The Scottish Association for Marine Science | NEWSLETTER 30

SCOTTISH ASSOCIATION *for* MARINE SCIENCE

SAMS

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MARINE BIOTECHNOLOGY IN SCOTLAND

RECENT ADVANCES IN AMNESIC SHELLFISH POISONING RESEARCH SEE PAGE 11

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Aquaculture Today 2005
13-14 April 2005
Edinburgh Mariott Hotel
www.aquaculturetoday.co.uk

37th International Liège Colloquium
Gas Transfer At Water Surfaces
2-6 May 2005
Liège, Belgium
www.ulg.ac.be/oceanbio/co2/2005.html

GLOBEC Symposium
Climate Variability and Sub-Arctic Marine Ecosystems
16-20 May 2005
Victoria, BC, Canada
www.globec.org

American Association of Pharmaceutical Scientists
National Biotechnology Conference
5-8 June 2005
San Francisco, CA, USA
www.aapspharmaceutica.com/meetings/biotec/bt05/index.asp

International Ocean Research Conference
6-10 June 2005
UNESCO Headquarters, Paris
www.tos.org/conference.htm

7th International Marine Biotechnology Conference
7-12 June 2005
St John's, Newfoundland, Canada
www.imbc2005.org/welcome_e.html

Canadian Institute of Marine Engineering
Green Marine Conference
17-18 June 2005
Victoria, BC, Canada
www.greenmarine2005.org/

GLOBEC, IMBER, SCOR, GEOHAB, NERC & CASIX
Advances in Marine Ecosystem Modelling Research
27-29 June 2005
Plymouth, UK
www.amemr.info/

International Festival of the Sea
30 June - 3 July 2005
Portsmouth
www.festivalofthesea.co.uk/

6th International Crustacean Congress 2005
18-22 July 2005
University of Glasgow
www.gla.ac.uk/icc6

CoastGIS 2005
Computer Mapping and GIS for Coastal Zone Management
21-23 July 2005
AECC, Aberdeen
www.abdn.ac.uk/~geo466/

Aquaculture Europe 2005
5-9 August 2005
Trondheim, Norway
www.easonline.org/agenda/en/AquaEuro2005/default.asp

Offshore Europe 2005
6-9 September 2005
Aberdeen
www.oe2005.com/

ICES Annual Science Conferences
20-24 September 2005
AECC, Aberdeen
www.ices.dk

Scottish Marine Group
Autumn Meeting
27 October 2005
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www.sams.ac.uk

11th Annual International Partnering Conference
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www.ebdgroup.com/bioeurope/

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Front cover: The DNA molecule was generated using Accelrys Vierwerlite
© Marcel Jaspars, University of Aberdeen

About SAMS

The Scottish Association for Marine Science (SAMS) is a Scottish charity (est. 1884), learned society, and company limited by guarantee committed to improve understanding and stewardship of the marine environment, through research, education, maintenance of facilities and technology transfer. SAMS is a Collaborative Centre of the Natural Environment Research Council, and hosts the National Facility for Scientific Diving, and the Culture Collection of Algae and Protozoa. It is an academic partner in UHI Millennium Institute under whose auspices SAMS delivers the BSc (Hons) Marine Science, and trains ca 20 PhD students.

As the owner and operator of the Dunstaffnage Marine Laboratory - three miles north of Oban - SAMS is an internationally renowned marine research establishment currently employing circa 120 staff. Our research activities encompass the entire breadth of marine science. SAMS focuses much of its scientific activities on multidisciplinary research questions from Scottish coastal waters to the Arctic Ocean.

SAMS is funded by an agreement with the Natural Environment Research Council for its Northern Seas Programme, by commissioned research for other public and private organisations, and by donations and subscriptions from its ca 600 members. SAMS operates SAMS Research Services Ltd, which delivers SAMS' commercial activities including the European Centre for Marine Biotechnology and Seas@SAMS, and SAMS Ardtoe, an aquaculture research unit.

SAMS Membership

Ordinary: anyone interested in marine science. Subscription - £12

Student: any person under 18, or registered students at Higher Education Institutes. Subscription - £5

Corporate: organisations interested in supporting marine science. Subscription - £60

Unwaged: anyone without a regular wage. Subscription - £5

For further information and application materials please contact the company secretary Mrs Elaine Walton (Elaine.Walton@sams.ac.uk).

Editor

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www.sams.ac.uk

SAMS Board

Dear SAMS member

Dr Anuschka Miller, EDITOR

Biotechnology may be a new buzz word, but it's certainly not a new activity. In fact people have been searching for useful applications, processes or products in nature since the dawn of civilisation, when healing properties of plants were discovered and when people realised that one plant tastes better than another. Later the active ingredients responsible for these effects - e.g. salicylic acid (aspirin) and sugar - were identified, isolated, and mass produced. Nowadays we have methodologies in molecular biology that make such bioprospecting easier and faster, and also allow for a more cost efficient and environmentally sustainable mass production of active ingredients, making their benefits affordable to a larger number of people.

The marine environment with its staggering phylogenic diversity is the least tapped bioresource for biologically active compounds, but is certain to harbour great solutions. The reason for the lack of attention awarded the marine environment by bioprospecting scientists may be partly due to difficulties of access, and partly because few researchers trained in molecular biology also have a solid background in marine science. The European Centre for Marine Biotechnology at SAMS has been developed to bridge this gap. Bringing emerging biotechnology development companies focusing on marine organisms together with an active, multidisciplinary marine research laboratory with resources such as specialised sampling equipment, expert knowledge and the largest and most diverse culture collection of algae and protozoa in Europe is thus not only a commercial project. It gives us the opportunity to make new inroads in understanding biochemical and molecular processes in marine organisms, and thereby to advance marine science.

This Newsletter focuses significantly on recent developments in marine biotechnology in Scotland, a sector carrying great hopes for regional development. I hope it particularly illuminates the many members without a clear understanding of what marine biotechnology actually is. It certainly did this for me.

SAMSnews

Professor Graham B. Shimmield, DIRECTOR

SAMS COMMITMENT TO MARINE BIOTECHNOLOGY

This edition of the SAMS Newsletter is in large part dedicated to the developments in marine biotechnology in Scotland, and the celebration of the opening of the European Centre for Marine Biotechnology at Dunstaffnage by Baroness Susan Greenfield CBE on 5th April 2005. The ECMB concept began back in 1998 with the help of Alasdair Munro from Highlands and Islands Enterprise (see article page 5), and before 'knowledge transfer' had become the strong driver for public outreach of science and technology development that it is today. SAMS Council appreciated the value of an incubator facility in marine biotechnology to confirm SAMS' commitment to new marine science directions, and to offer the scope for R&D development in the Highlands with our partners at Highlands and Islands Enterprise. At SAMS we have a growing awareness of the opportunities afforded through the synergy of basic and applied science, and welcome the co-location with commercial Small to Medium sized Enterprises (SMEs). European funding through the European Regional Development Fund consolidated the idea into reality, so that we are now in a position to celebrate the establishment of the first four tenants in the ECMB.

KNOWLEDGE TRANSFER

It is therefore no accident that in the recent quinquennial review of SAMS conducted by the Natural Environment Research Council (the 2005 Science and Management Audit) SAMS should be strongly commended for its approach to knowledge transfer. Across the organisation many examples of strong science find application in individual projects (so called 'Proof of Concept' ideas). SAMS also contributes significantly to collective approaches such as the Marine Foresight Panel's working group on

marine biotechnology, and has an active programme of public outreach for example through this Newsletter and our Open Days. While I am writing this, two newly appointed Knowledge Transfer Officers are settling into their offices at Dunstaffnage, effectively doubling the number of staff previously involved with these activities at SAMS. I would like to welcome Drs Kate Rowley and Jody de Brouwer to the team!

SAMS ARDTOE RESEARCH DIRECTIONS

At the turn of the year I was delighted to welcome Drs Dave Schoeman and Ben Wilson to SAMS Ardtoe. Dave and his family have arrived from South Africa, and Ben from British Columbia, Canada, although originally from here in Scotland. Both will have a key role to play in the development of SAMS Ardtoe and in the close scientific collaboration with SAMS staff at Dunstaffnage. Dave's work on climate impacts on marine ecosystems[1] is at the forefront of understanding the operation of large marine ecosystems.

> Dr Bob Batty of SAMS (centre) with his Ig Nobel Biology Prize (www.improbable.com) for showing that herring apparently communicate at night by farting.

IG NOBEL PRIZE FOR DRS BOB BATTY AND BEN WILSON

Ben has collaborated with colleagues at SAMS for a number of years, resulting in the award last year of an 'Ig Noble' for his joint work with Dr Bob Batty at SAMS on acoustic communication in herring shoals i.e. 'fish farts'! Although the Ig Noble represents the lighter side of public communication of science and knowledge transfer, it serves a very real purpose of making science accessible and fun. The work is not trivial, and the presentation of the award at Harvard in the USA in November was a significant achievement for both scientists. The award was shared with Professor Lawrence Dill (Simon Fraser University, Canada), Dr Magnus Whalberg (University of Aarhus, Denmark) and Dr Hakan Westerberg (National Board of Fisheries, Sweden). Congratulations to our Ig Nobel Laureates!

A YEAR OF CONSOLIDATION AHEAD

At the start of this year, I indicated to all staff at SAMS that this was the year of consolidation. For the membership of SAMS, we are looking at the new opportunities afforded by 'pooling', the euphemism coined by the Scottish Higher Education Funding Council to indicate the collective and collaborative approach to international quality science across the Scottish universities. We are at the early stages of developing a marine science pool that will serve the academic community well if we can harness the considerable talent that exists across many universities. SAMS aims to play a significant role in helping this coordination, befitting this learned society with over 120 years tradition in promoting research and education in marine science for the benefit of Scotland. ●

Reference
[1] Richardson, A.J. and D.S. Schoeman (2004) Climate change impact on the plankton community in the Northeast Atlantic. *Science* 305: 1609-1612.

Nothing new under the sun

THE DEVELOPMENT OF MARINE BIOTECHNOLOGY IN SCOTLAND

Alasdair Munro, Top Country Development, Inverness

> The European Centre for Marine Biotechnology at the SAMS Dunstaffnage Marine Laboratory links research and business support.

Marine biotechnology is poised for rapid growth. This is the view of pundits who have also pinpointed Scotland's potential to be a leading player. The opening of the new European Centre for Marine Biotechnology at SAMS is seen as the first tangible investment in Scotland in marine biotechnology. But is this the case? Perhaps surprisingly, the answer is No. There were two earlier generations of marine biotechnology, and this helps to explain why the current reincarnation makes so much sense.

What is marine biotechnology? There are various definitions, but the most succinct is: *Marine biotechnology is the use of marine organisms to provide solutions, thereby benefiting society*. To achieve these solutions, it is often necessary to use other technologies such as electronics and advanced engineering; many would bring these applications into the marine biotechnology family.

But, why should Scotland be in the forefront of marine biotechnology? The most obvious reason is that Scotland is a maritime nation, with a coastline of over 11,500 miles. Some 63% of the UK continental shelf area is in Scottish waters and this area is five times greater than that of landward Scotland. There are huge, diverse resources in these seas.

HISTORY OF SCOTTISH MARINE BIOTECHNOLOGY

The first generation of marine biotechnology involved the burning of kelp to extract soda, potash and iodine. It started in 1698 and continued spasmodically for two centuries, mainly in the Outer Hebrides and Orkney. Thus it provided employment entirely in impoverished rural communities, using a renewable local resource.

The pioneering second generation started in a hut in Campbeltown in 1934. A company called Cefoil was set up to extract alginate from seaweed to make a Cellophane type wrapping. Cefoil ran three government World War 2 factories, two near Oban, and at Girvan to extract alginate for making camouflage netting. After the war, Cefoil became Alginate Industries and the uses of alginates expanded rapidly, mainly in food and pharmaceuticals. There have been changes and closures over the years, but the industry still thrives at Girvan as ISP Alginates, where it employs over 150 people in the largest plant of its kind in the world.

As we enter Scotland's third generation of marine biotechnology, seaweed, or macroalgae, provides a common thread with the earlier generations. Macroalgae and their cousins, microalgae, are diverse and important sources for new products. The leaders for what we now understand to be marine biotechnology have been the USA and Japan, but other countries are now rapidly becoming involved. The potential for Scotland was pioneered by Heriot-Watt University which introduced the first BSc course in marine biotechnology.

THE EUROPEAN CENTRE FOR MARINE BIOTECHNOLOGY

Highlands and Islands Enterprise and SAMS began to assess the potential in 1998. What was particularly important was the promise for linking research and business start-ups. The first Scottish marine biotechnology company, Integrin Advanced Biosystems, was set up in 1999, as a spin-out from SAMS, in the former alginate plant at Barcaldine, near Oban.

The recent redevelopment of the SAMS laboratories at Dunstaffnage provided the opportunity to link research and business support in the shape of the new dedicated European Centre for Marine Biotechnology which opens this year. A state of the art building of some 1,340 square metres, it provides suites of laboratory and office space. Already it houses the national UK Culture Collection of Algae and Protozoa, and its first company, Aquapharm Biodiscovery. Two further tenants are in the process of moving in.

THE CHALLENGE AHEAD

The potential of marine biotechnology on a UK scale has now been recognised. In January this year, the Marine Foresight Panel published a comprehensive review.[1] This demonstrates the wide range of suitable marine raw materials - for example, algae, alginates, chitin and invertebrates, and the even wider range of applications which include food additives, nutritional supplements, pharmaceuticals, cosmetics and biomaterials. The strong Scottish dimension is highlighted: thirteen of the twenty-one companies so far established are Scottish, and ECMB is cited as the type of centre of excellence which should be encouraged elsewhere.

Our seas will form the basis of a new age of discovery. The goals of the Census of Marine Life state: 'The diversity of marine life is huge and may rival that of the rain forests in the number of species found there, and, yet, our knowledge of ocean life lags far behind that of terrestrial life.' Discoveries will undoubtedly result in a considerable expansion of marine biotechnology, with the resulting benefits of creating opportunities in rural coastal communities. But we will have to work hard to bring these benefits about in a responsible, self-sustaining way. That is the future challenge. ●

Reference
[1] A study into the Prospects for Marine Biotechnology Development in the United Kingdom. www.dti.gov.uk/pdfs/FMP_MBG_Volume_1_Final1.pdf

The language of bacteria

Dr Grant Burgess, Heriot-Watt University

> An isolate of *Bacillus licheniformis* from a Sottish seaweed, which only produces antibiotics when grown on a surface.

>The author with some of the seaweed used to isolate novel bacteria.

SYNCHRONISING BACTERIAL ACTIVITIES

They may be small and apparently simple in their construction, but bacteria are a highly successful group of organisms that communicate with each other. They do so by releasing small chemical messages, similar to pheromones, in a process known as 'quorum sensing'. These chemical messages allow bacterial populations to coordinate the behaviour of large populations of cells. When this system evolved, it empowered them to behave like large, multi-celled organisms.

We now know that quorum sensing controls many aspects of bacterial physiology including biofilm formation and antibiotic production.

As bacteria communicate to coordinate the production of bioactive compounds, we investigate the use of chemical signals to control this production.

BIOFILM BUSTERS

But marine bacteria don't only communicate and co-operate, they also compete with one another. And as they have been trying to kill each other for almost three billion years, they have developed some pretty sophisticated strategies. One of these is the recently discovered ability of some bacteria to destroy the biofilms of their competitors. Many bacteria rely on the formation of a biofilm in order to infect other cells – which is one of the causes for their ability to colonise humans. We are studying the mechanisms of biofilm busting, which may one day help develop effective ways of preventing infections.

ARE BACTERIA STIMULATING MICROALGAE?

The UK marine biotechnology sector has enormous potential, but is currently quite small, and so it is essential for those involved to work together to raise the profile of this new strategic sector and the commercial success stories that are currently emerging. The opening of the European Centre for Marine Biotechnology in Oban is a significant milestone in the emergence of a strong UK and European presence in marine biotechnology. There are currently joint projects being developed between my group at Heriot-Watt University and Dr Frithjoff Küpper's group at SAMS to investigate whether bacteria play a role in the release of antibiotics and toxins by microalgae. ●

Dr Grant Burgess is a Reader in Marine Biotechnology at Heriot-Watt University and was a participant in the steering group which oversaw the establishment of ECMB. Further information can be found at www.esmb.org.

Old friends?

THE RELATIONSHIP BETWEEN PHYTOPLANKTON AND HETEROTROPHIC BACTERIA

Drs David Green and Mark Hart, SAMS

The open ocean contains an estimated total of 1.2 x 10^{29} bacterial cells[1]. This equates to about 1 billion (1 x 10^{9}) cells per litre of seawater. And it is estimated that the ocean floor contains 10 times more than the pelagic ocean. What are they all doing? This is the fundamental question being addressed by current marine microbiology.

Marine bacteria found in the water column can be broken into two broad groups: photosynthetic cyanobacteria and heterotrophic bacteria. The heterotrophic bacteria are the 'biological pump' responsible for biogeochemical cycling of nutrients in the marine ecosystem. Our research at SAMS deals with heterotrophic bacteria, but instead of concentrating on the great majority of bacteria that live freely in the water column, we focus on bacteria that live associated with phytoplankton. Phytoplankton are at the base of the marine food chain, and bacteria that live with these primary producers are likely to represent the first stage in the global biogeochemical cycles that supply and re-supply the world's oceans with the nutrients that ensure continued biological production.

DIVERSITY OF PHYTOPLANKTON-ASSOCIATED BACTERIA

We are currently studying whether there are bacteria that only live with phytoplankton. For example, whether it is a random assemblage, a definable bacterial community that co-associates with one phytoplankton species, or a grouping common to algal genera or wider groups such as all diatoms or dinoflagellates.

We have found the bacterial diversity associated with several dinoflagellates, a group that includes species prone to forming harmful algal blooms, to be surprisingly conserved across both the species and the geographical divide. *Marinobacter algicola* (see image) for example was found to live with five different types of dinoflagellate from the UK, Spain, Canada, and Korea. We were puzzled to discover that assemblages living with coccolithophorids like *Emiliania huxleyi* display a remarkable similarity with those associated with dinoflagellates. This may be because dinoflagellates and coccolithophores both bloom during calmer weather periods and when nutrients become limiting, which may select for bacterial types that are adapted to these conditions.

What about bacterial consortia associated with the other main lineage of marine phytoplankton, the diatoms? We have so far only investigated one genus, the pennate diatom *Pseudo-nitzschia*, some species of which cause amnesic shellfish poisoning. While the same bacterial families are present, at the species level the bacteria are quite different from those found on dinoflagellates and coccolithophores.

These differences could reflect the environment, the physiology of the bacteria, the surface signals on the algal cell, or the algal excretory products. Alternatively it could be due to different evolutionary processes of different microalgal groups. By investigating more diatom species and comparing the results with those from dinoflagellates and coccolithophores, we hope ultimately to deduce how phytoplankton organize and structure their bacterial communities.

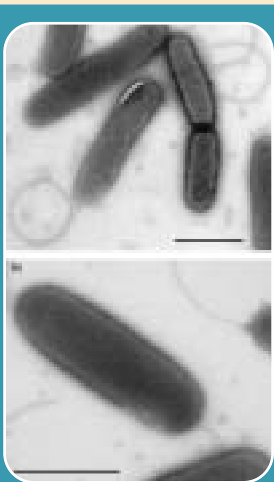
WHY BE FRIENDS WITH BACTERIA?

We are examining two questions regarding the function of different bacteria: What effects do they have on primary production? And what are their roles in the biogeochemical cycling of nutrients?

In this regard, our results and work published by others show that some bacteria are crucial to the growth of phytoplankton. We found that certain bacteria supply dinoflagellates with a 'growth factor', while the literature reports that bacteria can supply vitamin B12 to diatoms. We are also investigating whether bacteria aid the uptake and biological availability of iron, an essential element for phytoplankton photosynthesis.

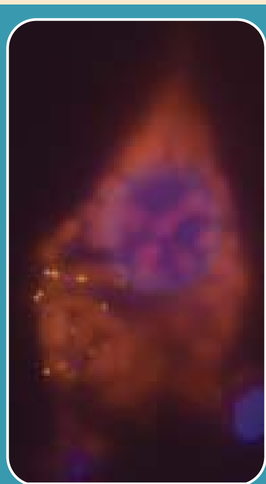
We think that there is a long and well-established 'friendship' between certain members of the marine heterotrophic bacterial community and phytoplankton. As prokaryotic and eukaryotic microbes have existed together for millions of years, this may not be surprising. However, such a view represents a paradigm shift. In the past bacteria were seen as simply feeding on the products of algal photosynthesis, while we now view this relationship as being about mutual benefits. The implications of symbiotic relationships between phytoplankton and their bacteria are profound: the bacteria may affect phytoplankton species succession in the ocean, and they may insulate or exacerbate the effects of environmental change felt by the phytoplankton. Our research is still in its infancy, although the relationship may be ancient. ●

Reference
[1] Whitman *et al.* (1998). Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci.* 95:6578-6583.



< Transmission electron micrograph of *Marinobacter algicola* isolated from the dinoflagellate *Gymnodinium catenatum*. (Bar = 1 µm). © David Green, SAMS

> Fluorescence microscopy shows bacteria – here alphaproteobacteria (bright orange dots) – attached to the surface of their algal host, the dinoflagellate *Ceratium furca*. The pale orange ovoid bodies are the algal chloroplasts, the large blue sphere is its nucleus. © Mark Hart, SAMS



Smuggling a culture collection into exile

LIFE AND WORK OF MICROBIOLOGIST ERNST GEORG PRINGSHEIM (1881-1970)

Dr Anuschka Miller, SAMS

Organisms grown in culture are a fantastic resource for biotechnology. The Culture Collection of Algae and Protozoa at the European Centre for Marine Biotechnology was founded by a refugee from Nazi Germany, Professor Ernst Georg Pringsheim, who had brought the founding strains in his luggage from Prague.

EARLY YEARS

Ernst Georg Pringsheim was born on 26th October 1881 near Breslau in Silesia as one of four sons of a wealthy landowner. Like his brothers he certainly was not a good pupil and had to retake four school semesters. Nevertheless all but the youngest brother, who was killed in World War I, eventually became professors.

> In front of Pringsheim's picture are Christine Campbell and Dr John Day from SAMS, today's curators of the Culture Collection of Algae and Protozoa that Pringsheim had started during exile in Cambridge.

Initially the young Pringsheim was torn between painting and botany, and spent two years in Munich taking lessons in art. But he took against the bohemian lifestyle of artists, and increasingly delved into science, especially plant physiology. He moved to Leipzig to study botany under the great physiologist Wilhelm Pfeffer. There he found his vocation and focussed intensely on his botanical studies, culminating in receiving 'summa cum laude' (the top grade) for his doctorate thesis on water movement and turgor regulation in wilting plants after just three semesters.

DISCOVERING THE MICROCOSM

He then began work in Breslau, where he was introduced to working with microorganisms. He married Lily Chun, daughter of a Professor in Zoology, but this was to be an unsuccessful relationship. In 1909 he became lecturer in Halle, where he deepened his interest in microbiology, worked on plant nutrition, and developed the first axenic culture of Cyanobacteria. He was made professor just before the breakout of World War I, and worked on bacteriology prior to being called up at the age of 36. He hated all things military, and after his initial training served in an army hospital.

Soon after the war Pringsheim moved to the Institute for Plant Physiology at the University of Berlin in Dahlem. There, in 1920, he published an influential paper on the nutritional value of acetic acid for saprophytic flagellates. But these were difficult years that saw national insecurity, inflation, and hunger, and - for Pringsheim - divorce.

He thus happily accepted a professorship at the German part of Charles University in Prague leading an impoverished institute for plant physiology. He adored the city, appreciated his improved economic situation, and embarked for 15 years on a career focussed on improving techniques to cultivate microalgae and developing a sizeable collection. He also worked on the phylogeny of bacteria. He married a pharmacy student that had assisted him in the laboratory, Olga Zimmermann, who was to work with him for the next 30 years.

HITLER'S SHADOW

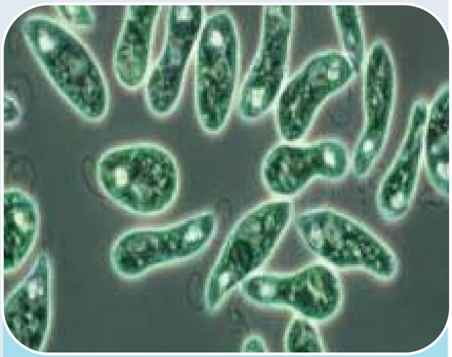
Pringsheim yearned to return to Germany, but when he was finally offered a prestigious professorship in Frankfurt in 1932, Hitler was rising, and Pringsheim, of Jewish origin (although Lutheran persuasion), could not be appointed. He thus remained in Prague until he was replaced by a Nazi late in 1938 just before the German invasion. Wisely, and supported by the London phycologist Professor FE Fritsch, the Pringsheims went into exile in England, taking cultures from their algal collection, but leaving behind their entire belongings. The Prague collection has since become part of what is today the Culture Collection of Algae at the Laboratory of Algology in the Czech Republic.

THE CULTURE COLLECTION OF ALGAE AND PROTOZOA

For the next 15 years Pringsheim worked at the Botany School at the University of Cambridge, and developed both his research and his collection of microalgae and protozoa. In 1946 he published 'Pure Cultures of Algae', a valuable practical text for phycologists. His Culture Collection of Algae and Protozoa survives to this day, now housed at SAMS. During these years Pringsheim worked with important students and colleagues including MR Droop (who was to bring CCAP to what is now SAMS), RC Starr (who later established what is now the Culture Collection of Algae at the University of Texas) and CB van Niels.

THE SAMMLUNG VON ALGENKULTUREN

Working five years beyond retirement age, he eventually had to vacate his laboratory at the Botany School aged 70. In 1953 he returned to Germany to work at the Botanical Institute in Göttingen. Finally receiving recognition for his work in his beloved Germany, Pringsheim developed a vibrant algal research laboratory based around a new collection of algae developed from cultures from Cambridge, the Sammlung von Algenkulturen. The scope of his research was broad, with particular interest in the nutrition of *Beggiatoa* and 'colourless algae'. He eventually retired aged 86, having published 180 titles including 4 books and having isolated 438 strains still available from the four culture collections he founded in person or through instruction and inspiration.



> Pringsheim was interested in algae, protozoa and bacteria. His research focus, however, was on colourless and heterotrophic flagellates, especially *Euglena*. Image shows *Euglena gracilis*. © John Day, CCAP.

Drugs from sea squirts?

ISOLATING PATELLAMIDES ONBOARD AN AUSTRALIAN RESEARCH VESSEL

Professor Marcel Jaspars, University of Aberdeen

> Diving to collect our model organism certainly added enjoyment! © Anke Klüter

WHY BOTHER WITH SEA SQUIRTS?

Ascidians – more illustratively known as sea squirts – are an interesting class of animals. Their adult form is that of a simple sessile hermaphroditic filter feeder while the swimming juvenile has a tadpole-like appearance with a notochord and a dorsal nerve cord that define them as chordates. Being thus related to vertebrates like ourselves suggests that ascidian biochemistry may show significant similarities with ours, but as the relationship is not close, also novel biochemical compounds and processes can be expected. Ascidians have been identified as prolific producers of biologically active compounds some of which may prove to be valuable active agents for pharmaceuticals.

We have been particularly interested in a colonial species, *Lissoclinum patella*, which occurs on the Great Barrier Reef and looks like green candle wax dripping from coral reefs. The green colour originates from a photosynthetic symbiont, *Prochloron*, which inhabits the cloacal cavity of the sea squirt host. We are investigating *Lissoclinum* as model organisms mostly because they contain patellamides which may act as primitive enzymes. It appears that they are synthesised by *Prochloron*. Patellamides are compounds of great promise for medical developments as they are likely to find applications in the treatment of multiple-drug resistant cancers or inflammatory diseases.

MASS PRODUCTION OF COMPLEX BIOMOLECULES

One of the challenges that hampers the commercial exploitation of *Lissoclinum patella* as that of many other marine organisms is the difficulty of accessing significant numbers of them. Collection from the wild is ecologically dubious and could result in overexploitation. Other options to increase and facilitate their availability include aquaculture or tissue culture. In our case, however, *Prochloron* could not be cultured independently as it relies on its ascidian host for survival. Alternatively the bioactive compounds, once isolated and identified, could be synthesised chemically, or with molecular biological techniques. None of these methods is unproblematic. Aquaculture may be a solution where a supply of just one compound is needed. Growing single cells in tissue culture on the other hand is still very difficult because we know too little about the physiology of these simple chordates – we know more about growing complex human cells! Chemical synthesis of the more complex active sea squirt compounds would be ideal, but many of these molecules take more than 20 steps to synthesise which can be difficult to achieve on a larger scale. We are therefore using molecular biological tools to produce the compounds of interest.

We initially isolated large stretches of *Prochloron* DNA, and we then inserted these sections into the bacterium *E coli* using a 'vector'. Now we are attempting to identify the particular colony of bacteria (from 1433!) that might contain the patellamide-coding DNA. To achieve this we are combining molecular biology and analytical chemistry techniques.

> Dr Paul Long from the London School of Pharmacy extracts DNA. © Marcel Jaspars

> The colonial sea squirt *Lissoclinum patella* is green due to an autotrophic symbiont, *Prochloron*, that is responsible for the production of patellamides. © Carsten Wolff

AND THE BEST: FIELDWORK ON THE GREAT BARRIER REEF

Most biotechnology research is conducted in clean laboratories in large cities, but it does not always have to be that way! As *Lissoclinum patella* occurs on the Great Barrier Reef, in the summer of 2004 my colleague Dr Paul Long, a molecular biologist from the London School of Pharmacy, and I teamed up with Drs Chris Battershill and Walt Dunlap from the Australian Institute of Marine Science to conduct this research aboard the AIMS Research Vessel *Lady Basten* on Davies Reef. This allowed us to double as SCUBA divers and collect our own organisms, which gave this work expedition a rather exciting edge! We were faced with some unusual challenges at sea, one of which was the need to transplant the guts of a chemistry and molecular biology lab to the rather limited space in a generic lab on a research vessel, a lab that rocked, yawed and pitched. Fortunately our Australian marine science colleagues - especially Walt - were veterans of many expeditions and knew exactly what to take. To us lab chemists it was unusual to say the least to heat our samples to 37°C in the cosy confines of a vessel's engine room and to conduct such sophisticated work as chemical extractions, chromatography, enzymic digestions and DNA extractions in such an environment. Fortunately all this work can be achieved with a limited amount of robust and reliable equipment. ●

This work has been supported by the Australian Institute of Marine Science, The London School of Pharmacy, The College of Physical Sciences, University of Aberdeen, The Carnegie Trust for the Universities of Scotland, The Royal Society of Edinburgh, and the Leverhulme Trust.

My PhD Project

REEFS ON RIGS

Susan Gass, SAMS UHI

Cold-water corals are globally widespread. In the NE Atlantic the dominant species is *Lophelia pertusa* which forms orange or white colonies. This coral can grow forming small, scattered colonies or groups of colonies as those west of Shetland, or construct large reefs like those found along the Norwegian continental margin. *Lophelia* was unreported in the North Sea until 1999 when coral colonies were spotted on the infamous Brent Spar platform as it was decommissioned.

> The orange and white colonies of the cold-water coral *Lophelia pertusa* are common on oil and gas platforms in the northern North Sea - here on the Heather Alpha platform. (Image courtesy of Lundin Britain Ltd)

They were next filmed live in the Beryl field and again in 2000 on the Brent Alpha drilling platform. This was unexpected because *Lophelia* was thought to be a sensitive organism unlikely to survive in the contaminated environments expected around a drilling platform.

As oil and gas exploration and production has moved into deep water in the NE Atlantic, concern has been raised about potential impacts on cold-water corals. Of greatest concern for corals, which feed by trapping passing prey from the water, are the drilling muds and cuttings which are discharged into the marine environment. Cuttings are the rocky rubble obtained when drilling into the seabed while muds are a mixture of water or oil with chemical additives that contain metals. Essential activities such as feeding and respiration - and consequently growth and reproduction - may be negatively impacted by living in silty environments. Also the chemical and metal additives in drilling muds may have a toxic, detrimental effect on *Lophelia*.

FIELDWORK BY VIDEO

My research project explores the puzzling occurrence of *Lophelia* on oil and gas platforms in the North Sea. First I had to establish whether *Lophelia* occurrence in the North Sea is widespread or a rare exception. As the oil industry regularly surveys all its platforms with remotely operated vehicles (ROVs) to check for structural integrity, I could use the recorded video surveys in my search for *Lophelia*. I found *Lophelia* on 13 platforms in the northern North Sea and thus conclude that *Lophelia* is common on oil and gas platforms in the northern North Sea.

In the videos I saw hundreds of round *Lophelia* colonies with densely packed polyps of both the white and orange variety. The majority of colonies had polyps fully expanded for feeding. But in some cases, where colonies were growing close to drilling discharge pipes, colonies were either partially or fully smothered in drill cuttings. After examining the full depth range of two platforms, *Lophelia* colonies were only found between depths of approximately 50 to 130 m which corresponded to depths with stable temperatures around 8°C and a salinity of 35. I could even follow the same colonies of *Lophelia* through time by analysing videos from 1994, 1998 and 2002 of the Tern Alpha platform. These images allowed me to calculate the corals' average yearly growth rates - the first repeated estimate for any species of cold-water coral.

LABORATORY STUDIES

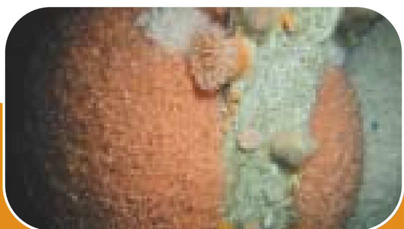
My project depends on a partnership with several oil companies who have assisted by sampling *Lophelia* from their platforms. ROVs can be used to scrape corals off platform legs, catch them in a net, and bring them to the surface. *Lophelia* samples were collected that way from the legs of six platforms. Some specimens were kept alive for experiments in our SAMS coral aquarium while others were frozen for skeletal analyses.

Experiments in the aquarium explore polyp behaviour in response to increased sedimentation rates, especially the mechanisms corals employ to clean themselves of sediment build up.

The frozen coral skeletons will be used to investigate whether *Lophelia* can act as an archive of conditions in its ambient marine environment. As a coral grows, it secretes a calcareous skeleton where calcium can be replaced by other elements present in seawater. For this part of the project, we targeted samples of *Lophelia* with visual evidence of exposure to drill muds as well as control specimens with no evidence of exposure. Inductively coupled plasma mass spectrometry (ICPMS) is used to determine whether metals from drilling muds, such as barium, cadmium, nickel, and zinc, occur in higher concentrations in corals exposed to drill muds.

DO CORALS NEED SPECIAL PROTECTION MEASURES?

Much debate has occurred about the potential impacts on cold-water corals from the oil and gas industry. In 1999, Greenpeace brought a court action against the government claiming that known coral areas newly licensed for exploration should have been considered for Special Areas of Conservation under the EU habitat directives. Although Greenpeace won the case, little research has been done to examine potential impacts from drilling activities on cold-water corals. The results from my research will not only increase our understanding of the biology of *Lophelia* but it will also help us understand its sensitivity to these high stress environments.



> While coral feeding is usually impaired by increased siltation, these corals on Heather Alpha have been growing well over time. (Image courtesy of Lundin Britain Ltd)

> A remotely operated vehicle samples *Lophelia pertusa* from the North Cormorant platform for further analysis. (Image courtesy of Shell U.K. Ltd)

Towards a prediction of toxic blooms

FACTORS REGULATING DOMOIC ACID PRODUCTION IN *PSEUDO-NITZSCHIA*

Drs Keith Davidson & Johanna Fehling, SAMS

Some species of marine planktonic diatoms of the genus *Pseudo-nitzschia* Peragallo produce domoic acid (DA) which can accumulate in filter feeding shellfish such as mussels and scallops without much appreciable negative impact on these invertebrates. However, if we or other vertebrate predators then eat these delicacies, we might develop amnesic shellfish poisoning (ASP) through the neurotoxic action of DA. This has led to illness and death of animals and humans in various regions worldwide.

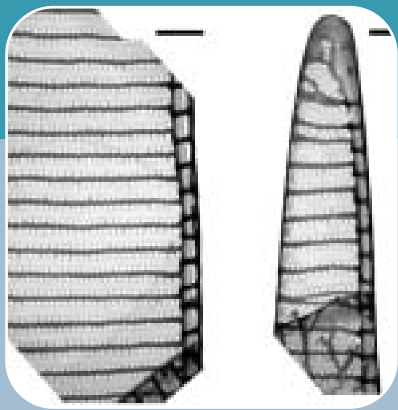
In Scottish waters, elevated DA concentrations have been recorded since 1998. Fortunately, there have been no recorded illnesses from ASP in the UK with large quantities of shellfish being eaten safely. However, this is achieved by an active monitoring program of phytoplankton and shellfish toxicity in various locations around the country. When monitoring has indicated elevated shellfish toxin levels, closures of the Scottish west coast scallop fishery have been necessary. This peaked in 1999 with the largest closure ever seen worldwide (an areas of 49,000km^2), causing severe economic losses to the industry and the region's economy as a whole.

At SAMS we have been seeking to gain a better understanding of the factors that govern the appearance and toxicity of *Pseudo-nitzschia* spp. in Scottish waters with the aim of minimizing future fishery closures. This work has involved the combination of a field sampling program with laboratory physiological and molecular studies of isolated *Pseudo-nitzschia* cells.

IDENTIFYING TOXIC *PSEUDO-NITZSCHIA* STRAINS

We isolated phytoplankton cells from the Firth of Lorne to establish clonal cultures of a variety of *Pseudo-nitzschia* species for toxicity investigations. Previously one species, *Pseudo-nitzschia australis*, isolated at the time of the 1999 ASP event, had been shown to produce DA toxin in Scottish waters. Our study confirmed the toxicity of *P. australis* but also identified *Pseudo-nitzschia seriata* as a local DA producer.

> *Pseudo-nitzschia seriata* – here a TEM image – is a diatom that produces domoic acid, the toxin responsible for amnesic shellfish poisoning (scale bar = 1 um). © Johanna Fehling.



> Shortages in dissolved inorganic phosphorus and silicon lead to enhanced production of the amnesic shellfish toxin domoic acid by *Pseudo-nitzschia.* © Johanna Fehling.

Identification of *Pseudo-nitzschia* to species level - especially discrimination between *P. australis* and *P. seriata* - is problematic. Light microscopy has insufficient resolution to discriminate between these two species. Higher resolution transmission electron microscopy (TEM) was also ambiguous on its own, as important differences in morphological fine structure existed in our cells compared to other published records. However, we managed to identify the strains of our isolated cultures unambiguously as *P. australis* and *P. seriata* by using molecular methods to amplify the internal transcribed spacer (ITS)1, 5.8S and ITS2 and the partial large subunit of the rDNA operon. Two strains of *P. seriata* isolated in successive years were found to have sequences identical to one another and also to the ITS and partial LSU rDNA sequences of other published *P. seriata* strains.

NUTRIENT LIMITATION INFLUENCES DA PRODUCTION

The stress imposed on *Pseudo-nitzschia* cells following exhaustion of a limiting nutrient has been implicated in DA toxin production. Not all *Pseudo-nitzschia* species produce DA, and for those that do, the factors that govern the amount produced and the rate of production remain poorly understood. We thus conducted controlled nutrient manipulation experiments on our isolated cultures.

As DA is an amino acid nitrogen limitation results in the repression of toxin production. A lack of dissolved inorganic phosphorus or silicon on the other hand leads to enhanced rates of toxin production. Moreover, we found that our Scottish *P. seriata* strain becomes significantly more toxic when it experiences silicon stress, indicating that the delicate balance of inorganic nutrients in our coastal seas is potentially critical to ASP events.

FUTURE RESEARCH CHALLENGES

Our medium term aim is to produce a mathematical model of the growth and toxin production of *Pseudo-nitzschia*. It is therefore necessary to determine how environmental factors other than nutrient stress influence these properties. Initial laboratory experiments have identified the importance of the duration of the period of daylight, one of the major factors in determining the seasons in high-latitude area such as Scotland. We found that photo-period influences cell growth of both toxic and non-toxic *Pseudo-nitzschia* species. For *P. seriata* total toxin and toxin per cell were also influenced by photo-period, suggesting that this parameter is a major factor governing the appearance of different *Pseudo-nitzschia* species throughout the year.

Such results suggest that predictions of blooms and their toxicity based on environmental conditions may indeed become possible. ●

Meeting surFace to surFace

BIOSURFACTANTS BRING THE WORLD CLOSER TOGETHER

Drs Tony Gutierrez and David Green, SAMS

A lot happens at surfaces. Imagine washing last night's fish and chip pan without using a detergent, or taking a bath without soap. The water would just run off, leaving behind most of the oils and grease stuck to your pan surface, or body, as the case may be. Molecules of oil tend to stick together, and the same applies equally to molecules of water. If you have ever mixed oil and water together you know that they just don't mix. But bring in a surfactant, and the repulsion between the two is broken, as though a new bond between old foes has formed. The molecules of oil and water now begin to meet face to face and mix in with each other. And the surface is where it all happens.

Detergents and soaps are essentially what scientists refer to as surfactants, substances that are able to interact with oils and water simultaneously and allow both to associate, so to speak. As the name suggests, surfactants act on surfaces, and it is here that they reduce the tension – i.e. surface tension – between two relatively non-mixable liquids. Lowering their surface tension at the interface between the two liquids allows the molecules of both parties to interact and mix together. But how or why does this happen? It all has to do with the intrinsic properties of the surfactant molecule: they are 'amphiphilic'. This means that they possess both water-loving and oil-loving components on the same molecule. With this, surfactants are able to grasp a hold of oil and water molecules simultaneously, acting as glue that binds two otherwise non-miscible liquids together.

With such a useful property, it is no wonder that surfactants find application in almost every sector of modern industry, from agrichemicals, to food ingredients, textiles, construction, healthcare, pharmaceuticals, and most importantly in our household washing-up liquid and bathroom soap. But there is a problem. Most surfactants are derived synthetically from petroleum, which brings to light environmental as well as health and safety concerns.

At SAMS we are investigating new types of surfactants that are produced naturally by biological processes, i.e. biosurfactants. Using the marine environment as our platform for discovery, we are targeting particular groups of bacteria that we have identified as likely producers of novel bioemulsifiers, a type of surfactant that can mix oils with water to form emulsions like ice-cream and mayonnaise. This research began due to our interest in the ecological roles of these compounds in the ocean, specifically with marine bacteria that degrade crude oil released by accident or from natural oil or gas seeps. Hydrocarbons are toxic to most organisms, and only relatively few species of bacteria have developed the knack to make a living from oil. By producing surfactants and emulsifiers, the oils can be made more soluble, so that the bacteria can enzymatically attack the oil and use it as a food source. It's one of nature's very own and effective clean-up processes. Furthermore, since surfactants sequester and act on surfaces, their accumulation at the ocean-atmosphere interface may influence the oceans' exchange of gases with the atmosphere.

> Dr Tony Gutierrez is developing biosurfactants from marine bacteria that one day may replace synthetic petroleum-derived emulsifiers for use in products such as ice cream and mayonnaise. (© Rory MacKinnon, SAMS)

From an initial screen for novel biosurfactants, we have found four highly promising emulsifiers with properties similar to - or better than - existing commercial emulsifiers. The challenges ahead are significant because these emulsifiers don't only have to be every bit as good as existing commercial products (which we think they are) but it must also be possible to produce them more cheaply if they are to 'cut ice' as commercial products, let alone, make it into ice-cream and mayonnaise! ●

> Surfactants reduce surface tension between different liquids, such as water and oil. This is measured using a tensiometer that can measure tension forces in milli-Newtons. (© David Green, SAMS)

> Emulsifiers are compounds that can interact both with polar water and apolar oil molecules, thereby producing emulsions. Shown is one of our emulsifiers mixing olive oil and water alongside its control without emulsifier. (© David Green, SAMS)