Marine clocks

from individual functionality to ecosystem complexity

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Individual "personalities" & hidden rhythmic complexity – The polychaete *Platynereis dumerilii*

The **annelid worm *Platynereis dumerilii*** is native shallow coastal habitats around Europe. Famous for its circalunar reproductive cycle synchronized by the interplay and interpretation of sun- & moonlight, the worm has become a model for marine clock systems and light receptors. **Here we focus on the worm's diel/circadian rhythmic complexity**, highlighting aspects that are often overlooked in model chronobiology.

Individual variability is an inherent feature of biological systems and rhythms. Yet, the **basis of rhythmic individual "personalities" is largely enigmatic**. Similarly, individual rhythmicity is often characterized via outputs like behavior or clock genes. However, looking only at **single outputs can miss the multi-layered complexity of organismic rhythms**.

Rhythmic worm behavior is highly diverse and individual-specific

P. dumerilii individuals show a diel/circadian behavioral continuum for highly rhythmic to fully arrhythmic. Strong difference exist even among siblings from incrossed strains. **Individual-specific rhythmic "personalities" are persistent over time**, even if incubation conditions vary (Fig.1). Individual diversity may arise from epigenetic modifications and/or neuronal remodeling. Rhythmic diversity may be an adaptation to the worm's life cycle, which involved broadcast spawning and larvae settlement in heterogenous coastal habitats.

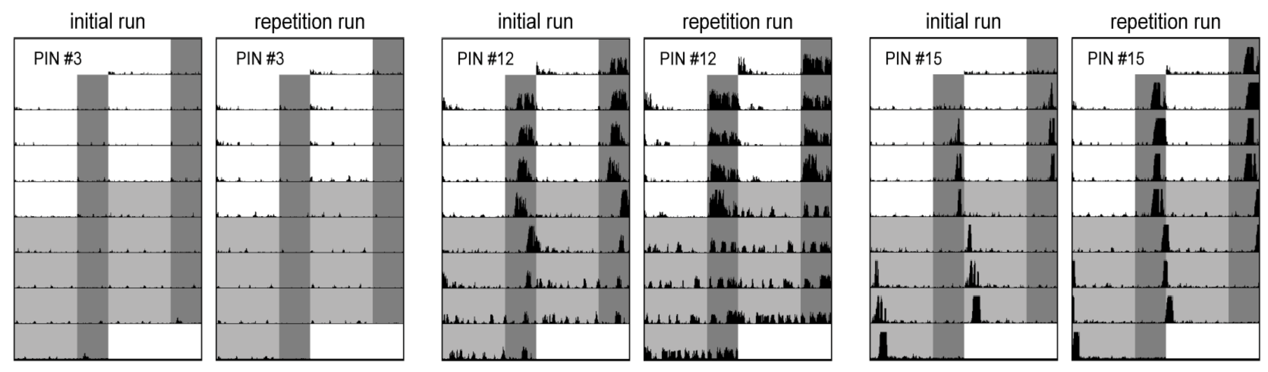
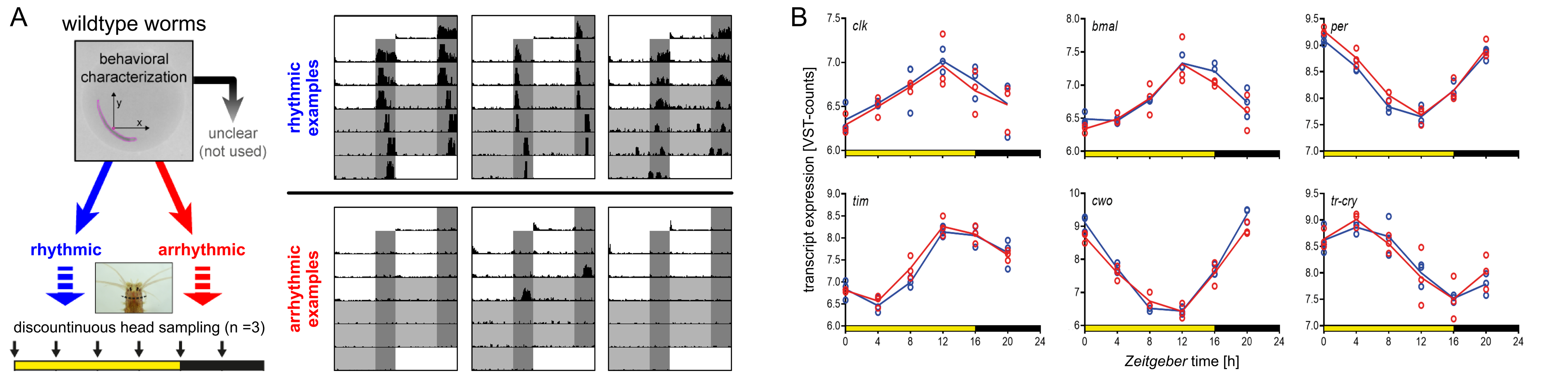


Fig. 1: Diversity & repeatability of individual worm diel/circadian phenotypes. Behavior of *P. dumerilii* wildtype individuals (3 examples shown) was recorded in LD & DD. 1-2 months later, the experimental runs were repeated with the same individuals. Häfker et al (2024) Plos Biol

Diel rhythmicity strongly differs between levels of biological organization

Considering the large individual behavioral diversity (Fig.1), we explored the molecular background of these patterns. We focused on extreme behavioral cases (high rhythmic vs. arrhythmic) and generated 24h head transcriptomes to compare transcript expression rhythms (Fig.2A). Surprisingly, both phenotypes show **identical clock gene cycling in spite of contrasting behaviors** (Fig.2B). Even more striking, GO-term analysis of cycling transcripts shows that behaviorally **arrhythmic worms are more rhythmic with regard to metabolism-related transcripts**. In general, rhythmic and arrhythmic phenotypes show no distinct "switch" in gene expression programs, but rather gradual adjustments in expression patterns, in line the observed continuum in behavioral phenotypes (Fig.2C). The contrasting patterns highlight the complexity of multi-level rhythms and the importance of integrative approaches in properly characterizing organismic rhythmicity.



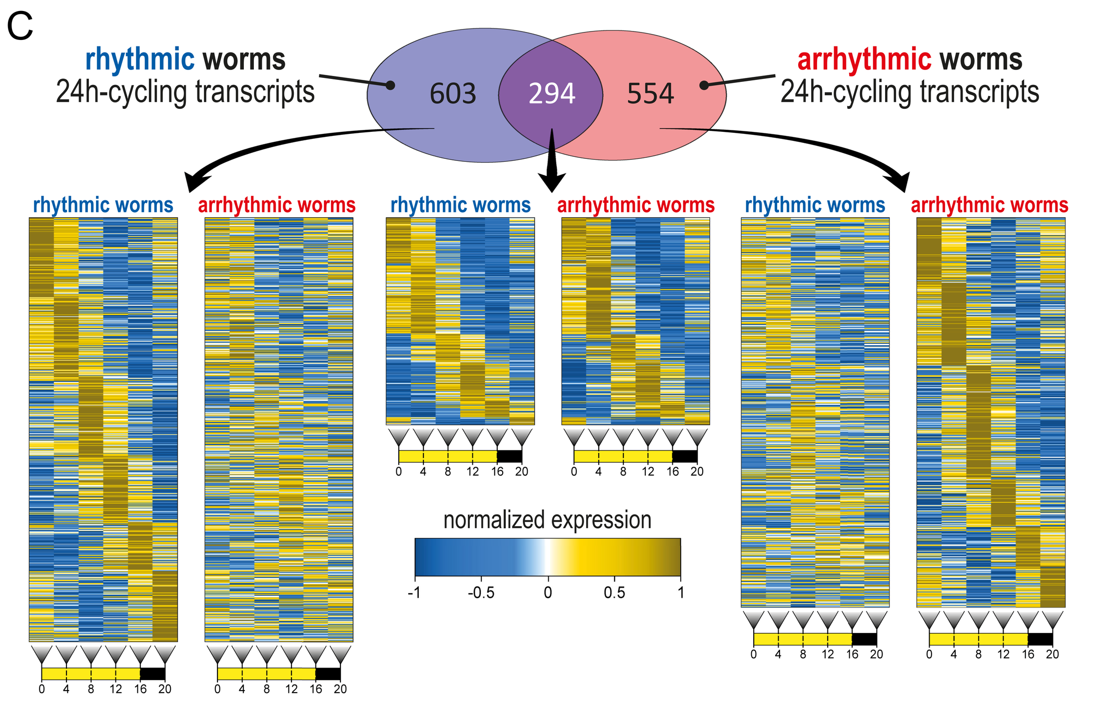


Fig. 2: Characterization of multi-level worm rhythmicity. (A) *P. dumerilii* wildtype worms were grouped into behaviorally rhythmic & arrhythmic diel phenotypes. Intermediated phenotypes (unclear) were excluded. Heads were sampled over 24h in LD for transcriptomics. (B) Clock gene transcripts show identical patterns in both phenotypes (lines = mean). (C) Heatmaps & GO-terms show similar numbers but limited overlap in cycling transcripts. Behaviorally rhythmic worms show increased cycling in transcripts related to locomotion and environmental responsiveness. In contrast, behaviorally arrhythmic worms show stronger rhythmicity in metabolic processes. Notably, there is not distinct "switch" in expression programs, but gradual expression differences mimicking the observed behavioral continuum (intermediate phenotypes). Häfker et al (2024) Plos Biol

PDF role in *P. dumerilii* circadian activity

The neuropeptide pigment-dispersing factor (PDF) is crucial to *Drosophila melanogaster* circadian activity. P. *dumerilii pdf* mutants were generated via targeted mutagenesis (TALENs), and circadian locomotor activity was recorded. ***pdf* loss does not alter worm circadian rhythmicity**, but causes elevated overall activity (Fig.3). This suggests a conserved role of PDF in behavioral control, but challenges it's role in rhythmicity beyond insects.

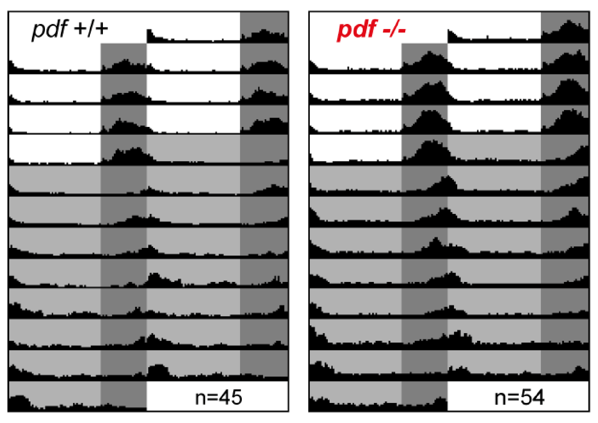


Fig. 3: Circadian activity patterns of *P. dumerilii pdf* wildtypes (+/+) & mutants (-/-). Worm behavior was recorded in LD & DD. Häfker et al (2024) Plos Biol

Mutant transcriptomic rhythms under natural sun- & moonlight

An L-cryptochrome (light-responsive) enables *P. dumerilii* to distinguish sun- & moonlight, thereby affecting circalunar reproduction. We entrained ***l-cry* wildtypes & mutants in field mesocosms and in the lab to create full & new moon 24h transcriptomes** (Fig.4) Early results suggest *l-cry* mutants struggle to distinguishing different lights, thus supporting previous findings.

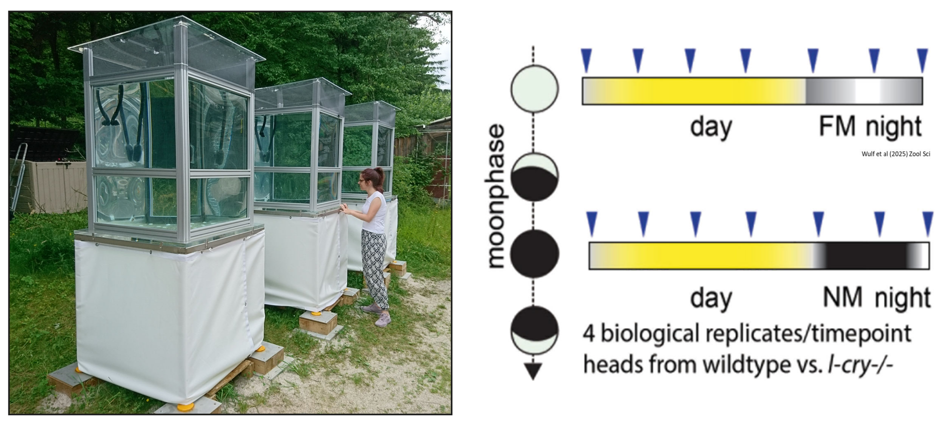


Fig. 4: Field setup and sampling. *l-cry* wildtype & mutant worms were exposed to natural sun- & moonlight in field mesocosms (640 L). After 3.5 months of entrainment, heads were sampled over 24h at full & new moon. An equivalent set of lab samples was collected. Wulf et al (2025) Zool Sci

Clock functioning in a "self-made" environment – The copepod *Calanus finmarchicus*

In the open ocean, **countless species perform diel vertical migrations (DVM)** that fundamentally shape ecosystem function. Because many environmental parameters, most prominently light intensity & spectrum, vary strongly with depth (Fig.5), DVM determines the ***realized* diel environmental cycles** that migrating organisms experience. Since circadian clocks influence DVM and are themselves entrained by *realized* cycles, a paradoxical feedback emerges: **biological clocks help generate the very entrainment conditions to which they synchronize** (Fig.6).

The **copepod *Calanus finmarchicus***, an ecological key species, performs DVM in shallow waters and seasonal diapause in deeper layers. With robust clock gene oscillations, it serves as a **valuable model to study clock functioning in "self-made" natural environments and in the lab**. As *C. fimarchicus*' habitat range is shifting poleward under climate change, mechanistic insights are critical to predict clock system responses/adaptations, and potential cascading ecosystem consequences.

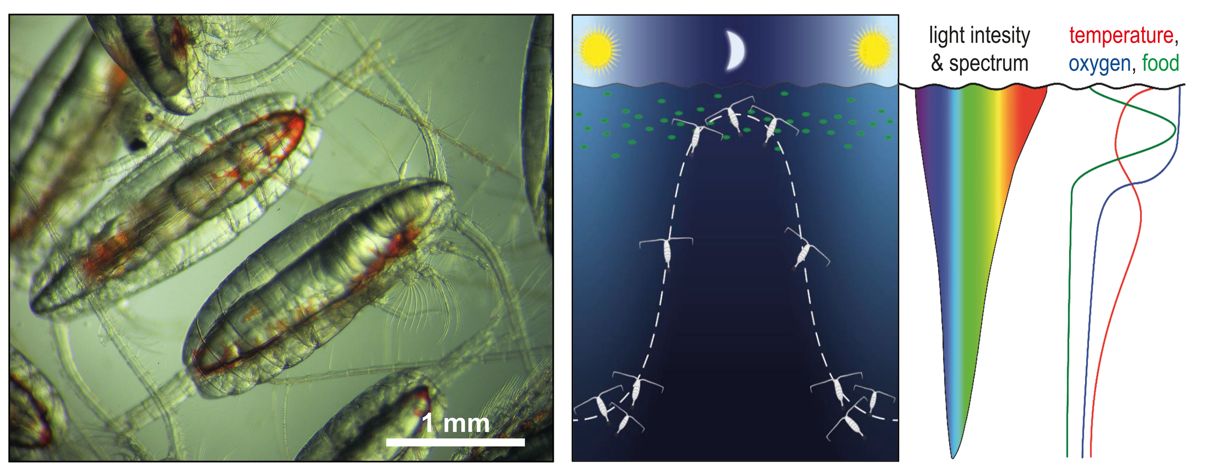


Fig. 5: *C. finmarchicus* DVM in a vertically structured water column. The planktonic copepod *C. finmarchicus* is a key species in the north Atlantic ecosystem, providing food for various species including commercially important fish stocks and whales. By performing DVM in the vertically strunctured pelagic water column, copepods and other migrators directly determine the *realized* environmental conditions and cycles that they experience. picture: © David Pond

The curious case of *C. finmarchicus*' two *periods*

Highly uncommon among invertebrates, *C. finmarchicus* has **two distinct *period* genes with strongly differing expression** (Fig.7). *per1* shows strong diel cycling during the active life phase, but overall expression peaks in diapause. *per2* cycling is dampened, but most robust at the time of diapause initiation, when overall expression peaks. **The functions two *period* genes, as well as triggers of diapause in *C. finmarchicus*, are unknown.**

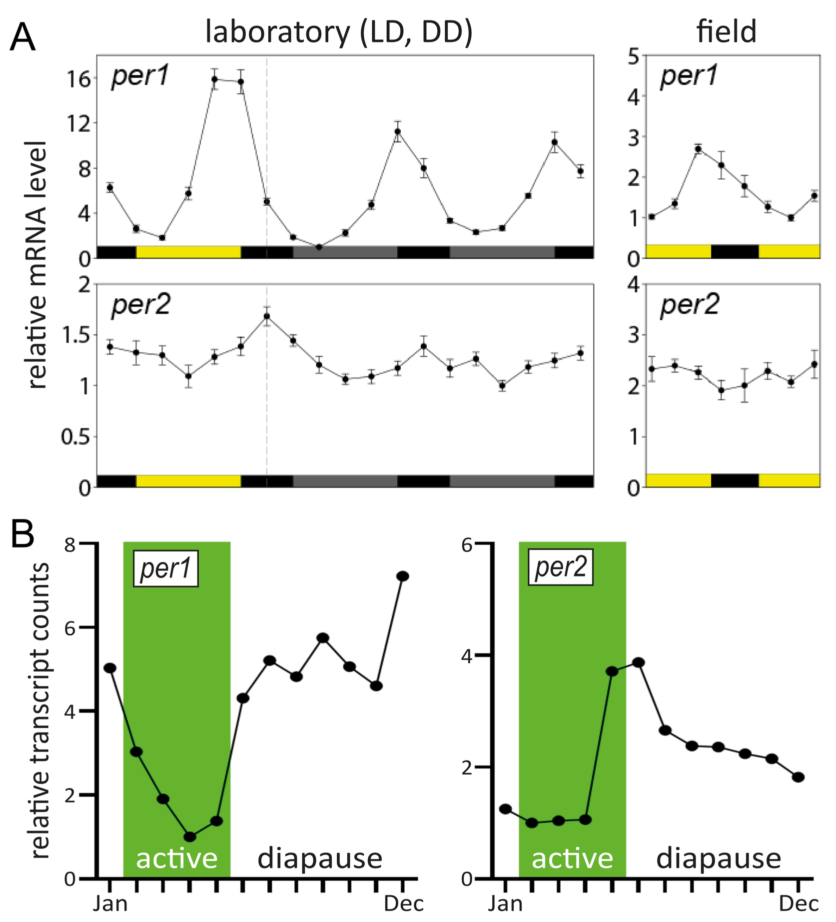


Fig. 7: *C. finmarchicus per1 & per2* temporal patterns. (A) Diel/circadian expression in the lab and in the field (May, Loch Etive, Scotland). (B) Seasonal expression during the active life phase in shallow waters and diapause in deep waters in the field. Häfker et al (2017) Curr Biol / Payton et al (2024) Mol Ecol (new *de novo* assembly)

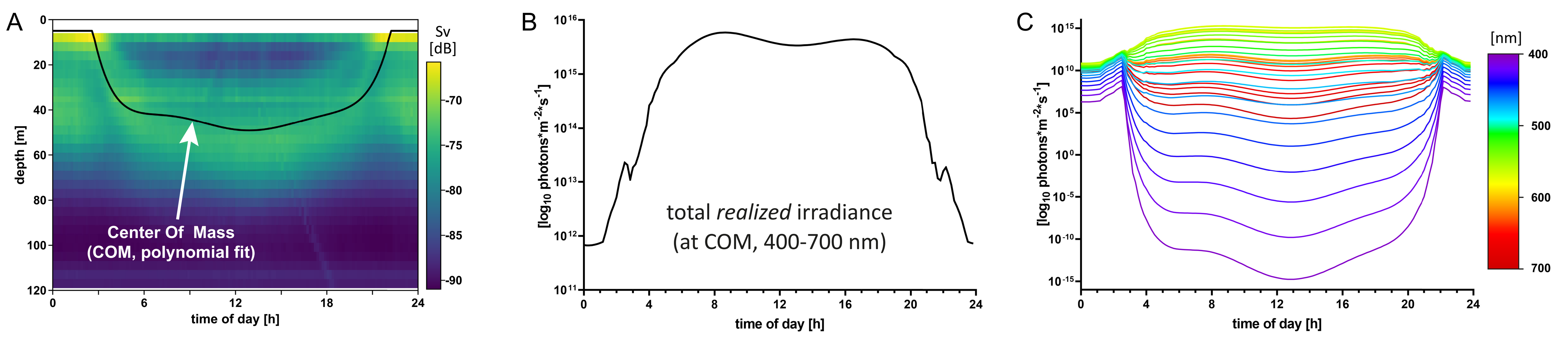


Fig. 6: *C. finmarchicus* diel vertical migrations (DVM) determines the *realized* light cycle. (A) Zooplankton vertical distribution measured as backscatter sound volume (Sv [dB]). Recorded via underwater echosounder in May in Loch Etive Scotland, a fjord with greenish/yellow water due to high organic substance content. (B) Total *realized* light irradiance as experience by *C. finmarchicus* at COM depth during DVM. (C) Wavelength-specific *realized* light irradiance experienced during DVM. Depending on wavelength, intensity cycles can be absent or even inverted, highlighting the interplay of DVM and spectral light attenuation with depth. Häfker et al (2022) Comms Biol, Häfker et al (in prep).

take home messages

- **Individual diversity & rhythms** are evolutionarily conserved. Their mechanistic basis & ecological significance are hardly explored.
- **Rhythms can strongly differ between functional levels** (behavior, circadian clock, metabolism). Integrative approaches are needed.
- **Natural (*realized*) environmental cycles** can be highly complex, likely requiring habitat- & species-specific adaptations of clock functioning & outputs.
- **Re-creating naturalistic cycles in the lab** depends on detailed knowledge of environmental conditions & organismic rhythmicity in the field.

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