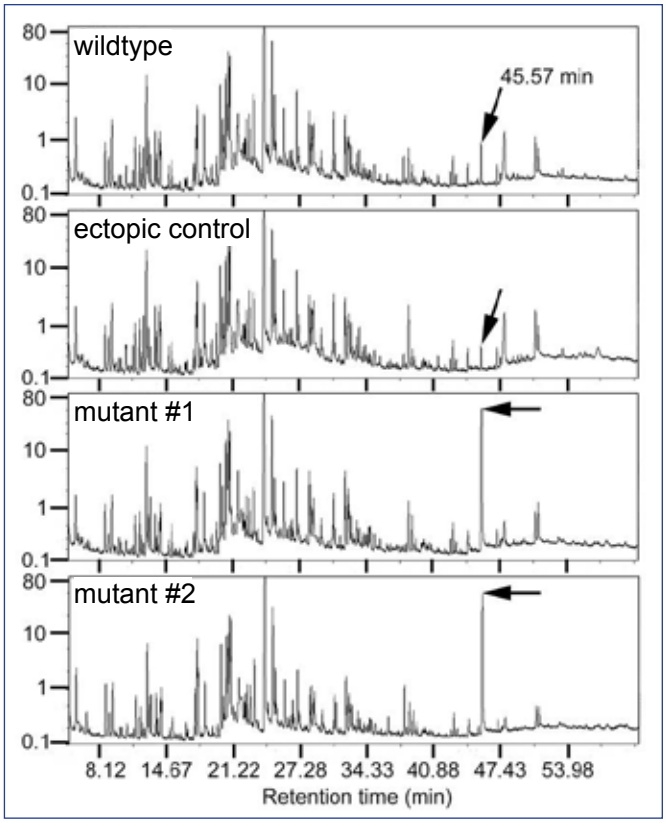

Figure 1. GC-MS total ion chromatograms (TIC) of polar extracts from *S. nodorum* strains harbouring Sch1 (wild-type and ectopic control) and also of mutant isolates (mutants #1 and #2).The y-axes are in a log-scale. The elution of RT4557 (alternariol) is marked with an arrow.

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Biography

Dr Peter Solomon completed his undergraduate degree and subsequent PhD studies at The University of Queensland investigating the role of molybdenum-containing enzymes in the photosynthetic bacterium *Rhodobacter capsulatus*. In 1998, he undertook a postdoctoral position at the Carlsberg Laboratory in Denmark investigating the nutritional basis of the tomato-*Cladosporium fulvum* interaction. In 2000, he moved to the Australian Centre for Necrotrophic Fungal Pathogens located at Murdoch University in Perth to further investigate fungal-plant interactions using the *Stagonospora nodorum*-wheat interaction. In 2008, Dr Solomon accepted a laboratory leader position in the Research School of Biology at The Australian National University, where he continues to investigate the molecular basis of necrotrophic fungal wheat diseases.

Microbial communities in Antarctic lakes: Entirely new perspectives from metagenomics and metaproteomics

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Driven by advances in DNA sequencing technologies, an astounding amount of data is being generated from genetic material sourced directly from the environment, and this exponential growth of data is set to continue. By surmounting the challenges of working with such vast datasets, a whole new level of understanding is being gained about microbial diversity, microbial evolution and whole ecosystem function. For precious, pristine and logistically difficult to obtain Antarctic samples, metagenomic and metaproteomic approaches are providing the basis for fundamental new discoveries about how Antarctic systems function.

The application of new technologies capable of large-scale

parallel high-throughput sequencing, such as those used by the Roche 454 and Solexa Illumina systems, have drastically increased the capacity to sequence environmental samples. As a result, there has been an exponential increase in DNA sequence data for targeted marker genes (for example, 16S rRNA) and whole microbial communities (metagenomes). Only a short time ago, Sanger-based sequencing of 16S rRNA genes provided diversity surveys based on a hundred or so full-length gene sequences representing the most abundant community members. In contrast, by examining thousands of molecules through "tag sequencing" of hypervariable regions of the 16S rRNA gene[1-3], tremendous gains have been made in resolving community structure at a fraction of the original cost[4]. Readers wanting a more detailed understanding of 16S rRNA sequences are directed to the May 2011 issue of *Microbiology Australia,* which focuses on microbial systematics. The first metagenome was for marine viruses and totalled ≈1.28 Mbp[5]. Merely eight years later, 55 Gbp of sequence data (1.2 billion paired reads, average length 50 bp) was generated for a marine sample from just two runs of an ABI SOLiD sequencer[6]. What is most apparent about this level of advance is that it is clear that this trend is set to continue into the foreseeable future.

Metagenomics makes it possible to determine the functional potential as well as phylogenetic composition of a population. By inferring cellular processes, metabolic pathways and adaptive mechanisms, functional capability can be mapped to individual taxa within whole community structures. By incorporating community level functional studies (for example, metaproteomics, metatranscriptomics, stable isotope probing), complementary information can be gained about gene expression and the activity of these cellular processes. Deriving meaning from such large datasets is inherently challenging due to the computational requirements for data processing, and the sheer scope of what the data describes. Metagenomic and metaproteomic analyses are also affected by the complexity of the community, which may vary by orders of magnitude in terms of total diversity and richness, in the genomic heterogeneity of individual species or in its structure at a "micro" (for example, biofilm) or "macro" (for example, stratified lake) scale (for example, see a review such as 7).

We previously highlighted in *Microbiology Australia*, the value of metagenomics for studying Antarctic communities[8] including the effects of climate change on Antarctic microorganisms[9]. In the process of studying Antarctic aquatic systems, we have developed sampling and analytical approaches that enable the diversity, function and evolution of microbial populations to be described to a high level of accuracy, detail and depth. For example, for Ace Lake, a marine-derived, stratified lake (Figure 1), >8 million trimmed DNA sequencing reads (Sanger and 454) were generated providing nearly 9 million gene predictions[10]. Metaproteomic analysis also generated >490 000 mass spectra leading to the identification of >1 800 proteins[10], and in a separate study >500 proteins assigned to a single species of green sulphur bacteria (GSB)[11]. The research developed new ways to derive food web relationships from the phylogenetic and functional data[10,11].

All our Antarctic samples have been collected by sequential size fractionation through a 20 μm prefilter directly onto 3.0, 0.8 and 0.1 µm pore-sized filters (Figure 1) using an approach developed for ocean sampling during the Global Ocean Survey[12]. Size fractionation has proven particularly useful for reducing the complexity of whole samples and for generating a greatly improved understanding of the ecosystem in the context of resource partitioning[10]. By evaluating metagenome and metaproteome data from six sampling depths of the lake, we have learned how the structure of the community and the interactions of microbial populations define biogeochemical fluxes and how the community responds to resource limitation (for example, nitrogen) by short-circuiting of biogeochemical cycles[10,11].

Due to the dominance and abundance of GSB at the oxycline in Ace Lake, we have also been successful in generating an almost complete genome sequence and high (31%) metaproteome coverage[11]. The species of GSB identified may have been dominant in Ace Lake for thousands of years[13] and it appears free of phage predators[10] – this latter fact is highly unusual given the rapid viral-bacterial dynamics typically observed in aquatic systems[14] and the diversity of GSB in other lake systems[15,16].

In polar environments, where large grazers are often absent, the importance of viral predation is hypothesised to be much greater than in temperate regions[17]. In Organic Lake, a hypersaline lake neighbouring Ace Lake (Figure 1), taxonomic assignments proved difficult, as 96% reads could not be confidently matched to anything in the RefSeq database. However, whole metagenomic assembly yielded the complete genome of a novel virophage, named the Organic Lake Virophage (OLV), as well as two near-complete genomes of phycodnaviruses (algal viruses)[18]. Fifty-nine per cent of reads were now explained by these novel genomes, illustrating how dependent the metagenomic approach can be on the nature of the sample. Virophages are a recently discovered virus family[19] which act as "viruses of viruses". They require a larger helper virus to replicate, but drastically reduce the infectivity of their helper[19-20]. In Organic Lake, OLVs are likely to use phycodnaviruses as their helper and infect the unicellular green alga *Pyramimonas*. Phytoplankton appear to be the only source of primary production in Organic Lake so viruses that regulate their numbers affect the rest of the microbial food web. Moreover, an analysis of globally sourced metagenomes found OLV signatures in diverse aquatic habitats suggesting they may have ecological significance in other environments.

One of the real values of metagenomics is letting the data describe the system and discovering what is present and important – not necessarily a match to preconceived expectations. Both Ace and Organic lakes revealed unexpected roles for viruses (for example, OLV) and dominant cellular species (for example, GSB). New knowledge of the cellular and viral species meant new ecological inferences could be teased out about the functioning of the Ace and Organic lakes communities. In Ace Lake, it became clear that the low diversity means the system depends upon a few key species for essential microbial processes[10]. Although this is likely an adaptation to the long polar light cycle, it also suggests the current lake assemblage is very fragile. Since the

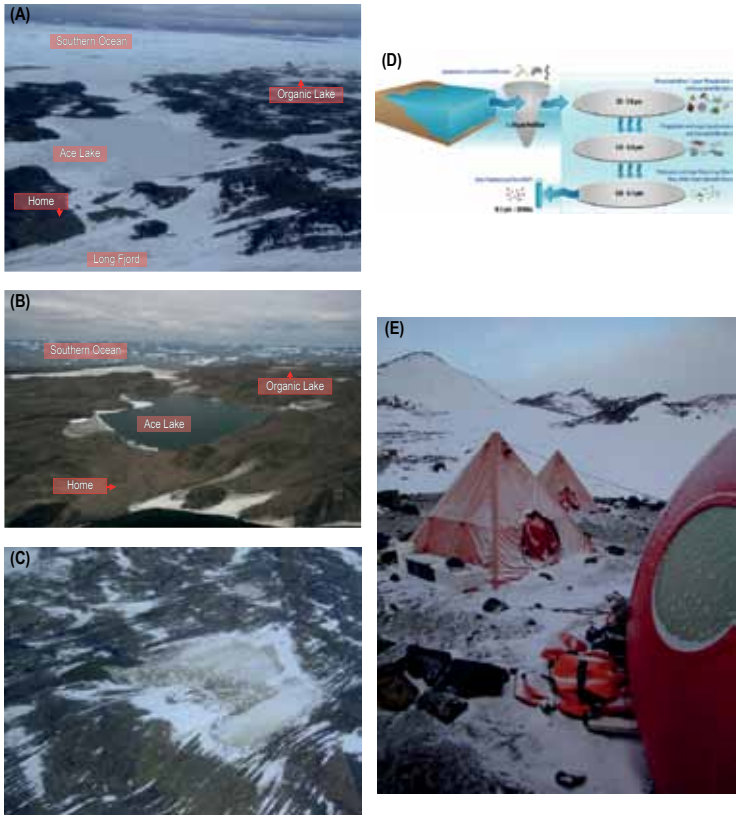

Figure 1. Antarctic metagenomics. (A) Aerial view showing Ace Lake (foreground) and Organic Lake with frozen surfaces. The lake surfaces are frozen for the majority of the year. The ocean waters of the Southern Ocean, including Long Fjord are shown. The lakes were marine basins rendering life in the lake of marine origin. The lakes separated from the ocean 3,000–6,000 years ago. (B) Aerial view showing the surface ice melted on both lakes. (C) Aerial view of Organic Lake with a corner section melting. (D) Cartoon depicting the sequential filtering process (courtesy of the J Craig Venter Institute). (E) Expedition camping near Ace Lake.

GSB are essentially clonal, mathematical simulations predict if they are challenged by environmental perturbation or phage predation the population would require many years to recover[10]. A similar model of virophage-phycodnavirus-host population dynamics revealed that virophage predation would increase the frequency of algal blooms and drive higher rates of primary production[18].

Our studies of Ace and Organic lakes point to an unanticipated and important role for specific components of the microbial loop and the apparent "peculiarities" of polar ecosystems – knowledge we have only been able to gain by using modern 'omic' approaches to generate a new level of information about microbial communities, and linking that information to associated chemical, physical, geological and biological data. Our analyses provide platforms for learning how environmental perturbations affect the microbial dynamics over time and we are now in a position to study intra- and inter-annual variation in community composition and associated microbial processes in the lakes. On one hand, the use of omic techniques, multidisciplinary teams of researchers and access to long-term data records have highlighted just how much we can learn about these unique systems and, on the other hand, the extent of our lack of knowledge and the requirement for learning more. Importantly, this also teaches us that these amazing Antarctic systems need real protection so society can continue to learn and prosper from them for years to come.

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Biographies

Rick Cavicchioli is a Professor at UNSW whose group studies environmental microorganisms, particularly cold adapted archaea and oligotrophic marine bacteria, utilizing "omic" technologies applied to model species through to microbial populations representing entire ecosystems – e.g. Antarctic lakes, the Southern Ocean. The focus on cold and extreme adaptation fosters a biotechnology program focused on developing enzymes with enhanced performances for application to a broad range of industries, particularly water recycling.

Sheree Yau is a PhD student working to understand the microbial dynamics of populations in Antarctic lakes, particularly the diversity and function of viruses.