Marine Research Sub-Programme

(NDP 2007–'13) Series

SeaChange

Casadh na Taoide

Proceedings of the 2nd Annual Beaufort Marine Biodiscovery Research Workshop

10-11 December 2009, Queens University Belfast

NDP

National Development Plan

Transforming Ireland

Marine Institute

Foras na Mara

The Marine Institute is the national agency which has the following functions:
"to undertake, to co-ordinate, to promote and to assist in marine research and development and to provide such services related to research and development that, in the opinion of the Institute, will promote economic development and create employment and protect the marine environment" Marine Institute Act 1991.

Sea Change:A Marine Knowledge, Research & Innovation Strategy for Ireland

Sea Change—A Marine Knowledge, Research & Innovation Strategy for Ireland 2007-2013—was launched in early 2007 and was the outcome of extensive analysis and consultation with government departments, state agencies, industry and the third-level sector. It outlines a vision for the development of Ireland's marine sector and sets clear objectives aimed at achieving this vision, namely to:

1. Assist existing, and largely indigenous, marine sub-sectors to improve their overall competitiveness and engage in activity that adds value to their outputs by utilising knowledge and technology arising from research.
2. Build new research capacity and capability and utilise fundamental knowledge and technology to create new marine-related commercial opportunities and companies.
3. Inform public policy, governance and regulation by applying the knowledge derived from marine research and monitoring.
4. Increase the marine sector's competitiveness and stimulate the commercialisation of the marine resource in a manner that ensures its sustainability and protects marine biodiversity and ecosystems.
5. Strengthen the economic, social and cultural base of marine dependant regional/rural communities.

The Sea Change strategy was developed as an integral part of the government's Strategy for Science, Technology and Innovation (SSTI) and the Marine Institute as the lead implementation agency is working within SSTI policy and with government departments and agencies to deliver on the Strategy.

The Marine Institute managed Marine Research Sub-Programme, one of eight sub-programmes within the Science, Technology and Innovation (STI) Programme of the National Development Plan 2007—2013, targets funding to meet the objectives of the Sea Change strategy.

Over the lifetime of Sea Change, funding will beprovided for:

- Project-Based Awards
  - o Strategic Research Projects
  - o Applied Research Projects
  - o Demonstration Projects
  - o Desk/Feasibility Studies
- Researcher Awards
  - o Strategic Research Appointments
  - o Research Capacity/Competency Building
  - o Post-Doctoral Fellowships
  - o PhD Scholarships
- Industry-Led Research Awards
  - o Company Awards
  - o Collaborative Awards
- Infrastructure Awards
  - o Infrastructure Acquisition
  - o Access to Infrastructure

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Proceedings of the 2nd Annual

Beaufort Marine Biodiscovery Research Workshop

Queen's University Belfast, Northern Ireland

McClay Pharmacy Building, Medical Biology Centre Complex

10-11th December 2009

Organised by:

Prof. Christine Maggs
Queen's University Belfast, Northern Ireland;

&

Marine Institute
Rinville, Co. Galway

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Marine Institute Foras na Mara

SeaChange Casadh na Taoide

Queen's University Belfast

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Foreword

Marine environments are diverse, and organisms that occupy the many ocean niches are exposed to various extremes of pressure, temperature, salinity and available nutrients. Because of this diversity the marine sector offers a greater scope for discovery and associated breakthrough technologies compared to terrestrial ecosystems.

The progress made by the Beaufort Marine Biodiscovery project team is clear in the proceedings of the 2nd Annual Biodiscovery Workshop. Since the project was initiated in 2007, marine biotechnology continues to draw the attention of policy, research and industry interests. Increasing these various stakeholders recognise the massive scientific and economic potential of the sector. Recent reports position marine biotechnology as a rapidly growing global business sector, set to be worth €3.78 billion by 2012.

Irish and European marine biotechnology activity continues to gain momentum. In 2011, the ESF Marine Board will launch a Strategy for Marine Biotechnology; and the EC, through a collaborative working group of which Ireland is a key player, is spearheading initiatives to inform policy and create wider networks of researchers and funding agencies; with the long-term goal of strengthening Europe's position in marine biotechnology.

Activity within the Beaufort project contributes new knowledge to help Irish industry to base next generation products and processes on marine origin materials. The project has brought new expertise to Ireland and is building new capacity through the training it provides to PhD students. Progress is rapid and researchers are now bidding for major research grants from Europe as well as contributing expertise to policy making. The Marine Institute continues to engage with industry and the research community in seeking to identify opportunities to commercialise project research outputs.

A landmark event for Irish Marine Biodiscovery in 2010 is the planned biodiscovery cruise to the Porcupine Trough; this cruise will use the Marine Institute RV Celtic Explorer and the new ROV, Holland I, in collecting samples of sponges and other marine organisms from depths close to 3000 metre.

Demand for novel marine materials is principally from cosmeceutical, nutraceutical, medical and pharmaceutical industries, but demand for marine based products from biomaterials and for functional bioactive substances derived from micro algae, bacteria and seaweed is also high. It is clear that advancement in scientific disciplines such as molecular biology, bioinformatics and genomics have enabled and contributed to market growth.

Continued successes of the Beaufort Biodiscovery project and in other marine biotechnology-related projects is essential. Such projects form the backbone of Ireland's Marine Biotechnology Programme.

The National Programme in Marine Biotechnology (NMBP) was conceived in Sea Change -Ireland's Marine Research and Innovation Strategy 2007-2013, to support the national economic development by exploiting the potential of the marine biotechnology market area. The diverse range of projects and initiatives which are being developed under this programme are being co-ordinated by the Marine Institute.

Ireland has secured vibrant multinational pharmaceutical and biotechnology industries. Successive strategic investments in scientific research have enabled core areas of biological and life sciences to develop. Ireland has a strong, rapidly developing research capability in areas of science that support marine biotechnology including algal research, marine sciences, animal sciences, microbiology, systems biology, bioinformatics, pharmacology, toxicology, genetics, molecular biology, biochemistry and cell biology.

The National Marine Biotechnology Programme is helping to develop a wider research capability in Ireland to exploit marine biological resources in food, health, materials and industrial processes. Transforming our natural marine resources into commercial products requires a strong network of research centres linked to innovation-oriented industry partners. Acting as a national co-ordination point the NMBP will bring a focus to marine biotechnology research and contribute to developing Ireland's knowledge-based marine bio-economy.

Only by focusing on research excellence, as well as capturing early stage industrial inputs, will Ireland's significant on-going investment in marine biotechnology lead to commercial success.

Dr Dermot Hurst
Sea Change Management Unit
Marine Institute

Introduction

Dr. Ilaria Nardello, National Coordinator –National Marine Biotechnology Programme. Sea Change Management Unit, Marine Institute, Ireland.

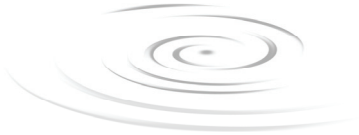
The Beaufort Marine Biodiscovery Research Project

The Beaufort Marine Biodiscovery Research Project is designed to explore the extraordinary biodiversity of our marine life, especially from a functional point of view and through genetic and biochemical techniques, and utilize this information for the production of services and goods.

This seven-year programme was funded via the Marine Research Sub-Programme of the National Development Plan 2007-2013, with the aim of developing new drugs and advanced biomaterials from marine animals, plants and micro organisms, thereby contributing to the objectives envisioned under *Sea Change - A Marine Knowledge, Research & Innovation Strategy for Ireland* 2007-2013, led by the Irish Marine Institute.

The *Beaufort Marine Biodiscovery Research Project* brings together research teams from the **National University of Ireland, Galway** (NUIG), **University College Cork** (UCC) and the **Queen's University Belfast** (QUB), and includes six new Postdoctoral researchers and twelve PhD students, to build a world class capacity in marine biodiscovery and bioprospecting. In NUIG, the research activities are focussed on sampling of bioactive compounds from marine algae, plankton and benthic invertebrates. Researchers from UCC are studying the microbial biodiversity of various marine organisms, particularly marine sponges, with a view to identifying micro organisms that may produce potential antibiotics/bioactives. The QUB team are researching marine-derived biomaterials, sponge taxonomy, microbial metabolism and antimicrobial activities.

In conjunction with the project partners, the **Marine Institute** has established a pilot-scale biodiscovery laboratory for processing, extraction, fractionation and storage of samples for bioassay screening.



The 2nd Annual Beaufort Marine Biodiscovery Research Workshop

The second annual Beaufort Marine Biodiscovery Research Workshop was held at Queen's University Belfast on 10-11th December, 2009, organised by Prof. Christine Maggs - one of the Principal Investigators of the Beaufort Marine Biodiscovery Research project, in association with the Marine Institute of Ireland.

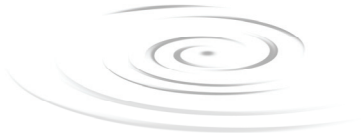
The aims of the workshop were the presentation of the progress achieved in the marine biodiscovery research area through the Irish Beaufort Marine Biodiscovery Research Awards, and the exchange of information among the project partners. Representatives from national and cross-border funding agencies were also invited to provide an overview of their strategies and funding schemes.

The workshop presented a means of discussion on cross-institute collaboration that may overcome capacity gaps and funding needs. It also created a bridge towards funding opportunities by gathering researchers and programme managers in the same audience.

The scientific sessions covered most of the conference programme and presentations were given by all the Beaufort-awarded Marine Biodiscovery postdoctoral researchers and PhD students. Researchers working in partner laboratories also introduced their work. Guest speakers included Professor Bill Baker, from the University of South Florida (USA), and prospective Biodiscovery Professor in NUIG; Prof. Russell J. Stewart from the University of Utah (USA); and Mr. Bernard Picton from the Ulster Museum (UK).

The final session was dedicated to key national and cross-border funding agencies, with speakers from InterTrade Ireland (ITI), Invest Northern Ireland (INI), the National Health Service (NHS) and Science Foundation Ireland (SFI).

The presentations given at the workshop have been captured in extended abstracts, which follow.



Database Construction and Integration

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The Biodiscovery Database is aimed at managing data generated by the National Marine Biodiscovery Programme and linking the data collected by the project's stakeholders to existing biodiversity, genetic and chemical resources. The data management system currently implemented for the tracking of specimens and sub-samples is based on the Australian Institute of Marine Science system – an in-house system designed to support the Australian Biodiscovery programme. This system has been customised for the needs of the Biodiscovery Programme in terms of data compatibility and exchange.

The database system includes software tools that have been developed for merging data collected within the Biodiscovery Project with other Marine Institute resources and external databases. These tools are for the data mining and visualisation of biogeographical, genetic and chemical information aimed at the identification of potential biodiversity and bioactivity "hotspots". The purpose of these tools is both to assist in the strategic planning of where to target sampling effort as well as to reduce the time and cost of the bioactivity discovery process.

Capacity Building at the Marine Institute Biodiscovery Laboratory

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The Marine Institute Biodiscovery Laboratory was set up to process samples according to the requirements contained in Work Packages 2 (Sampling, Extraction & Identification) and 5 (Data Management) of the Beaufort Marine Biodiscovery Research Programme.

The methods and processes developed and optimised in the Biodiscovery Laboratory for chemical extraction of biologically active compounds from marine specimens are based on those contained in the scientific literature and those at other marine biodiscovery laboratories. The constraints of cost (cost per unit sample), time (time from start to finish to obtain a crude extract), chemistry (stability of extracts and bioactives, prevention of cross-contamination, etc) and robustness (can be equally applied to all marine specimen types e.g. algae, ascidians, sponges etc) as well as sample tracking and tracing were also incorporated. An overview of setting up the laboratory itself, the equipment purchased, health and safety infrastructure and the biodiscovery processes are presented and the number and type of quality specimens collected to date are described. The initial work involved in obtaining extracts and preliminary screening for bioactives is broken down into 8 Steps. Steps 1 – 7 currently require 6-8 working days based on the current equipment and personnel resources. Some natural product databases are presented. Finally the procedure which must be followed by researchers wishing to obtain samples and/or extracts is described detailing the material transfer and confidentiality agreement.

Algal Systematics and Taxonomy in the Marine Biodiscovery Programme

Fabio Rindi, Mónica B.J. Moniz and Jazmin Hernandez-Kantun

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Reliable identification and a detailed understanding of species boundaries are a mandatory requirement for the selection of species with useful biological activities. Algal taxonomic research in the Marine Biodiscovery Program is currently focusing on the collection of seaweeds for biological screening and on the systematic investigation of some algal groups of interest for biotechnological purposes. The systematics of the Prasiolales, a group of green algae distributed in the upper intertidal zone, is being investigated combining morphological studies and sequences data of several molecular markers. Genera of maerl-forming red algae (*Lithothamnion, Lithophyllum* and *Phymatolithon*) are being investigated using the same approach. Similar investigations will be performed in the near future on other red algal genera (*Nemalion, Peyssonnelia, Scinaia*).

Exploring an Unknown World: Diversity and Taxonomy of Algal Epibionts on Sponges of Irish Shores

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In general very little information is available about the diversity of algae growing as epibionts on sponges, and most studies are restricted to the southern hemisphere. We are currently analysing the epibiontic algal flora of the sponges of Irish shores, with the purpose of enumerating species diversity, examining possible associations between species of algae and species of sponges, and selecting algal epibionts suitable as sources of pharmaceuticals. So far, we have recorded 48 algal species, of which 33% have been reported to produce molecules with medical and pharmaceutical properties. Most algal specimens growing on sponges were of small size, and some species could be identified only after isolation in culture. A number of species are currently maintained in culture; among them some have been selected as promising candidates for production of bioactive compounds. For these species it is planned to scale up the production in order to obtain sufficient amounts for bioactivity screening.

A Vision for a Marine Biodiscovery Programme in Ireland

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Despite Ireland's early entry into the marine chemistry field, with seminal studies of algal polysaccharides and practical uses of algal extracts, much of the biopharmaceutical potential of Irish marine invertebrates, algae and microbiota remain unstudied. Stimulated by renewed national interest in marine biodiscovery, including substantial funding via the 2007 Sea Change initiative, Irish academia now has the resources to undertake a significant research effort in marine chemistry. A programme to collect, inventory, screen and analyze these marine resources, utilizing expertise and facilities at the National University of Ireland Galway, the Marine Institute in Galway, Queen's University Belfast, University College Dublin, University College Cork, and Trinity College, as well as other Irish institutions, will capture significant biomedical discoveries. To be sure, biodiversity begets chemodiversity, and the complex Irish geography is ideal for the development of niche habitats; from the rugged western coast with stunning cliffs of Moher, the long, deep inlets of Counties Kerry and Cork, and innumerable islets of Mayo and Donegal, to the rocky shores of the Irish and Celtic Sea coasts, unstudied and understudied biodiversity can be accessed to supply the raw material for a biodiscovery programme. Described herein is just such a biodiscovery programme, focused on development of a repository of Irish biodiversity upon which chemical and biological analyses can be undertaken. Long term objectives of this programme include new chemical compounds and biological organisms for medical and agrochemical applications, biotech-nicological spin-off companies based on intellectual property derived from that research, and workforce development to support commercial exploitation of programme discoveries.

Metagenomic Strategies to exploit Marine Molecular Biodiversity

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Marine sponges have generated much interest due to the large range of novel and potent biologically active metabolites that have been isolated from them, with many of these sponge-derived compounds currently being investigated in clinical trials. The source of many of these metabolites is ultimately microbial; with sponges hosting a large and extremely diverse microbial community that can constitute up to 40% of total sponge volume. The sponge / microbial symbiosis have generated much interest from evolutionary, ecological and biotechnological standpoints. This association is probably the oldest existing association between microbes and metazoans, and the microbial community is implicated in many aspects of sponge biology, including both primary and secondary metabolism.

A major bottleneck in the development of sponge derived metabolites into useful pharmaceuticals is the lack of a sustainable supply. Neither sponges nor the vast majority of the sponge microbiota are amenable to culture, and wild harvest of sponges is not sustainable. To overcome this problem we have developed a functional metagenomics approach to study and exploit the microbiota of bioactive producing marine sponges. The culturable and uncultured microbiota associated with the sponge *Haliclona simulans* were analysed and compared using 16S rRNA analysis. The metagenomic analysis of microbial diversity showed that many unique bacteria were present in the sponge and that the vast majority of these micro-organisms were unrepresented in the cultured isolates. The functional metagenomics approach which we have adopted will allow access to the entire microbiota of the sponge.

Sponges were also collected from Lough Hyne Marine Reserve and these were analysed by HPLC - High Resolution FT-Mass Spectrometry. Comparison of these data showed that each sponge collected had a diverse array of metabolites present with each sponge having a different set of metabolites present. Together this metagenomic and metabolomic data shows that sponges collected from Irish coastal waters have both diverse chemistry and diverse microbiota – making these sponges an ideal target for a metagenomic based biodiscovery approach.

Exploiting the Diverse Microbial Ecology of Marine Sponges

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Marine sponges (Porifera) host a diverse assemblage of microorganisms from all kingdoms of life: *Archaea*, *Prokaryota* and *Eukaryota*. Amongst the symbiotic roles of sponge-associated microbes is host defence – through the production of bioactive secondary metabolites. Previous culture-based sponge–microbe investigations have revealed cultivable bacteria from seven different phyla whilst culture-independent investigations have identified members of 23 bacterial phyla. Sponges were collected from Lough Hyne Marine Nature Reserve, Cork, Ireland, and bacteria were cultured from the sponges on three different isolation media to access as wide a variety of diversity as possible. Over 1,400 cultures were isolated from 11 sponge species. Phylogenetic characterisation of the bacterial isolates was performed through sequence analysis of the 16S rRNA gene. The cultured isolates were affiliated to four bacterial phyla *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* (α-, β- γ- and ε-classes) and. Isolates of particular interest are members of a phylum known to include producers of bioactive secondary metabolites (*Proteo-bacteria*), members of a phylum known to include prolific producers of bioactive secondary metabolites (*Actinobacteria*) and isolates whose 16S rRNA sequence is very divergent from known sequences hosted in sequence databases, implying possible novel species and/or genera. Future work will include culture-independent metagenomic analyses of the bacterial diversity present in the sponges using next generation sequencing technology.

Culture Dependent Approaches to Identify Bioactive Compounds from Irish Marine Sponges

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Samples of 13 marine sponges were collected from Irish coastal waters and bacteria and fungi were isolated from a number of these samples. For the bacteria, 114 isolates were obtained from a *Suberites carnosus* sample and 158 isolates from a *Leucosolenia* sp. sample. 55 antibiotic resistant isolates were obtained from a *Haliclona simulans* sample. Phylogenetic analyses of the cultured bacterial isolates showed that four different bacterial phyla were represented; Bacteroidetes, Actinobacteria, Proteobacteria, and Firmicutes. For the fungi, 98 isolates were obtained from 12 of the sampled sponges. The sponge bacterial and fungal isolates were assayed for the production of antimicrobial substances in an overlay assay, and biological activities against Gram-positive bacteria and a Gram-negative bacterium were observed. 50% of isolates from *Suberites carnosus*, 38% of isolates from *Haliclona simulans*, 3% of isolates from *Leucosolenia* sp. and 33% of all fungal isolates showing antimicrobial activity against at least one of the test strains. In total, 117 bioactive isolates were discovered in the initial bioactivity screen and these were also tested for the production of antibiotics in a well diffusion assay. Two antibiotic-resistant isolates showed activity in this assay and supernatants from liquid cultures of these strains were extracted for subsequent analysis. These compounds are currently under investigation for their identity and novelty using NMR and LC-MS. Apart from the isolation of bacteria and fungi from the sponge samples, crude extracts were prepared from all 13 sponges and analysed via LC-MS. From all the peaks obtained during the analysis only a few of them have been identified as being previously known chemicals, among them known antibiotics and compounds with anticancer activity such as Hennoxazole D and Herbimycin which are likely to be produced by microbes. The traces of an interesting derivative of the calcium signal blocker Xestospongin were

found in an extract from *Haliclona simulans*. The structure of this compound is currently being determined using NMR. Apart from this, many ions detected by MS could not be linked to any known compound. Additionally, antimicrobial assays, among them an anti-Trypanosome assay, have been carried out with 9 of the extracts and 8 of them showed activity in at least one of the assays employed. Thus it is clear that the collected sponge samples and their derived microbes and fungi are a good source from which to search for novel bioactive compounds.

Anti-Fungal Activity from Marine Sponge Microbiota

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It is predicted that marine microbes may be a source of novel bioactive molecules and this potential reservoir remains to date largely unexplored. We have been studying the microbial ecology associated with the sponge *Haliclona simulans* using both culture - dependent and culture-independent approaches (Baker et al., 2008; Kennedy et al., 2008). The culture-dependent approach has identified bacteria from a number of genera, many of which display varying degree of antimicrobial activity. We report here on the screening of 130 bacterial isolates from *H. simulans* for anti-fungal activity using deferred - antagonism and disc – diffusion assays. 25 of these strains showed anti-fungal activity on plates, with culture supernatant from 15 of those strains retaining activity. The supernatant from these 15 strains was subsequently analysed using a variety of extraction and analysis procedures, and it was determined that 8 were extractable by organic solvent indicating that the activity was likely to reside with a small metabolite rather than a protein. One of the strains, a Streptomycete named SM8, was selected for further analysis. This strain secretes an activity that is inhibitory to yeasts and fungi, including the pathogens *Candida albicans* and *Aspergillus fumigatus*. The supernatant from a culture of this bacterium was treated with an XAD-16 resin which binds hydrophobic metabolites, which can subsequently be eluted with methanol. The crude extract was then fractionated by reverse-phase HPLC and fractions assayed for anti-fungal activity using the NCCLS assay. Fractions were collected by preparative HPLC and two peaks were observed which were active. Peak A was then analysed further by HPLC and it was found that there four prominent peaks were present which were active. These were analysed by high-resolution LC-MS with 2 major peaks (m/z of 585 and 601) and 2 minor peaks (m/z 587 and 443) being observed. We are currently undertaking LC-MS and NMR to determine the chemistry of this antimicrobial metabolite. Future work will expand the analysis to determine the potential cytotoxicity of the metabolite and genetically characterise the biosynthetic pathway for this anti-fungal compound.

Isolation and Characterisation of Spore-forming Bacteria from the Marine Sponge *Haliclona Simulans* with Promising Bioactive Properties

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Recent interest in marine habitats is being driven by the search for novel antimicrobial compounds as a result of a worldwide increase in antibiotic resistance and the emergence of untreatable multidrug-resistant strains of bacteria. Marine sponges have an abundant, complex, and in cases, specific microflora, where bacteria may represent as much as 40% of the sponge's biomass. These symbionts are emerging as important producers of bioactive metabolites. While sporeformers do not appear to be part of the most abundant sponge-associated bacteria, the isolation of *Bacillus* strains is frequently reported from different sponge samples, but very little is known on how these may differ from terrestrial isolates. *Bacillus* offer several advantages over other marine bacteria, including a good track-record as producers of bioactive compounds and an extensive array of molecular tools. The aim of this study was to isolate and characterise the culturable spore-forming population of the marine sponge *Haliclona simulans*, with the goal of elucidating how these may differ from their soil counterparts and to establish their potential as producers of compounds with industrial and biomedical interest. Aerobic sporeformers, isolated following heat treatment of sponge material, represented approximately 1% of the total culturable bacterial population of *H. simulans*. Isolates were identified as *Bacillus* and *Paenibacillus* strains on the basis of their physiological properties and 16S rDNA phylogenetic analysis, and were seen to belong to a variety of previously classified and potentially new species. Some *H. simulans* sporeformers display growth and sporulation properties distinct from terrestrial isolates. A significant percentage of the isolates showed protease activity and strong antimicrobial activity against indicator strains that included important food-pathogens, such as *Clostridium perfringens, Listeria monocytogenes* and *Staphylococcus aureus*. Thus it is clear that the microflora of *H. simulans* includes a diverse sporeforming bacterial population, which in some instances, demonstrate promising industrial and antimicrobial properties.

Isolation and Identification of Marine-derived Anti-biofilm Agents for Medical Device Applications

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Bacterial quorum sensing (QS) is the cell-to-cell signalling mechanism responsible for the expression of genes for virulence factors and gene products required for bacteria–host interactions and regulating the ability to form surface-associated, structured and cooperative consortia known as biofilms, which play another important role in bacterial pathogenesis and constitute a common cause of persistent infections. As QS does not seem to influence growth, it is unlikely to constitute a selective pressure for the acquisition of resistance, representing a crucial target for non-antibacterial therapy and biofilm eradication on indwelling medical devices. The aim of this research is to screen marine micro and macro-organisms for the production of quorum sensing inhibitors (QSI) and to evaluate their effects on biofilm formation and maturation of several medically relevant strains.

80 bacterial isolates were grown and purified from marine samples collected in the Strangford Lough intertidal zone based on colony morphology and stored using the Microbank system at -80°C. 65 isolates were identified based on 16S rRNA sequence analysis following DNA extraction, 16S rRNA gene amplification and sequencing. Sequences were aligned and used to construct a Maximum Parsimony phylogenetic tree. Isolates were initially screened for caseinolytic activity on skim milk agar. Isolates were further screened using the overlay method reported in 2004 by McLean et al., for the inhibition of quorum-sensing regulated Violacein production in the indicator strain *Chromobacterium violaceum* ATCC 12472 and the quorum-sensing regulated Prodigiosin production in the indicator strain *Serratia marcescens* p48 NCIMB 11857. 3 strains KS6, KS8 and LL67 showed putative QSI in the overlay assays.

Strain LL67 was grown in large 2-L seeded tray-cultures for 7 days and extracted using ethyl acetate. Disc diffusion assays on *C. violaceum* ATCC 12472 using the re-suspended LL67 organic fraction identified both an antimicrobial and a QSI effect present in the extract. The organic fraction was separated further using silica column MPLC and 72 fractions were obtained. Screening of the individual fractions with *C. violaceum* ATCC 12472 revealed antimicrobial activity in fraction 1. Screening of the fractions for QSI did not identify any active fraction. Studies using ^{1}H NMR, ^{13}C NMR, and Gas chromatography are underway to attempt to identify and characterize the bioactive molecule(s) involved.

Limits of Cell Function and Stress Responses of Marine Microbes: Biotechnological Applications

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About 70% of the Earth is covered by ocean; much of the planet's terrestrial environment is underlain by ancient salt deposits of marine origin; furthermore, numerous types of habitats occur at the coastal margins. Collectively these marine environments contain a vast reservoir of microbes with diverse phylogenetic identities, cellular phenotypes, and stress tolerances. We aim to explore the marine microbes of the island of Ireland in the context of their biotechnological potential. The focus of our approach will be to design novel sampling and isolation strategies based on investigations of marine-relevant stress parameters and the geochemistry of marine habitats and their micro-niches around Ireland. The Culture Collection produced will be used to characterise stress tolerances, to manipulate cellular metabolism, and to probe cellular extracts for proteins/ metabolites with potentially useful activities during the project period. One approach will be to use the extremophilic (and mesophilic) microbes collected to make comparisons with known model halophile/ marine microbe species in a range of growth-rate and metabolic assays in order to identify novel or exceptional phenotypic properties. Screens for antimicrobial activity will be conducted using a range of model pathogens, for antibiofilm activity and for other pharmaceutically relevant activities.

Functional Metagenomic Based Approaches to Identify Novel Enzymes with Biotechnological Application from Marine Invertebrates

David Lejon [1], Jonathan Kennedy [1], Fergal O'Gara [1,2,3] , Alan Dobson [1,2]

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In the last decade, marine sponges have been the focus of much interest, both from an ecological point of view due to their close association with a large variety of microorganisms and from a biotechnological point of view since they are a rich source of biologically active secondary metabolites. The occurrence of structural similarities between compounds from sponges and from microbes has led to the hypothesis that some of these bioactive compounds may in fact be of microbial origin. Since chemical synthesis of these natural products can be difficult and expensive, their production by microbes would be very advantageous. However, the vast majority of the sponge microbial community currently remains uncultured or uncultivable and the full diversity of the sponge microbial consortia is therefore largely inaccessible using traditional microbiological methods.

Metagenomic based approaches provide a means whereby access can be gained to the aforementioned sponge microbial consortia. These approaches involve the genomic analysis of unculturable microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms The marine sponge *Haliclona simulans* was chosen as a model system for this study, given that this sponge is found throughout Irish coastal waters and that the genus *Haliclona* has been found to be a good source of new and diverse biologically active natural products; with over 100 bioactive compounds having previously been isolated from this sponge genus. Moreover, the microbiota of the sponge has previously been shown to contain diverse microorganisms and a variety of polyketide synthase genes which are typically involved in the biosynthesis of biologically active natural products. In order to access the metabolic diversity of the sponge, a functional metagenomic based strategy has been developed. This talk will be presenting data on the construction of a metagenomic library from the sponge, containing around 40,000 clones, with an average insert size of 30-40kb. Results will be presented on the subsequent screening of this library for novel enzymatic activities; thereby confirming the sponge metagenome as a valuable resource for obtaining novel enzymes including proteases, lipases, and phosphatases.

Discovery and Application of Novel Bioactive Substances from Marine Sponges for the Control of Major Food Pathogens

John A. O' Halloran[1,2,3], Jonathan Kennedy[2,3], Burkhardt Flemer[2], Lekha Margassery[2], Marlies J. Mooi [1,3], Teresa Barbosa[3], John Morrissey[3], Alan D.W. Dobson[2,3] and Fergal O' Gara[1,3] .

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Infection caused by multi-drug resistant food-borne pathogens, such as *Salmonella* spp., *Campylobacter jejuni* and *Escherichia coli*, are a major public health concern. Therefore, there is an urgent need to identify novel antimicrobial agents to combat these pathogens. Marine sponges have been shown to be a rich source of novel bioactive compounds, many of which are thought to be produced by sponge-associated microorganisms. 522 bacterial strains from the marine sponges *Haliclona simulans*, *Axinella dissimilis*, *Polymastia boletiformis*, *Suberites carnosus* and *Leucosolenia* sp. were isolated on various media types and assayed for the production of compounds with antimicrobial activity against various Gram-positive and Gram-negative food and clinical pathogens, including *S. typhimurium*, *Listeria monocytogenes*, *Pseudo-monas aeruginosa* PAO1 and *Clostridium difficile*. While 136 (26%) of the bacterial isolates showed activity against at least one of the pathogens tested, the number of bioactive isolates varied significantly between the sponge species. *Pseudovibrio* and *Streptomyces* isolates were numerically the most abundant producers of antimicrobial activity and PCR screens of *Pseudovibrio* and *Streptomyces* isolates from *H. simulans* revealed the presence of potential antibiotic-encoding polyketide synthase and nonribosomal peptide synthase genes. Antibacterial activity of 180 cultured fungal isolates from the marine sponges was also demonstrated, with 56 (31%) active against *E. coli*, *Staphylococcus aureus* or *Bacillus subtilis*. Our study reveals that the culturable microflora of marine sponges may represent a rich source of novel antimicrobial compounds, a number of which are currently being characterised.

Biomaterials Requirements for Bone Repair – Structural Aspects

Fraser J. Buchanan

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Bone substitutes provide alternatives to autograft but do not provide equivalent *in vivo* performance. Bone tissue engineering may offer a solution in the long term but optimised scaffolds which support *in vivo* or *ex* vivo bone generation are required.

Applications for these type of bioresorbable biomaterials are in four main areas:

- tissue engineering of bone/cartilage (e.g. porous scaffolds)
- fracture fixation (e.g. screws, pins, plates, cements, adhesives)
- bone grafting (as alternatives to allograft/autograft)
- delivery of bioactive agent or drug through a controlled release mechanism.

This paper considers the opportunities available for use of marine-derived biomaterials in the above applications.

In tissue engineering it has been possible to produce porous tissue scaffolds from hydroxyapatite (HA, the mineral found in bone) by replication of a natural marine sponge (e.g. *Spongia agaricina*). This has been shown to have the ideal pore size and interconnectivity for cellular invasion [*Cunningham E. et al., Proc IMechE, 2009, Part H, Vol. 223 (6) 727, 2009*].

In terms of fracture fixation calcium phosphate bone cements are commercially available for direct injection into fracture sites. The cement is too brittle for some applications such as spinal fracture (vertebroplasty). Research is underway to address this issue through incorporation of short collagen fibres into the cement. The fibres have a tendency to bridge cracks, thereby improving the fracture toughness [*O'Hara R, et al. GRIBOI, Martinique, March 2009*]. Collagen fibres that can be sourced from marine sponges have been investigated, for example from *Chondrosia reniformis*.

Bone grafting involves compaction of a granular filler into a bone void or defect. Historically marine coral have been used for this application and are still commercially available. However there are issues regarding sustainability and bioresorption rate. Ongoing work has involved development of an algae-derived bone filler from sustainably sourced

Corallina officinalis. This material can be hydrothermally converted to HA and shows promise as a bone graft material [*Walsh P, et al. Chemical Engineering Journal, 137, 173-179, 2008*]

Preliminary investigations are underway into the potential application of coccoliths (from *Emiliania huxleyi* phytoplankton) as a surface for the adsorption/desorption of drugs, such as antibiotics. The studies will include culturing the *E. huxleyi* as well as the potential to hydrothermally concert the calcium carbonate structure to HA. An objective of this work is to engineer bone tissue constructs that will facilitate a stable drug release rate over a prolonged period of time in order to prevent infection.

In Vitro Evaluation of a Novel Porous Scaffold Based on a Natural Sponge Template for Bone Tissue Engineering

Susan Clarke[1], Eoghan Cunningham[2], Seung-Yeol Choi[2], M. McKechnie[1], George Burke[4], Nicholas Dunne[2], Gavin Walker[3] and Fraser Buchanan[2]

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Porous calcium phosphate ceramics have excellent osteoconductive properties. Pore size, distribution and interconnectivity may all affect bone cell attachment, proliferation and differentiation and there is evidence that cells prefer a degree of non-uniformity and a structure that closely resembles that of natural bone. Therefore it is hypothesised that using a naturally occurring porous structure as a template may provide a scaffold with enhanced design. Using the marine sponge, *Spongia agaracina*, as a template, a method was developed to produce hydroxyapatite scaffolds. The aim of this study was to compare cell attachment and proliferation on these scaffolds with that on HA scaffolds templated from synthetic poly-urethane sponge.

Human foetal osteoblasts (hFOBs) were seeded at $1x10^5$ cells/sample and incubated for 4 or 14 days. Outcome measures were cytotoxicity at d4 and cell number at d4 and d14. Cell morphology at d7 was examined by SEM and confocal microscopy for which cells were labelled with CelLuminate™ fluorescent micro-spheres. To determine that cells were penetrating the scaffold, cells were labelled with Live/dead™ kit, and samples were cut in half in cross section and viewed under a confocal microscope. To investigate if differences in cell response between the scaffolds were related to their physical or chemical characteristics, conditioned medium (CM) experiments were performed. Materials were soaked in complete medium (1g/ml) for 14 days. hFOBs were seeded at $1x10^5$ cells/cm^2 and exposed to the CM at concentrations of 0, 1, 10, 25, 50 and 100% for 14 days. Outcome measures were cytotoxicity (d1), cell number and alkaline phosphatase activity.

SEM and CelLuminate™ showed that cells are able to attach to both scaffolds and were able to penetrate into the pores. Live cells were found in the middle of both the natural and synthetic derived materials and there was no evidence of dead cells. Quantitatively, there were more cells on the marine scaffold than on the synthetic derived material; 0.07 µg/ml

DNA compared to 0.03 and 1.24 compared to 0.81 at d4 and d14 respectively. These differences were statistically significant at d14 ($p=0.006$). There were no differences in cell number or alkaline phosphatise activity of hFOBs cultured with CM from the two materials but there was more cell death when exposed to 100% CM from marine-derived materials.

The pore architecture of the scaffolds may be the key to the greater cell number on marine-derived scaffolds at d14. Further investigations are required to optimise bone cell attachment and differentiation. In conclusion, both scaffolds were capable of supporting cell growth and viable cells were found in the centre of the materials however more cells were found in the marine-derived scaffold.

Mechanisms of Cell Signalling in Micro Organisms Relevant to Marine Environments

Marlies J. Mooij[1,3], John O'Halloran[1,3], Teresa Barbosa [1,3], Jonathan Kennedy[3], John Morrissey[2,3], Alan Dobson[2,3], Fergal O'Gara[1,2,3].

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Cell signalling systems and the various genes under its control are crucial for bacterial virulence. As such, these signalling systems could serve as novel targets in the development of new therapeutic agents. Many bacteria use the cell density dependent cell-to-cell signalling system, quorum sensing (QS), to coordinate their virulence, including motility, the formation of biofilms and the expression of drug resistance efflux pumps. Therefore, quorum sensing is a promising target for antimicrobial drugs. In this study, *Pseudomonas aeruginosa*, a ubiquitous environmental bacterium that is among the top three opportunistic human pathogens, has been used as a model organism for studying cell signalling. Reporter assays are being used to identify novel QS and biofilm inhibitors from marine sponges, which are a rich source for structurally novel biologically active compounds. To date, 100 sponge isolates and ~14,000 clones from a metagenomic library from the marine sponge, *Haliclona simulans*, have been screened, revealing 4 very interesting candidate clones. LysR-type transcriptional regulators are another potential target for the development of novel therapeutic agents, since they are often involved in virulence. Recently, we have identified a novel set of genes, besides the MexEF-oprN efflux pump that is modulated by the LysR-type transcriptional regulator, MexT. We are currently studying if MexT and/or the genes under its control could serve as novel therapeutic targets, since MexT is involved in several virulence phenotypes, including antibiotic resistance, secretion of virulence factors through the Type 3 Secretion System, biofilm formation and swarming motility.

Recently, we have established a new zebrafish facility within the BIOMERIT Research Centre at the Microbiology department at University College Cork. We are planning to employ Zebrafish embryos as an *in vivo* biological model, to screen for drug interventions on bacterial infections, as well as for effects that bioactive compounds may have on the development of the zebrafish embryo.

Sponge biodiscovery – first get the framework right!

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The aims of this project are: to use a molecular approach to try and resolve ordinal classification of sponges, to build taxonomic capacity for sponges in Ireland and to investigate how DNA sequences can help to distinguish between sibling species and phenotypic variability in sponges.

Levi in 1957 pointed out that the Demospongiae were one of the last groups where the classification at the levels of subclasses, orders and families was still unresolved. This is still true today. In *Systema Porifera*, 2002, all genera were described in detail and the classification revised in advance of molecular results. Molecular studies have contradicted this classification in a number of ways.

The present study is based on three regions of 28S rDNA: the D1-D2, D3-D5, and D6-D8 regions. Currently, sequences have been obtained from 115 species. In addition CO1 sequences have been obtained for species from families poorly represented in Genbank i.e. Axinellidae and Raspailiidae. A D1-D8 tree of 52 species is presented here. Some of the changes have considerable importance for sampling for biodiscovery as they show that species such as *Axinella damicornis* are not related to other *Axinella* species but to Agelasidae. Agelasidae have been shown to have certain categories of bioactive molecules, but without accurate taxonomic insights some related species would not necessarily be considered for investigation.

It is concluded that molecular phylogeny is particularly useful in groups such as sponges where morphological characters are few. CO1 sequences from this study will contribute to the Consortium for the Barcode of Life (CBOL). Sibling species can be detected by using sequence data with maximum variation in the large ribosomal subunit occurring in the D2 region.

Molecular Systematics, Biogeography and Biodiscovery of Keratose Sponges

Carsten Wolff[1], Margaret Rae[2], Grace P. McCormack[1]

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Dictyo- and dendroceratid sponges have long been targeted as candidates in biodiscovery due to the high incidence of bioactive metabolites. However, these marine sponge species are a group notoriously difficult to classify. Due to the existing ambiguities in morphology-based classical systematics and ongoing difficulties in the more recent field of chemotaxonomy, the focus of this project is on applying molecular methods to investigate phylogenetic relationships of this group of sponges alongside investigations of their morphology and chemistry. Initial phylogenetic analyses of 28S rRNA gene sequences have indicated the possible presence of cryptic species. However, further work on additional gene loci is required to confirm this observation. Chemical extracts have also been prepared from a number of specimens and await testing. Few studies have been carried out at the population level in sponges, thus investigating micro-evolutionary processes in this group will contribute greatly to our growing understanding of sponge evolution. To date, 145 *Dysidea fragilis* biopsies from locations along Ireland's coast and abroad, along with a smaller number of other keratose species, have been collected for population genetic work. We have shown that the ribosomal intragenic regions (ITS1 and ITS2) commonly used in population genetics are not suitable for use with *Dysidea fragilis*. The high levels of intragenomic polymorphisms in sequenced clones make it impossible to distinguish individuals from each other. Current focus is on developing microsatellites for this species.

Appendix 1 – Workshop Agenda

Day 1: Thursday 10th December

11h00	*Welcome* Professor Christine Maggs, Queen's University, Belfast.
11h05	*Introduction and overview of the Beaufort Biodiscovery Research Award* Professor Alan Dobson, University College, Cork.

Day 1 - Morning Session - 1 - "Sampling, Extraction and Identification"

11h15	Chairperson: Professor Mark Johnson, NUI, Galway.
11h20	*Ireland's Marine Biodiversity* Bernard Picton, Ulster Museum.
12h00	*Database construction and integration* Dr. Helka Folch, Queen's University, Belfast/ Marine Institute.
11h40	*Co-ordination of sample collection, storage, extraction and characterisation* Dr. Margaret Rae, NUI, Galway/ Marine Institute.
12h20	*Algal systematics and taxonomy in the Marine Biodiscovery program* Dr. Fabio Rindi, NUI, Galway.
12h40	*Molecular systematics, ecology and bioactivity of keratose sponge* Carsten Wolff, NUI, Galway
12h50	Molecular and morphological phylogeny of sponges Christine Morrow, Queen's University, Belfast
13h00	*Exploring an unknown world: diversity and taxonomy of algal epibionts on sponges of Irish shores* Mónica Moniz, NUI, Galway

Day I - Afternoon Session -2- "Screening and Culture"

14h20	Chairperson: Professor Alan Dobson, University College, Cork.
14h25	*A Vision for a Marine Biodiscovery Programme in Ireland* Professor Bill Baker, University of South Florida.
14h45	*Metagenomic strategies to exploit marine microbial biodiversity* Dr. Jonathan Kennedy, University College, Cork
15h05	*Exploiting the diverse microbial ecology of marine sponge* Steve Jackson, University College, Cork
15h15	*Culture dependent approaches to identify bioactive compounds from Irish marine sponges* Burkhardt Flemer, University College, Cork
15h25	*Identification of novel inhibitors of pathogenic yeasts and fungi from marine microbes* Lekha Margassery, University College, Cork
15h35	*Spore forming Bacillus spp. with potential bioactivities isolated from the marine sponge Haliclona simulans* Rob Phelan, University College, Cork
15h45	*Isolation and identification of marine-derived anti-fouling agents for medical device applications* Alessandro Busetti, Queen's University, Belfast
15h55	*Limits of cell function and stress responses of marine microbes: biotechnological applications* Julianne Megaw, Queen's University, Belfast

Day 1 - Afternoon Session - 3 - "Related Biodiscovery Projects"

16h30 Chairperson: Professor Oliver Dolly, Dublin City University.

16h35 *Biotechnological methods applied to the isolation, purification and characterisation of functional food bioactive components from marine sources*
Dr. Maria Hayes, Teagasc.

16h55 *Functional metagenomic based approaches to identify novel enzymes with biotechnological application from marine invertebrates*
Dr. David Lejon, University College, Cork.

17h15 *Discovery and application of novel bioactive substances from marine sponges for the control of major food pathogens*
Dr. John O'Halloran, University College, Cork.

Day 2: Friday 11th December

Day 2 - Morning session – 4- "Funding Agencies Insight"

09h00 Chairperson: Dr. Dermot Hurst, Marine Institute.

09h05 *Innovation Island*
Aiden Gough, InterTrade Ireland.

09h25 *Northern Ireland Life Sciences*
David Bell, Invest Northern Ireland.

09h45 *Funding Opportunities in Health & Social Care R&D*
Janice Bailie, NHS.

10h05 *Science in a Connected World: building collaborative research in Ireland*
Dr. Stephen Simpson, Science Foundation Ireland.

Day 2 - Morning session – 5- "Application of Research Results"

10h45	Chairperson: Dr. Brendan Gilmore, Queen's University, Belfast.
10h50	*Extraction of higher value products from algae* Dr. Gary Sheldrake, Queen's University, Belfast
11h10	*Biomaterials requirements for bone repair – structural aspects* Dr. Fraser Buchanan, Queen's University, Belfast
11h30	*Novel biomaterials development* Dr. Susan Clarke, Queen's University, Belfast.
11h50	*Mechanisms of cell signalling in micro organisms relevant to marine environments* Dr. Marlies Mooij, University College, Cork.
12h10	*Barnacle bioadhesion* Dr. Anne Marie Power, NUI, Galway

Day 2 – Afternoon Session – 6- "Closing Session"

13h40	Chairperson: Professor Alan Dobson, University College, Cork.
13h45	*Marine bioadhesives: The sticky kisses of Phragmatopoma californica* Professor Russell J. Stewart, University of Utah, Utah, USA.
14h05	*Open discussion*
14h30	*Key issues and opportunities from the workshop* Ms. Yvonne Shields, Marine Institute.

Appendix 2 – List of participants

Attendee	Organisation
Dr. Janice Bailie	National Health Service, Northern Ireland
Prof. Bill Baker	University of South Florida, Florida, USA
Dr. Teresa Barbosa	University College Cork
Mr. David Bell	Invest NI Ulster (UK)
Dr. Fraser Buchanan	Queens University Belfast, Northern Ireland
Mr. Alessandro Busetti	Queens University Belfast, Northern Ireland
Dr. Benjamin Clark	University College Dublin
Dr. Susan Clarke	Queens University Belfast, Northern Ireland
Mr. Eoin Cunningham	Queens University Belfast, Northern Ireland
Prof. Alan Dobson	University College Cork
Prof. Oliver Dolly	Dublin City University
Dr. Nicholas Dunne	Queens University Belfast, Northern Ireland
Dr. RuAngelie Edrada-Ebel	University of Strathclyde, Scotland (UK)
Ms. Kathryn Fee	Queens University Belfast, Northern Ireland
Mr. Burkhardt Flemer	University College Cork
Dr. Barbara Fogarty	Marine Institute, Ireland

Attendee	Organisation
Dr. Helka Folch	Queens University Belfast, Northern Ireland
Dr. Gillian Gardiner	Waterford Institute of Technology
Dr. Brendan Gilmore	Queens University Belfast, Northern Ireland
Mr Aiden Gough	Intertrade Ireland
Dr. John Hallsworth	Queens University Belfast, Northern Ireland
Ms. Catriona Harrington	University College Cork
Dr. Maria Hayes	Teagasc, Ireland
Dr. Dermot Hurst	Marine Institute, Ireland
Mr. Stephen Jackson	University College Cork
Prof .Mark Johnson	National University of Ireland, Galway
Jaimie-Leigh Jonker	National University of Ireland, Galway
Dr. Jonathon Kennedy	University College Cork
Ms. Patricia Killian	Marine Institute, Ireland
Dr. Tassos Koidis	Teagasc, Ireland
Leonid Kulakov	Queens University Belfast, Northern Ireland
Dr. David Lejon	University College Cork
Prof. Christine Maggs	Queens University Belfast, Northern Ireland

Attendee	Organisation
Ms. Lekha Menon Margassery	University College Cork
Mr. Martin McAleese	Agri-Food & Biosciences Institute, Northern Ireland
Ms. Marion McAneney	Intertrade Ireland
Ms. Ciara McCarthy	Dublin City University
Mr. Paul McEvilly	National University of Ireland, Galway
Ms. Helena McMahon	Institute of Tralee
Ms. Julianne Megaw	Queens University Belfast, Northern Ireland
Ms. Monica Moniz	National University of Ireland, Galway
Dr. Marlies Mooij	University College Cork
Dr. John Morrissey	University College Cork
Ms. Christine Morrow	Queens University Belfast, Northern Ireland
Mr. Cathal Murphy	University College Dublin
Dr. Ilaria Nardello	Marine Institute, Ireland
Ms. Margaret Mary Nimoh	Queens University Belfast, Northern Ireland
Prof. Fergal O'Gara	University College Cork
Mr Donal O'Gorman	Dublin City University
Dr. John O'Halloran	University College Cork

Attendee	Organisation
Mr. David O'Neill	Waterford Institute of Technology
Dr. Geoffrey O'Sullivan	Marine Institute, Ireland
Dr. Laurie O'Sullivan	Waterford Institute of Technology
Dr. Iwan Palmer	Queens University Belfast, Northern Ireland
Mr. Rob Phelan	University College Cork
Mr. Bernard Picton	Ulster Museum, Northern Ireland
Dr. Anne Marie Power	National University of Ireland, Galway
Dr. Margaret Rae	National University of Ireland, Galway
Mr. Carl Reddin	Queens University Belfast, Northern Ireland
Mr. Paul Ricketts	Queens University Belfast, Northern Ireland
Dr. Fabio Rindi	National University of Ireland, Galway
Dr. David Rooney	Queens University Belfast, Northern Ireland
Dr. Gary Sheldrake	Queens University Belfast, Northern Ireland
Ms. Yvonne Shields	Marine Institute, Ireland
Dr. Julia Sigwart	Queens University Belfast, Northern Ireland
Dr. Stephen Simpson	Sience Foundation Ireland
Dr. Dagmar Stengel	National University of Ireland, Galway

Attendee	Organisation
Prof. Russell J. Stewart	University of Utah, Utah, USA
Ms. Lucille Stuani	Marine Institute, Ireland
Mr. Declan Troy	Teagasc, Ireland
Dr. Pamela Walsh	National University of Ireland, Galway
Mr. Carsten Wolff	National University of Ireland, Galway

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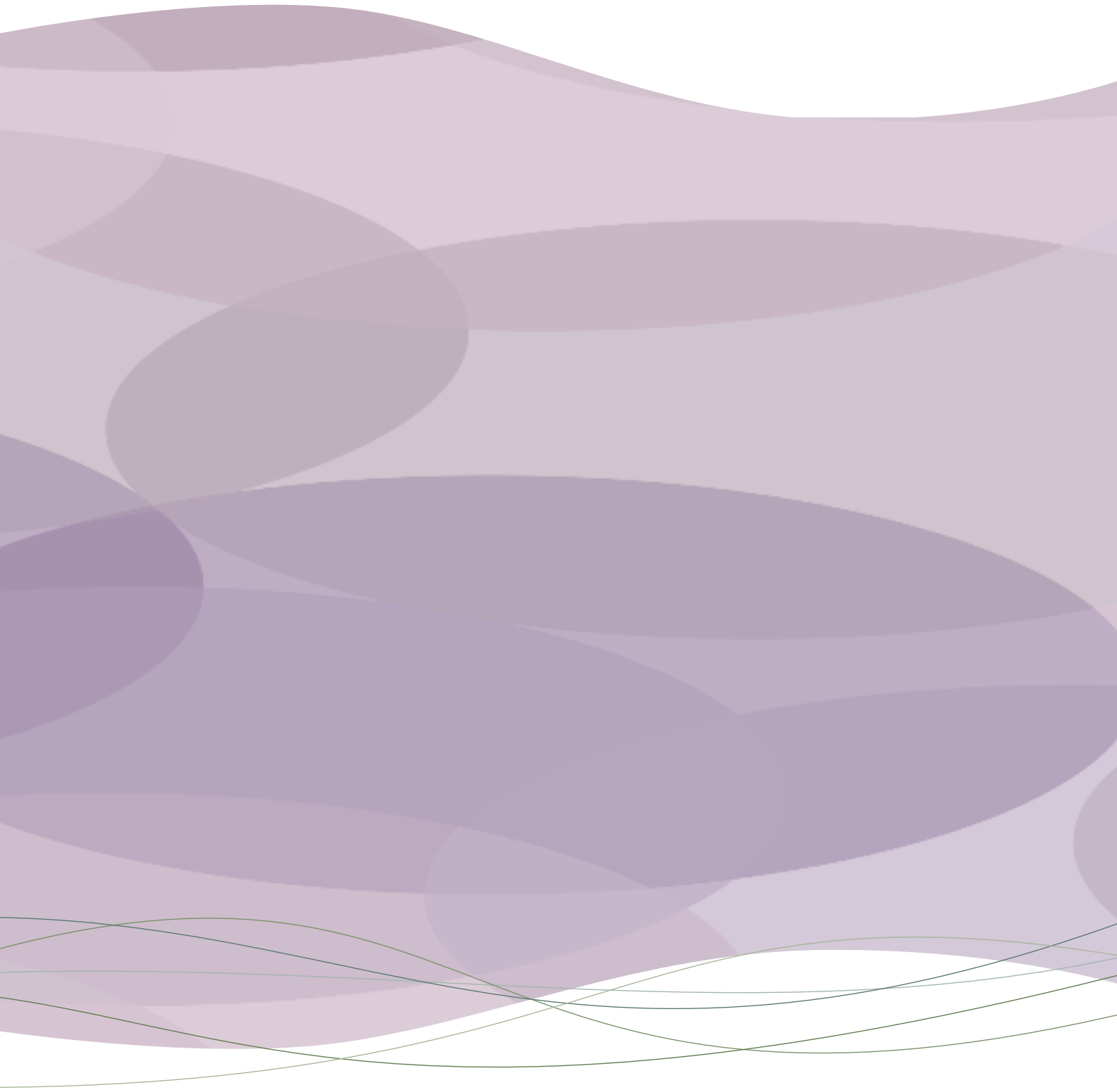

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