Project: AFI 7/06

Gene function in Antarctic krill: determining the role of clock-genes in synchronised behavioural patterns

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Introduction

Antarctic krill (*Euphausia superba*) are the key species in the Southern Ocean, acting as both the main consumer of phytoplankton and the principal food source of many of the animals that define that ecosystem. This project examines rhythmic behaviour patterns in this species and the functioning of the genes that underlie these behaviours. The two behavioural rhythms being investigated are diel vertical migration and the moult cycle.

Vertical migration occurs in many planktonic species, with the animals moving towards the surface at night to feed on phytoplankton and into deeper water during the daytime to avoid visually-guided predators. The extent of vertical migration in Antarctic krill is unclear (Godlewska, 1996). Polar animals (van Oort et al., 2005) often show differences in the extent of their circadian activity profiles dependant on the latitude from which the animals are taken. Animals from higher latitudes experience shorter nights during the summer and diel variations in activity are not normally apparent. The same species at lower latitudes experience a significant period of dark and usually show circadian variations in activity.

Little is known about the moult cycle in Antarctic krill, but it is thought to be around 20 days long, varying with the temperature. This project will be the first to attempt to describe the endogenous clock mechanisms that are presumed to underlie both the moult cycle and vertical migration behaviour.

Aims

The association between circadian rhythms and vertical migration will be investigated by both behavioural and molecular experiments. Vertical migration behaviour patterns of individual krill will be recorded in an activity monitor, based on the design of apparatus used in the study of northern krill (*Meganyctiphanes norvegica*) (Velsch & Champalbert, 1994). Video analysis of krill behaviour in aquaria will also be carried out.

Molecular biology techniques will be used to isolate the canonical circadian clock genes and to investigate the spatial and temporal expression of these genes. It is hoped to also isolate the genes associated with the moult cycle using microarray technology.

Field Work

The animals used for this project were caught during the Discovery 2010 cruise JR177 aboard the RRV *James Clark Ross*. Krill from two areas were used, one from around 60° South from the vicinity of the South Orkney Islands, and the second from North-West of South Georgia at around 52° South (Figure 1).

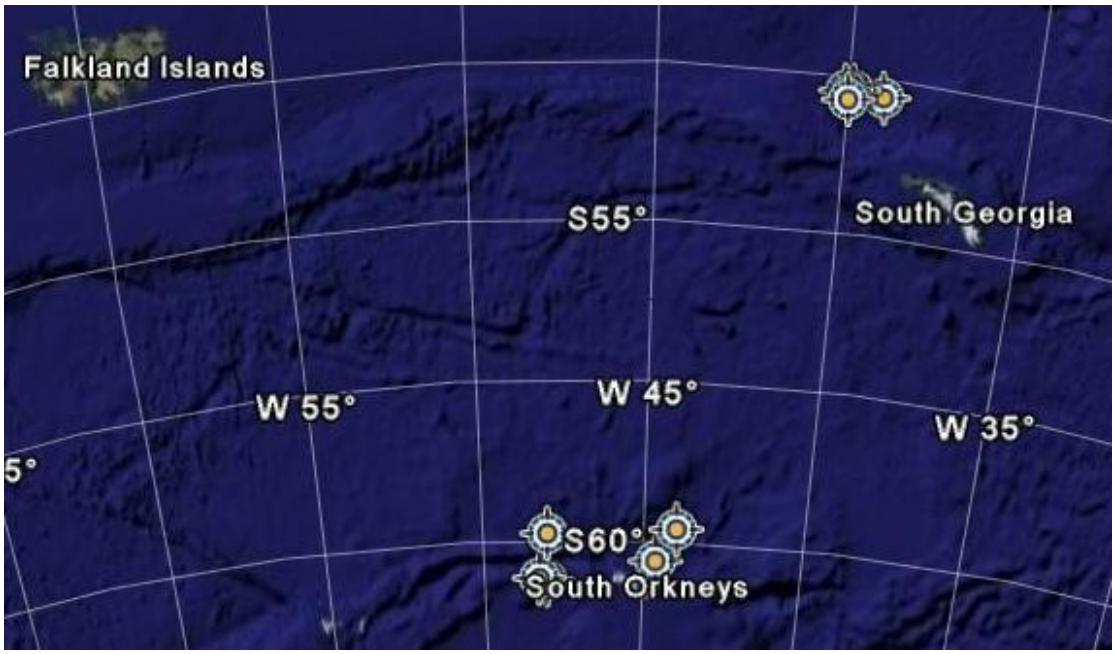

Figure 1: Sampling stations (yellow bullets) from which krill were taken for use in this project.

All of the krill were taken by target fishing mainly using the RMT8 net (Figure 2), based largely on observations of krill swarms using the EK60 echo sounder. The nets were towed for only a short time and, after hauling, the krill were transferred as quickly as possible to the cold room for sorting.

Figure 2: RMT8 net being deployed.

Activity monitor. The activity monitor is a purpose-built apparatus comprising 12 vertical tubes, each containing 5 l of seawater, retained upright in a light-tight box. Each tube has infra-red barriers 5 cm from the top and bottom of the tube and the output from these barriers is recorded continuously. The lighting within the apparatus is adjusted to that experienced by the krill at normal daytime depths and the temperature in the cool-room kept at the level of the seawater supply. The movements of the krill over a 10-day period were recorded. The krill spend the first 5 days under a light/dark cycle matching that of the region from which they were taken in order to record their vertical migration patterns under normal lighting conditions. This is followed by 5 days in total darkness to see whether the activity pattern is maintained in total darkness, indicating control by an endogenous circadian rhythm. After 10 days, the animals were measured, the moult stages assessed, and the heads fixed in paraformaldehyde for 2 hours. The brain and optic tracts were dissected from each specimen and stored in methanol at -80° for later analysis. Three runs were carried out, using animals from 60.44° S, 59.66° S and 52.75° S. When not required for krill, the activity monitor was used to assess its suitability for monitoring copepod activity. Specimens of *Rhincalanus gigas* were used, either singly in each tube or in groups over a 2-day period under a light/dark cycle.

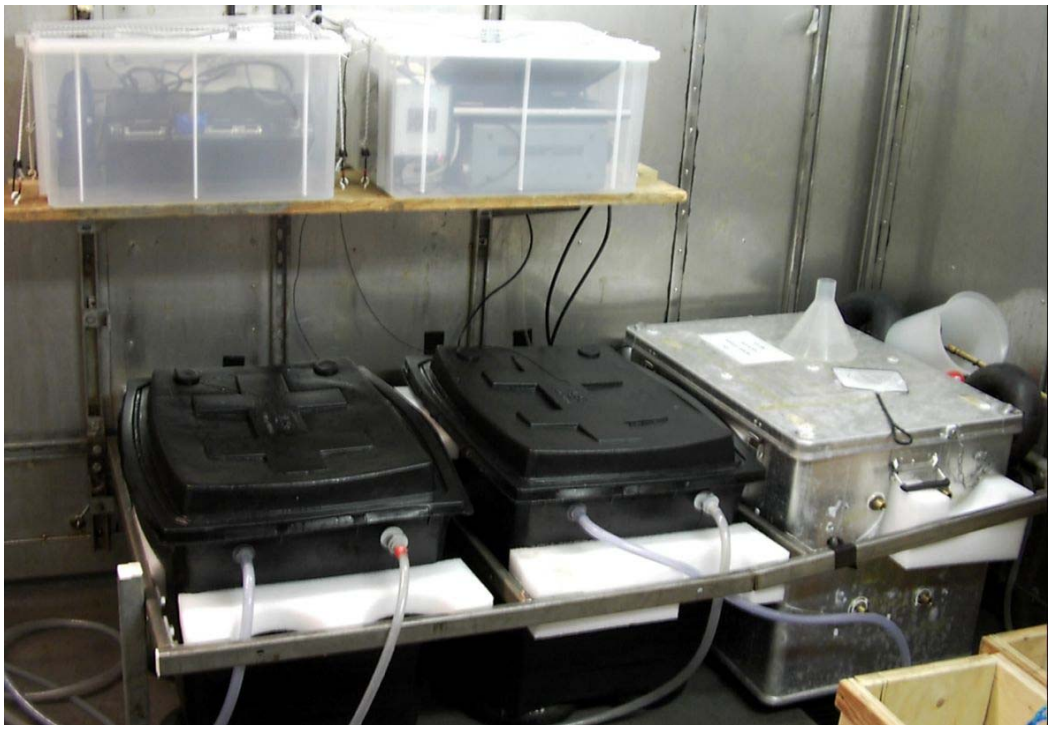

Figure 3: Cool room containing two entrainment tanks (black) and the activity monitor.

Video analysis. Due to a lack of space in the cold room, it was not possible to attempt any video analysis during cruise JR177.

Molecular biology. A sample of krill (usually 30 animals) was taken from each net and stored in *RNAlater* (a commercially available solution for long term storage of tissues without deterioration of their RNA) to be used for the isolation of canonical clock genes. These were left at 3° C overnight and then stored at -20° C for subsequent analysis. A sample of krill (usually 10 animals) was also taken from each net and the heads fixed in paraformaldehyde (PFA) for 2 hours and dissected as above for later analysis using in-situ hybridization and immuno-cytochemistry. Where abundant krill were taken in the net, up to 200 animals were rapidly frozen in a methanol freezing mixture at -80° and then stored in the -80° freezer for further clock gene studies. A total of 1,820 krill were preserved from 16 net hauls for clock gene identification.

Figure 4: Antarctic krill *Euphausia superba*.

An entrainment tank containing around 250 l of flowing seawater was used for studying the spatial and temporal expression of clock genes. The animals were maintained under a controlled light regime that mimicked that normally experienced by swarms at the latitude from which they were taken. After 2 days entrainment, a sample of krill (usually 10 animals) was taken at 3-hourly intervals over a period of 48 hours; these were either preserved in *RNAlater* or were frozen directly at -80° as detailed above. During the next 24 hours samples were taken at 6-hourly intervals and fixed in PFA as above. The lights were switched off and on the 2nd, 3rd and 4th day in total darkness, the above sampling regime was repeated. The circadian entrainment experiment was carried out twice, using animals from nets at latitudes of 60° S and 52° S. A total of 1,090 preserved specimens were returned to the UK for subsequent analysis.

A second entrainment tank was used to study the moult cycle genes. Up to 20 animals were removed each day at the same time, measured, moult staged and the heads preserved in *RNAlater* for subsequent use in the microarray studies to isolate the genes involved in the moult cycle. A total of 590 krill in RNAlater were returned to the UK at -20° for further analysis.

Preliminary results

The activity monitor did not reveal any obvious sign of vertical migration within the tubes, although more detailed analysis will be necessary before any conclusions can be drawn. All of the other experiments rely on molecular analysis at the home laboratories, either at the University of Leicester or at BAS, Cambridge. However, all of the experiments were completed and sufficient specimens preserved so it is anticipated that at least some of the molecular questions will be answered in the fullness of time.

Acknowledgements

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References

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