Sink or swim: drivers of copepod migration behaviour (SinkSwim)

Final Report for

Marine Alliance Science Technology Scotland

Visiting Fellowship

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Loch Etive, Argyll, field site for the MASTS SinkSwim project

Summary

Diel vertical migration (DVM) of marine zooplankton is one of the most profound coordinated movements of organisms on the planet, and it contributes fundamentally to ecological interactions in both freshwater and marine habitats, and to global biogeochemical cycles. Yet, as this behaviour has been studied in more detail at higher spatiotemporal resolution, it has become apparent that behaviours of individuals do not necessarily reflect the apparent movements of the population. What controls variability in this behaviour at the individual level? To what extent do environmental factors such as light and physiological factors such as metabolism influence the migration behaviour of individuals? The primary aim of this fellowship was to answer these questions by deconstructing DVM patterns of individual organisms into component parts of activity, photobehaviour, and metabolism, using as a model one of the most abundant and ecologically important species within the mesozooplankton community, *Calanus finmarchicus*. This copepod has large lipid reserves and is the main trophic link between phytoplankton and higher trophic levels in the North Atlantic and Arctic. Through a series of field collections and laboratory experiments with the *C. finmarchicus* population of Loch Etive over the summer-winter seasonal gradient, we characterized behavioural phenotypes of shallow (50-5m) and deep (140-50m) CV individuals in activity and photopbehaviour assays, and measured metabolism through respiration rates and lipid reserves. We found diurnal phenotypes to increase as day-length decreased, and these day-active copepods had higher activity levels during their active phase than did nocturnal copepods. Photobehaviour was similar among behavioural phenotypes. Subsequent analysis of metabolism data will allow us to test whether these predict the observed activity patterns. We have secured additional funding to continue this work. Visiting Fellow Cohen gave two departmental seminars, and three presentations at U.K. scientific meetings. One manuscript has been submitted using techniques developed in this project, and submission of three additional manuscripts are expected in 2018-2019.

Background and Objectives

Diel vertical migrations (DVM) are central to zooplankton behaviour and structure their populations in time and space (Hays 2003, Brierley 2016). The migrations are primarily light driven (Cohen and Forward 2009, Ohman and Romagnan 2016) and recent evidence has shown that even exceptionally low levels of light from the sun (or the moon) at high latitudes are sufficient to maintain migrations (Last et al. 2016, Ludvigsen et al. 2018), testament that this behaviour is probably highly adaptive in predator avoidance (Gliwicz 1986). Net sampling and acoustics have revealed great variation in the extent of migrations, from a few metres to one kilometre (Osgood and Frost 1994, Ochoa at al. 2013) and a complexity of scattering layers in acoustic data hints at species-specific migrations or ontogentic variation in migration speed and DVM depth, and even individual variability within a species/stage (Cottier et al. 2006, Falkenhaug et al. 1997, Klevjer et al. 2012). Indeed, when individual animals have been assessed in laboratory studies, great variation between species is realized – for example in the life-stages of copepods (Båtnes et al. 2015) and between the sexes in krill (Tarling 2003). Understanding migration behaviour is also important in understanding the oceans biogeochemical cycles (Steinberg and Landry 2017) and the prey field for foraging fish and other planktivores under climate change (Johnsen 2014).

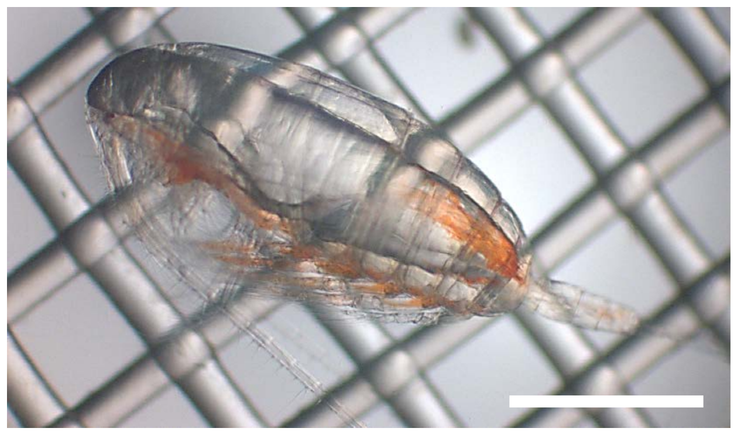


Figure 1. *Calanus finmarchicus*. The fifth copepodite stage of this species (CV stage) overwinters at depth (50-150 m) in the Bonawe Deep of Loch Etive. Stored lipid is visible here as the greyish deposit filling most of the body. Scale bar is 1 mm.

This visiting fellowship therefore aimed to deconstruct DVM patterns of individual organisms into their component parts, focusing on one of the most abundant and important species within the mesozooplankton community, *Calanus finmarchicus.* This copepod, with its large lipid reserves, constitutes the main trophic link between microplankton through to higher trophic levels such as fish, seals, whales and birds, and is virtually cosmopolitan in its distribution. It also has become a model copepod for molecular studies with a *de novo* assembly of its transcriptome (Lenz et al. 2014). It is however hard to distinguish from its congeners *C. glacialis* and *C. helgolandicus*, where populations overlap, and where the common morphological discriminator, size, is unreliable. We have focused our research effort on a near shore population of *C. finmarchicus* in Loch Etive (station RE5, Bonawe Deep) recently genetically screened as pure *C. finmarchicus* (pers. comm. J. Søreide) and already well-characterized in terms of life-cycle and lipid energetics (Clark et al. 2012) and population DVM using acoustics and clock gene transcriptional activity (Häfker et al. 2017).

Through two recent pilot studies (Dr Last in Loch Etive, Häfker et al. 2017; Drs Last and Cohen in the Arctic Ocean) we have shown that *Calanus* copepods demonstrate measurable behavioural and physiological variations within populations. In order to determine which environmental and/or biological processes contribute to conserving such variation the following research objects were addressed in this Visiting Fellowship:

Obj. 1) A large scale behavioural screening programme of wild-collected *C. finmarchicus* over a critical lifecycle period (July to December 2017) coupled with environmental measurements (e.g. water column characteristics, light, oxygen); identification of dominant phenotypes in the activity patterns expressed by these wild copepods as: daily (diurnal), nightly (nocturnal), and randomly (arrhythmic);

Obj. 2) Determine thresholds for light-induced swimming behaviour of the groups identified in Obj. 1 using photo-responsive tests in a laboratory apparatus that simulates the underwater light field;

Obj. 3) Determine the metabolic status (respiration rate, lipid sac volume) of the groups identified in Obj. 1 and;

Obj. 4) Incorporation of behavioural/metabolic data into the "Coltrane" life-history model.

Activities and Achievements

Towards objectives 1-3, we made monthly measurements of water column hydrography by CTD casts at RE5 from July – December 2017 (Figure 2). In the upper 50m, the water column showed a seasonal progression of decreasing temperature, salinity, Chl-a, DO, and light. Apart from seasonally decreasing DO, the water mass below 50m was stable with salinity of ~28psu and temperature of ~11.5 °C. Additional optical sampling of the water column was consistent with the stratified and stable water column (Figure 3).

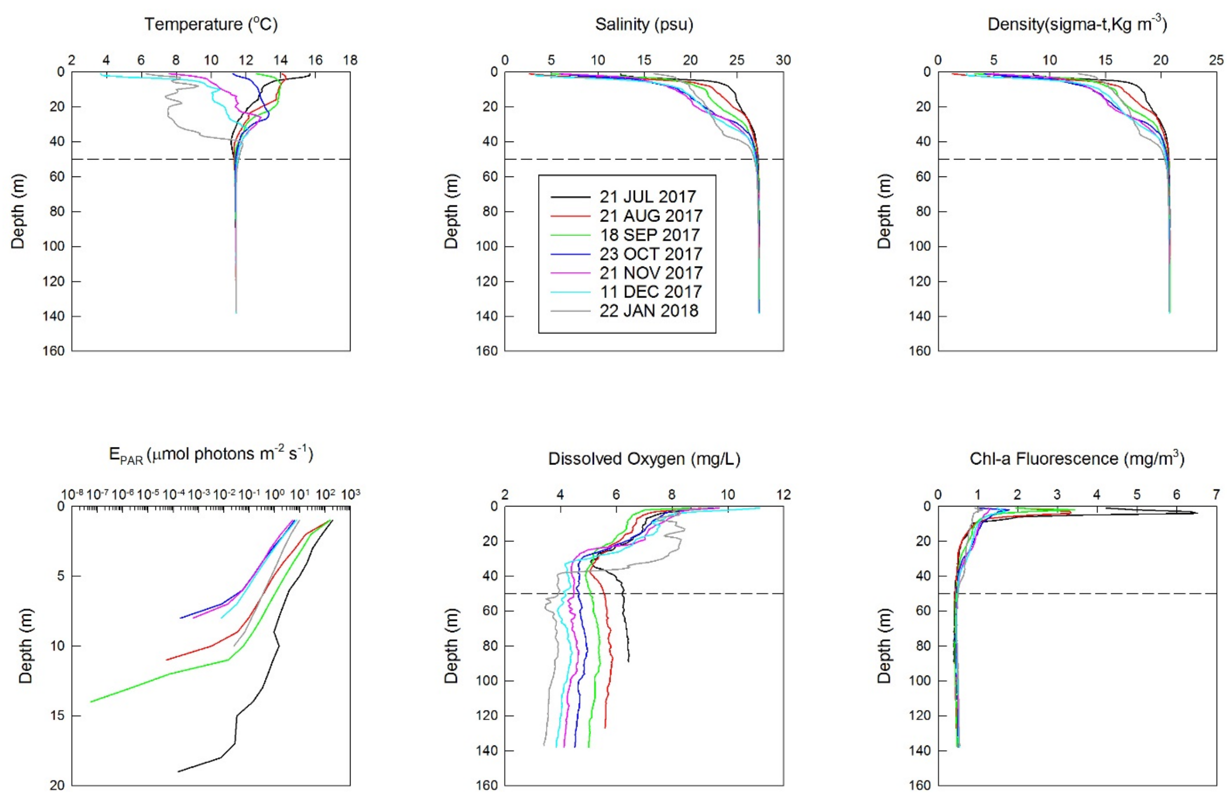


Figure 2. Monthly CTD profiles in Loch Etive at RE5. We used a SeaBird SBE19 CTD with chl-a, DO, and PAR sensors. Dashed lines indicate the 50m depth. While the visiting fellowship ended in December 2017, we have continued sampling at RE5 and data for January 2018 are shown.

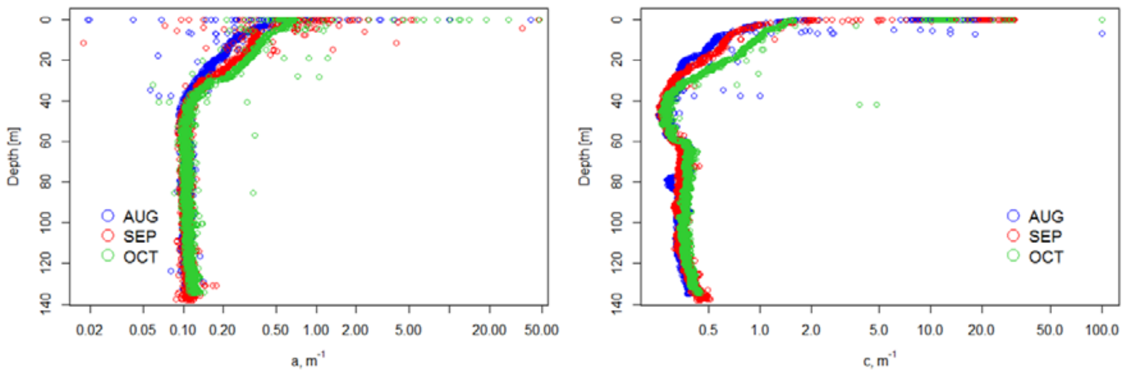


Figure 3. Seasonal optics profiles with a WetLabs AC-S *in situ* spectrophotometer. Absorption (*a*) and beam attenuation (*c*) profiles are consistent with a stratified water column over the study period.

In conjunction with monthly CTD and optics profiles, we collected *C. finmarchicus* from 140-50 m (deep) and 50-10m (shallow) using a 0.5m close-open-close plankton net of 200 µm mesh. Samples were diluted with water collected from 50m depth at RE5 and maintained in a dark cool box for transport (<2 hour) to the laboratory for behaviour and metabolism experiments. For behaviour experiments, copepods were held at 11.5 °C in 3ml test tubes filled with 28 psu seawater collected from RE5, and dim ambient photoperiod. Test tubes were maintained within Locomotor Activity Monitors (LAMs, Trikinetics) which recorded bouts of swimming activity. For each month of the study period, 192 copepods were assayed (96 shallow, 96 deep). After ~6 days, activity rhythms for each individual copepod (e.g., Figure 4) were visually inspected for behavioural phenotype (nocturnal, diurnal, arrhythmic), and assigned a “rhythm index” value using autocorrelation. A subset of individuals expressing diurnal and nocturnal phenotypes were selected for metabolism experiments. Routine metabolic rate was measured in a microrespirometer (Loligo) for diurnal copepods during the day with dim light (July), an additional treatment of nocturnal copepods in darkness during the night (August), and an additional treatment of diurnal/night and nocturnal/day in later months (September – December). Following these experiment all copepods were photographed for subsequent measurement of body length and lipid sac volume, and then frozen at -80 °C.

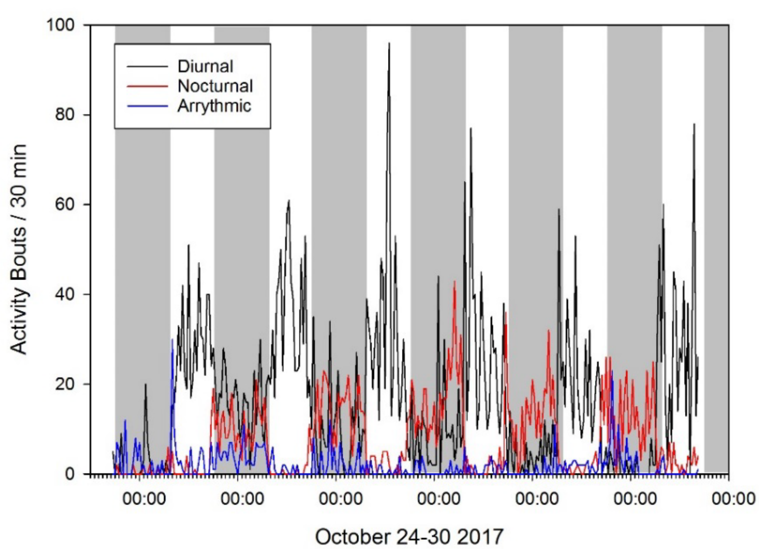


Figure 4. Representative copepod activity rhythms showing the three behavioural phenotypes. Shading represents the time of night (lights off).

As day-length decreased over the course of the experiments (July – December 2017) we observed a decrease in the proportion of copepods with nocturnal phenotypes and an increase in diurnal phenotypes (Figure 5). For shallow-collected copepods this occurred only during July-August, whereas in deep-collected copepods diurnal phenotypes progressively reached ~60% of the population. We are in the process of measuring body size and lipid area from photographs of copepods, which will be analyzed with these behavioural data to determine if energy storage predicts behavioural phenotype. Interestingly, we continued these experiments in January 2018 when day-length increases, and the proportion of copepods expressing diurnal phenotypes seems to be decreasing in the deep samples.

The time series of activity for each copepod was also analyzed for activity level by summing the number of activity bouts and relating these to the rhythm index (Figure 6). Diurnal copepods had daytime activity levels that increased with rhythm index, while nocturnal copepods had nighttime activity levels that increased with rhythm index. Interestingly, diurnal copepods had higher levels of activity during the day than did nocturnal copepods at night.

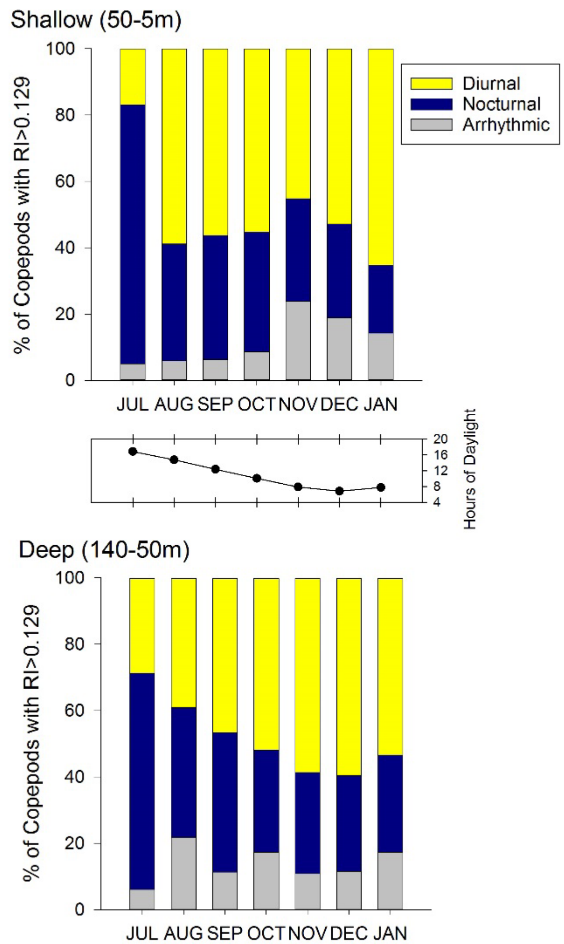
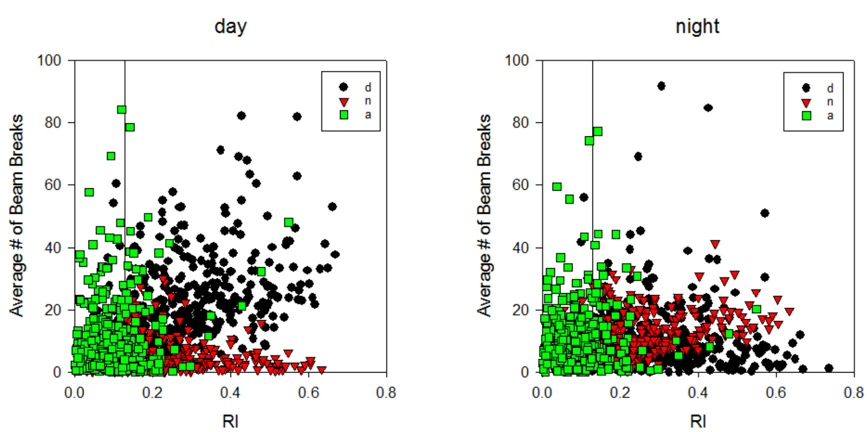


Figure 5. Proportion of copepods in shallow (upper panel) and deep (lower panel) collections exhibiting diurnal, nocturnal, and arrhythmic phenotypes.

Figure 6. Activity levels of *C. finmarchicus* during behavioural experiments as a function of rhythm index (RI). "day" (left panel) shows activity for individual copepods summed over the light phase, while "night" (right panel) shows activity summed over the night phase. Higher RI equals stronger cyclic activity in the rhythm time series for that individual.



For all months, respiration rates were consistently lower in darkness at night, irrespective of the behavioural phenotype (Figure 7). Final analysis and interpretation of these data will require correction of rates by body size determined from copepod photographs along with a separate experiment in which we derived the relationship between dry weight, carbon, and body size for shallow- and deep-collected *C. finmarchicus* from RE5. Two SAMS undergraduate students (Maxwell Bennie and Jack Waldie) are assisting with body size measurements using the methods of Vogedes et al. (2010).

We assessed photosensitivity of copepods in a horizontal trough as opposed to a simulated angular light field in order to increase sample size of each behavioural phenotype. We ran these experiments twice (September, October). While data in September suggested some elevated positive phototaxis in arrhythmic copepods (Figure 8), we discontinued phototaxis experiments to put this research effort elsewhere.

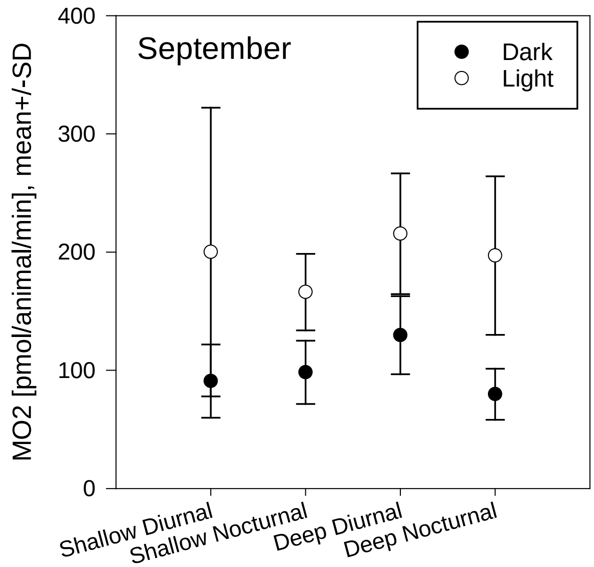


Figure 7. September respiration rates for shallow and deep *C. finmarchicus* with diurnal and nocturnal behavioural phenotypes tested under low light during the day (open circles), and in darkness at night (closed circles).

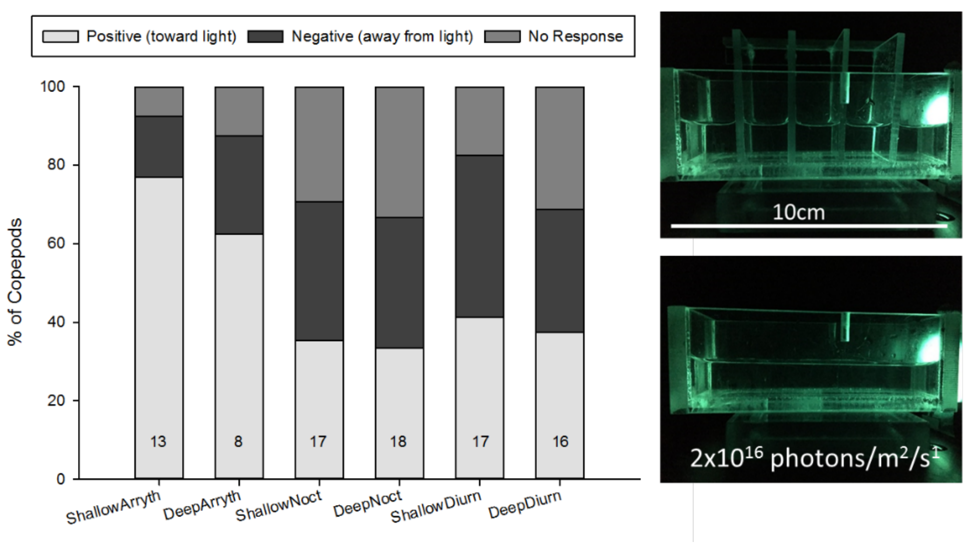


Figure 8. Phototaxis assay. Using a horizontal trough with removable partitions, we collected data on *C. finmarchicus* swimming toward or away from a stimulus light at a set intensity (2x10^{16} photons m^{-2} s^{-1}).

Working with Prof. David Pond (SAMS), we conducted a related series of experiments on *C. finmarchicus* metabolism and buoyancy. Experiments consisted of osmoregulation, respiration, and swimming behaviour under a range of salinities (24-34 psu), temperatures (5 - 12.5 °C) and hydrostatic pressures (1 and 10 bar), along with two transects of zooplankton and hydrography along Loch Etive into the Firth of Lorn. While these data await further analysis, preliminary observations suggest that for *C. finmarchicus* living at 28 psu and 11.5 °C in Loch Etive, small changes in salinity and temperature affect buoyancy of individual copepods. Respiration and osmoregulation experiments suggest potential metabolic costs of hyperosmotic regulation at lower salinities. Further, respiration data suggest copepods may be awaking from diapause under laboratory conditions, which has implications for interpreting the behavioural experiments described above.

Collaborating with Dr. Phil Anderson and Shane Rodwell (SAMS), Cohen performed two field calibrations in Loch Etive of a light sensor that is being developed at SAMS.

We held two formal project meetings (among other informal discussions) to update collaborators on progress and discuss research plans. One meeting was at the Univ. of Strathclyde (Faculty: Neil Banas, David McKee, Douglas Speirs; Graduate student: Euan McRae; Post-doc: Ina Lefering) and the other was at SAMS (Faculty: Finlo Cottier; Post-doc: Laura Hobbs).

Last and Cohen met with chronobiolgists at the Univ. of Glasgow (Faculty: Barbara Helm; Graduate student: Nicholas Huffeldt) to discuss mutual research interests and potential collaboration.

Cohen presented four talks in seminar series and scientific meetings while supported by MASTS, and Last/Cohen presented an e-poster on the visiting fellowship research:

> Cohen, J.H. (2017) Visual Ecology and Behaviour of Marine Zooplankton: from the deep sea, to the Arctic, to Loch Etive. Seminar Series - Scottish Association for Marine Science, Oban, Scotland UK.
>
> Cohen, J.H. (2017) Light as an ecological factor for marine zooplankton: diel vertical migration. Department of Biology Seminar Series, University of St Andrews, St Andrews, Scotland UK.
>
> Cohen, J.H. (2017) Photic Ecology in the Epipelagic High Arctic Polar Night: A Deep Sea Analogue? Oral presentation. MASTS Annual Meeting, Glasgow, UK.
>
> Cohen, J.H., G. Johnsen, J. Berge, M.A. Moline, K.S. Last (2017) Light climate of the high Arctic Polar Night and its implications for biology. Oral presentation. NERC Arctic Science Meeting, Oban, UK.
>
> Last, K.S. and J.H. Cohen (2017) Sink or swim: drivers of copepod migration behaviour. E-poster. MASTS Annual Meeting, Glasgow, UK.

Future Prospects

We are continuing the monthly collection of *C. finmarchicus* from RE5, along with behavioural and respiration experiments. PI Last (with Cohen as international collaborator) was recently awarded NERC funding for a proposal [Chronobiology of changing Arctic Sea Ecosystems (CHASE)] that builds upon the findings of this visiting fellowship. Through that award we will unravel the molecular mechanisms controlling the observed behavioural phenotypes.

We are working with collaborators at Univ. of Strathclyde (Banas, Hobbs) to parameterize a population model for Loch Etive *Calanus* using behavioral phenotypes. The modeling work is ongoing and requires morphometric and normalized respiration data to be finalized.

Cohen was invited to be an international collaborator on a NERC proposal being led by Dr Bhavani Narayanaswamy (SAMS) focusing on microplastics in the marine environment. Their mutual interest in this topic emerged during discussions throughout the visiting fellowship.

Cohen and D. Pond will be submitting a proposal in 2018 to the Human Frontiers Science Program to continue investigating biophysical aspects of buoyancy in *Calanus*.

Manuscripts directly resulting from research and/or analytical tools made possible by the visiting fellowship and acknowledging MASTS will include:

Last et al. – seasonality in *C. finmarchicus* behavioural phenotypes

Cohen et al. – buoancy in *C. finmarchicus*

Daase, M., K. Kosobokova, K.S. Last, J.H. Cohen, M. Hatlebakk, and J.E. Søreide (submitted, *Marine Ecology Progress Series*) New insights into life history traits of male *Calanus* spp. in the Arctic.

Cohen, J.H., K.S. Last, C.L. Charpentier, G. Johnsen, and J. Berge (in preparation for *PLOS Biology*) Extreme Arctic light influences endogenous visual sensitivity in krill.

Acknowledgements

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