SCAR Prince of Asturias Fellow Final Report

The Diversity and Trophodynamics of Mixotrophic Protists in Antarctic Coastal Waters

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Brief introduction & aims of the project

Mixotrophy is the ability of organisms to form body substance both autotrophically and heterotrophically. Despite the inherent physiological and ecological implications of this nutritional strategy, there is very little known about the abundance, distribution and importance of mixotrophy in the marine environment, and virtually no information exists from Antarctic waters. Evidence suggests that mixotrophs may play a vital role in "extreme" polar aquatic environments but because mixotrophic behaviour cannot be determined by taxonomic affinity alone, complementary grazing, growth and production studies are needed to determine the contribution of mixotrophs to the carbon flow in aquatic food webs.

This study aimed to determine the abundance, temporal, taxonomic and environmental distribution of mixotrophs in coastal waters off Davis Station and a saline lake in the Vestfold Hills, Eastern Antarctica. Once analysis is complete, the results will offer insight into the relative, seasonal contribution of mixotrophs to the structure and function of coastal polar marine and lacustrine ecosystems and add to growing evidence that mixotrophy promotes the survival of protists in polar aquatic environments.

Period of Antarctic field & laboratory research: December 2003 – December 2004
Laboratory analyses at Australian Antarctic Division Hobart: 10 January - 06 March 2005

Work performed within context of original fellowship application

The study was conducted during an 11 month period at the Australian Davis Station, Vestfold Hills, eastern Antarctica. Weekly sampling trips to one of two focal study sites: O'Gorman Rocks, a coastal site approximately 1.5 km offshore from Davis station and Ace Lake, a meromictic, saline lake of marine origin known to support mixotrophic phytoflagellates (*Pyramimonas gelidicola* and *Cryptomonas* sp.), were carried out. A second marine site, Bayly Bay, a meromictic embayment approximately 15 km from Davis in which a population of potentially mixotrophic cryptophytes existed, was also visited on a number of occasions. Water was collected from a range of depths, as well as sea-ice cores. On each occasion the following was conducted/determined: analysis of water chemistry (total dissolved and particulate organic carbon, NO_3, NO_2, NH_4, PO_4); physical profiling of water column (temperature, salinity, oxygen, PAR, pH); taxonomic classification of the microbial community; abundance & biomass of the bacterial, protozoan and metazoan components; primary production; bacterial production, particle (fluorescently labelled microspheres and bacteria) uptake rates of both mixotrophic and heterotrophic components; osmotrophic uptake of carbon substrates (fluorescently labelled dextrans and growth on carbon substrates); isolation and culturing of protists; growth rates of cultured organisms.

Additional research conducted

1. All prasinophytes share a similar ultrastructure to *P. gelidicola*, implying that mixotrophy may be common in the genus but is "switched off" unless conditions are sufficiently extreme. Therefore, work conducted during the study period additionally aimed to test whether most, if not all, *Pyramimonas* species can and do indulge in phagotrophy when conditions are "right". Ideally such an investigation will extend beyond the Antarctic but the opportunity was taken to collect

Antarctic marine and lacustrine pyramimonads for electron microscopic analysis of their ultrastructure and culture clonal material for sequencing of the 18s gene. If phagotrophy in *P. gelidicola* and other prasinophytes can be proved ultrastructurally and phylogenetically, the phylogeny of green algal evolution would potentially require revision.
2. Further genetic sequencing is underway for the mixotrophic cryptophyte (putative) species collected from our study sites. It is speculated that this is a new species but phylogenetic analyses are underway to confirm this.
3. During screening of Ace Lake samples collected during this study by scientists at the University of Tasmania, a signature of the toxic dinoflagellate *Pfisteria* was detected. Further work is underway to confirm what could prove to be a significant finding.
4. Due to logistic constraints, ship passage to Davis Station was restricted to joining a two month marine science voyage. During this period extensive marine surveys of water chemistry, Chl *a*, plankton abundance, fish abundance and mega-faunal abundance were carried out. The opportunity was also taken by the P.I. to collect deep sea sediment samples for analysis of microbial diversity and abundance.

Significant findings to date

The data are still being analysed. However, the significant findings to date include:

1. The mixotrophic phytoflagellates, *Pyramimonas gelidicola* and the cryptophyte, which dominate in Ace Lake and in the sea-ice at certain times of year, employ a gradient of nutritional strategies from autotrophy, through osmotrophy, to phagotrophy. The algae feed over a wide size spectrum from 4×10^3 daltons to 1 μm fluorescently labelled bacterial prey. In previous years, up to 47% of the *P. gelidicola* population in Ace Lake was phagotrophic. However, in 2004 this figure was only 5%. Instead, *P. gelidicola* was shown to be actively taking up dissolved carbon substrates (osmotrophy). The proportion of algal cells from both species engaging in osmotrophy was highest during the period of total winter darkness and again when ice thickness was maximal and PAR levels in the water column low. Cultured algae (both *P. gelidicola* and cryptophytes) grown in the laboratory with glucose as a carbon source grew at higher rates than those without.
2. Protist biomass was extremely low in the winter water column at the marine study sites. However, there was an abundant heterotrophic/mixotrophic community in the sea-ice all year. Both *P. gelidicola* and cryptophytes dominated the phytoflagellate community at different times. Both were observed graze bacteria in nearby lakes, but neither grazed in the sea-ice. They did, however, engage in osmotrophic behaviour. A further potentially mixotrophic Euglenid was present most of the year. Only in the summer did autotrophic diatoms become dominant.
3. Similarly, known mixotrophs dominated the protist biomass in Ace Lake.
4. As mentioned above, mixotrophic behaviour in both *P. gelidicola* and the cryptophyte would seem on initial inspection to be linked to environmental parameters such as PAR and ice thickness. However, the marked inter-annual differences in the dominant nutritional strategy adopted by the algae warrants significant effort to analyse existing long term data sets and determine which environmental cues influence the nutritional strategy adopted. This work is underway.

Expected outcomes

Papers nearing submission

1. **Bell, E.M.** & Davidson, A.T. Heterotrophic and mixotrophic growth of two Antarctic phytoflagellates, *Pyramimonas gelidicola* (Prasinophyceae) and a cryptophyte. *In prep*.
2. **Bell, E.M.**, Pearce, I. & Davidson, A.T. Growth and grazing of an Antarctic sea-ice Euglenid. *In prep*.

3. Pearce, I., **Bell, E.M.** & Davidson, A.T. Seasonal changes in the species composition and abundance of marine microbes at an Antarctic coastal site. *In prep*.
4. Pearce, I., **Bell, E.M.** & Davidson, A.T. Seasonal changes in the concentration and metabolic activity of bacteria and viruses at an Antarctic coastal site. *In prep*.
5. McKenna, K., Moorhead, D., **Bell, E.M.**, Maden, N., Marshall, W.J. & Laybourn-Parry, J. Carbon cycling models in a saline Antarctic Lake: top-down versus bottom-up controls. *In prep*.

Papers anticipated by end 2006 (titles subject to change)

6. **Bell, E.M.,** Pearce, I. & Davidson, A.T. The seasonal dynamics of the sea-ice community at an Antarctic coastal site.
7. **Bell, E.M**., Belbin, L., Davidson, A.T. Environmental cues "switch on" the mixotrophic behaviour of the Antarctic phytoflagellate, *Pyramimonas gelidicola*.
8. **Bell, E.M**., Laybourn-Parry, J., Belbin, L. & Davidson, A.T. Inter-annual changes in the microbial community of an Antarctic saline lake.
9. **Bell, E.M.** & Rubino, F. The microbial abundance and diversity of Antarctic deep sea sediments.
10. **Bell, E.M**., Harlow, L., Van den Hoff, J., & Wright, S. Genetics and morphology of an Antarctic cryptophyte (species name to be added).
11. **Bell, E.M**., Van den Hoff, J. & Pearce, I. The distribution and ecology of the Antarctic cryptopyte, (species name to be added), in the lakes and off shore environment of the Vestfold Hills, eastern Antarctica
12. **Bell, E.M**., Harlow, L. & Davidson, A.T. The genetics and ultrastructure of the mixotrophic Antarctic phytoflagellate, *Pyramimonas gelidicola*.

Presentations given to date

- Invited speaker at Tjärno Marine Laboratory, Göteborg University, 3 June 2005: The nutritional versatility of the Antarctic phytoflagellate, *Pyramimonas gelidicola*.
- McKenna, K., Moorhead, D., **Bell, E.M.**, Maden, N., Marshall, W.J. & Laybourn-Parry, J. Carbon cycling models in a saline Antarctic Lake: top-down versus bottom-up controls. ASLO, 21-24 June 2005, Santiago, Spain.

Future presentations

- Oral and poster presentation of this project and Antarctic deep sea sediment results at the XXIX SCAR Open Science Conference, 12-20 July 2006, Hobart, Australia

Data archiving & culture collection

Once published, the data generated in the project will be entered onto the Australian Antarctic Division (AAD) metadata facility and made openly available to the scientific community and general public. An extensive culture collection of mixotrophic, autotrophic and heterotrophic Antarctic protists from the O'Gorman, Bayly Bay and Ace Lake study sites has been established at the AAD. This collection is available to all interested parties.

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