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

May 8-10 2016, Time and Light: Novel Concepts and Models in Sensory and Chronobiology

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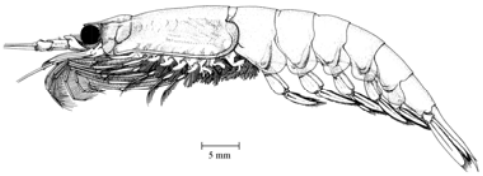
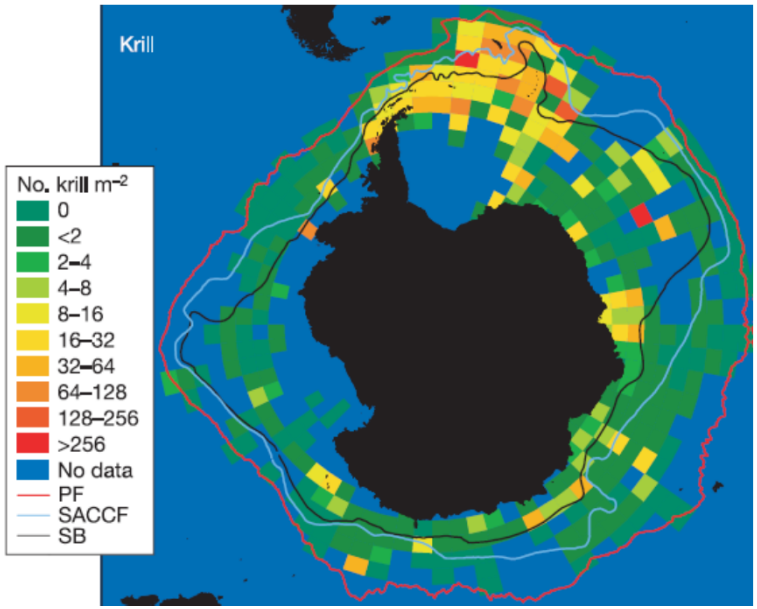
Photo: J. v. Franecker

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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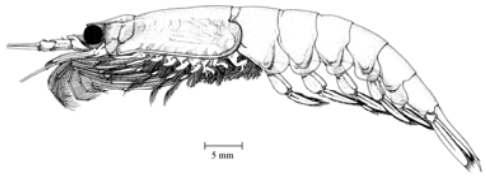

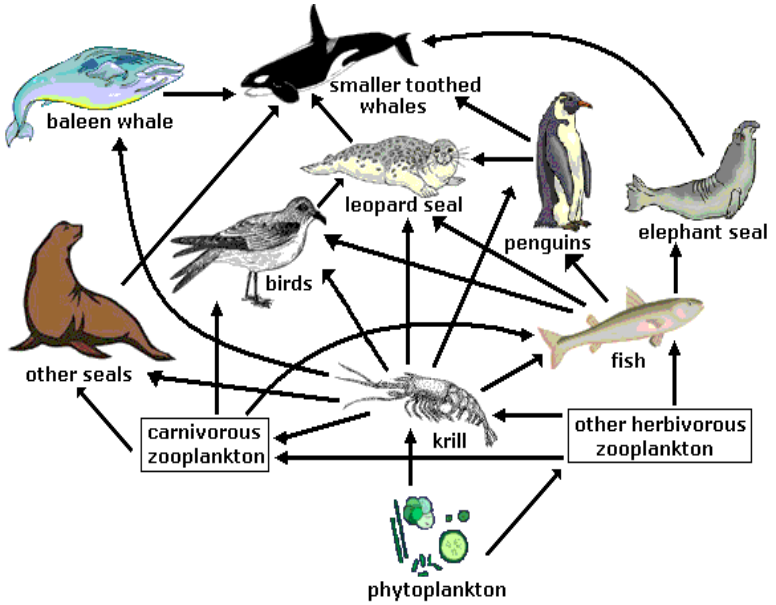

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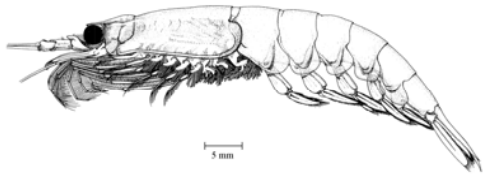

from www.supplementquanda.com

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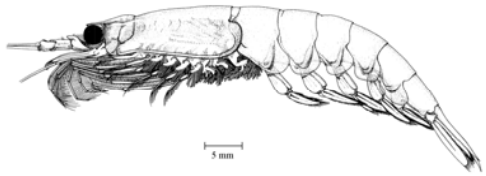
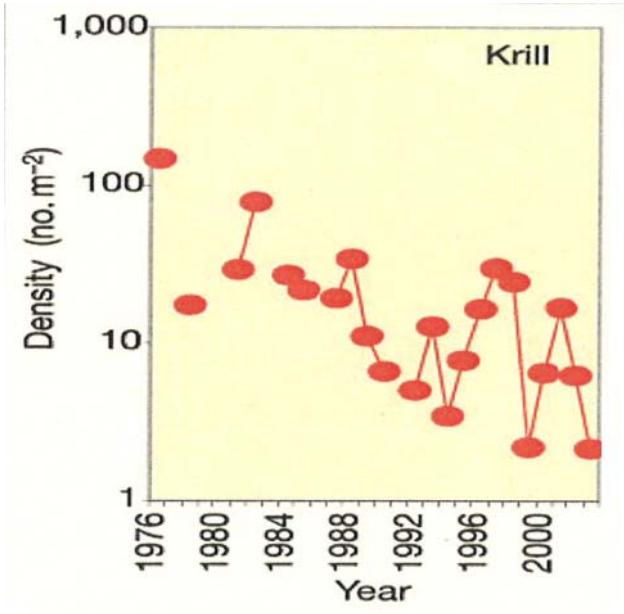
  - Long-term decline in biomass

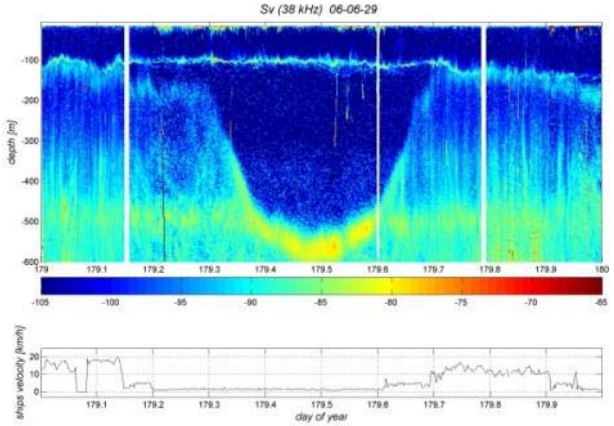




Atkinson et al. 2004

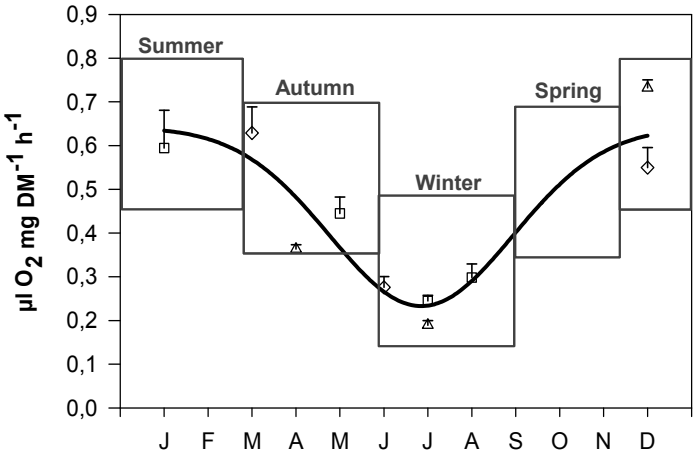
Biological rhythms of krill at the daily and annual scale

Daily behavioral functions



→ diel vertical migration (DVM)

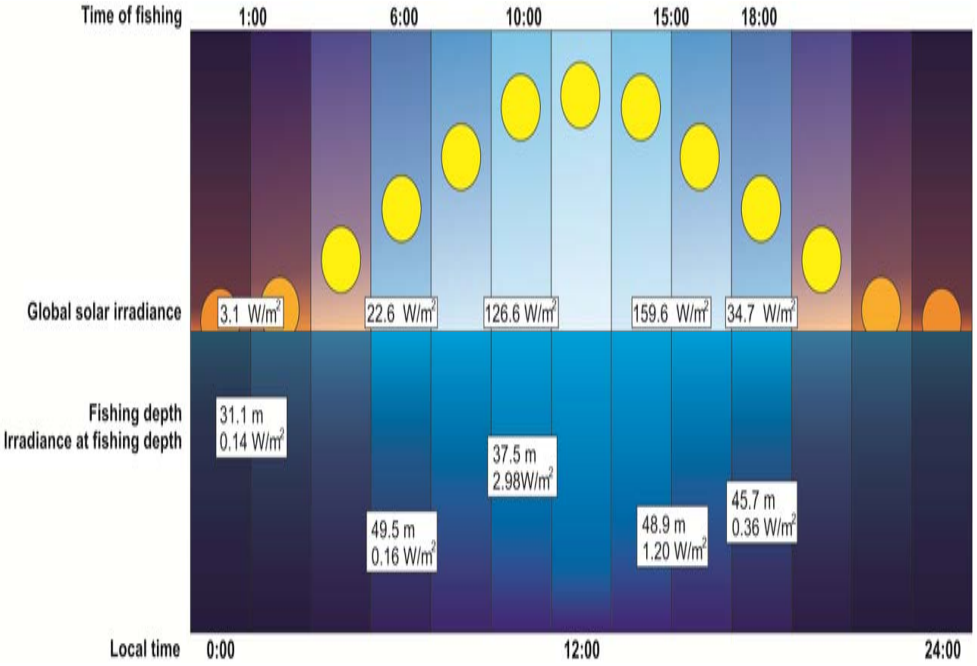
Annual physiological functions



→ metabolic depression

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

Diurnal transcriptome characterisation in natural conditions

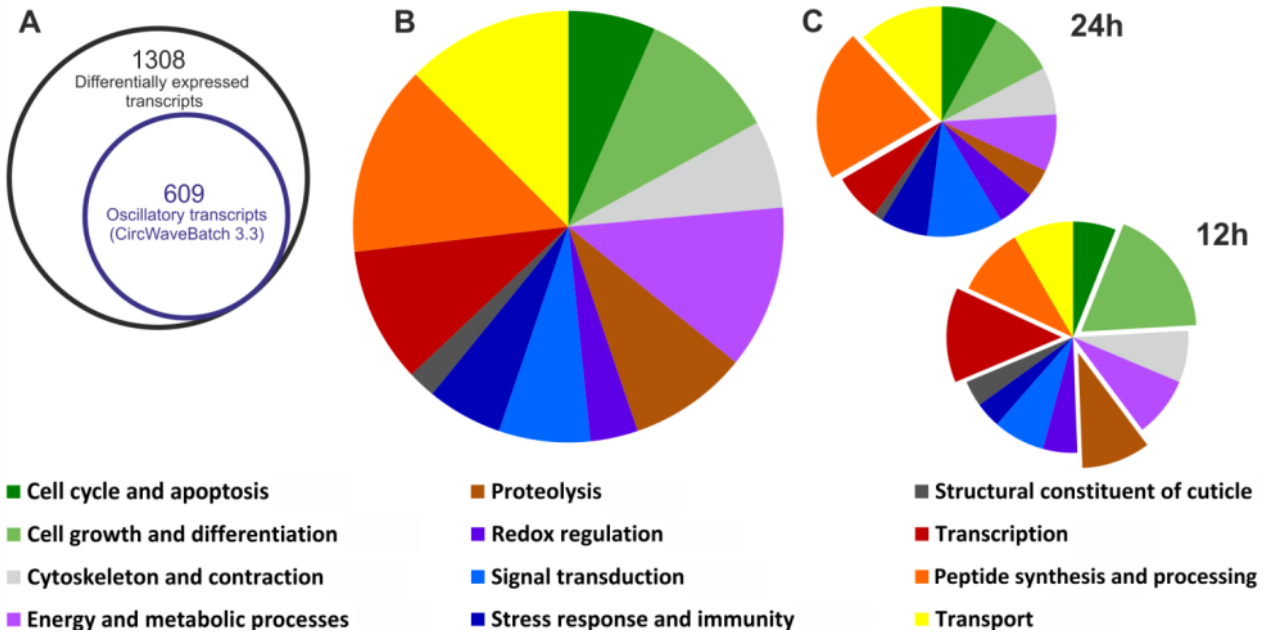


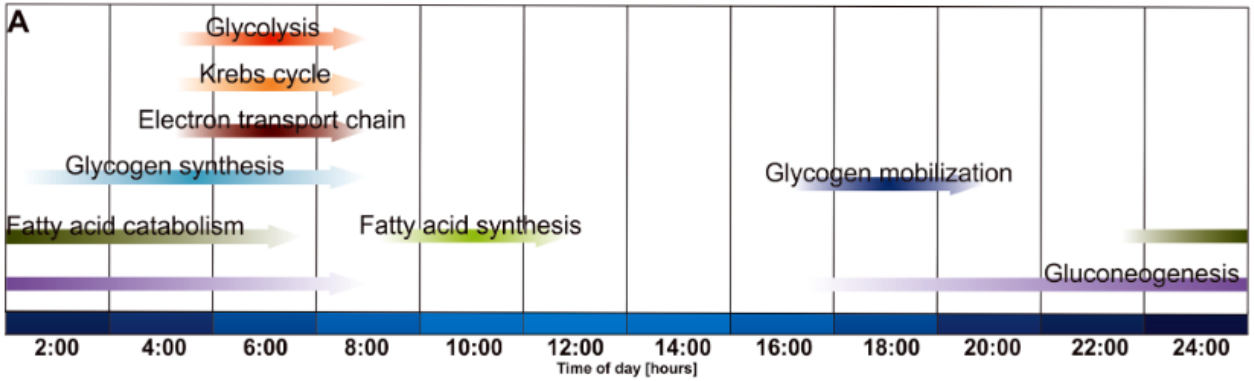
De Pittà et al. 2013

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





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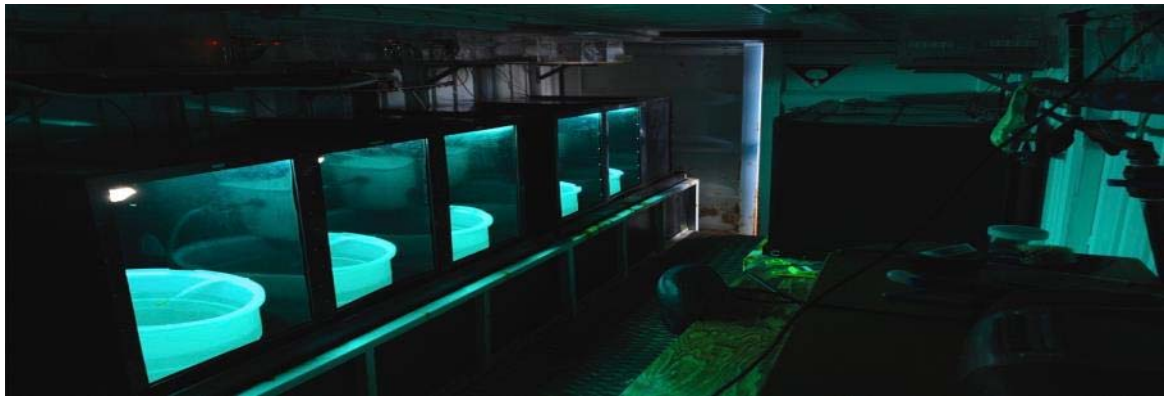
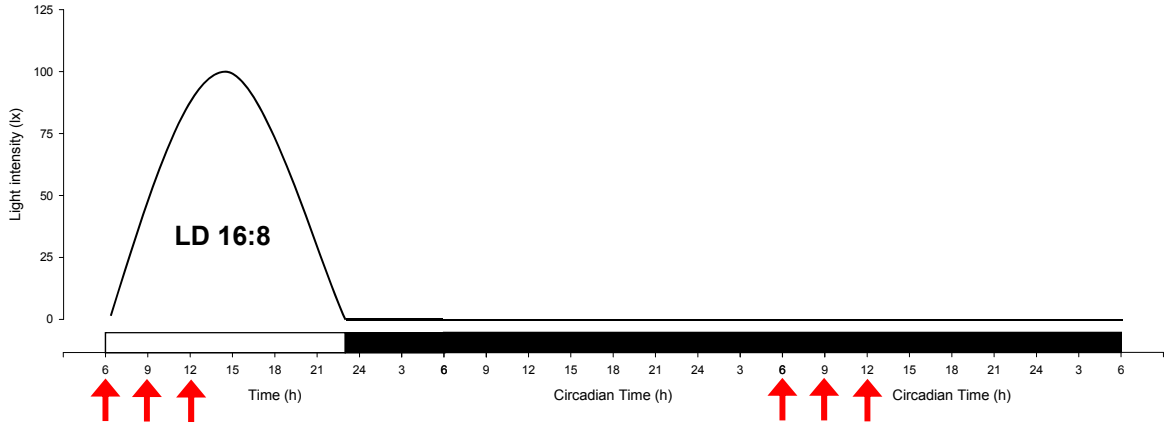
Circadian cycles of transcription?

De Pittà et al. 2013

Circadian transcriptome characterisation under laboratory conditions



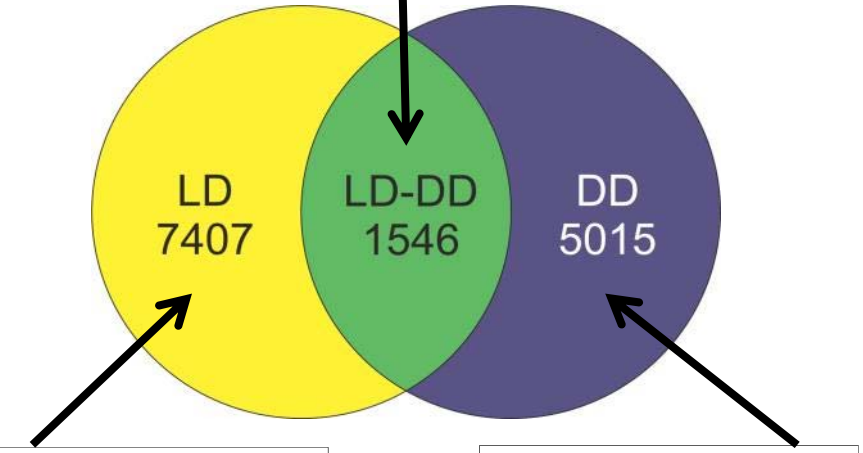


24-hour time series of LD/DD entrained krill

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

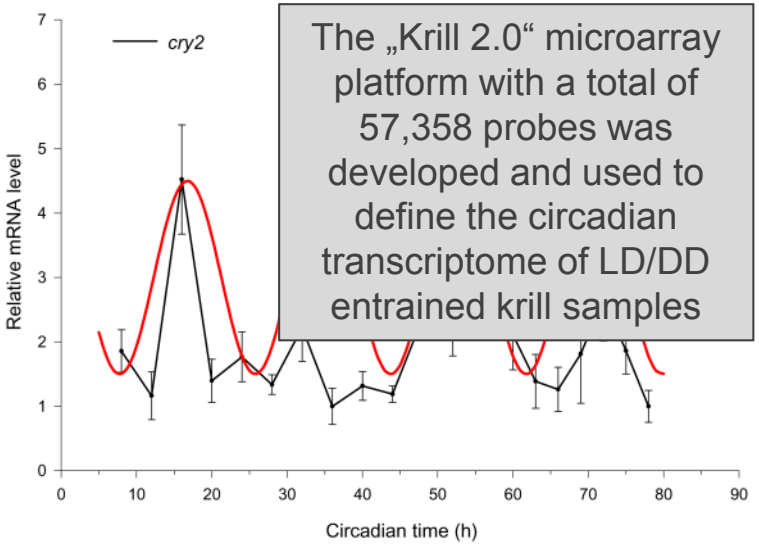
Circadian transcriptome characterisation under laboratory conditions

414 transcripts showed the same oscillatory period in LD and DD



Period in **LD**:
- τ = 12h (11 %);
- τ = 15h (9 %);
- τ = 18h (13 %);
- τ = 21h (15 %);
- **τ = 24h (52 %).**

Period in **DD**:
- τ = 12h (14 %);
- τ = 15h (28 %);
- τ = 18h (17 %);
- τ = 21h (18 %);
- **τ = 24h (23 %).**



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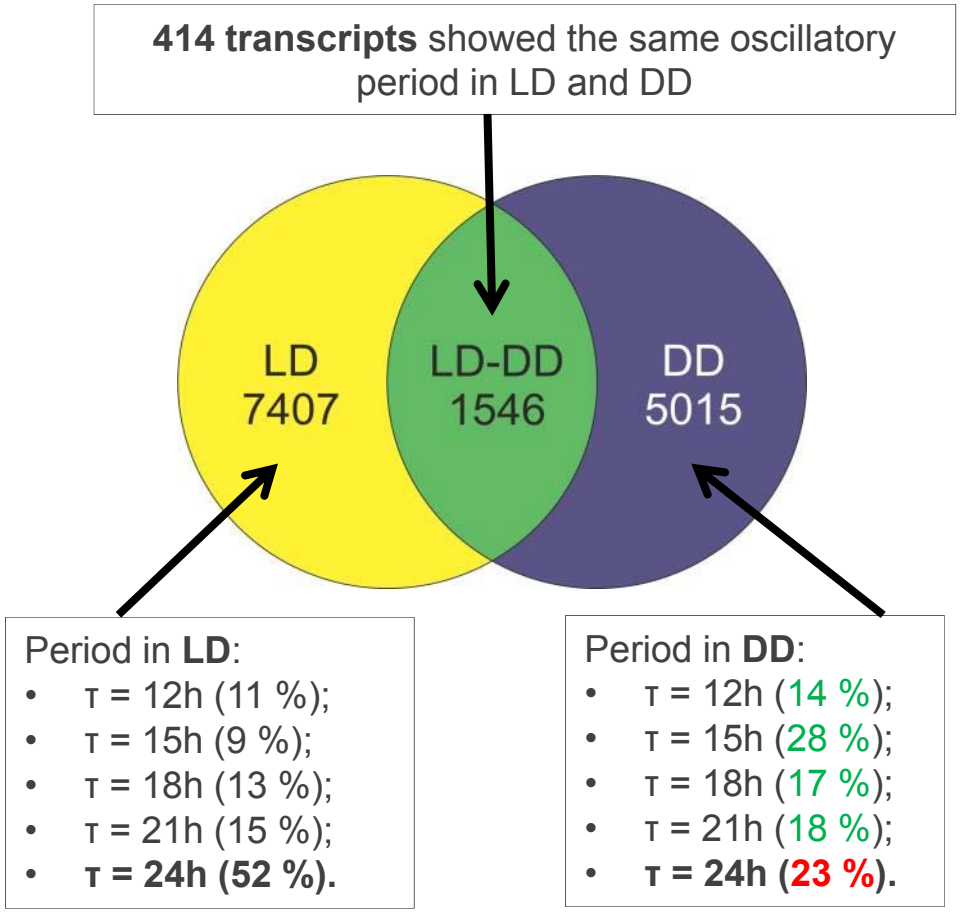
- *Euphausia superba* exhibits a surprisingly short circadian period of *cry*2 expression levels: ~ 18 hours

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

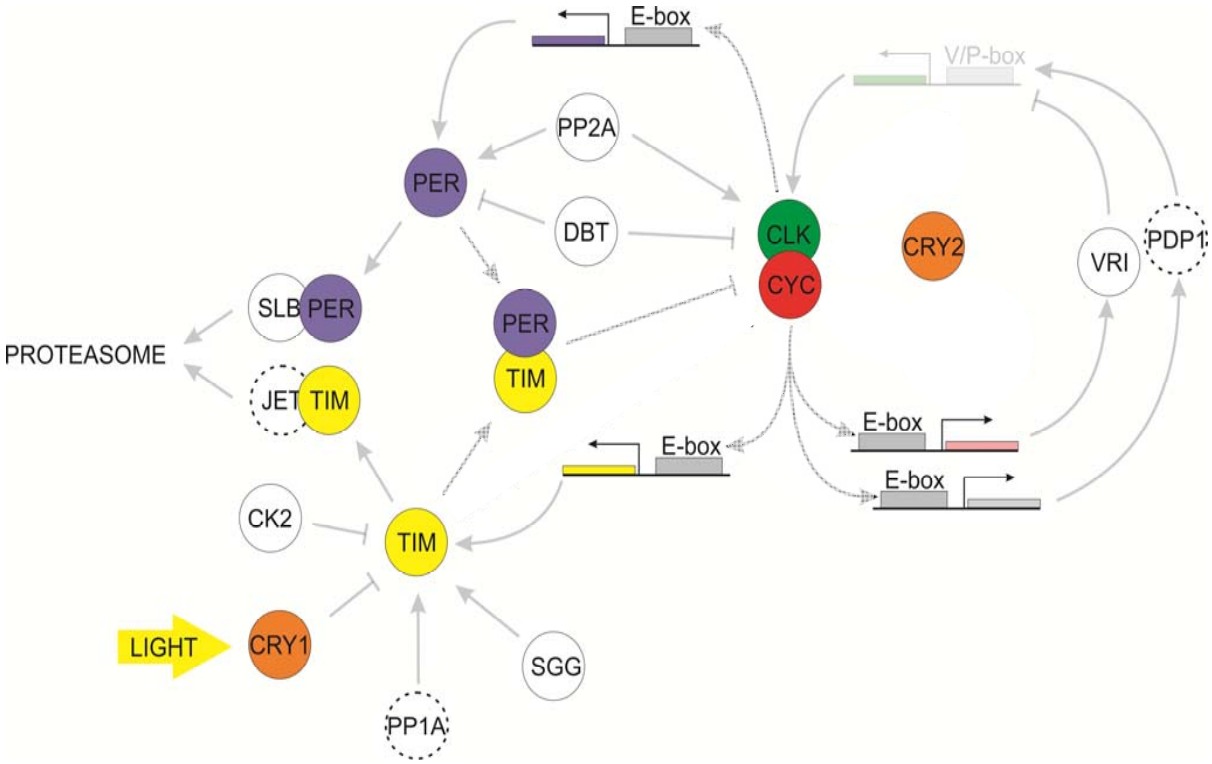
Meyer lab, AWI
Costa lab, University of Padova

↓

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

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

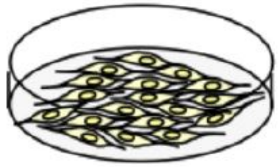
Costa lab, University of Padova



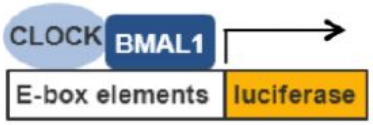
Putative architecture of the circadian clockwork in *Euphausia superba*

Principle: Co-transactivation assay

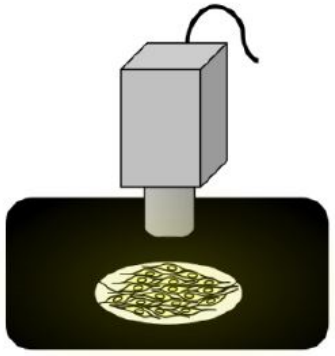
HEK293 cells

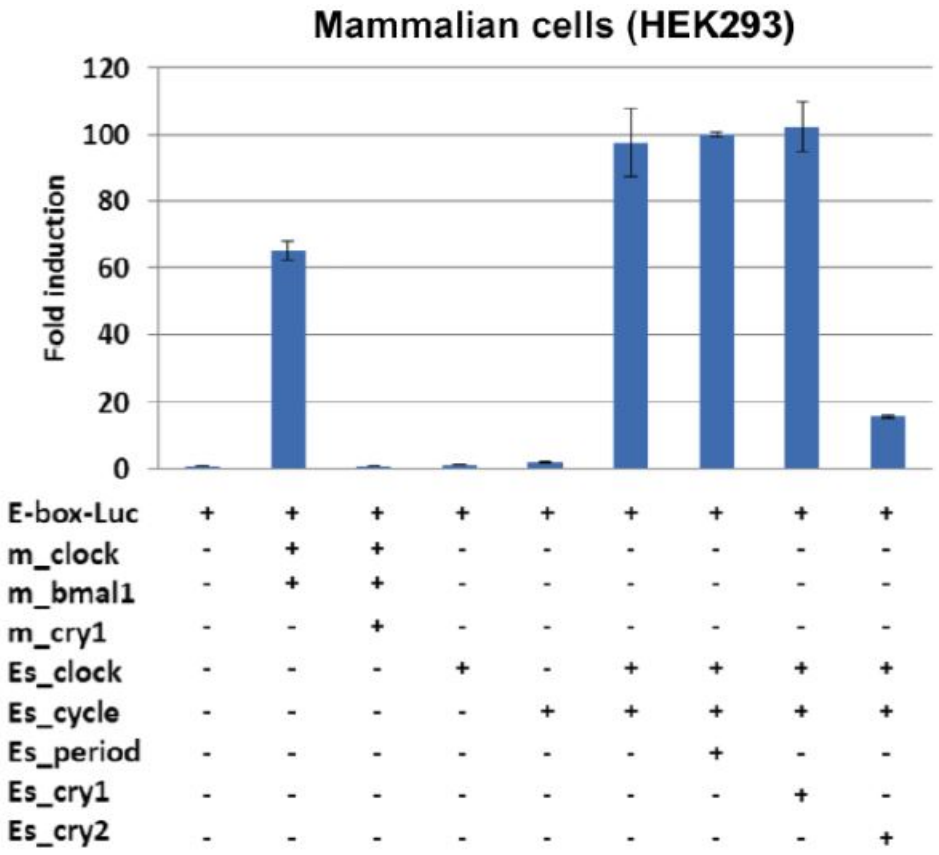


Transfection



Detection





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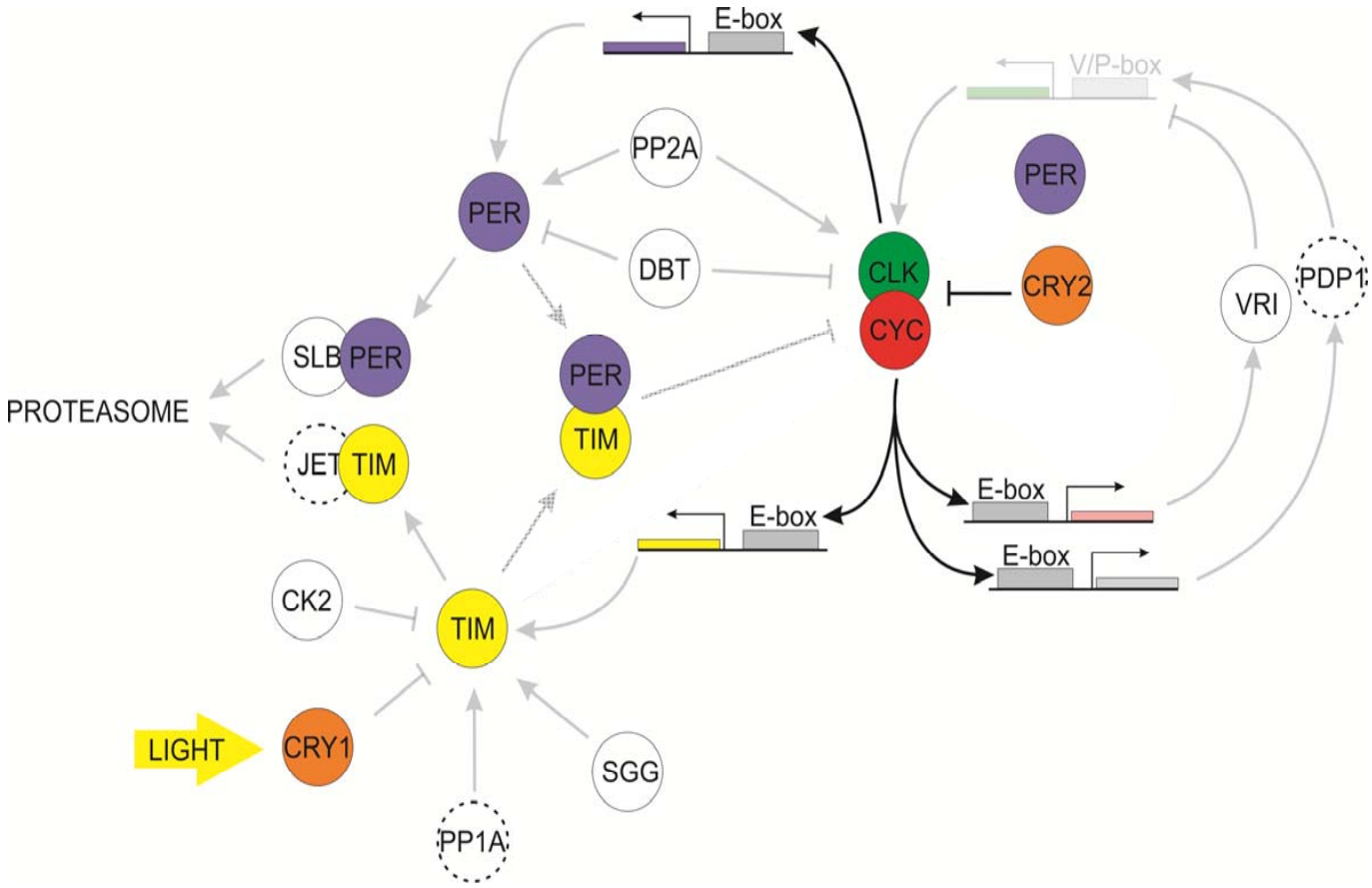
Putative architecture of the circadian clockwork in *Euphausia superba*



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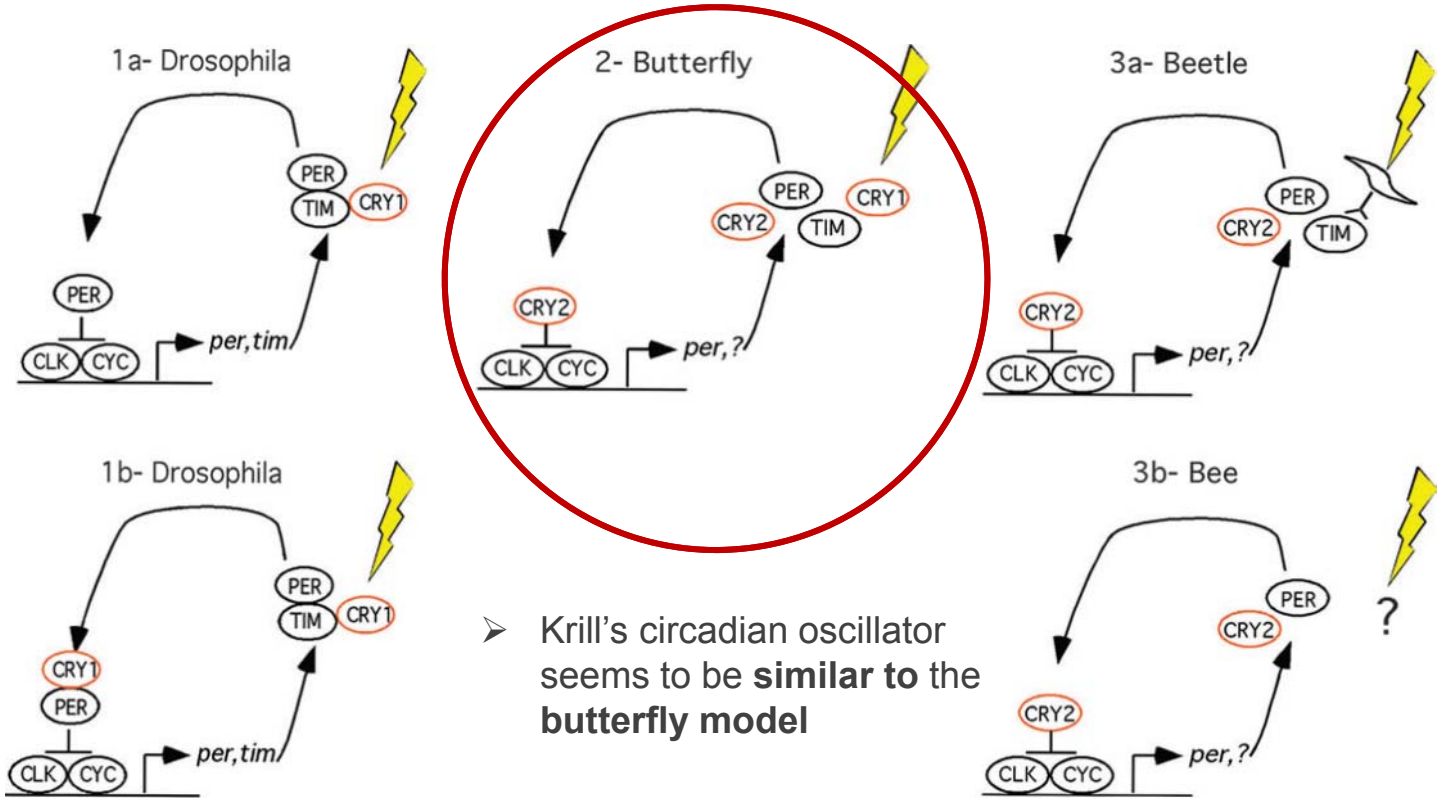


- Krill's circadian oscillator seems to be **similar to** the **butterfly model**

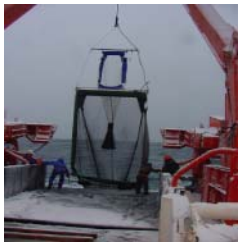
modified after Reppert 2015

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

LD and DD entrained 24 h time series in different seasons

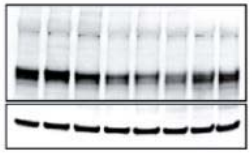
Laboratory

Field



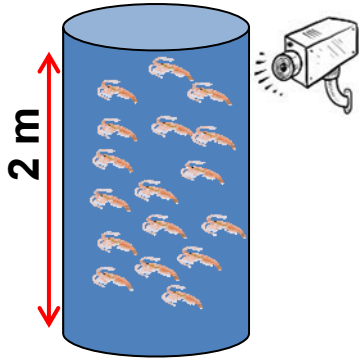
Better readout for the krill clockwork

Antibodies



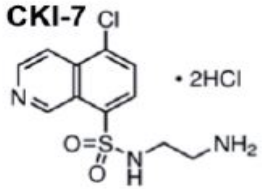
Bioluminescence

Diel vertical migration



Manipulation of the krill clockwork

Pharmacological (kinase inhibitors)



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Biological rhythms of krill at the daily and annual scale

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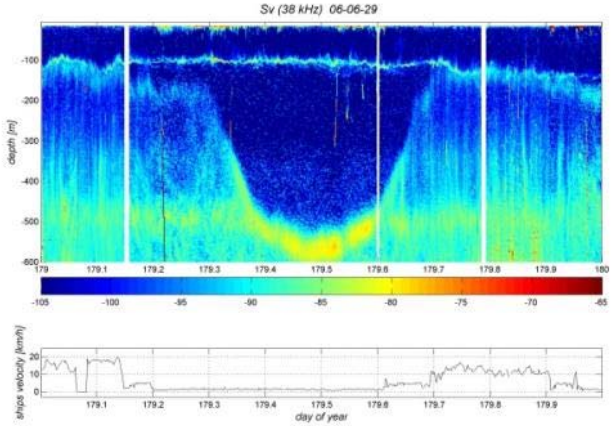

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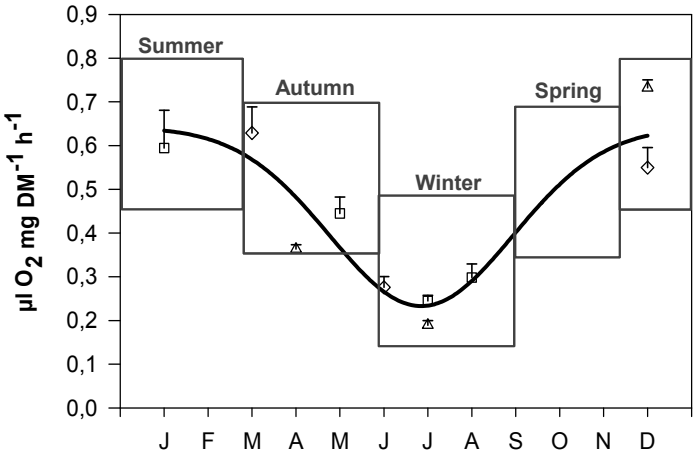

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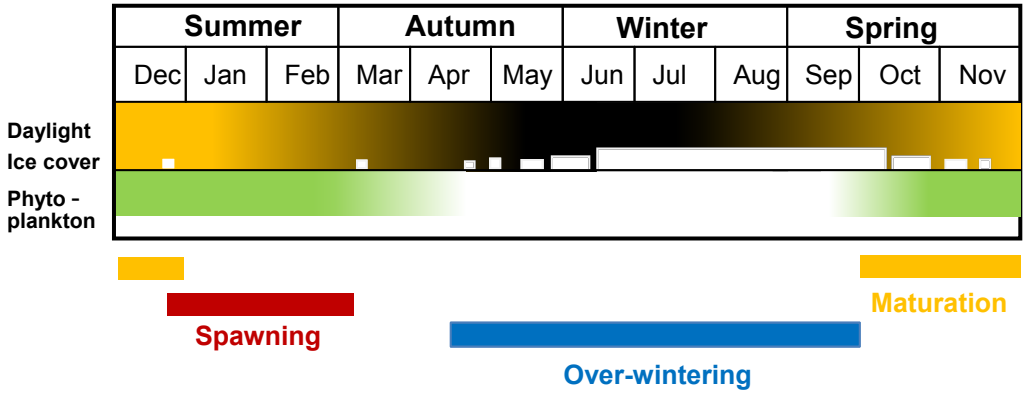
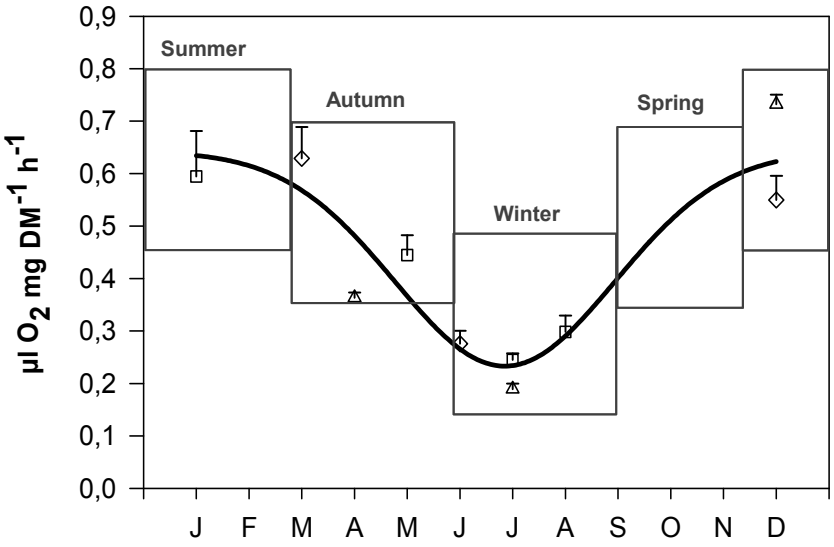
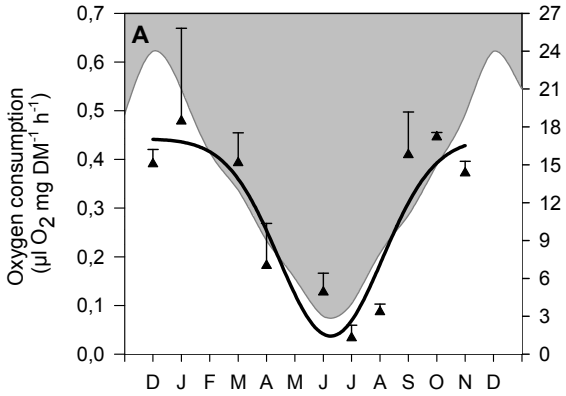
Adaptations to a highly seasonal environment





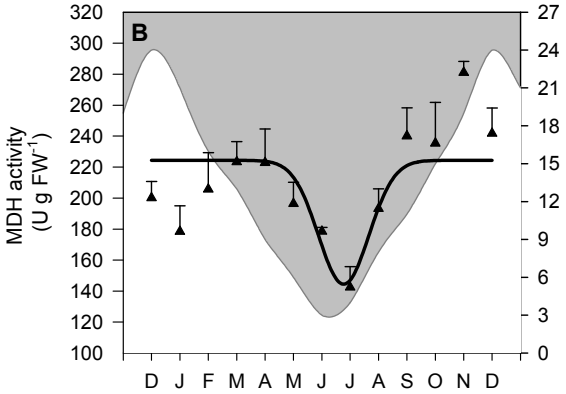
- What is the role of photoperiod?

Long-term laboratory experiment on life krill



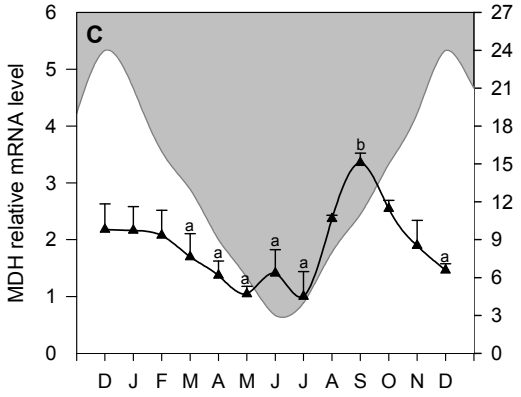
Respiration

- Overall metabolic activity



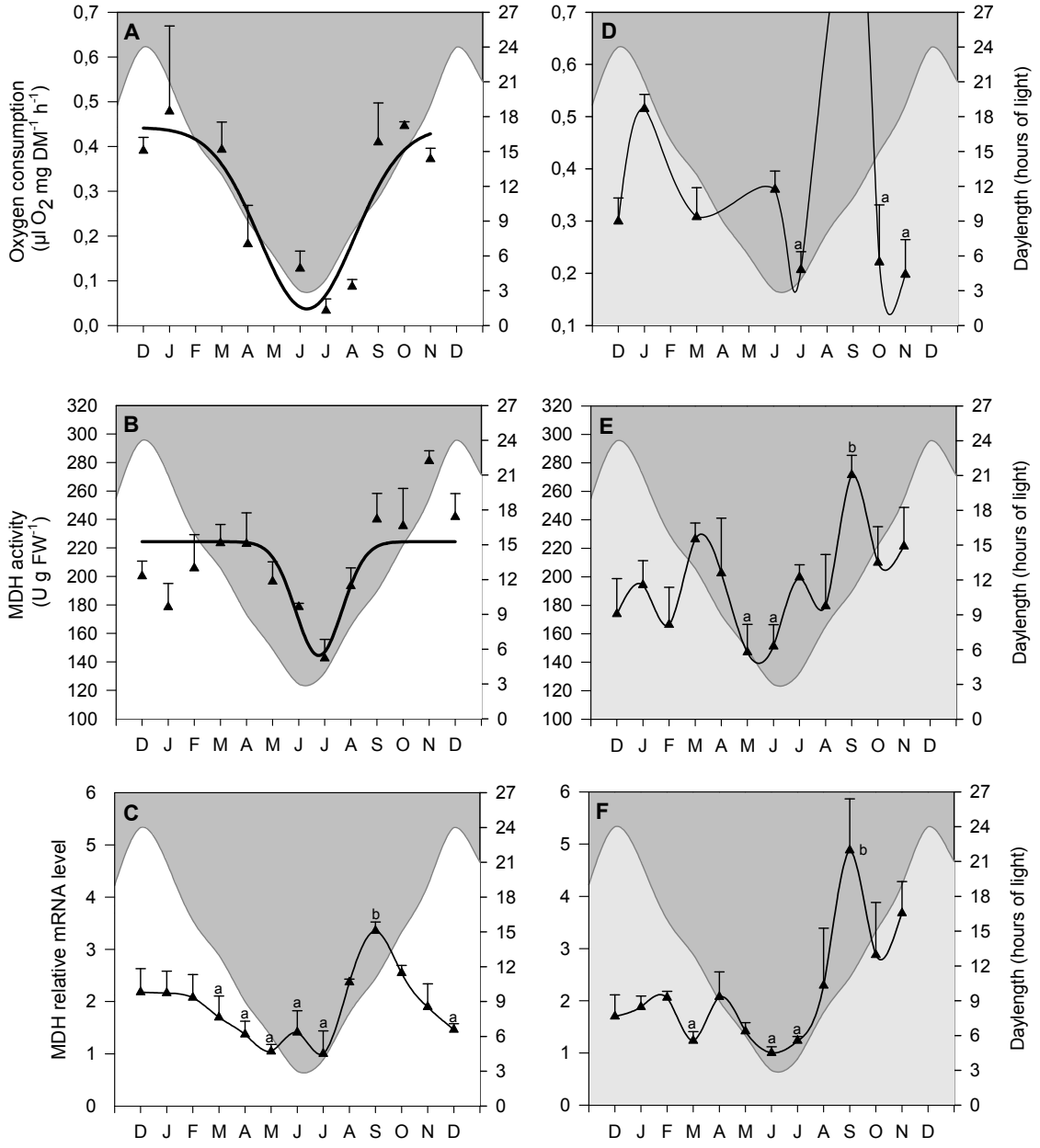
Malate Dehydrogenase MDH

- Key enzymatic proxy for overall metabolic activity



MDH relative expression levels

Long-term laboratory experiment on life krill



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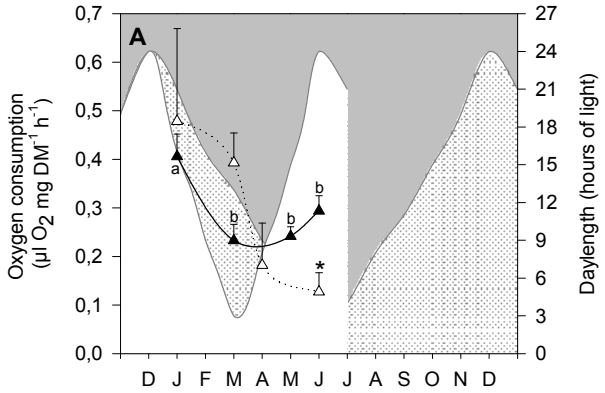


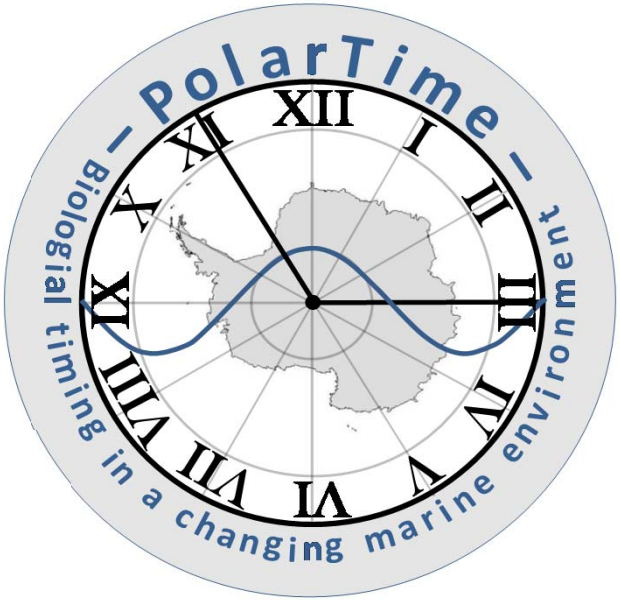
Respiration

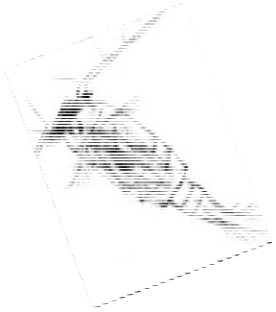
- Overall metabolic activity

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





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Australian Antarctic Division

UNIVERSITÀ DEGLI STUDI DI PADOVA

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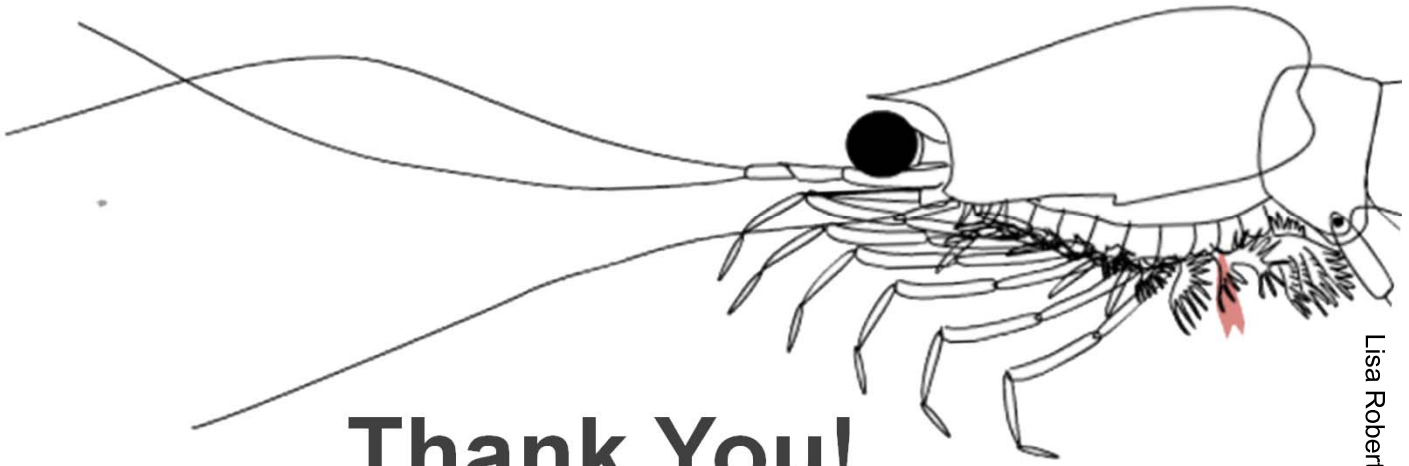

Thank You!