# Circadian Physiology in Zooplankton

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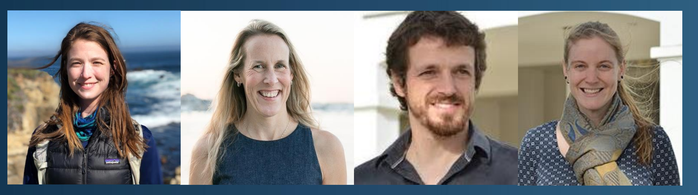


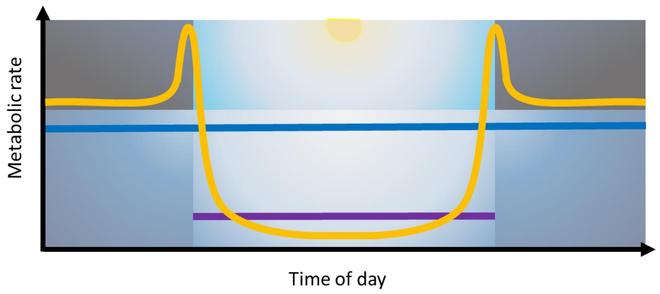
## Background

### Diel Vertical Migration (DVM)

Common pattern where individuals spend the night in surface waters and retreat to depth during the daytime.

Estimated to be responsible for 15 to 40% of the total organic carbon and nitrogen export from surface to the mesopelagic (Steinberg *et al.*, 2000; Steinberg *et al.*, 2002; Bianchi and Mislan, 2016).

Physiological consequences of DVM poorly studied beyond investigations of speed and the energetic cost of swimming. Many other processes are likely influenced, such as fuel use, energetic trade-offs, underlying diel (circadian) rhythms and antioxidant responses.

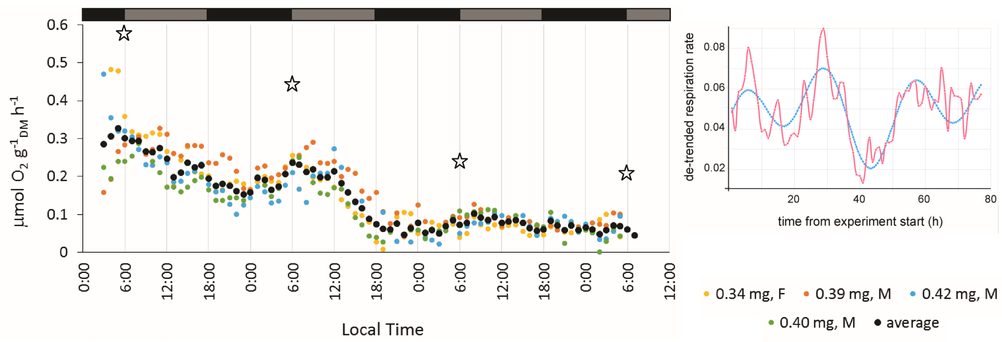



### Biogeochemical estimates and assumptions about respiration for DVM organisms.

- Metabolic rates are averaged over the full 24 h period (blue line).
- Temperature, and sometimes oxygen, coefficients are applied to characterize environmental effects of physiology (purple line).
- Although not included in models it is hypothesized that there are peaks in respiration rate associated with migration and a theorized lower metabolism during the daytime while animals are non-feeding at depth (yellow line).

**Physiological drivers of deviation from basal metabolism:**
Surface: temperature **(+)** specific dynamic action of feeding **(+)**
Depth: temperature **(-)** circadian rhythm linked to low food **(-)**
Migration: swimming effort **(++++)**

## Results







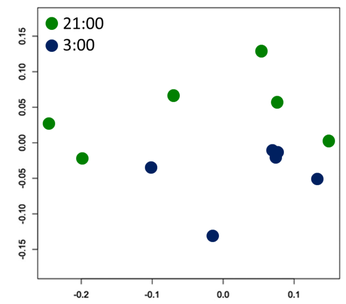



### Circadian changes in respiration

- Respiration rate of *Pleuromamma xiphias* showed a strong circadian rhythm in the absence of a light cue (on top of an overall trend of decreasing metabolic rate).
- Peak metabolism was at ~6:00, which corresponded with local sunrise (6:23), while lowest metabolism was documented at 0:00.
- Daytime rates were higher than those published for the same species previously.
  50(M) and 116(F) µmol $O_2$ $g^{-1}_{DM}$ $h^{-1}$ for adult (Teuber et al 2013)
  139 µmol $O_2$ $g^{-1}_{DM}$ $h^{-1}$ (Steinberg et al 2000, assuming RQ 0.87)

### Changes in proteome

- 1653 proteins identified, 11% differentially abundant between time points.
- No significant difference in full proteome profile between time points (ANOSIM; R = 0.193, p = 0.076) although some clustering.
- Differential abundance analysis revealed marked physiological signatures suggestive of changing metabolic processes, respiration and antioxidant enzyme activity.




- The proteome of the copepods that had recently arrived at the surface after migrating up from depth (21:00 time point) showed evidence of increased oxygen transport, reproductive effort and bioluminescence.
- Later in the evening (03:00) there was a higher abundance of a number of metabolic proteins, consistent with the carbohydrate, lipid and general oxidative metabolism.

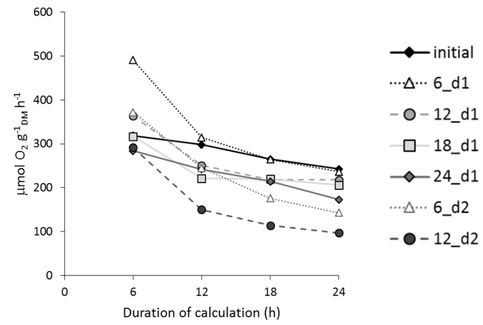
### Development of transcriptome for future work

- 458,857 Trinity 'genes' with 94.08% mapping success. N50 of 469 bp, E90N50 of 980. BUSCO similar to model invertebrates but with higher duplication C: 89.3% [C:73.1%, D: 16.2%], F: 9.0%, M 1.7% (978 genes).

## Biogeochemical Implications

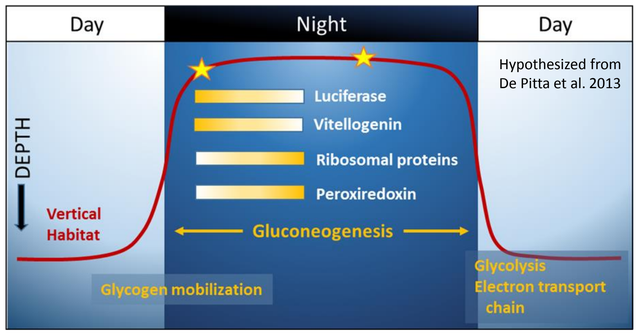
### Respiratory Flux Implications

Classical respiration calculations (24 h) for *P. xiphias* can underestimate daytime respiration by ~6-24%.




Migrators of the genus Pleuromamma make up ~10% of the night time biomass (<200 µm size fraction) at BATS, with the species *P. xiphias* making up ~50% of the Pleuromamma biomass (Steinberg et al., 2000).

In the literature, although there is variation, there seems to be a consistent trend of increased respiration near dawn and dusk (Häfker *et al.*, 2017; Pavlova 1994).




### Proteome Implications

Proteomics analysis suggest diel changes in physiology that could influence when and where nitrogen and other dissolved organic compounds are excreted.

These pathways may respond differently to various environmental factors (i.e. food, light, temperature). Predictive understanding will require a detailed analysis.

## Methods

**Animal Sampling:** Respiration individuals were collected May 2017 via small boat with a Reeve net from 0-150 mwo between 21:00-23:00. Transcriptomics and proteomics individuals were collected April 2016 from the R/V Atlantic Explorer with a Reeve net from 0-180 mwo at 20:30 and 2:45. *Pleuromamma xiphias* were removed from the tow, identified via microscope and flash frozen within 35 minutes of capture.

**Respiration:** Copepods were allowed 1 h for acclimatization in the dark in 1-L 0.2-micon fsw. Copepods were individually placed in 60 mL BOD bottles of 0.2 micron fsw with a Pyrosciences Firesting optical oxygen spot sensing system (as in Maas et al. 2018). The respiration rate of each individual (n=4), and one control was monitored continuously (every 2 min) for 3 days. At the end of the experiment the animals were checked for swimming then dried and weighed. The respiration rate was calculated using a sliding window approach of 2 h. Rhythmicity was evaluated with BioDare2 and significance tested with R-package RAIN (Zielinski *et al.*, 2014; Thaben and Westermark, 2014).


**Transcriptome generation:** Six copepods (M&F from both time points) were pooled and RNA extracted using Qiagen universal RNA mini kit. RNA was sequenced on an Illumina HiSeq2000 with 125-bp paired-end reads and Trinity assembly conducted following typical protocols (v.2.1.1; Grabherr *et al.*, 2011). Assembly was clustered to 95% similarity using CD-HIT EST (v.4.6.1; Li and Godzik, 2006). Annotation of the transcriptome with BLASTX versus nr (cutoff $1e^{-6}$) and Swiss-Prot (cutoff $1e^{-10}$). The assembly was reciprocally blasted against other crustacean and the fly assemblies and homologues for gene families of specific interest (i.e. circadian, molting, metabolic, stress response) were identified.

**Proteomics:** Proteins from female copepods (n=6 per time point) were extracted as per Timmins-Schiffman *et al.*, 2013. Mass spectrometery was performed at UW on a Q-Exactive mass spectrometer in triplicate in DDA mode. Peptide identifications and protein inferences for tandem mass spectra for each copepod were found through Comet (2016.01 rev. 2; Eng et al., 2015) searches and the Trans Proteomic Pipeline (TPP; Deutsch et al., 2010) against *P. xiphias* predicted protein sequences, translated from the assembled transcriptome. Abacus was used to coordinate protein inferences across replicates and calculate normalized spectral abundance factor (NSAF) (Fermin et al., 2011) for proteins with a minimum combined probability of at least 0.9. Individual copepod protein abundance profiles were compared using non-metric multidimensional scaling (NMDS) and ANOSIM using the vegan library in R v. 3.3.2 (Oksanen et al., 2016, RCoreTeam, 2016). Differential abundance of proteins was determined using QSpec (Choi et al., 2008).

## Acknowledgements

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## Data Accessibility

Genbank BioProject PRJNA352670. This Transcriptome Shotgun Assembly is GFCI00000000. Mass spectrometry proteomics data is shared by the ProteomeXchange Consortium via PRIDE with dataset identifier PXD005778.