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Clock genes in a north Atlantic key zooplankter

Expression during overwintering in a high Arctic fjord

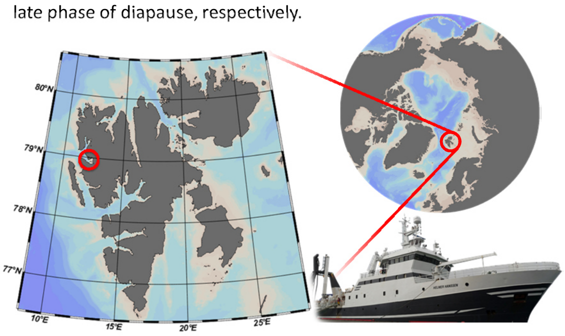
Introduction

The copepod *Calanus finmarchicus* plays a crucial role in the north Atlantic food web, channelling energy from phytoplankton primary production to higher trophic levels including commercially important fish stocks like herring or cod[1]. Whereas the species performs diel vertical migration in spring/summer, its seasonal cycle is characterised by a phase of diapause in deeper waters during winter. Although known for more than a century, the exact regulation of these rhythms – diel and seasonal – is yet unclear.

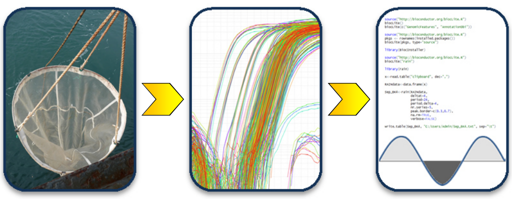
Recent annotations of clock genes in *C. finmarchicus*[2] enabled us to investigate their expression over the 24 hour cycle and to compare the patterns between two different phases of the seasonal cycle resembling LD & DD conditions in the field.

Materials & Methods

Samples were collected in Kongsfjorden, Svalbard (78.6°N, 11.6°E) on board of RV Helmer Hanssen. Samplings were done in September 2014 (LD 10:14) and January 2015 (DD) representing the early and late phase of diapause, respectively.

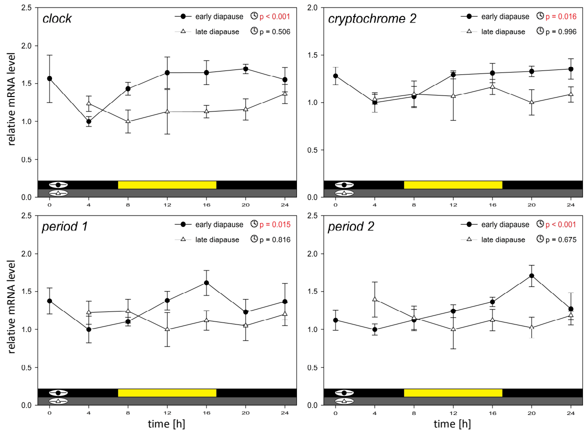


In September and January, *C. finmarchicus* juveniles (CV stage) were sampled by vertical net hauls in depth >200 m. Samples were collected over a 24h period with 4h intervals. After RNA-extraction, the expression of 9 clock genes was measured via Taqman® quantitative PCR. The data was then analysed for 24h rhythmicity using the R-package "RAIN".



Results

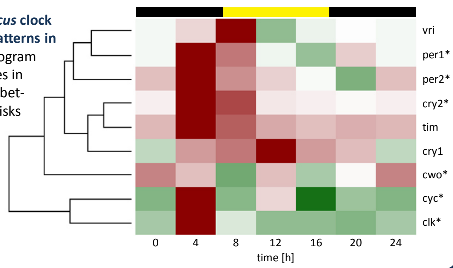
In September (early diapause, LD), 6 of the 9 investigated clock genes showed a significant 24h rhythm (*clock*, *cycle*, *cryptochrome 2*, *period 1&2*, *clockwork orange*). 3 Genes showed no rhythmicity (*timeless*, *cryptochrome 1*, *vrille*). No rhythmicity was evident in January (late diapause, DD).



Expression levels of *clock*, *cryptochrome 2*, *period 1&2*. Samples of *Calanus finmarchicus* (stage CV) were collected in Kongsfjorden (78° 6′ N, 11° 6′ E) below 200 m depth. Colour bars indicate surface light conditions (LD, DD) in September (early diapause) and January (late diapause). Mean ± SE. 5 replicates per time point, 15 pooled copepods per replicate.

September expression *of cryptochrome 2* and *period 1&2* is lowest before sunrise and peak around sunset. Weak *timeless* oscillation might explain its insignificant rhythmicity. *clockwork orange*, *cycle* and *clock* show highest activity at daytime with the latter ones showing very similar patterns. Despite being arrhythmic, *vrille* shows a distinct trough after sunrise followed by a sharp increase. *cryptochrome 1* expression is higher at night but stays arrhythmic.

***Calanus finmarchicus* clock gene expression patterns in September**. Dendrogram indicates similarities in expression patterns between genes. Asterisks indicate significant 24h rhythmicity.



Conclusions & Perspectives

- There is strong indication for a light-entrained circadian clock in *Calanus finmarchicus*.
- Under LD, several clock gene patters (*period 1&2*, *clock*, *cycle*) resemble previous findings[3,4], while others show shifted (*cryptochrome 2*, *clockwork orange*) or no rhythmicity (*timeless*, *vrille*)[3,5]. Rhythmicity is lost under long-term DD.
- Next step will be linking clock gene expression to phenotypic rhythms like diel vertical migration or the seasonal life cycle to identify possible circadian control mechanisms.

The presented work shows how the mechanistic knowledge about endogenous timekeeping gained from terrestrial model organisms can be transferred to field research on non-model species of high ecological relevance.

References

[1] Runge J.A. (1988): Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. *Hydrobiologia* 167-168, p. 61-71

[2] Christie A.E., Fontanilla T.M., Nesbit K., Lenz P.H. (2013): Prediction of the protein components of a putative *Calanus finmarchicus* (Crustacea, Copepoda) circadian signaling system using a de novo assembled transcriptome. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* 8, p. 165-193

[3] Zantke J., Ishikawa-Fujiwara T., et al. (2013) Circadian and Circalunar Clock Interactions in a Marine Annelid. *Cell Reports* 5, p. 99-113

[4] Meireles-Filho A.C.A., Amoretty P.R., et al. (2006): Rhythmic expression of the cycle gene in a hematophagous insect vector. *BMC molecular biology* 7, p. 38

[5] Richier B., Michard-Vanhée C., et al. (2008) The Clockwork Orange Drosophila Protein Functions as Both an Activator and a Repressor of Clock Gene Expression. *Journal of Biological Rhythms* 23, p. 103-116

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