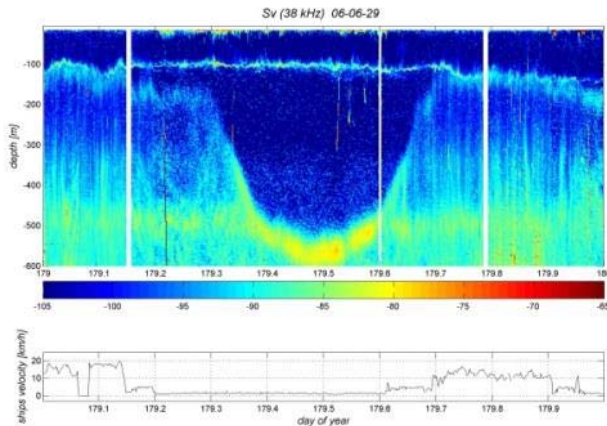


Atkinson et al. 2004

Biological rhythms of krill at the daily and annual scale

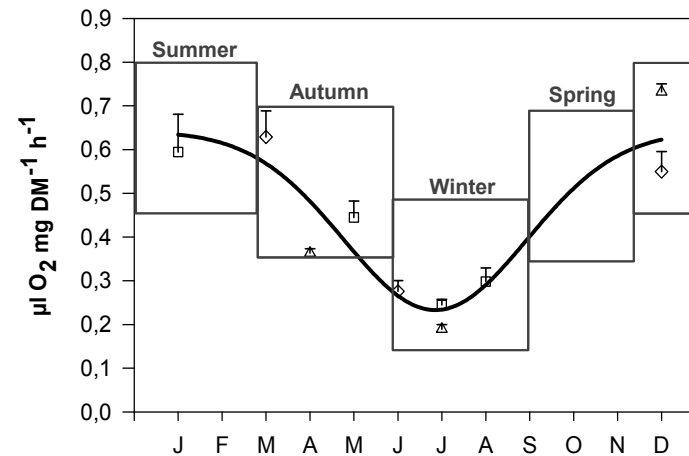


Daily behavioral functions



→ diel vertical migration (DVM)

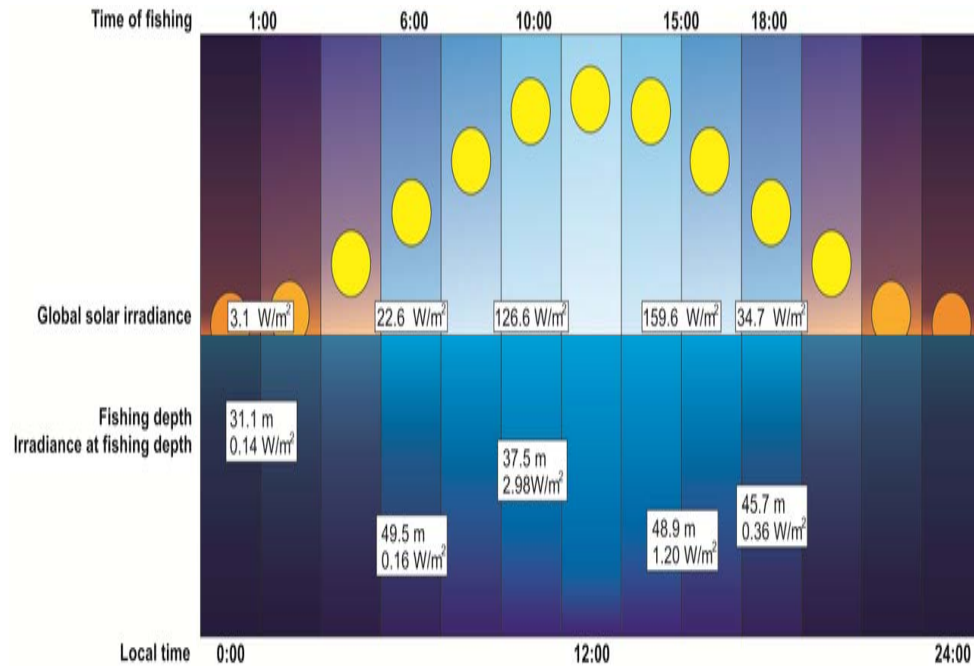
Annual physiological functions



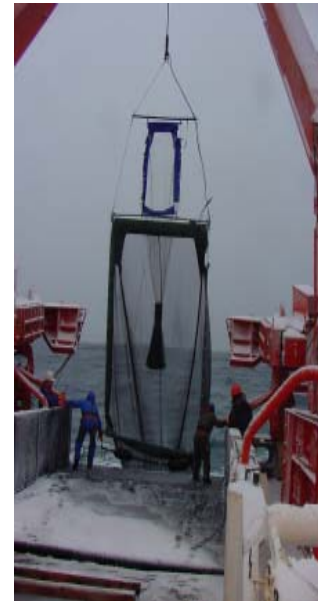
→ metabolic depression

➤ Are these processes in krill regulated / mediated by endogenous clocks?

Diurnal transcriptome characterisation in natural conditions



De Pittà et al. 2013

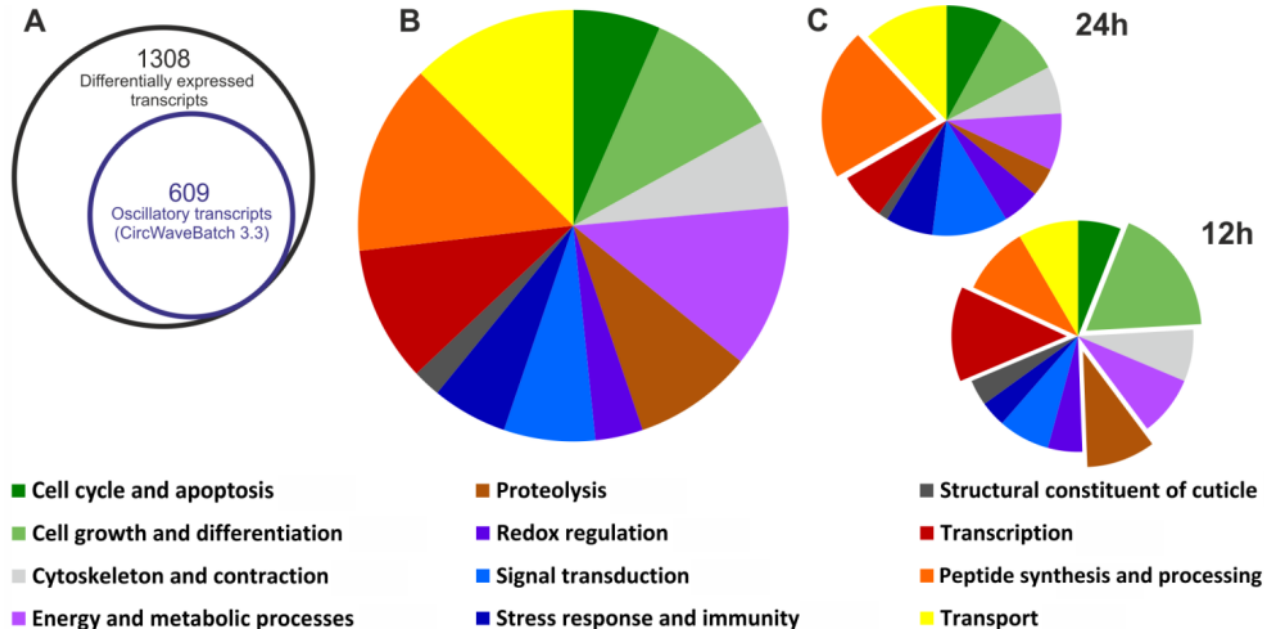


24-hour in-situ time series



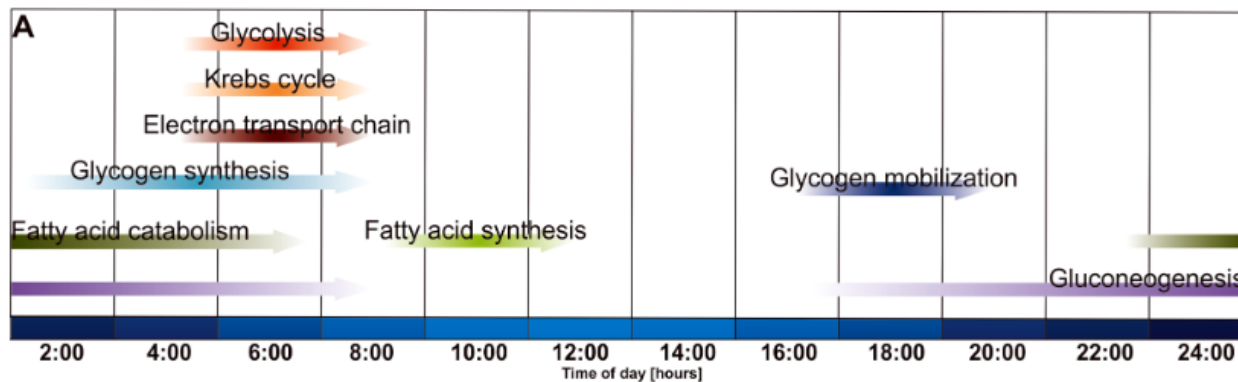
The „Krill 1.0“ microarray platform with a total of 32,217 probes was created to analyse gene expression signatures

Diurnal transcriptome characterisation in natural conditions



The „Krill 1.0“ microarray platform with a total of 32,217 probes was created to analyse gene expression signatures

Circadian cycles of transcription?



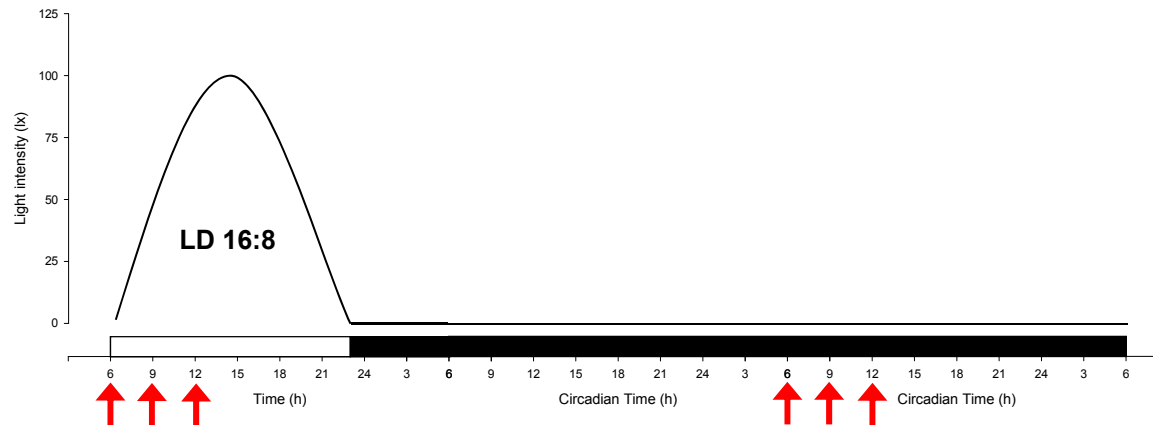
Circadian transcriptome characterisation under laboratory conditions



24-hour time series of LD/DD entrained krill



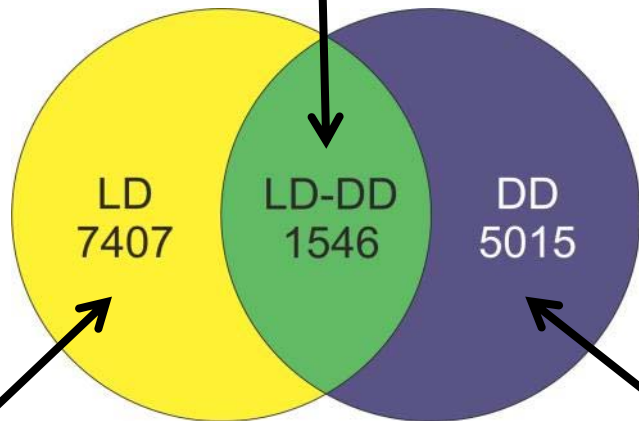
The „Krill 2.0“ microarray platform with a total of 57,358 probes was developed and used to define the circadian transcriptome of LD/DD entrained krill samples



Circadian transcriptome characterisation under laboratory conditions

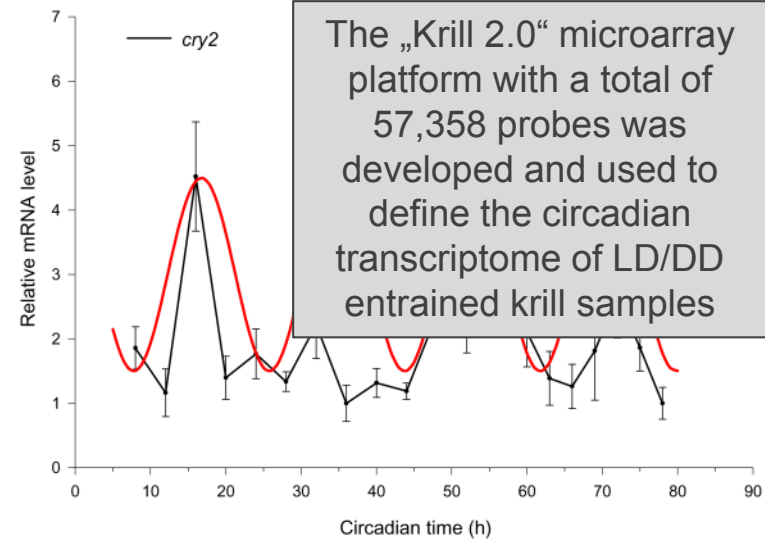


414 transcripts showed the same oscillatory period in LD and DD



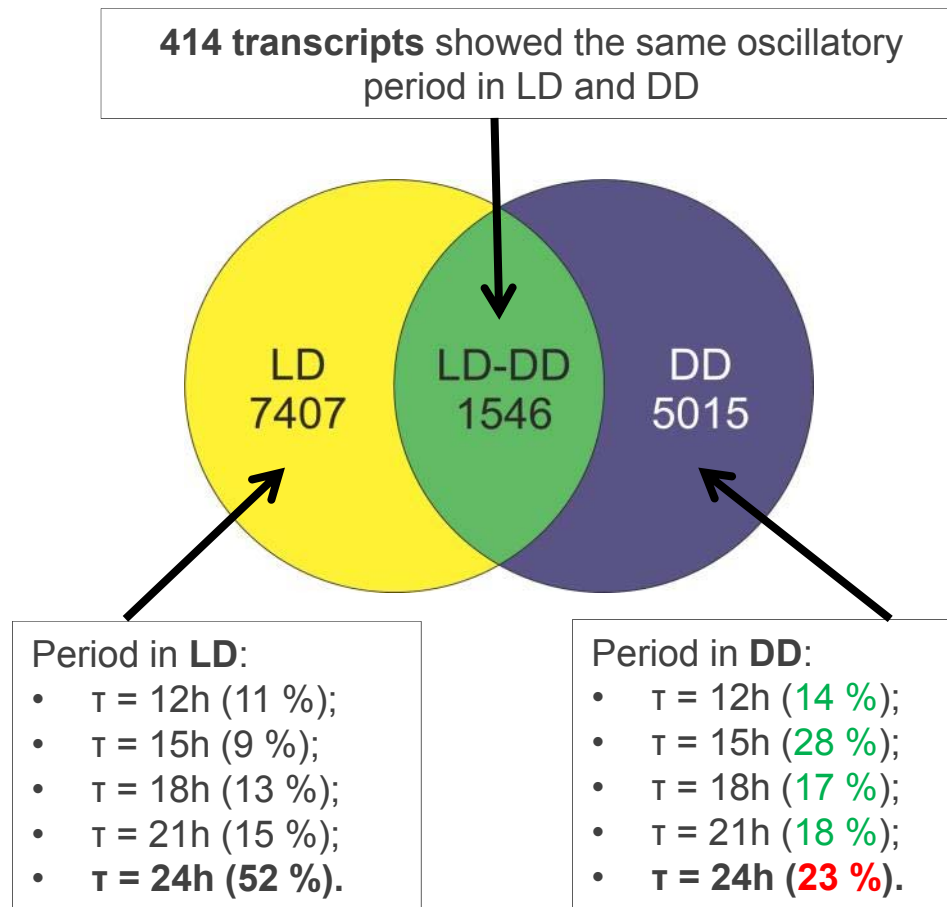
- Period in **LD**:
- $\tau = 12\text{h}$ (11 %);
 - $\tau = 15\text{h}$ (9 %);
 - $\tau = 18\text{h}$ (13 %);
 - $\tau = 21\text{h}$ (15 %);
 - $\tau = 24\text{h}$ (52 %).

- Period in **DD**:
- $\tau = 12\text{h}$ (14 %);
 - $\tau = 15\text{h}$ (28 %);
 - $\tau = 18\text{h}$ (17 %);
 - $\tau = 21\text{h}$ (18 %);
 - $\tau = 24\text{h}$ (23 %).



➤ *Euphausia superba* exhibits a surprisingly short circadian period of *cry2* expression levels: ~ 18 hours

Circadian transcriptome characterisation under laboratory conditions



Conclusions

- A **functional circadian clock** in krill controls a chronological progression of biochemical and physiological events throughout the 24-hour cycle
- **Circadian periods** of the majority of oscillating genes in DD are **deviating from 24 h**
- This might be a feature of a „**high latitude**“ circadian clock with a **wide entrainment range**

Putative architecture of the circadian clockwork in *Euphausia superba*



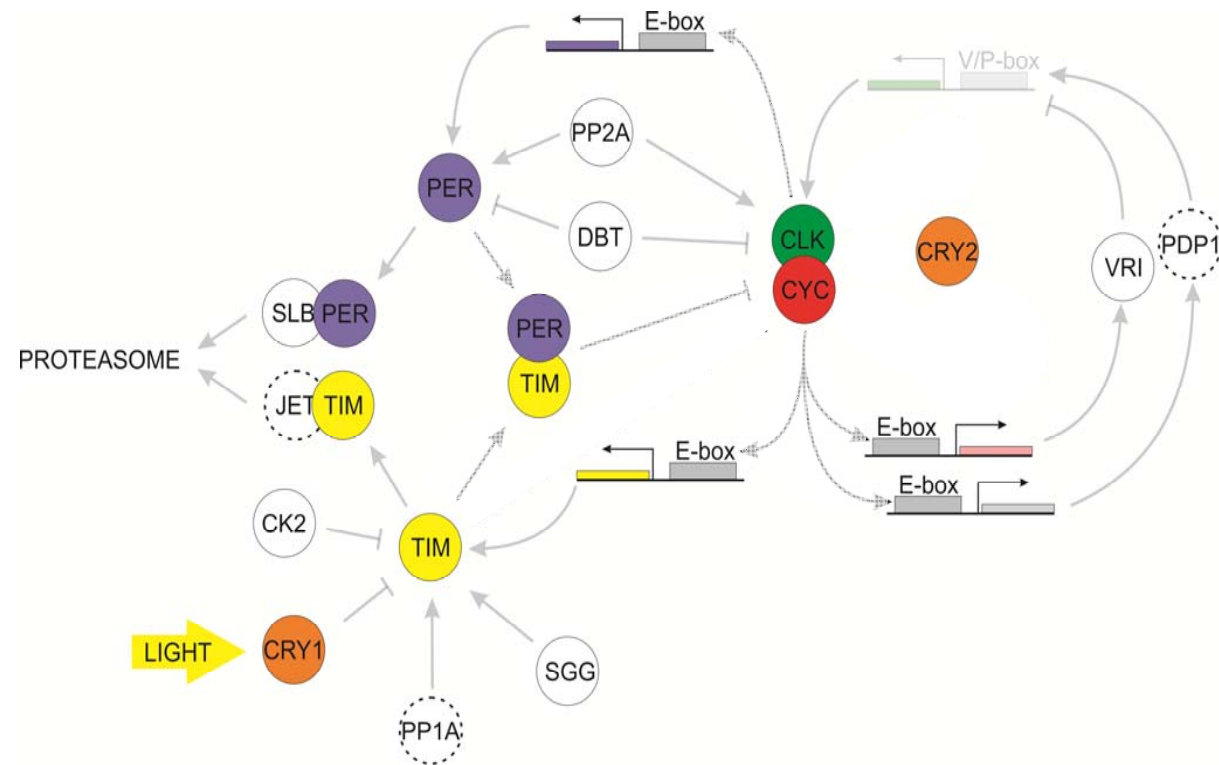
RNA sequencing of the krill transcriptome
 Meyer lab, AWI
 Costa lab, University of Padova

↓

Primer design for 21 putative *Es* clock genes based on available transcript information

Identification of 17 clock genes transcripts including *clock*, *cycle*, *per*, *tim*, *cry2*

Costa lab, University of Padova

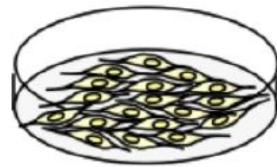


Putative architecture of the circadian clockwork in *Euphausia superba*



Principle: Co-transactivation assay

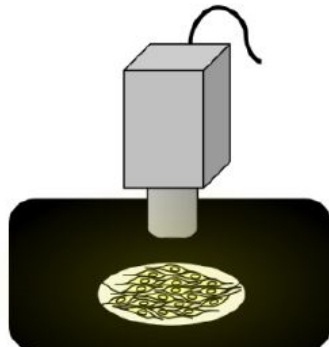
HEK293 cells



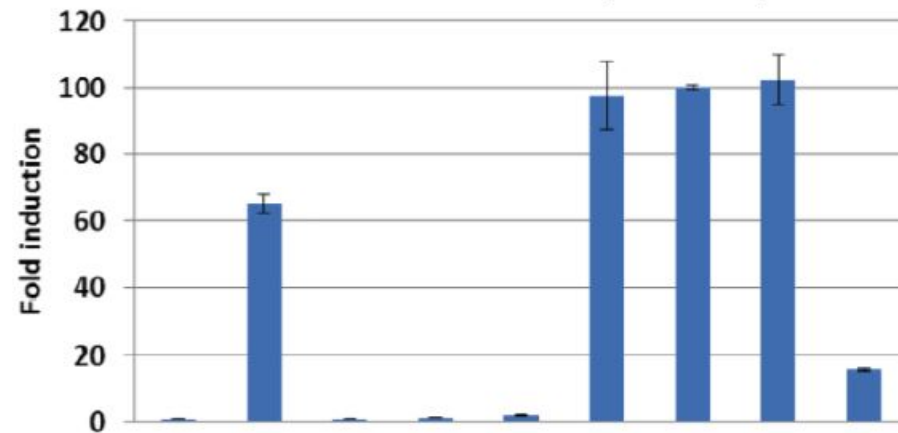
Transfection



Detection

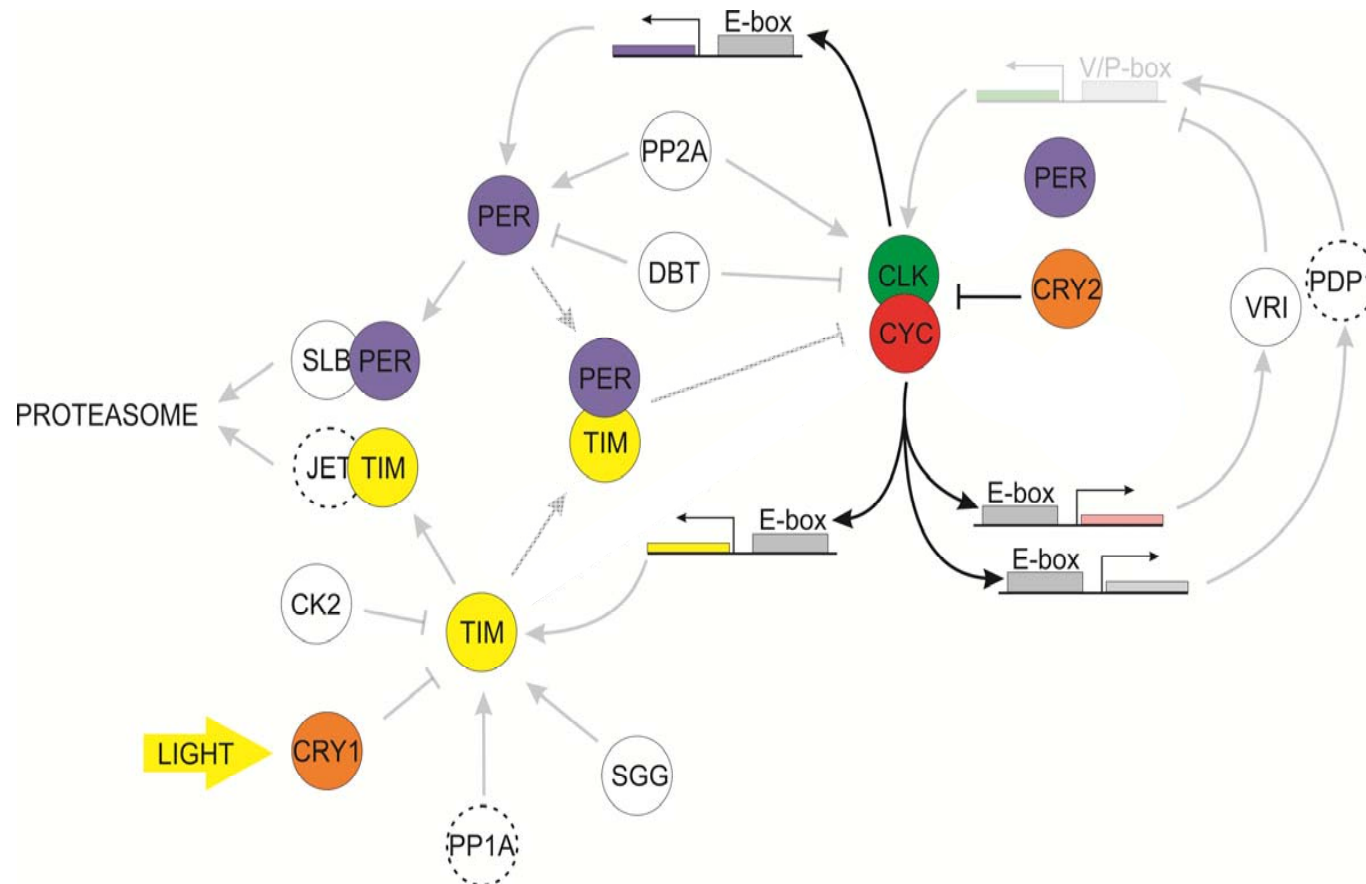


Mammalian cells (HEK293)

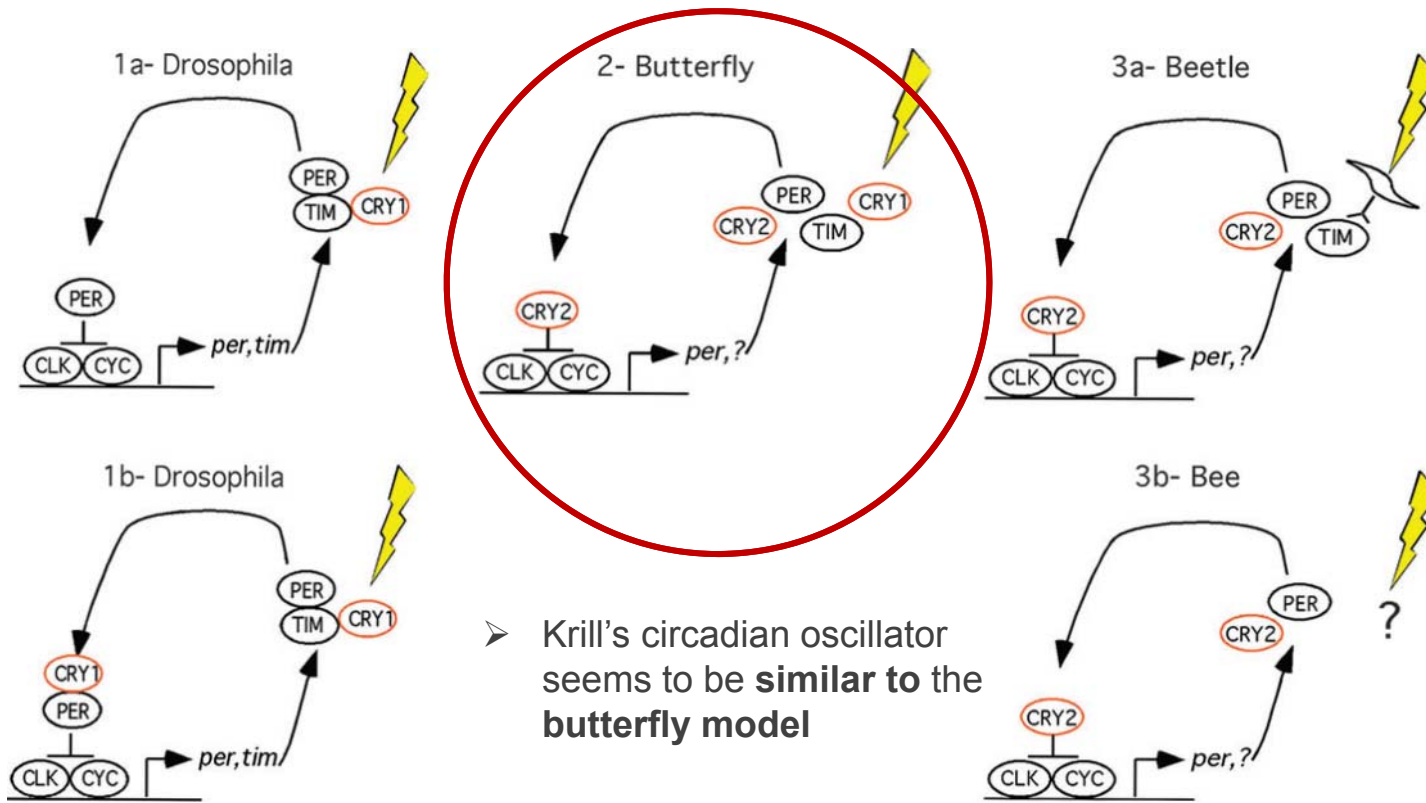


E-box-Luc	+	+	+	+	+	+	+	+	+
m_clock	-	+	+	-	-	-	-	-	-
m_bmal1	-	+	+	-	-	-	-	-	-
m_cry1	-	-	+	-	-	-	-	-	-
Es_clock	-	-	-	+	-	+	+	+	+
Es_cycle	-	-	-	-	+	+	+	+	+
Es_period	-	-	-	-	-	-	+	-	-
Es_cry1	-	-	-	-	-	-	-	+	-
Es_cry2	-	-	-	-	-	-	-	-	+

Putative architecture of the circadian clockwork in *Euphausia superba*



Putative architecture of the circadian clockwork in *Euphausia superba*



modified after Reppert 2015

Future work will focus on the seasonal plasticity of the circadian clockwork in *Euphausia superba*

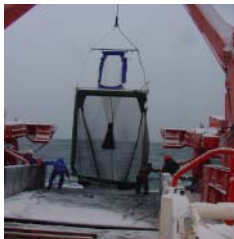


LD and DD entrained
24 h time series in
different seasons

Laboratory

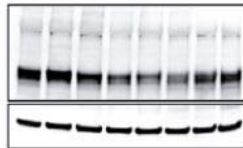


Field



Better readout for
the krill clockwork

Antibodies

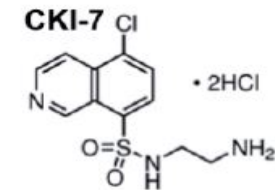


Bioluminescence

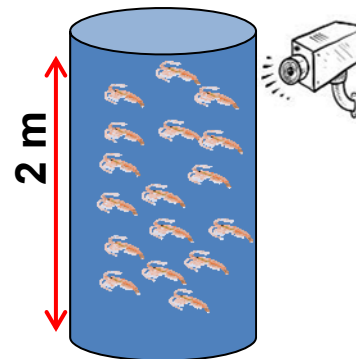


Manipulation
of the krill
clockwork

Pharmacological
(kinase inhibitors)



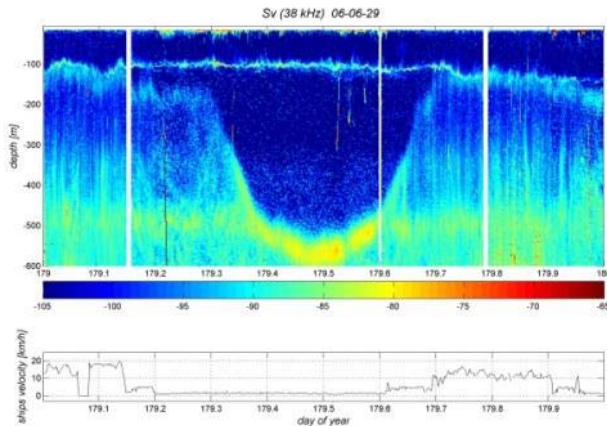
Diel vertical migration



Biological rhythms of krill at the daily and annual scale

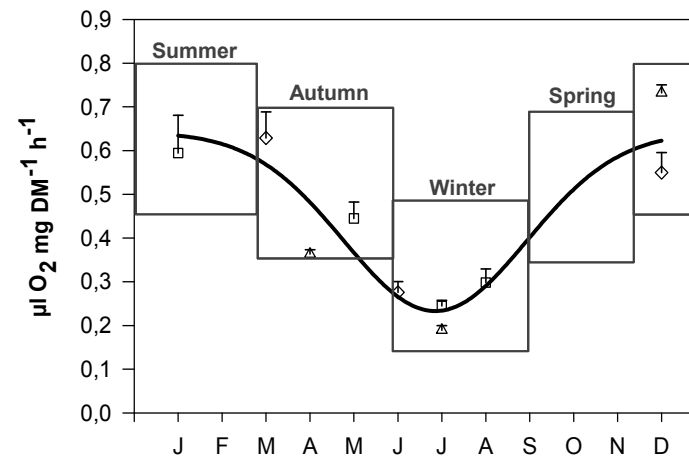


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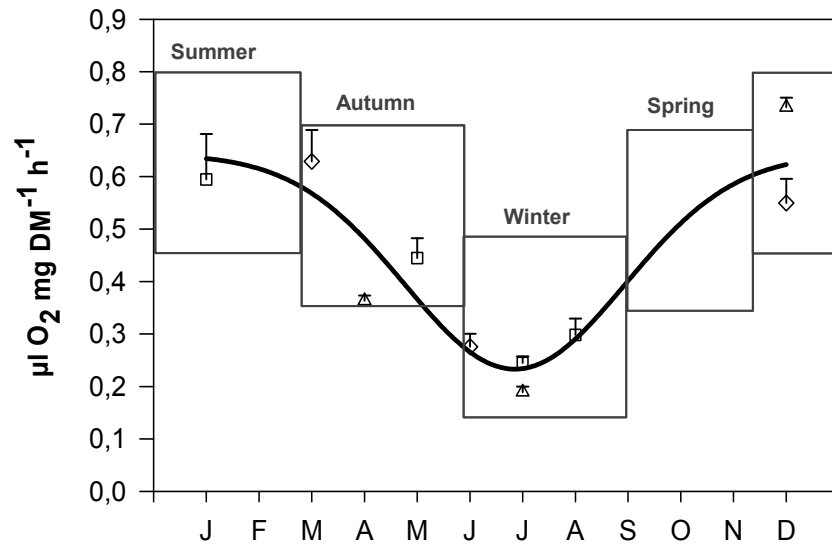
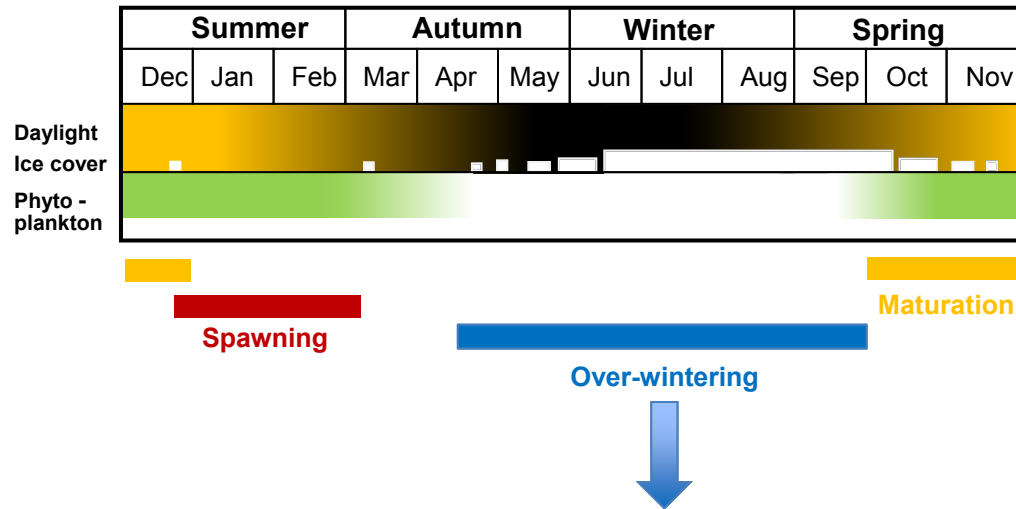
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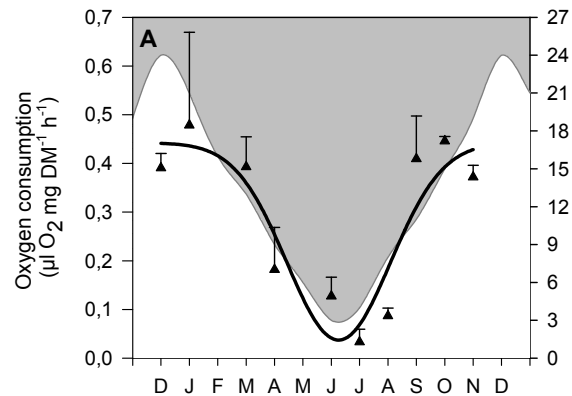
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Adaptations to a highly seasonal environment



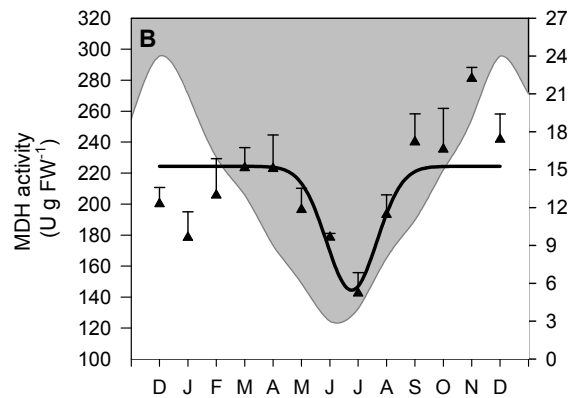
➤ What is the role of photoperiod?

Long-term laboratory experiment on life krill



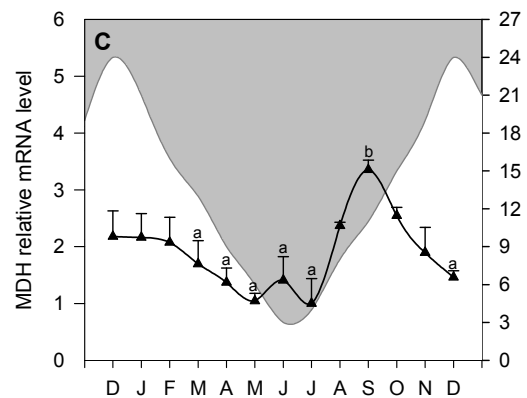
Respiration

- Overall metabolic activity



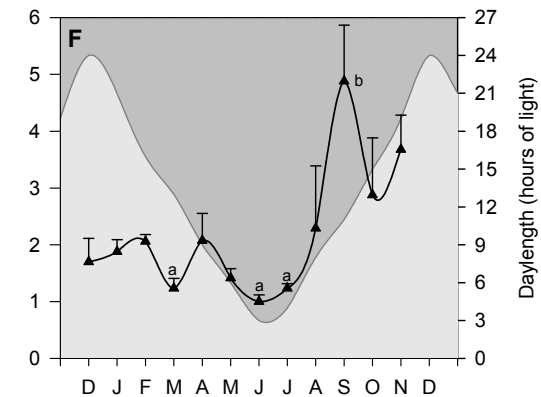
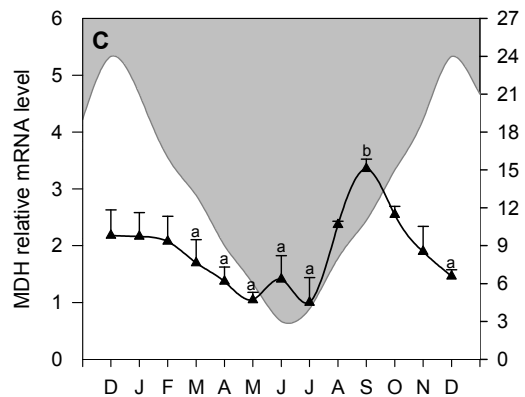
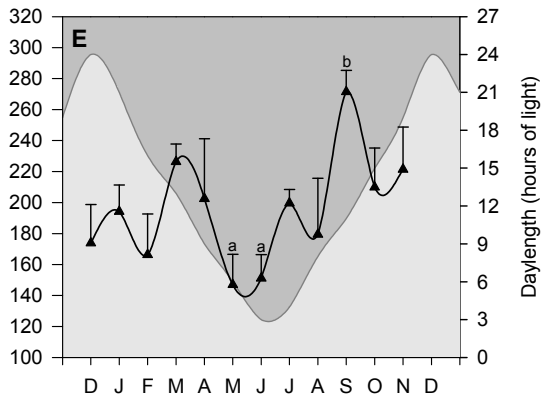
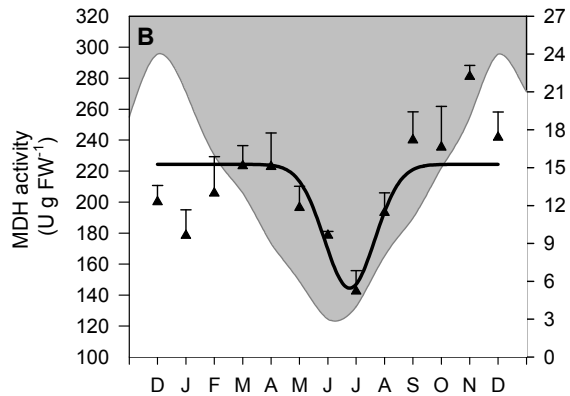
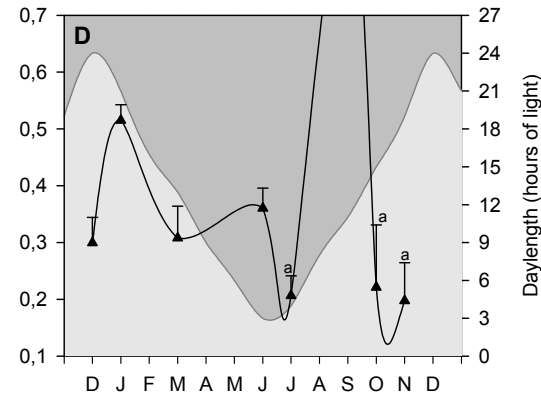
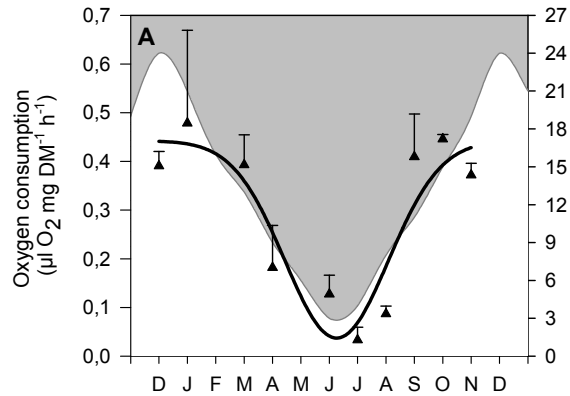
Malate Dehydrogenase MDH

- Key enzymatic proxy for overall metabolic activity



MDH relative expression levels

Long-term laboratory experiment on life krill



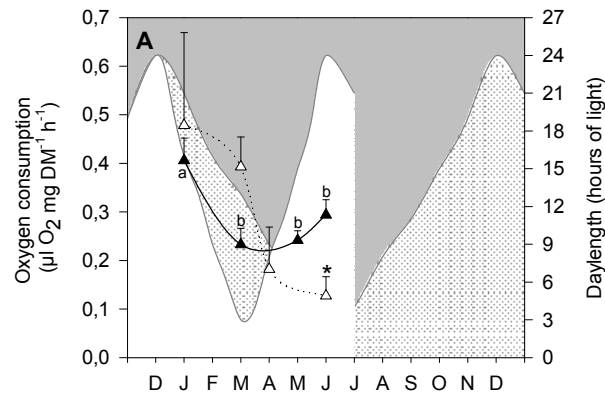
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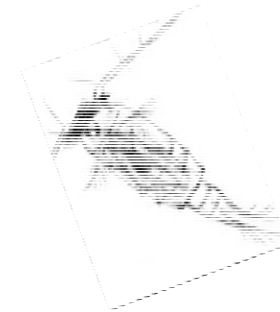
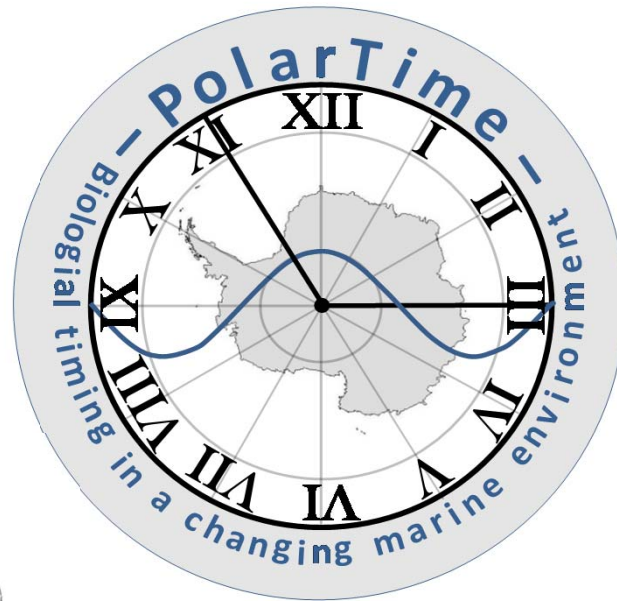
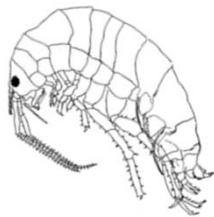
Respiration

- Overall metabolic activity

Conclusions

- **Photoperiod is an essential environmental *Zeitgeber*** for the modulation of krill's seasonal cycles
- The **clock mechanism** that seems to be involved is yet **not understood**
- A **complex interplay between internal clocks** (circadian, circannual) and **external entrainment signals** may be required to maintain a complete annual cycle of physiological functions

Biological timing in a changing marine environment: clocks and rhythms in polar pelagic organisms



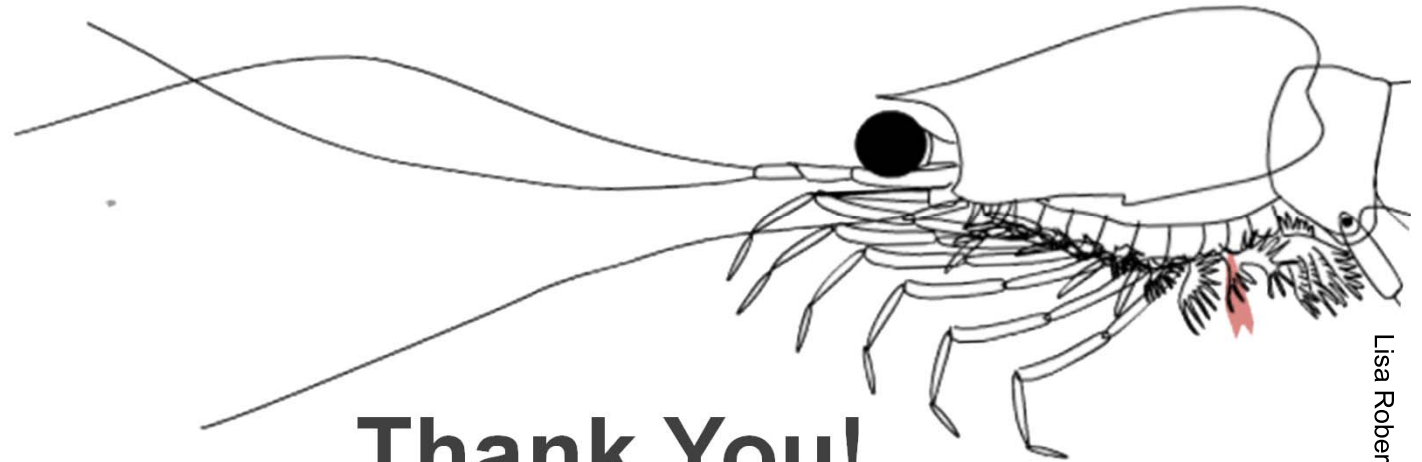
Helmholtz Virtual Institute (HVI)
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Thank You!

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