QMB Abstracts Marine Biology

MB1: Two sides of the same coin: promoting or avoiding the evolutionary consequences of domestication in marine aquaculture

Naish, K.A.[1], Waters, C.D[1], Brieuc, M.S.O.[1,3], Martinez, V.[2]

[1]School of Aquatic and Fishery Sciences, University of Washington, Seattle, USA
[2] Faculty of Veterinary and Animal Sciences, University of Chile, Santiago, Chile
[3] Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway

Marine aquaculture takes many forms and has different goals, from closed culture systems that enhance production, to hatchery releases that support declining wild populations. At one end of the spectrum, domestication is deliberately encouraged, whereas at the other it is optimistically avoided. Most marine populations in culture are recently derived from their wild progenitors, affording a powerful means to characterize evolutionary processes underlying domestication. This information in turn has significant potential to inform their management. In production systems, key loci involved in domestication may be used to accelerate selective breeding or develop new lines, while in conservation systems, management approaches aimed at avoiding adaptation to culture may be evaluated. Pacific salmonids provide useful models for such studies, because they are extensively cultured in production and conservation-based hatcheries, and genomic resources are readily available for most species. Here, three case studies will be used to examine the genetic consequences of domestication, and its relevance to ongoing management in marine aquaculture. The first example compares several production strains of Coho salmon to their wild source population to identify both shared and unique evolutionary processes involved in their domestication. The second examines the evolutionary consequences of disease management strategies in conservation hatcheries that release anadromous Steelhead salmon. The third describes the evaluation of “best case” versus “worst case” scenarios in avoiding evolutionary change in captive rearing of a Chinook salmon population, where there are reduced options for population recovery. Throughout, the comparative aspects of the studies will be examined, with the aim of exploring how different culture systems and species may be used to inform common goals in the management of marine populations.

MB2: New Zealand aquaculture breeding and genomics, progress and opportunities for three flagship species

Symonds, J.E. [1], King, N.[1], Walker, S.P.[1], Roberts, R.D.[2], Malpot, E.[3], Clarke, S.M.[4], Dodds, K.G.[4], Blanchard, B.[5], Sutherland, D[5]., Tate, M[6]., Buxton, P.[6]

[1]Cawthron Institute, 98 Halifax Street East, Nelson, NZ, [2]SPATnz, 139 Glen Rd, Glenduan, Nelson, NZ, [3]Moana New Zealand, 139 Glen Rd, Glenduan, Nelson, NZ,
[4]AgResearch, Invermay Agricultural Centre, Mosgiel, NZ, [5]Mount Cook Alpine Salmon, Twizel, NZ,
[6]Sanford Limited, 119 Riverside Rd, Kaitangata, NZ

Aquaculture is a significant primary industry in New Zealand (NZ) and currently produces over NZD $400 million p.a. in export revenue (www.aquaculture.org.nz) with a target to increase revenue to $1 billion p.a. by 2025. Although tonnage is small on a global scale, the industry has built an international reputation for the supply of high quality seafood based on three flagship species, Greenshell™ mussels (*Perna canaliculus*), Pacific oysters (*Crassostrea gigas*), and king (Chinook) salmon (*Oncorhynchus tshawytscha*).

Aquaculture of all three species began in the 1960-70s, but it was not until the mid-1990s that stock improvement through selective breeding was implemented. The first king salmon family breeding programme was established in 1994 and the Cawthron Institute initiated a Pacific oyster breeding programme in 1999 using a combination of between- and within-family selection. In 2002 Cawthron produced the first Greenshell™ mussel families using wild parents and has since established a family-based breeding programme, now operated and managed by BreedCo Ltd. In 2007, the second largest salmon farming company, Sanford Ltd., moved away from mass selection and developed a combined between- and within-family selection programme. These programmes have provided significant benefit to the industry and have also allowed industry to respond effectively to new challenges, such as the mass mortalities of Pacific oysters, which first occurred in 2010 due to a highly pathogenic variant of the oyster herpes virus (OsHV-1 µvar). Genomics resources have been developed to support these programmes based on SNP genotyping-by-sequencing and SNP array approaches. This has enabled evaluation of the genetic health of the breeding populations and identified the potential benefits of genomic selection. Genomics represents an opportunity to increase genetic gain and more effectively utilise the potential for within family selection in all three flagship species.

MB3: Developing Snapper for Aquaculture: The roadmap of genetic research from wild to farmed fish

Morrison-Whittle, P.[1], Ashton, D.[1], Wellenreuther, M.[1,2],

[1] The Institute of Plant and Food Research Limited
[2] School of Biological Sciences, University of Auckland

Developing a species for aquaculture through selective breeding is a continually evolving science. Traditional breeding systems, while ultimately effective, can be slow, inefficient and may yield highly inbred populations that are vulnerable to disease and resistant to further improvement. These problems are greatly reduced through modern breeding systems which harness extensive genomic research to inform and enhance selective breeding of target species to maximize generational gains. As a result of this genomic focus, modern breeding programmes generate a considerable amount of data that we can use to further our understanding of commercially and culturally important species.

In this talk, we will examine the genetic resources generated as a part of the accelerated breeding programme for New Zealand Snapper at PFR and explore some of the insights gleaned on one of New Zealand's most important fin-fish species.

MB4: A review of recent advances in reproductive technologies of fish: the dawn of a new era?

Lokman, P.M.[1]
[1]Department of Zoology, University of Otago, Dunedin, NZ

Reliable availability of fingerlings for finfish farming is crucial for the commercial success of a farming venture. Artificial propagation, whether or not achieved using hormonal manipulation of broodstock with fertility drugs, has been key to ensuring fingerling supply. More recently, new technologies, centered around germ cell manipulation ("surrogate broodstock") and genetic modification, provide promise for further development of reproductive control and fingerling quality. Thus, the transplanting of germ cells from slow-maturing donors into fast-maturing (preferably sterilized) recipients of related species provides scope for shortening life cycles and hence, generation intervals, with benefits to broodstock handling and genetic selection. External fertilization further enables interfering with primordial germ cell survival and/or migration to the gonadal ridge. As a result, sterile offspring can be produced in which the trade-off between growth and reproduction is no longer affecting animal growth rate and farm profitability. Similarly, application gene editing technologies may prove valuable for the production of sterile fish and/or for modifying protein-encoding genes with known relation to desirable traits. The use of sterile, modified salmon is already approved for several Northern hemisphere markets and is likely to drastically alter the aquaculture landscape in the future.

MB5: A whole genome-level analysis of New Zealand tarakihi stock structure (*Nemadactylus macropterus*)

Papa, Y.[1], Morrison, M.A.[2], Wellenreuther, M.[3,4], Ritchie, P.A.[1]
[1]Victoria University of Wellington, Wellington, NZ, [2]National Institute of Water and Atmospheric Research, Auckland, NZ, [3]New Zealand Institute for Plant & Food Research, Nelson, NZ, [4]University of Auckland, Auckland, NZ.

Tarakihi (*Nemadactylus macropterus*) is widely distributed around the inshore areas of New Zealand and South of Australia. It supports an important commercial fishery with annual landings in New Zealand averaging over 5000 t for the past 40 years. Very little is known about its stock structure and previous low-resolution genetic studies have sampled around Australia but only analysed one sample site from New Zealand. The aim of this research is to use whole-genome sequencing of a large population sample (1400 specimens from 20 New Zealand locations and two locations in Australia) of *N. macropterus* to determine the population genetic structure. A high-coverage draft de novo genome will be assembled as the reference genome and coupled with a sample of several hundred low-coverage tarakihi genome sequences. This will be one of the first genome-level studies of a New Zealand fishery species and will enable precise population genetic testing for differentiation and the discovery of adaptive variation. The results of this study will be compared to a parallel study that will sample mitochondrial DNA.

MB6: Studying whole body regeneration and evolution in marine chordates

Megan J Wilson

Department of Anatomy, University of Otago, Dunedin, New Zealand.

Tunicates are filter feeding marine invertebrates that compose the closest phylogenetic group to the vertebrates. This chordate subphylum contains a particularly diverse range of reproductive methods, regenerative abilities and life-history strategies. Consequently, tunicates provide an extraordinary perspective into the emergence and diversity of chordate traits. To gain further insights into both the evolution of tunicates and their regenerative properties, we have sequenced the genome and the whole-body regeneration (WBR) transcriptome of the colonial ascidian *Botrylloides leachii*. Additionally, we are studying the process of WBR through gene expression studies and small molecular inhibitory assays. Here, I will give an overview of our current research in tunicate evolution and biology at Otago.

Comparative genomics between 5 sequenced tunicate genomes revealed a set of proteins unique to colonial tunicate genomes that function in circulation, wound healing and cell communication. Further analysis of ancient gene gene clusters, identified many examples of multiple cluster breaks and gene dispersion, suggesting several lineage-specific genome rearrangements occurring during tunicate evolution.

Epigenetic processes are known to play an important role in development, healing and regeneration. To determine if histone deacetylases (HDACs) is required for WBR, we inhibited its action using valproic acid (VPA) and Trichostatin A (TSA). HDAC inhibition prevented the final morphological changes normally associated with WBR and resulted in aberrant gene expression. Additionally, atypical expression of a stem cell marker, *Bl_Piwi* was found in immunocytes upon HDAC inhibition. Together, these results show that HDAC function, specifically HDAC I/IIa class enzymes, are vital for *B. leachii* to undergo WBR successfully.

Future work using transcriptomics, siRNA and cell tracing experiments aim to unravel why *B. leachii* can regenerate a new adult in less than 10 days, whereas most chordates including other ascidians, have much poorer regenerative abilities.

MB7: The implications of gene editing technology for New Zealand

Barry Scott
Institute of Fundamental Sciences, Massey University, Palmerston North, NZ

The revolution in gene editing technologies is making it easier to make targeted changes in the genomes of animals, plants and microorganisms. The development of these new technologies has huge potential benefits in many sectors including healthcare, agriculture and conservation. However, the technology to carry out gene editing and the ideas about how it might be applied are, in many cases, moving well ahead of public understanding and consideration of the proposed changes, and any consensus on how this technology might be used.

To explore the implications of gene editing technology for New Zealand, the Royal Society Te Apārangi has convened a multidisciplinary panel of experts, supported by a Māori reference group, to consider the social, cultural, legal and economic implications of gene-editing technologies for New Zealand.

The terms of reference for the panel are to:

- Raise awareness of the current gene editing technologies, their recent development and what they are being used for
- Outline the technologies' opportunities and risks, including current global practice
- Provide insight and advice for public, business and government audiences on the future implications of these new technologies for New Zealand

The approach taken by the panel has been to generate a series of discussion papers containing various gene-editing scenarios to initiate a conversation with the NZ public. To date, papers on gene editing in healthcare and pest control have been released and a series of meetings held to canvas views of policy makers, industry, scientific and community organisations, and high school students. Two additional pieces of work are in progress on *The use of gene editing in the primary industries* and the *Implications for the New Zealand regulatory framework*.

This talk will provide an overview of the activities of the panel, progress to date, major issues encountered and future challenges that lie ahead.

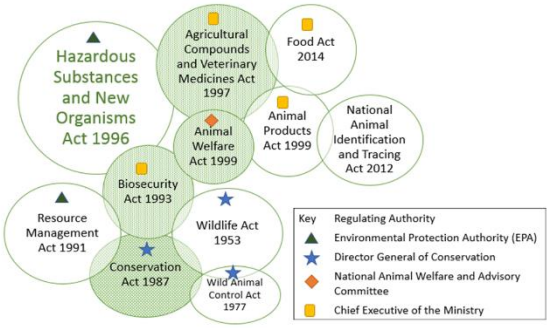
Reference

- https://royalsociety.org.nz/major-issues-and-projects/gene-editing-in-aotearoa/

MB8: Gene editing in Aotearoa – legal considerations

Julie Everett-Hincks and Mark Henaghan. Law Faculty, University of Otago, Dunedin, New Zealand.

Gene edited crops and animals pose significant new challenges for regulation. Under current NZ legislation (Hazardous Substances and New Organisms Act, 1996) and a judicial ruling on interpretation of the legislation and regulations, the status of gene-edited crops and animals in New Zealand are considered genetically modified. A precautionary approach is employed for regulating these new organisms. The implications from the effects of differing legislation and regulatory authorities has been investigated, identifying legal and policy issues requiring consideration for gene editing use in our primary industries.



Professor Mark Henaghan and Dr Julie Everett-Hincks have been working with the Gene Editing Panel for Royal Society Te Apārangi, providing legal advice as to the potential use of gene editing in the areas of human healthcare, pest control and primary industries. Royal Society Te Apārangi is encouraging New Zealanders to consider and share their views on some potential uses of gene editing in New Zealand. To assist public discussion, two papers have been produced outlining scenarios for the use of gene editing for both pest control and healthcare. A further paper with scenarios for the use of gene editing in primary industries will be published soon, along with a paper examining current legislation and regulation. The papers have been produced by a multidisciplinary expert panel convened by the Society and co-chaired by Professor Barry Scott, who is also Vice President of the Society and a Professor of Molecular Genetics at Massey University.

For further information please contact Dr Julie Everett-Hincks jeh@otago.ac.nz
More information can be found by visiting the Royal Society Te Apārangi website: https://royalsociety.org.nz/major-issues-and-projects/gene-editing-in-aotearoa/

MB9: Social License or Cultural License: Is there a difference?

Maui Hudson
University of Waikato

Gaining a social license from the public for the adoption of new biotechnologies has become the focus of increasing attention. The level of trust the public has in the scientific community to make responsible choices in the public interest has seemingly declined over a number of years. Gene editing is the most recent addition to the genetic engineering toolbox to challenge public perceptions and ethical sensitivities. Understanding and mitigating their concerns is an important part of social license but how are cultural rights and interests reflected in this dialogue? This presentation will reflect some of the emerging Māori perspectives on gene editing and discuss what would be required to gain a cultural license.

MB10: Social license in the marine environment: Dissecting the discourse

Sinner, J.[1], Newton, M.[1], Farrelly, T.[2]
[1]Cawthron Institute, [2]Massey University

The term “social license to operate”, or SLO, has increasingly featured in public discussion about commercial operations in the marine environment. Choice of wording and sentence structure can affect power relations between people and groups, so how the term is used matters. We analysed grey literature documents such as company reports, government policy documents and press releases to examine the implications of how SLO is defined and deployed with respect to NZ’s marine industries. We show that this discourse has been dominated by industry and central government voices, who frequently vest agency over SLO with industry and then state or imply that industry already has SLO and just needs to maintain or improve it. Whether inadvertent or intentional, this choice of language empowers industry at the expense of communities and iwi. Industry and government could change their wording to send a different, more empowering message to iwi and community groups about seeking their acceptance and trust. This would help achieve the vision of a blue economy, increasing benefits from the ocean environment while sustaining communities and marine ecosystems.

MB11:Conflicts between agricultural and tourism sectors: evidence for solutions

Damien Mather
University of Otago
[1]Department of Marketing, University of Otago, Dunedin, NZ

Synthesising our research findings on international importers' trust in NZ agricultural exports[1, 2], crises recovery in food products[3], international market acceptance of food production technologies[4, 5] and country food production technologies on inbound tourism markets[6] with pervasive current affairs discourses surrounding NZ tourism sector and proposed agricultural production strategies[7] we noted evidence for solutions that might met all sectors' needs. We found no evidence that the choice of either agricultural, or any other sector amongst production technologies supressed inbound tourist demand[6].

Evidence for consumer or inbound tourist resistance to some agricultural production technologies offered by actors outside of the agricultural sector based on stated preferences we found to be seriously flawed[8]. This has long distracting discourse from more substantial issues such as damage to recreation land and water ecologies by pests and other agricultural management strategies which are far riskier to inbound tourist demand and highly incongruous with the positioning of New Zealand to international tourists. We find that, paradoxically, opportunities to efficiently deal with those real problems with biotechnology have been needlessly overlooked.

Furthermore, we find evidence that the profitability of both sectors can be substantially enhanced and protected with the judicious application of biotechnology to agricultural production, land and water ecology management.

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3. Chen, S., Knight, J., Gao, H. & Mather, D. 289-290 (Springer International Publishing, Cham; 2018).
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5. Knight, J.G., Mather, D.W., Holdsworth, D.K. & Ermen, D.F. Acceptance of GM food - an experiment in six countries. *Nature Biotechnology* **25**, 507-508 (2007).
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7. Knight, J., Holdsworth, D. & Mather, D. 1-89 (University of Otago, Dunedin, NZ; 2003).
8. Mather, D.W. et al. Social Stigma and Consumer Benefits Trade-Offs in Adoption of Genetically Modified Foods. *Science Communication* **34**, 487-519 (2012).

MB12: The regulation of GMOs in New Zealand

Strabala, T. J.
New Organisms, Environmental Protection Authority, Wellington, NZ.

The concept of a 'new organism' in the New Zealand context came into being with the Hazardous Substances and New Organisms Act, 1996 (The HSNO Act), and is defined as any organism not present in New Zealand on or immediately before 29 July 1998, as well as any genetically modified organism. The Environmental Protection Authority is responsible for administering the HSNO Act, and as such regulates all New Organisms, including GMOs.

A GMO in New Zealand has a broad definition, but refers to Regulations specifying those organisms that meet the definition, but are considered exempt. These regulations were reviewed and redrafted in 2016 in response to a High Court challenge to the result of another of EPA's functions, the statutory determination of whether or not any organism is a New Organism under the HSNO Act.

The EPA has the power to grant various types of approvals for GMOs, which mostly involve the importation into containment, or the development of GMOs in containment. However, the EPA also has given approvals for the field trials and release of various GMOs since the inception of the HSNO Act. I will discuss the aspects of the law that EPA must consider in coming to a decision for any approval for the release of a GMO, or a determination as to whether or not an organism is or is not a GMO for the purpose of the HSNO Act, using recent EPA decisions as examples.

MB13: Gene editing technology: how it fits in New Zealand's policy framework
Gene editing technologies: how they fit in New Zealand's policy framework

Wouters, M.
Hazardous Substances & New Organisms Policy team, Ministry for the Environment, Wellington, NZ

Withdrawn

MB14: Genomic Selection for Improvement of Populations

Garrick, D.J.[1]
[1]AL Rae Centre of Genetics and Breeding, Massey University, Ruakura Research Centre, Hamilton, NZ.

Genetic improvement is easy to achieve – it is a simple matter of selecting above average candidates to use as parents in each successive generation. However, the challenge in practice is in cost-effectively predicting the merits of the candidates at as young an age as possible. Mass prediction uses individual phenotypes to predict genetic merit, pedigree selection relies mostly on phenotypes from close relatives sometimes including the selection candidate itself to predict genetic merit, whereas genomic prediction sums up the predicted effects of all the chromosome fragments inherited by the selection candidate. The accuracy of genomic prediction is influenced by our ability to reliably predict the effects of all the chromosome fragments.

Predicting the effects of chromosome fragments depends upon population aspects such as effective population size, which determines the size and number of segregating chromosome fragments; the size of the so-called training population used to predict the effects of each and every chromosome fragment; the heritability of the traits which effects the amount of data required to partition phenotypic performance into genetic and residual effects; the structure of the training population which affects the extent to which chromosome fragments inherited by the selection candidate were confounded in the training population; and the genetic architecture of the selection traits, which influences the genomic location and number of chromosome fragments influencing the trait as well as their mode of gene action.
Beyond these biological, genetic, genomic and statistical factors, the single most important factor determining the utility of genomic prediction in a given circumstance is the value proposition. The value proposition determines the level of investment that can be attracted to collect relevant phenotypic and genomic information that is critical to effective genomic prediction. These aspects will be described in the context of implementing genomic selection in some agricultural circumstances.

MB15: Optimised selection strategies in New Zealand aquaculture breeding schemes

Hely, F.S.[1], Symonds, J.E.[2], Walker, S.P.[2], Amer, P.R.[1]
[1]AbacusBio Ltd, Dunedin, NZ, [2]Cawthron Institute, Nelson, NZ

Selective breeding schemes have been implemented successfully in both research and commercial aquaculture species in New Zealand over the past 20 years. In aquaculture breeding schemes with high levels of individual fecundity compared with many other organisms, there is a significant challenge to maintain a genetically diverse population within limited infrastructure, while also driving genetic gains as rapidly as possible. An elegant solution is to deploy optimised parent and mating selection pair selection methods which maximise genetic gain while constraining inbreeding accumulation. A simulation model was initially developed to test the efficacy of applying a two-stage optimisation approach, combining optimised parent selection with minimum inbreeding mating[1], and showing the superiority of this approach in terms of increased genetic gain at similar or lower rates of inbreeding accumulation when compared to non-optimised methods. A challenge in aquaculture breeding schemes can come from the difficulty in maintaining equal proportions of offspring from mating pairs with potentially high, but very erratic levels of fecundity. The simulation model was extended to model these unequal family proportions proportions within an aquaculture breeding scheme, to test the impact on inbreeding accumulation and the efficacy of the optimised parent and mate selection methods under these conditions. The simulation results showed that it was possible to use optimal parent selection and minimum inbreeding to maintain reasonable levels of inbreeding in a small aquaculture breeding scheme with moderate to severely unequal family proportions, although at the cost of some genetic gain.

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MB16: Delivering Genomic solutions to New Zealand's Biological Economy

Clarke, S.M., Dodds, K.G., Brauning, R., Hess, A.S., Ashby, R.L., Van Stijn, T.C., Anderson, R.M., Caulton, A.J., Rowe, S.J., Bilton, T.P., McCulloch, A.F. and McEwan, J.C.

AgResearch Ltd, Invermay Agricultural Centre, Private Bag 50034, Mosgiel 9053, New Zealand

To enhance the value, productivity and profitability of the New Zealand biological economy, AgResearch has developed a suite of genomic tools. In addition to developing a suite of SNP array based genotyping tools, AgResearch has also invested in genotyping by sequencing (GBS) methods, both targeted and restriction enzyme based. For restriction enzyme based GBS, combining low-depth sequencing with algorithms that produce bias free genomic relationship matrices we can estimate: breed composition, pedigree, traceability, inbreeding and co-ancestry as well as using directly in existing mixed models (GBLUP) to estimate breeding values. In addition, further developments for GBS analysis has established methods to undertake GWAS, linkage mapping, estimation of linkage disequilibrium and derivatives such as the effective population size, Ne. I will present the development and implementation of genomic tools in the NZ livestock, forage and aquaculture industries which have been extended for use in genetic diversity studies.

MB17: Genomics of New Zealand trevally: identifying the genetic basis of quantitative traits to inform a newly developed breeding programme.

Valenza-Troubat, N.[1,2], Ashton, D.[1], Ritchie, P.[2], Wellenreuther, M.[1,3]
[1]The New Zealand Institute for Plant and Food Research, Nelson, NZ, [2]School of Biological Sciences, Victoria University of Wellington, Wellington, NZ, [3]Faculty of Science, University of Auckland, Auckland, NZ.

Most diversity in phenotypic traits is due to a combination of variation alleles at multiple Quantitative Trait Loci (QTL) and environmental effects (Mackay, 2001). Understanding the complex network of genes underlying phenotypic variation and their external modulation has been a major and longstanding challenge in genetics (Fisher, 1930). In animal breeding, one of the main goals is to identify individuals that have high breeding values for traits of economic interest and use them to produce offspring carrying these particular traits within short time frames (Dekkers, 2012). Modern genetic marker-informed breeding programmes can help accelerate these gains by focusing directly on the inherited components of traits and using parentage assignment to maximise family representation and control inbreeding (Vandeputte and Haffray, 2014).
Aquaculture has been slow to add genomic information to breeding programs. Here, we present the first efforts to identify the key genes that influence growth-related traits in a new aquaculture species, the white trevally (*Pseudocaranx georgianus*). We will generate the first genotype-phenotype map for this species to identify QTLs and understand their distribution and effects sizes. Ultimately, our research will combination of genome-wide DNA sequence information and phenotypic trait data to gain fundamental insights into the genetic architecture of traits and the extent to which these by genetic variation.

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3. Dekkers JCM (2012) Application of genomics tools to animal breeding. Current Genomics, 13, 207–212.
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MB18: The genetic consequences of past climate change on marine mammals inferred from ancient DNA and genomics

Foote, A.D.
Molecular Ecology and Fisheries Genetics Laboratory, School of Biological Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK

Ancient DNA and coalescent analyses of modern genomes provide a window into the demographic changes undergone by populations in response to the past climate change of the Late Pleistocene – Holocene transition. This period was characterised warming temperatures, rising sea level and the retreat of the ice sheets at high latitudes; changes that are also associated with present-day ongoing global warming. Thus, hindcasting to the changes of past can provide some predictive power of how populations may respond to the current challenges of climate change.

In this talk, using examples from studies of marine mammals, including bottlenose dolphins, killer whales and bowhead whales; I will highlight how ancient DNA studies have highlighted differences between the terrestrial and marine mammals in response to past climate change. The low cost of movement in the marine environment has enabled some populations to track their habitat as it shifts to higher latitudes during periods of warming. However, although some populations may be able to expand their range to colonise emerging habitats, this process is often accompanied by the extinction of lineages at the expansion edge and a subsequent increase in genetic drift and reduced efficacy of selection. This can result in populations at the high latitude extremes of the species range having increased deleterious mutation loads and reduced fitness relative to the source populations. Thus, the impacts of the climate change on populations can be both complex and subtle.

MB19: Environmental DNA for biodiversity, biosecurity and monster hunting

Gemmell, N.J.
Department of Anatomy, University of Otago, Po Box 56, Dunedin 9054, New Zealand.

Environmental DNA, or eDNA, is a smorgasbord of organic materials left behind by living things as they pass through their environment. This trace material is increasing being used to make sense of previously hard to study species, communities and ecosystems on land, in the air, and in the water. In marine systems we are testing the power of eDNA approaches for rapid and accurate assessments of biodiversity and ecosystem health - key enablers of ecosystem-based management. While recent studies, including our own, have shown the potential of eDNA to detect even rare, highly mobile, marine organisms, questions remain about the spatial and temporal resolution of marine eDNA. We have explored these questions in a number of marine settings around New Zealand and find extraordinary spatial resolution in our eDNA work. Temporal effects also appear to be surprisingly modest. I will discuss our findings in the context of future plans to monitor our aquatic systems for fisheries, conservation, biosecurity, and other purposes including the occasional monster hunt.

MB20: Bursting the Limits of Time: Ancient Population Genomics of Adélie penguins

Lambert, D.M.[1], Parks, M. [1], Bilge, A. [2], O'Rorke, R.[1], McComish, B.[3], Kumar, M.[1], Drummond, A.[4], Holland, B.[3], Baroni, C. [5,6], Salvatori, M.C. [5,6], Subramanian, S.[7], Millar, C.D.[8]
[1]Environmental Futures Research Institute, Griffith University, Nathan, Australia
[2]Department of Statistics, University of Washington, Seattle, Washington, United States of America
[3]School of Physical Sciences, University of Tasmania, Hobart, Tasmania, Australia
[4]Department of Computer Science, University of Auckland, Auckland, New Zealand
[5] University of Pisa, Dipartimento di Scienze della Terra, via S. maria n., 53 Pisa, Italy
[6]CNR-IGG, Institute of Geosciences and Georesources, Pisa, Italy
[7]GeneCology Research Centre, University of the Sunshine Coast, Australia
[8]School of Biological Sciences, University of Auckland, Auckland, New Zealand

The ecological study of individual species (autecology) once dominated the field of ecology. However, since the 1970's there has been an emphasis on synecology (population and community ecology). The advantages/disadvantages of these two approaches have been the subject of recent debate. We report here the results of six genomic, ecological and behavioural studies of a single marine / terrestrial species: the Adélie penguins (*Pygoscelis adeliae*) of Antarctica and consider our results in relation to both ecology and evolution.
We have sequenced complete genomes of 56 ancient Adélie penguins (including 22 at 8x coverage) from breeding colonies along the Ross Sea coast of Antarctica. These remains date to 46,500 yrBP. In addition, we have sequenced 24 contemporary Adélie penguins to 21× coverage from around the continent. We have used these data to estimate comprehensive molecular rates in Adélie penguins by comparing entire genomes, intergenic, intronic regions, protein-coding genes and putative regulatory regions genomic sequences from serial time points.
Using known family material, we report mutation rates for many loci including microsatellites that are widely used in both evolutionary and conservation biology studies. In addition, we used 27 million microsatellites loci from modern and ancient Adélie penguin genomes and 63 published chordate genomes, to identify any changes in these important loci. From an ecological perspective, we report ancient DNA studies aimed at mapping changes in penguin diet over the Holocene and Pleistocene periods. Antarctica is the classic example of food webs and by using guano of different ages we are able to illustrate changes in diet of individuals over this remarkable range of ages. Finally, from our field studies we are able to show the remarkable stability of mating systems in this species, in the face of extraordinary environmental challenges.

MB21: Subsistence practices, past biodiversity, and anthropogenic impacts revealed by New Zealand-wide ancient DNA survey

Seersholm, F.V.[1], Cole, T.L.[2], Grealy, A.[1], Rawlence, N.J.[3], Greig, K.[4], Knapp, M.[5], Stat, M.[6], Hansen, A.J.[7], Easton, L.J.[3], Shepherd, L.[8], Tennyson, A.J.D.[8], Scofield, R.P.[9], Walter, R.[4], Bunce, M.[1]
[1]Trace and Environmental DNA Laboratory, School of Molecular and Life Sciences, Curtin University, Perth, Australia; [2]Long-Term Ecology Laboratory, Manaaki Whenua Landcare Research, Lincoln, NZ; [3]Otago Palaeogenetics Laboratory, Department of Zoology, University of Otago, Dunedin, NZ; [4]Department of Anthropology and Archaeology, University of Otago, Dunedin, NZ; [5]Department of Anatomy, University of Otago, Dunedin, NZ; [6]Department of Biological Sciences, Macquarie University, Sydney, Australia; [7]Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark; [8]Museum of New Zealand Te Papa Tongarewa, Wellington, NZ; [9]Department of Natural History, Canterbury Museum, Christchurch, NZ.

New Zealand's geographic isolation, lack of native terrestrial mammals, and Gondwanan origins make it an ideal location to study evolutionary processes. However, since the archipelago was first settled by humans 750 y ago, its unique biodiversity has been under pressure, and today an estimated 49% of the terrestrial avifauna is extinct. Current efforts to conserve the remaining fauna rely on a better understanding of the composition of past ecosystems, as well as the causes and timing of past extinctions. The exact temporal and spatial dynamics of New Zealand's extinct fauna, however, can be difficult to interpret, as only a small proportion of animals are preserved as morphologically identifiable fossils. Here, we conduct a large-scale genetic survey of subfossil bone assemblages to elucidate the impact of humans on the environment in New Zealand. By genetically identifying more than 5,000 non-diagnostic bone fragments from archaeological and paleontological sites, we reconstruct a rich faunal record of 110 species of birds, fish, reptiles, amphibians, and marine mammals. We report evidence of five whale species rarely reported from New Zealand archaeological middens and characterize extinct lineages of leiopelmatid frog (*Leiopelma* sp.) and kākāpō (*Strigops habroptilus*) haplotypes lost from the gene pool. Taken together, this molecular audit of New Zealand's subfossil record not only contributes to our understanding of past biodiversity and pre-contact Māori subsistence practices but also provides a more nuanced snapshot of anthropogenic impacts on native fauna after first human arrival.

MB22: Marine Bioactives: Exploiting flexible biosynthetic pathways to fine tune desirable modes of action.

Battershill, C.N.[1]
[1] Environmental Research Institute, Coastal Science, University of Waikato, Tauranga, New Zealand.

From the National Cancer Institute international marine shallow water biodiscovery program (1986 and 2000), of the top anti-tumour active compounds that resulted in late phase clinical trial or beyond, approximately a third were sourced from the Australasian region [1]. Anti-tumour active compounds Halichondrin B (Halaven®), and Peluroside A from New Zealand sponges; Salicylihalamide A and Lobatamide A from a Western Australian sponge and ascidian, are prime examples. The reason for such a high incidence of success was due, in part, to the approach taken on collection. Knowledge of chemical ecology linked to phylogeny of encrusting invertebrates and algae, directed collection effort [2].

A legacy of this effort is a detailed bioinformatics database that permits hypothesis driven discovery approaches for new sectors. An example is application of anti-biotic activities from human health screening programs to agricultural targets such as Psa-V, a kiwifruit pathogen. Furthermore, extended research on the chemical ecology of species that elicited lead compounds, has permitted greater understanding of the roles of these bioactives in nature together with biosynthetic processes. In many instances, biosynthesis of frequently very complex molecules, is achieved via shared metabolic pathways with microbial symbionts [3]. Understanding this can lead to novel applications for production of target metabolites in vitro, providing options for large scale production of bioactives [4].

We can go further still: it is proposed that advance in developing more desirable applied modes-of-action can be made through examination of the flexibility of biosynthetic pathways invoked by chemical ecologically inspired manipulations of the holobiont. Hence, by harnessing the extraordinary biosynthetic abilities of marine invertebrates and their symbionts, the relationship between structure and function of bioactive secondary metabolites can be examined, possibly directing subsequent synthesis of more effective chemical entities. A number of experiments toward this end are discussed focusing on the Halichondrins from *Lissodendoryx n.sp*., the Mycalamides, Pateamine and Peloruside A from *Mycale hentscheli*, and the Dischorhabdins from *Latrunculia spp*.

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MB23: Ancient aquaculture and the influence of early-Māori on the distribution and dynamics of toheroa.

Ross P.M.[1]
[1]Environmental Research Institute, Coastal Science, University of Waikato, Tauranga, New Zealand.

The impacts of modern human society on marine ecosystems are both significant and well understood. In contrast, the influence of early humans on marine ecosystems is virtually unknown. New Zealand, the last major landmass settled by humans, provides an unparalleled opportunity to determine the significance of early human-ecosystem interactions. Māori settled New Zealand as late as the 14th century, meaning that evidence of early-human impacts are less obscured by time in New Zealand than in countries with more prolonged occupation. Genetic analyses (supplemented with Māori environmental knowledge) of toheroa, an endemic shellfish of cultural importance to Māori, has led us to hypothesise that the present-day distribution of this taonga (treasured) species is influenced by historical aquaculture, in the form of human-mediated translocations. This hypothesis is being tested through a multidisciplinary research programme combining archaeology, anthropology and molecular ecology with Mātauranga Māori (Māori knowledge). By examining Māori oral histories alongside archaeological records and toheroa population genetics, we are gaining a better understanding of the extent to which early Māori manipulated their marine environment.

MB24: Application of eDNA for Marine Biomonitoring: Lessons learned from freshwater systems

Gleeson, D. M, [1] , Bylemans, J[1], Hinlo, R[1]., Furlan E. M.[1]
Institute for Applied Ecology, University of Canberra, Bruce ACT, 2617, AUSTRALIA

Advances in DNA related technology hold significant potential for application to biomonitoring but also hold risks and challenges. An important advance has been the use of environmental DNA (eDNA), which is the DNA shed by organisms and finds its way into the environment. This provides a non-invasive approach to biodiversity surveillance and holds considerable promise for applications such as the detection of rare species, presence of invasive species in the early phase of the invasion curve, reproductive status of particular species, whole community inventory, and estimates of biomass. The main risks lie in inadequate delineation of the limits of detection leading to the potential for incorrect diagnoses of species presences or absences and in the inadequate databases and systems to fully interpret the information that emerges from multispecies DNA profiles. In order to overcome some of the implicit challenges, we have developed a framework to estimate the sensitivity of both the field and laboratory components eDNA survey methods, and we have been able to demonstrate how these can be used to estimate the overall sensitivity[1]. We have applied this framework to species-specific eDNA surveys to estimate the sensitivity, or probability of detection, for invasive aquatic species present in Australia in both freshwater[2,3] and marine settings. We have also demonstrated how eDNA can also detect spawning within a threatened species[4], has potential to estimate biomass, and how single eCells could be used for population size estimates. Examples from each of these applications will be presented along with future advances in eDNA technology can potentially transform species monitoring.

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MB25: Environmental DNA metabarcoding from the path of New Zealand's critically endangered Māui dolphins

Steel, D[1], Constantine, R.[2], Baker, C.S.[1,2]
[1]Marine Mammal Institute, Oregon State University, Newport, Oregon, USA, [2]School of Biological Sciences, University of Auckland, Auckland, NZ.

Genetic sampling for DNA barcoding or DNA profiling of whales and dolphins at sea remains challenging. Most samples have been collected with a biopsy dart requiring a close approach of a vessel to the individual as it surfaces. Here we have adopted droplet digital (dd)PCR and next-generation sequencing technology for the detection and species identification of cetaceans using environmental (e)DNA collected from seawater. ddPCR is a powerful new technology for detecting and quantifying low copy number eDNA by fractionating a PCR reaction into thousands of droplets using a water-oil emulsion. Based on success with eDNA sampling of other cetacean species, we collected seawater from the proximity of New Zealand's critically endangered Māui dolphin, a subspecies of the endemic Hector's dolphins. Māui dolphins are found only along the northwest coast of the North Island while Hector's dolphins are found around the South Island, with the occasional migrant identified genetically in the range of Māui dolphins. The detection of eDNA by ddPCR was confirmed by conventional PCR amplification and by next-generation metabarcoding with an Illumina MiSeq. From nearly 100,000 paired-end reads, we confirmed a large majority of reads representing the single mtDNA haploytpe, considered diagnostic of the Māui dolphins but also a low frequency of a haplotype characteristic of the Hector's dolphin, representing a potential immigrant. Although, with current technology, eDNA metabarcoding cannot provide a full DNA profile for individual identification, it could augment conventional biopsy sampling for identifying mixed groups of the Māui and Hector's subspecies or estimating haplotype frequencies in populations of Hector's dolphins.

1. Baker, C.S., D. Steel, S. Nieukirk and H. Klinck (2018) *Environmental DNA (eDNA) from the wake of the whales: Droplet digital PCR or detection and species identification*. Frontiers in Marine Science doi: 10.3389/fmars.2018.00133

MB26: From alpine lakes to open oceans: using metabarcoding to enhance aquatic biomonitoring and biodiversity assessments

Xavier Pochon[1,2], Anastasija Zaiko[1,2], Susie Wood[1]
[1] The Cawthron Institute, Coastal and Freshwater Group, Nelson, New Zealand
[2] University of Auckland, Institute of Marine Science, Warkworth, New Zealand

Recent technological advances in molecular methodologies provide opportunities to develop innovative diagnostic tools that can streamline and reduce costs associated with biological monitoring and characterising biodiversity across biomes. High-throughput sequencing (HTS) coupled with metabarcoding is increasingly being touted for analysing large DNA or RNA sample sets, producing vast datasets which can then be used to provide an in-depth understanding of spatio-temporal changes in biological communities. The technique can be applied to a diverse range of organisms expanding the scope of monitoring programs into biota and/or habitats that are currently not being surveyed due to technical limitations or poor taxonomic knowledge. Despite all the promises there are still significant challenges to be overcome before these methods can be applied with confidence to routine biomonitoring programmes. In this presentation three research projects will be used to illustrate the pros and cons of using HTS-based metabarcoding for characterising aquatic ecosystems in New Zealand. The first project showcases how metabarcoding can be incorporated into paleo-environmental reconstructions to understand how and why biological communities have change in 10% of our lakes (n=380) over the last millennium. The second project is part of a quadrilateral scientific collaboration in marine biosecurity surveillance and focuses on the potential biases occurring during HTS libraries preparation in an international experiment involving 12 laboratories from New Zealand, Australia, Canada, Europe and the USA. The third project highlights the power of citizen-science for gathering unique open ocean plankton community samples, which can then be used to generate unprecedented amount of critical data on largely uncharacterised pelagic environments. Metabarcoding has tremendous power for providing valuable new knowledge that can be used to improve environmental management of a variety of freshwater and marine ecosystems in New Zealand.

MB 27: Quantification of *Bonamia exitiosa* infection levels in the flat oyster *Ostrea chilensis* by ddPCR

Bilewitch, J.P.[1], Sutherland, J.[1], Hulston, D.[1], Michael, K.[2]

[1]NIWA, Centre for Coasts & Oceans, Wellington, NZ, [2]NIWA, Centre for Fisheries, Wellington, NZ.

The intracellular parasite *Bonamia exitiosa* causes significant and recurrent mortality in the Foveaux Strait oyster population that results in significant economic loss to the fishery. Fisheries model predictions of oyster populations are significantly impacted by summer mortality of oysters due to infection with *B. exitiosa* (Bonamiosis). Accurate forecasting of population trends therefore requires sensitive and accurate quantification of levels of *B. exitiosa* present in oyster populations during the summer months.

The categorical scoring of histological specimens has historically been used to measure disease prevalence and intensity in the oyster fishery, but it is time-consuming and is known to underestimate Bonamia infection levels. A specific, sensitive and rapid qPCR method for Bonamia detection was implemented by NIWA in 2011, but it was insufficient to quantify infection levels due to inherent variation in our high-throughput sampling methodology. We have subsequently used levels of oyster β-actin DNA as a normalisation factor for qPCR-quantification of *B. exitiosa* levels, allowing us to reliably correlate infection intensity measurements from genetic tests with those from histological scores. Significant variation between replicate qPCR reactions (particularly for the highest and lowest intensities of infection) required samples to be run in triplicate to counteract inherent qPCR variation. This reduction in cost-effectiveness was circumvented by transferring the assay to a droplet-digital PCR (ddPCR) platform. Both qPCR and ddPCR displayed similar relationships to histological scorings of infection levels, but ddPCR demonstrated superior sensitivity, repeatability and cost-effectiveness. We are now implementing ddPCR-based quantification of Bonamiosis in all future *B. exitiosa* surveys to build our understanding of the temporal dynamics of the disease.

MB28: New breeding technologies for fruit trees

Allan, A.C.[1,2] and Varkonyi-Gasic, E.[1]

[1]The New Zealand Institute for Plant & Food Research Limited (PFR), Private Bag 92 169, Auckland, New Zealand
[2]School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

Many studies have focused on manipulating levels of secondary metabolites in fruit through both conventional breeding and GM approaches. However, New Breeding Technologies (NBTs) offer ways of providing large changes consumer traits (in addition to grower traits) if the public will accept the resulting fruit.
Annualization of woody perennials has the potential sped the breeding and production of fruit crops and rapidly improve horticultural species. Kiwifruit (*Actinidia chinensis*) is a recently domesticated fruit crop with a short history of breeding and tremendous potential for improvement. We used CRISPR/Cas9- mediated manipulation to target mutation of CENTRORADIALIS (CEN)-like genes. Targeting these genes transformed a climbing woody perennial, which develops axillary inflorescences after many years of juvenility, into a compact plant with rapid terminal flower and fruit development. These changes have made kiwifruit amenable for accelerated breeding, indoor farming and cultivation as an annual crop. Using these plants, crossing has begun targeting higher levels of anthocyanins and carotenoids for future cultivars.

MB29: Biotechnology and Forest Trees in New Zealand

Charleson Poovaiah, Sanjeev Raikar, Lorelle Phillips, Steffi Fritsche, Catherine Reeves and Glenn Thorlby.

Scion, Rotorua, NZ,

The large stature of forest trees, long periods of juvenile (pre-reproductive) growth, and delayed expression of traits, such as wood quality result in breeding programmes being long and expensive. Biotechnology provides solutions to mitigate these breeding challenges, particularly through the use of new breeding technologies such as gene editing which allow rapid and precise trait modifications. We will illustrate the potential benefits of integrating gene editing into forest tree breeding programmes using engineered sterility as an example trait. Wildings, non-native invasive conifer tree species that have spread from planted forests, are a major problem in New Zealand. Their control costs in excess of $15M per year and they have been described by the government as “the most significant weed problem New Zealand faces”. The production of trees that are not able to generate wildings would provide a tool to mitigate the environmental and socio-economic damage they cause. Uncertainly over the global regulatory status of gene editing remains a barrier to its integration into breeding programmes. New Zealand is one of the few countries where the regulatory status of gene editing has been clarified. In 2014 the NZ Environmental Protection Authority ruled that plants produced via gene editing, where no transgene was used in the editing process, would not be regulated as GMOs. However, following a challenge in the High Court, this decision was overturned such that NZ currently regulates the products of gene editing as GMOs. The regulatory process which led to gene editing’s current GMO classification in NZ will be discussed.

MB30: Developing Gene editing technology in conifers

Charleson Poovaiah[1], Sanjeev Raikar[1], Glenn Thorlby[1]
Scion, 49 Sala Street, Rotorua, NZ.

The CRISPR/Cas9 nuclease system is a powerful and flexible tool for genome editing. CRISPR/Cas9 has been demonstrated to edit genomes in various plant species including woody species but has not yet been developed in conifers. Scion is developing gene editing technology in conifers, as we believe it will be an invaluable tool in accelerating breeding in these relatively undomesticated species. For a commercial release, it is likely that it will be beneficial to produces edited trees that do not contain a transgene. The long breeding cycles of conifers would make it challenging to uses crossing to remove a transgene. We are, therefore, developing protocols to directly edit protoplasts using a non-transgenic procedure and to regenerate trees from these edited protoplasts. We have consistently isolated protoplasts and have carried out successfully transformation. We will test the efficiency of various RNA polymerase III promoters for use in gene editing. Preliminary results show protoplasts can be used for gene targeting in conifers and will provide mutations for tree improvement and functional studies.

MB31: CRISPR Fruit: three bites at the problem

Drummond, R.S.M.[1], Wang, T.[1], Foster, T[2].

[1]The New Zealand Institute for Plant and Food Research, Auckland, NZ. [2]The New Zealand Institute for Plant and Food Research, Motueka, NZ.

Actinidia is a long lived woody perennial with excessive vegetative vigour that can contribute to an unacceptably low yield in an otherwise promising cultivar. Creating a dwarfed cultivar via GA insensitivity is a potential route to improving yield in such a plant. We are using three CRISPR-Cas9 based strategies that will produce respectively; a small deletion event, a gene replacement event and a base editing event, that lead to the removal or inactivation of the DELLA domain from an otherwise functional RGL gene. In this plant the T-DNA locus is likely to be unlinked to the RGL gene allowing its segregation away from the edited allele during subsequent breeding. The regulatory constraints on the use of these plants will vary between both the methods used and the jurisdictions where the plants are grown despite similarly precise and limited changes to the plant's genome.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
MB32	Detecting population-level marine diversity with environmental DNA methodology	Clare Adams	Department of Anatomy, University of Otago, Dunedin
MB33	Novel insights into the early regeneration response of *Botrylloides leachii*.	Rebecca Clarke	Department of Anatomy, University of Otago, Dunedin
MB34	Dissecting the discourse of social license to operate	Jim Sinner	Cawthron Institute, Nelson
MB35	Identification and structural prediction of pituitary gonadotropins in trevally (Pseudocaranx georgianus)	Matt Wylie	Plant and Food Research, Nelson

POSTER MB32: Detecting population-level marine diversity with environmental DNA methodology

Adams, C.I.M.[1], Jeunen, G.[1], Knapp, M.[1]

[1]Department of Anatomy, University of Otago, Dunedin, NZ.

The need to understand population dynamics for conservation and management purposes drives the development of effective and non-harmful sampling methodology. Non-invasive environmental DNA (eDNA) techniques, which allow for extracting organismal DNA from an environmental sample such as water, soil, or air, have shown great promise in this field. However, in the marine environment in particular, the development of eDNA tools has so far, with few exception, mainly focussed on reconstructing species diversity. We aim to develop an eDNA approach for obtaining population level allelic diversity in marine species of commercial and conservation interest. Our initial research focusses on the commercially important Pāua (*Haliotis iris*) and the New Zealand fur seal (NZFS) (*Arctocephalus forsteri*), a taonga species under active conservation management. Using a combination of controlled laboratory and field experiments, we are developing a field, laboratory and bioinformatics pipeline to reliably retrieve population genetic data of target species from seawater samples. Through developing and validating these methods, we hope to open the door to non-invasive genetic monitoring for New Zealand marine species.

POSTER MB33: Novel insights into the early regeneration response of *Botrylloides leachii*.

Rebecca Clarke[1], Miles Lamare[2] and Megan J Wilson[1]

[1]Department of Anatomy, University of Otago, Dunedin, New Zealand, [2]Department of Marine Science, University of Otago, Dunedin, New Zealand

There is an inverse relationship between an animal's complexity and their ability to regenerate, with no vertebrates being able to undergo WBR. One exception to this relationship is the tunicates. *Botrylloides leachii* is a colonial tunicate which has the remarkable ability to regrow a fully functional adult in about 8 days from a small section of blood vessels. This process is known as whole body regeneration (WBR). This process is not well understood and many questions about this process remain unanswered. For regeneration to occur there must be no zooids (adults) in the colony or it will revert to wound healing rather than regeneration. There is currently little known about the triggers which lead to WBR except for the presence or absence of zooids.

We have investigated the process of WBR through the expression patterns of known pluripotency genes by RT-qPCR and *in situ* hybridisation. Pluripotency genes are expressed in pluripotent stem cells which play a large role in WBR, allowing the formation of new tissues. The differences between WBR and wound healing are also being investigated to determine what genes are used solely in regeneration or wound healing to help us understand the mechanism that controls successful regeneration responses.

POSTER MB34: Dissecting the discourse of social license to operate

Jim Sinner[1], Mark Newton[1], Trisia Farrelly[2]

[1]Cawthron Institute
[2]Massey University

The term “social license to operate”, or SLO, has increasingly featured in public discussion about commercial operations in the marine environment. Choice of wording and sentence structure can affect power relations between people and groups, so how the term is used matters. Our poster presents an analysis of public documents related to NZ’s marine industries and examines the implications of how SLO is defined and deployed. We show that this discourse has been dominated by industry and central government voices, who frequently vest agency over SLO with industry and then state or imply that industry already has SLO and just needs to maintain or improve it. Whether inadvertent or intentional, this choice of language empowers industry at the expense of communities and iwi. We discuss implications for industry-community-iwi relations.

POSTER MB35: Identification and structural prediction of pituitary gonadotropins in trevally (*Pseudocaranx georgianus*)

Wylie, M.J. (1), Valenza-Troubat, N. (1), Storey, R.(1), Elizur, A. (2), Nocillado, J. (2), Lokman, P.M. (3), Wellenreuther, M.(1)

(1) Seafood Production Unit, New Zealand Institute for Plant & Food Research, Nelson, New Zealand. (2) Faculty of Science, Health, Education and Engineering, GeneCology Research Centre, University of the Sunshine Coast, Sunshine Coast, Queensland, Australia. (3) Department of Zoology, University of Otago, Dunedin, New Zealand email: Matthew.Wylie@plantandfood.co.nz

Trevally *(Pseudocaranx georgianus)* is of significant interest for aquaculture in New Zealand and a selective breeding programme is underway. To enable rapid breeding of this species we seek to 1) shorten generation intervals using recombinant gonadotropins, 2) gain knowledge about gametogenesis under culture condition, and 3) apply this knowledge to control broodstock spawning. The pituitary gonadotropins follicle-stimulating hormone and luteinizing hormone play a fundamental role in the endocrine cascade in vertebrates that enables gametes to be formed and released. Here we characterise and predict the structure of these glycoprotein hormones, examine their phylogenetic relationships with other fish species using a *de novo* genome assembly of trevally. We identified and described for the first time the three cDNAs encoding trevally *lhβ, fshβ* and *gpα*. Complete cDNAs within the coding region were obtained for *lhβ* (436 bp) and *gpα* (346 bp), while only a partial sequence has been identified for *fshβ* (285 bp). Upon identification of open reading frames for all gonadotropins, structural predictions will be presented. Consensus trees suggest that the Japanese jack mackerel *(Trachurus japonicus)* is closely related to trevally along with other carangoid fishes (i.e. *Seriola* species and cobia *(Rachycentron canadum)*). The identification and structural prediction of trevally pituitary gonadotropins advances our understanding in the reproductive physiology of this species and provides a future basis for the development of recombinant gonadotropins.