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Enzyme assay from frozen abdominal krill tissue

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CONTEXT

The goal of this study was to investigate whether **Antarctic krill** (Fig.1) possesses a circadian clock and whether such a clock controls key physiological processes of krill. To this end, we investigated temporal mRNA expression levels of the canonical clock gene *cry2* in individual krill that were maintained both under a light-dark cycle and constant darkness. In addition, we tested whether **gene expression of metabolic key enzymes** in these krill show daily or circadian oscillations, and to what extent transcriptional oscillations of these enzymes also persist at the protein-activity level [1].

MATERIAL

- **Precellys 24** homogenizer with **Cryolys** cooling device to have a constant temperature of 4°C within the homogenization chamber using liquid nitrogen.
- Precellys lysing kit: 013961-1-009 (CKmix).
- Sample: ~100 mg (fresh weight) of frozen abdominal krill tissue.
- Extraction solvents: ice-cold deionized water at a concentration of 100 mg fresh weight (fw) mL^{-1}.

PROTOCOL

- Precellys 24: 5000 rpm, 2x15sec, 10s break.
- Enzyme assay: Centrifugation of homogenate, spectrophotometrically analysis of supernatant in a specific buffer, data analysis.

Figure 1: Antarctic krill Euphausia superba.

RESULTS

Overall, our results showed that a **krill endogenous circadian clock** governs metabolic and physiological output rhythms (Fig.2). **Enzyme activity assays** revealed oscillatory patterns with roughly 9-12 h period under both lighting conditions that correlate with the relative changes in transcript abundance. Further studies will be necessary to investigate the contribution of the circadian clock to rhythmic variations in expression and activity of metabolic enzymes in krill and thereby will give a better understanding of how rhythmic physiology and behavior in krill will be regulated.

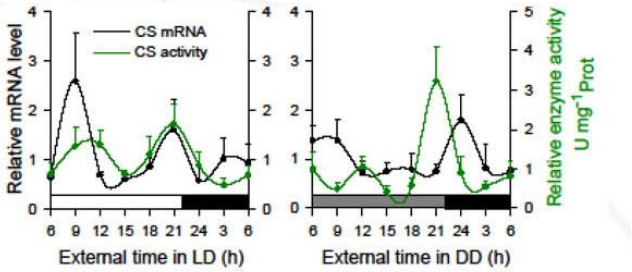

Figure 2: Oscillatory rhythms of the metabolic key enzyme citrate synthase (CS) in light-dark (LD) cycle and in constant darkness (DD).

Time course experiments require **a standardized and equably homogenization of biological tissues** that guarantee a reliable temporal analysis of data. In particular, **enzyme assays require a low temperature during the homogenization process to maintain enzyme activity.**

During this study, **the Cryolys guaranteed a constant temperature of +4° C** within the homogenization chamber and thus avoided an uncontrolled defrosting of the frozen samples.

[1] Teschke, M. et al., PLoS ONE doi:10.1371/journal.pone.0026090

CONCLUSION

Our **temporal enzyme activity** time course assays proved the high quality and efficiency of **Precellys 24 in combination with the Cryolys** cooling option.

Efficient and equably homogenization of cells and tissues with the Precellys 24 is also a prerequisite for the subsequent isolation of **highly purified total RNA** and will form the basis for our temporal **mRNA expression studies** in the future.

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