



Version 7 September 2021

Abstracts are ordered alphabetically by last name of presenter and include those provided to the programme committee for publishing.

Vortex Fluidic Device tailored alginate biomaterial for 3D bioprinting

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Biography:

Reinu has completed her PhD from Deakin University on Bioprocessing of hemp hurds for biofuel production in 2014. She won 3-minute thesis award and best student poster award in her PhD. She started teaching at Melbourne University from 2016. Since 2018, she is working as research associate at Flinders University on an ARC funded project - Vortex Fluidic Device assisted bioprocessing of seaweed in collaboration with an international industry partner. Reinu was successful in continuing this collaboration and got funding for further three years (2019-2022) to develop marine based biomaterials for 3D bioprinting and pharmaceutical application. In 2019, she completed business modelling and management studies from Harvard University. In 2021, she was awarded Flinders University cross-college grant on developing novel biomaterials. Her research interests are in biomass bioprocessing, biofuels, bioproducts, biomaterials, bioprinting. She has got more than 20 publications including research articles, book chapters and conference proceeding.

Alginate based biomaterials with tailored properties and advanced features are in great demand but due to its poor mechanical strength the scaffold collapses and results in the failure of environment responsive behaviour_{1,2}. Despite of these challenges alginate is considered as an excellent biomaterial due to its unique biological and biocompatibility properties to formulate 3D-bioprinting bioinks for tissue and implant constructs and drug delivery systems. Alginate is a biopolymer derived from brown seaweed which is composed of β -D-mannuronic acid (M-block) and α -L-guluronic acid (G-block) monomers. It forms weak polymeric hydrogel structure by chemically crosslinking with cation, so the physicochemical properties of natural alginate need to be tailored for improved mechanical strength₃. This study aims to investigate in tailoring its physicochemical properties by using an advanced in-house patented technology-Vortex Fluidic Device (VFD) for the molecular transformation of alginate and cleave into tuneable molecular weight and viscosity₄. The integration of VFD allows 'controlled alginate slicing' at varying temperature and rotational speed which modifies its molecular weight and viscosity. With an increase of every 10 °C from 30 - 80 °C alginate with ≥ 1000 kDa size can be controlled sliced up to ≤ 100 kDa in 30 min of reaction under the presence of hydrogen peroxide. This technology can tune the properties of alginate which can further be used to create customised bioink library for 3D-bioprinting.

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Phenotypic plasticity and acclimation capability in sea urchins (*Paracentrotus lividus*) under long-term exposure to seawater acidification

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Biography:

Davide Asnicar is a postdoc in the ecophysiology and ecotoxicology lab at the Department of Biology of the University of Padova. The topic of his researches has always been the study of human impact on marine organisms. He completed and successfully defended his PhD project in May 2021. In particular, he studied the intra- and trans-generational response of sea urchins to ocean acidification and contaminants, investigating the effects at the adult, gamete and offspring level.

Ocean acidification (OA) responses in marine organisms are often not univocal due to non-uniform exposure setups or the different ecological history of the specimens. We aimed at assessing the response to OA in sea urchins Paracentrotus lividus from sites with different environmental features, the Lagoon of Venice and a coastal area in the Northern Adriatic Sea. In a long-term experiment, animals were maintained in both natural seawater (pHT 8.04) and the end-of-the-century predicted pH (-0.4 units). For six months physiological (respiration rate, ammonia excretion, O:N ratio) endpoints were investigated monthly. After eight months of exposure, the antioxidant (superoxide dismutase, catalase, glutathione reductase, protein carbonyl content, lipid peroxidation) and detoxification (glutathione-S-transferase) capability were assessed in gonads and digestive tract, whereas immunosurveillance was investigated in the coelomic fluid (coelomocyte characteristics, cytotoxicity, lysozyme and acid phosphatase activity). During the first months under reduced pH, ammonia excretion increased and O:N decreased in coastal specimens, suggesting higher protein catabolism. Interestingly, as the exposure continued, animals from both sites were able to acclimate. Instead, low pH had slight effects on the antioxidant and immunological responses, but differences between sites of origin were highlighted. The coastal site had higher levels of antioxidant and immune-related biomarkers. These findings suggested a higher adaptation ability in sea urchins from a more variable environment such as the lagoon. Results revealed plasticity in the responses of sea urchins and demonstrated that eight months of exposure are enough for adult P. lividus to acclimate to the future predicted OA conditions.

Identification and characterization of pathogenic bacteria from New Zealand Green-lipped mussels (*Perna canaliculus*)

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Biography:

Awanis Azizan is a PhD student at the Aquaculture Biotechnology Research Group, Auckland University of Technology. Her current research focuses on biological functions and molecular mechanisms involved in New Zealand Green-lipped mussels and in response to environmental influences, such as summer mortality and pathogen co-infections. Other research interests include the development of new tools for assessing aquatic animal health and identifying mechanisms of disease susceptibility, infection and resistance.

Summer mortality events affecting *Perna canaliculus*, some associated to *Vibrio* spp., have depleted many populations of this marine mollusc. The objective of this study was to demonstrate pathogenicity of six strains of bacterial isolates in the host *P. canaliculus* by performing a bacterial challenge to understand if the isolates have specific virulence in this host. To assist this objective, a DNA diagnostic test was performed. *P. canaliculus* collected from a mussel farm were used in a challenge experiment with six bacterial isolates at three different doses (low, medium, high). The virulence in the host background of *P. canaliculus* was demonstrated at doses of 10⁷ CFUs per animal. One bacteria strain was found to be pathogenic to *P. canaliculus*. PCR was used for species identification using blast homology and phylogenetic trees targeting the atpA gene and gyrB gene. The virulence genes vsm and vso were investigated to identify the specific bacterial strain. Sensitivity and specificity of the atpA and gyrB PCR are useful for diagnosis of disease in shellfish. The presence of previously described virulence genes has been confirmed in this strain. The specific atpA and gyrB PCR assay will aid management of future epizootics of this emerging pathogen of aquatic fauna, and improve surveillance capabilities for mortality events where pathogenic bacteria are suspect.

POSTER

Beyond relaxed: Magnesium chloride anaesthesia alters the circulatory metabolome of a marine mollusc (*Perna canaliculus*)

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Biography:

Awanis Azizan is a PhD student at the Aquaculture Biotechnology Research Group, Auckland University of Technology. Her current research focuses on biological functions and molecular mechanisms involved in New Zealand Green-lipped mussels and in response to environmental influences, such as summer mortality and pathogen co-infections. Other research interests include the development of new tools for assessing aquatic animal health and identifying mechanisms of disease susceptibility, infection and resistance.

The New Zealand Green-lipped mussel industry is well-established providing vastly to aquaculture exports. To assess mussel health and reproduction status, visual examination of organs and/or collection of haemolymph is commonly applied. Anesthetics, such as magnesium chloride (MgCl₂) can be utilized to prevent muscle contraction and keep shells open during sampling. The specific effects of muscle relaxing agents on baseline metabolism in invertebrates is unknown, but it is evident that molecular, cellular and physiological parameters are altered with these chemical applications. To this end, metabolomics approaches can help elucidate the effects of relaxing agents for better assessment of their use as a research tool. Adult Green-lipped mussels were anaesthetized for 3 h in a MgCl₂ bath, whereafter haemolymph samples were collected and analyzed via gas chromatography-mass spectrometry applying methyl chloroformate alkylation derivatization. Anesthetized mussels were characterized as non-responsive to manual manipulation, with open valves, and limited siphoning function. Metabolite profiling revealed significant increases in the abundances of most metabolites with an array of metabolic activities affected, resulting in an energy imbalance driven by anaerobic metabolism with altered amino acids acting as neurotransmitters and osmolytes. This research is the first to use metabolomics approaches to identify the metabolic consequences of this commonly used bivalve relaxing technique. Ultimately the use of MgCl₂ anesthetization as a sampling strategy should be carefully evaluated and managed when performing metabolomics-related research.

Dislodgement events of the mussel *Mytilus galloprovincialis* under raft cultivation: mussel tenacity versus anemone *Actinothoe sphyrodeta* blooms

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Biography:

Dr. Jose MF Babarro is a scientist at the IIM-CSIC (Vigo, NW Spain). Driven by his ecophysiological expertise with marine bivalve molluscs, his interest is the understanding of resilience and adaptive patterns with regard to global change scenario, especially for those species with commercial interest. Jose MF Babarro has worked across academic and aquaculture-related sectors like Galician mussel cultivation, being his doctoral research focused on distinct mussel spat performance under raft-cultivation. At the present, he is investigating the impact of heterogenous environment on key tissues like byssus filaments (mussels) and calcium carbonate shell, both being threaten under future coastal environmental changes.

Aquaculture of marine bivalve molluscs represents an important economic sector worldwide. A total amount of 17.1 million tons of molluscs were produced in 2016, mainly clams, oysters and mussels, which are the most commonly cultivated bivalve molluscs (FAO 2018). With the aim to develop such successful industry, several instruments and infrastructure is needed (cultivation ropes, nets, or buoys) that together with cultivated organisms themselves represent an available surface for settlement and attachment of many different species i.e. biofouling. Eventually some of these fouling species may cause a very negative impact on bivalve's performance under cultivation i.e. higher presence of epibionts may derive either in dislodgements of cultivated biomass or difficulties to attach properly in clusters (for example marine mussels).

We have explored the impact of key fouling organism, the anemone *Actinothoe sphyrodeta* that secrete an oily substance and interfere with mussel responses e.g. biomass dislodged under cultivation through the weaken byssus tenacity developed on cultivation ropes when anemone blooms are notorious. Through qualitative assessments of fouling blooms, we have been able to estimate a disappearance rates up to 40% of mussel biomass under cultivation by combination of abiotic (hydrodynamic regime) and ecophysiological responses (mussel tenacity) with regard to anemone presence.

Such a new monitoring (fouling and its potential impact) is very relevant beyond the taxonomic point of view because can explain massive dislodgements under cultivation within the heterogeneous environment in a global change scenario. Beside a better inter-annual variability understanding of environmental changes promoting that fouling blooms are needed.

Development and validation of molecular biomarkers for the green-lipped mussel (*Perna canaliculus*) to characterize the effects of environmental contaminants

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Biography:

Camille Baettig is a PhD student working in the field of ecotoxicology. Prior to beginning the PhD previous work has included investigating the impact of nitrogen and phosphorus concentrations on Bavarian lake community structures (phytoplankton, zooplankton, white fish). Her current focus is to investigate and develop molecular techniques for the green-lipped mussel, Perna canaliculus. The aim of the PhD is to determine whether or not P. canaliculus is a suitable ecotox species for environmental hazard assessment. This is done through a comparative study looking at the biological effects of contaminants of emerging concern towards P. canaliculus and the Mediterranean mussel, Mytilus galloprovincialis, using an omics based approach.

In New Zealand there is a desire to use local, native species for ecotoxic hazard assessments rather than internationally standardized species for the characterization of environmental hazards. This includes using species with cultural relevance and those of commercial value, for example the NZ green-lipped mussel, Perna canaliculus. Due to New Zealand historically having low levels of contamination relative to the rest of the world sublethal endpoints, or biomarkers, may be necessary to predict potential environmental impact before it occurs. Although the internationally established mussel, *Mytilus galloprovincialis*, coexists across New Zealand, P. canaliculus, has a broader ecological niche and is capable of thriving in soft sediment, which may lead to exposure to sediment bound contaminants that *M. galloprovincialis* may not interact with. The specific aim of this research was to develop and validate RT-qPCR primers for *P. canaliculus*, targeting a suite of genes for various mechanistic responses (oxidative stress, xenobiotic transfer, membrane transportation, cellular and DNA response/repair, and endocrine disruption). Perna canaliculus was exposed to two reference contaminants, benzo[α]pyrene and copper, that are well studied and well known to induce sublethal responses in M. galloprovincialis. The results of the present study found modulation of oxidative stress, xenobiotic transfer, and genotoxicity genes which is consistent with previous results reported for other mussel species. These results demonstrate that P. canaliculus could be used as an indicator species for environmental risk assessment of ecotoxicological hazard assessments. We also found that membrane transport genes were modulated after exposure to both copper and benzo[α]pyrene, suggesting that these chemicals may have other mechanisms of toxicity that have not been fully investigated. This study developed 11 novel biomarkers for P. canaliculus and is the first experiment to implement gene expression for *P. canaliculus* in an ecotoxicological context.

Investigating the ecotoxicological impacts of microplastic additives towards New Zealand native species

<u>Dr. Andrew Barrick</u>¹, Dr Olivier Champeau, Dr Louis Tremblay ¹Cawthron Institute, New Zealand

Biography:

Andrew Barrick is an ecotoxicologist with experience using multiple levels of biological organization (molecular, enzymatic, behavioral, energetic and population) to investigate the potential impact of anthropogenic contaminants on an array of different marine and estuarine invertebrates. Previous work has included work on the French national project GIP SeineAval (part of the ECOTONES research cluster) which investigated the health status of the Seine estuary and project NanoReg2 (part of the European Commission's Horizon 2020 initiative) which investigated ecotoxicological hazards of manufactured nanomaterials and the application of safe(r)-by-design. He is currently involved in the emerging organic contaminants (EOCs) and microplastics (AIM2) MBIE projects investigating hazards associated with both types of anthropogenic pollutants towards New Zealand species.

Aotearoa Impacts and Mitigation of Microplastics (AIM2) is New Zealand's first comprehensive national program aimed at characterizing the threat microplastics pose towards ecosystems, animals, and people. AIM2 aims to address knowledge gaps surrounding the severity of New Zealand's microplastic problem and determine if they pose a significant risk to New Zealand's relatively pristine environments. One facet of the 'microplastic problem' are potential hazards associated with additives incorporated in plastics. While some of these additives have been previously identified as persistent organic pollutants others have not been fully investigated for ecotoxic potential. As part of AIM2 a short list of additives of emerging concern towards the New Zealand environment was compiled including available ecotoxicity data. Copepods, as primary consumers providing an essential link between phytoplankton and higher trophic levels, represent an important link in marine food webs that makes them ideal test organisms for investigating potential hazards associated with plastic additives. New Zealand's native copepod species Gladioferens pectinatus was used in the present study due to its wide distribution across the countries estuaries, ease in which it can be maintained in laboratory conditions, high reproductive rate, and relatively short life cycle. The project investigated: i) the capacity of copepods to biologically uptake plastic particles at different sizes ii) the ecotoxic potential of 8 different plastic additives iii) ecotoxicity of microplastics prepared with additives iv) multigenerational effects of microplastics and associated additives. The study provides a first look at the potential environmental hazards associated with plastic additives towards New Zealand's unique ecosystems.

Marine Bioprocessing - Linking New Zealand and Australia

Professor Colin Barrow¹

¹Deakin University, Geelong, Australia

Biography:

Dr Colin Barrow is Alfred Deakin Professor and Chair of Biotechnology at Deakin University. His research is focused on marine biotechnology and the application of nanomaterials and enzymes to Industrial Biotechnology. He is Program Leader for Innovative Bioprocessing Technology in the recently funded Marine Bioproducts CRC and collaborator on the MBIE funded Cyber-physical seafood systems program. Professor Barrow has a Ph.D. in chemistry and an MBA, more than 300 peer-reviewed publications, several patents, and has presented at numerous conferences and workshops. He has served as a member of the Expert Advisory Committee for Canadian Natural Health Product Directorate (NHPD), the TGA Advisory Committee for Complementary Medicines and is a founding member of International Society for Nutraceuticals and Functional Foods (ISNFF). Professor Barrow has two joint laboratories in China, one at Qingdao University and the other at Yunnan Minzu University, where he is a Guest Professor.

New Zealand and Australia have both committed significant funding and resources to developing products from marine bioresources, including microalgae, seaweed and seafood byproduct. MBIE has funded \$15M over 5 years for developing cyber-physical seafood systems for manufacturing marine co-products, primarily from seafood waste and byproduct. Australia has recently funded a Marine Bioproducts CRC with a total value of \$270M to drive the development of the marine bioproducts industry. Advanced bioprocessing technology and associated manufacturing capability is required to deliver on the promise of creating new bioproducts from marine biodiversity. This presentation will discuss how New Zealand and Australian researchers and industries can work together to create and strengthen the marine biotechnology capabilities of both countries.

Impacts of mono and mixed blooms of *Alexandrium catenella* on the life cycle of the Japanese pearl oyster, *Pinctada fucata* martensii

<u>Dr. Leila Basti¹</u>, Jiyoji Go, Dr. Satoshi Nagai, Dr. Kiyohito Nagai ¹Tokyo University Of Marine Science and Technology, , Japan

Biography:

Leila Basti is an Aquatic Environmental Engineer (INAT, Tunsia) and Doctor of Applied Marine Environmental studies (Tokyo University of Marine Science and Technology, Japan). Her research on coastal ecology and stock assessment, using GIS, earned her the double Engineer Laureate Prize from the Ministry of Higher Education and the Ministry of Agriculture, Hydraulic Resources and Fisheries, Tunisia. She is the recipient of the prestigious Japanese Governmental Scholarship (2004, 2007), the Strategic Young Researcher Fellowship (TUMSAT, 2010), and Japan Society for the Promotion of Science Fellowship (JSPS 2013). Dr. Basti was a Research Fellow at the Fisheries Research and Education Agency, Japan, and was appointed an assistant professor, in 2015. She was a visiting scientist and professor to several world-leading research institutes and universities in USA, UK, Netherlands, France, Germany, Japan, and New Zealand. In 2017, she joined the world aquaculture society as the international program chair of WA2020 Singapore.

Harmful algal blooms constitute a risk to human health, and coastal ecosystems and economies. There has been an increase in the occurrence and incidence of the blooms at regional and global scales due to climate change and anthropogenic activities. Coastal ecosystems and coastal aquacultures have been witnessing recurrent blooms associated with impacts on aquatic production, including in shellfish farms, due to mass mortalities of produce and/or due to sustained closures of production associated with the contamination of shellfish with toxins of human health concerns.

We present our results on the effects of one of the most widespread species of HAB, *Alexandrium catenella*, on the life cycle of an important shellfish species, the Japanese pearl oyster. We considered experiments with mono-blooms as well as with mixed blooms of this species, as most studies to date have not considered the impacts of mixed blooms of HAB. We tested the impacts of *A. catenella*, at several cell densities and in mixture with other HAB species, based on a 3-decade monitoring data of HAB in Japan. We tested the effects on gametes, fertilization, embryogenesis, larvae and spat of Japanese pearl oyster. Our results suggest differential negative effects depending on the mixed species tested and point to synergy and antagonism between the co-occurring HAB species.

Genetics and genomics of the European flat oyster (*Ostrea edulis*): progress and prospects to contribute to the restoration of populations in the framework of the Native Oyster Restoration Alliance (NORA)

<u>Dr. Pierre Boudry</u>¹, Dr. Tim Beam², Dr. Manu Gundappa², Dr. Jakob Hemmer Hansen³, Dr. Paulino Mártinez⁴, Dr. Arnaud Tanguy⁵, Dr. Sylvie Lapègue⁶

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Biography:

Research scientist, French Research Institute for the Exploitation of the Sea (Ifremer), Project manager, Department of Biological Resources and Environment, Ifremer, France. Since 1994, Pierre's research is dedicated to genetics and genomics of bivalves. Most of his research is connected with aquaculture or conservation issues and has led to over 150 publications, resulting in his designation in a bibliographic analysis (DOI 10.1007/s10499-015-9928-1) as the most productive author in of oyster research worldwide from 1991 to 2014. Since January 2021, Pierre is the Director of Biogenouest, the network of life sciences technology core facilities in Western France.

Over the past 50 years, studies dedicated to population genetics of Ostrea edulis have aimed to document spatial and temporal differences and to identify major driving evolutionary forces, such as genetic drift, local adaptation or impact of stock translocations, while a second major area of research has been dedicated to identify stocks with higher resistance to bonamiosis, mass or family-based selective breeding and disease testing. The impact of this research on farmed and wild populations has remained limited until now, due to low or short-term investments and/or limited commercial or environmental interest. Hence, aquaculture production and fisheries collapsed in the 1970's and hardly recovered since. Therefore, in 2001, O. edulis and flat oyster beds have been nominated for inclusion in the OSPAR list of threatened and/or declining species and habitats. This is, however, likely to change. Recent efforts to counter the decline of this species in Europe, as illustrated by the engagement of the NORA community (Native Oyster Restoration Alliance: https://noraeurope.eu/), have resulted in new initiatives and progress in breeding, genetics and genomics. This recent progress of different research groups across Europe will be reviewed in the light of increasing demand for disease-free or disease-resistant seed to restore populations and reviving aquaculture production and fisheries. Concerns about effective population size of hatchery-propagated seed, genetic impact of transfer of wild seed between evolutionary significant units will be addressed in view of the potential gains and of emerging genome-based selective breeding programs.

Unveiling immunity trade-offs at critical developmental stages in scallops: role of dietary HUFAs and its potential use in aquaculture

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Biography:

Marine Biologist (Universidad Católica del Norte - UCN, Chile) and Ph.D. in Biology (Laval University, Canada). Currently, Professor at the Aquaculture Department, Marine Science Faculty at the UCN. My broad scientific interests are the physiology and genetics applied to aquaculture of shellfish. My research is focused on understanding the responses of marine organisms to changes in their environment from their physiology and genetics. Specifically, I study how the physiological capacities are modulated by external and internal factors, such as the environment, development, reproductive, nutritional, stress and immune statuses; as well as their genotype. I look for these responses at different levels of biological organization, from main effect genes through molecules, organelles, the complete cell, tissues, the whole animal and population level. For this my work is characterized by an interdisciplinary approach, which has been possible due to the permanent collaboration with several other researchers.

Due to their crucial role in the protection against infectious diseases, important advances in scallop immunology have been made in recent years. These include the characterization of the cascades of cellular and humoral events coordinated by messengers, cell receptors, signaling pathways, and the expression regulation of antimicrobial effectors. In vertebrates, mounting the initial immune response is highly energy demanding; however, this aspect has been barely studied in mollusks. Massive outbreaks are more frequent during the reproductive season and in specific larval stages in hatchery settings. Thus, we focused our work on understanding how some physiological processes with high energy demand, such as those associated with reproductive investment and early ontogeny development, can affect the immune response capacities. We further evaluated whether an improvement in the nutritional quality (in terms of HUFAs content) of the diet enhances the ability to establish the immune response at these critical stages. Argopecten purpuratus, a species of great economic importance for Chile and Peru, was the animal model of these studies. A multiapproach methodology using parameters at molecular, cellular, and individual levels, under routine-basal status and after the exposure to a pathogenic Vibrio strain, was applied. Energy parameters measured were: (1) metabolic demand, determined as oxygen consumption rate; and (2) ATP supplying capacity, via key mitochondrial enzymes activities, mitochondrial membrane potential, and mitochondrial density, using invivo image analyses. Immune parameters were: (1) number of circulating and tissue-infiltrating hemocytes, and their phagocytic capacities (for reproductive adults); and (2) molecular immune responses via the transcriptional levels of several immune genes. Overall, results suggest a compromise between the allocation of energy in reproduction and the immune responses in adult scallop, where the energy metabolic capacity of hemocytes would be at the basis of the observed diminished immune responses in mature and spawned scallops. The administration of microalgae high in HUFAs during reproductive conditioning enhanced immune capacity. At the level of early ontogeny, results suggest that the veliger larval stage exhibits the lowest metabolic capacity to face a bacterial challenge, which together with an underdeveloped immune system could explain the susceptibility of this stage to bacterial infections and mortalities. The application of microalgae rich in HUFAs also enhanced metabolic and immune capacities at this critical larval stage. In this talk, I will discuss the observed immunity-tradeoffs and the potential role of dietary HUFAs in scallop aquaculture. This study was supported by FONDECYT-1170118 and Doctoral Scholarship by CONICYT-PFCHA/DOCTORADO NACIONAL/2017-21170162.

Sustainable aquafeeds to boost abalone nutrition in land-based farming

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Biography:

Natalia Bullon is a PhD student in the School of Science, at the Auckland University of Technology. Her research interests focus on aquaculture nutrition, aquafeed development, aquafeed delivery design and sustainable aquafeeds.

Overpopulation and reduced agricultural land areas have driven the aquaculture sector to increase its production from 7% to 40% since the 1980s. To address such demands, new aquafeed technologies have been developed to maximise productivity of commercial farming relying on natural and artificial ingredients. However, global sustainable aquaculture initiatives prioritize the development of feed technologies that are not only commercially viable, but also do not increase pressure on limited wild fisheries, maintain the health benefits of the seafood and minimise negative environmental effects. Although aquafeeds for abalone have been successfully produced and trialled in laboratory settings, the inclusion of the unsustainably sourced fishmeal as the main protein source remains a common practice due to its high digestibility and excellent amino acid profile. This study aims to explore the feasibility of using alternative ingredients and agriculture by-products at different inclusion levels for replacing fishmeal in diets of *Haliotis iris*, a New Zealand marine gastropod.

POSTER

Nutritional and metabolomic changes of juvenile farmed abalone (*Haliotis iris*) in New Zealand

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Biography:

Natalia Bullon is a PhD student in the School of Science, at the Auckland University of Technology. Her research interests focus on aquaculture nutrition, aquafeed development, aquafeed delivery design and sustainable aquafeeds.

Water temperature plays a crucial role in the physiology and immune responses of aquatic animals. Climate change scenarios, especially unpredictable water temperature fluctuations may negatively affect nutritional requirements and feed consumption resulting in growth deficiencies and decreased survival of many species, including abalone. Despite the well-known effects of water temperatures on aquatic animal performance, our understanding of the metabolic and nutritional changes across different seasons for farmed abalone remain limited. This study aimed to investigate biochemical and metabolic alterations in both whole body and foot muscle of healthy *Haliotis iris* during one year of grow-out. Proximate analysis was used to identify nutritional variations and a gas chromatography–mass spectrometry (GC–MS)-based metabolomics approach was used to identify metabolic changes. It is envisaged that the results of this study may provide a basic framework for physiological research into *Haliotis iris* and support the development of targeted nutritional strategies according to the environmental demands.

Eating contest: potential trophic niche overlap between native and nonindigenous species

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Biography:

Sara Cabral is a PhD candidate at the MARE - Marine and Environmental Sciences Centre (University of Lisbon) in partnership with the ISMER - Institut des Sciences de la Mer de Rimouski (UQAR). She completed the ERASMUS MUNDUS Master in Marine Biodiversity and Conservation (Ghent University) in 2014 and graduated in Marine Biology (Universidade do Algarve) in 2012. She published 5 research papers in international peer reviewed journals and 1 book chapter. Her scientific research activity has been developed in natural sciences and biology, particularly in aquatic ecosystems ecology, marine biodiversity, fisheries management, marine invertebrates and non-indigenous species.

The introduction of non-indigenous species (NIS) is considered one of the of the main drivers of biodiversity loss, representing one of the major threats to ecosystems. Competition for both space and resources are some of the interactions occurring when NIS and native species inhabit a similar ecological niche, leading to a potential overlap of their trophic niches and consequently, to food limitation. Understanding these competitive feeding interactions is crucial to assess the impacts of NIS on native species. This study aimed at using complementary approaches to address the potential trophic niche overlap between the NIS Manila clam (Ruditapes philippinarum) and pleated sea squirt (Styela plicata) and the natives cockle (Cerastoderma edule) and blue mussel (Mytilus edulis). This research was conducted in situ at the Albufeira coastal lagoon (Portugal), where since 2017 mussel farmers have been facing significant production losses due to tunicates biofouling. Three approaches were used: the analysis of stable isotopes and fatty acid trophic markers and the biodeposition method. The first two consisted of comparing the stable isotopes and fatty acids present on both organisms of the target species and the potential food sources collected seasonally. The third method consisted of comparing the Total Particulate Matter (TPM) and the Particulate Organic Matter (POM) of the seawater with the TPM and the POM of feaces and pseudo-feaces deposited by the organisms to determine clearance rates based on biodeposition. The use of complementary techniques is a promising approach, providing cumulative information on potential trophic overlap between native and non-indigenous species.

Toward environmentally-benign marine antifouling – an intractable problem requiring holistic thinking and multiple tactics

<u>Dr Patrick Cahill¹</u>, A. Prof. Joahan Svenson¹, Dr Grant Hopkins¹ ¹Cawthron, Nelson, New Zealand

Biography:

Patrick is a Scientist and Team Leader at Cawthron. He has specialist technical expertise in marine pest control, including unique capabilities for biological development and assessment of marine antifouling technologies.

Marine biofouling is an age-old problem that reduces hydrodynamic performance of vessels, accelerates deterioration of materials, increases loading of static structures, and facilitates marine bioinvasions. Traditional approaches to biofouling management, namely coatings laced with broad-spectrum and often persistent toxins, are increasingly unacceptable due to collateral environmental harm. Many antifouling biocides and co-biocides have been banned or specified for phased removal from the market. Even copper, the current antifouling mainstay, has been banned in some jurisdictions and is facing scrutiny from regulators the world over. New approaches are urgently needed to ensure biofouling can be effectively managed in the future. The challenge is considerable, requiring effective control of the large taxonomic diversity found in biofouling communities under varied conditions in the ocean but with low or no risk to the environment (including adjacent natural biofouling communities). A 'silver bullet' is unlikely to exist and a range of technologies that can be applied in isolation or combination present the most reliable and robust option. Exploiting knowledge of biofouling ecology and biology can maximise the likelihood of fit-forpurpose antifouling approaches and leveraging cross-disciplinary expertise can yield innovative new options. Antifouling research at Cawthron follows this paradigm, and we are applying our biological expertise to direct development and assessment of a holistic package of antifouling technologies. These technologies include: natural antifouling compounds from marine organisms; synthetic biocides with benign breakdown products; selective inhibitors of biofouling settlement designed in silico; foul-release coatings and functionalised materials; mechanical disruption using bubble streams; and reactive treatments protocols. This talk will provide an overview of our varied work on marine antifouling and a synthesis of intended implementation and future research priorities.

POSTER

Larval Resilience to Decreased Salinity in Acanthaster cf. solaris (CoTS)

Mr Matthew Clements¹

¹The University of Sydney, Sydney, Australia

Biography:

I am a current honours student at the University of Sydney supervised by Maria Byrne. Our research investigates the resilience of Crown of Thorns starfish larvae to environmental factors like salinity and food density. We've also focussed on COTS larval cloning, regeneration success, and settlement cues.

Previous research on asterinid species investigated the asexual propagation of Ailsastra heteractis, a geographically isolated population of seastars on the Great Barrier Reef, and the growth and recruitment of Marthasterias glacialis from Lough Hyne, Ireland.

Growing up on the Northern Beaches of Sydney with Sydney's rocky shores and beautiful beaches at my fingertips, as well as with environmental consultancy experience collecting invertebrate sedimentary samples in the North Sea and British Isles, my journey to date has gifted me a passion for inquiry into marine invertebrate world and for the protection of marine habitats.

COTS is an opportunistic boom-and-bust species with periodic outbreak populations that cause widespread coral mortality across the Great Barrier Reef (GBR). When their larvae are in the plankton, coastal runoff events generate plumes of simultaneous decreased salinity and increased terrestrial nutrients and larval food. While the relationship between increased larval food and greater larval settlement success is considered to be an explanation for outbreaks, their tolerance to combined low salinity and variable food conditions is not known. We investigated survival and the proportion of normal larval phenotypes in the two larval stages, bipinnaria and brachiolaria in response to 6 salinity (between 15 ppt and 34 ppt) and 3 algal food density (no food, 10 000 cells ml-1, and 20 000 cells ml-1) treatments, in all combinations. Both larval stages exhibited similar tolerance to salinity across all food density groups with 19ppt, 22 ppt, and 25 ppt treatments significantly reducing the percentage of normal phenotypes and survival. With time, lower incidences of normal phenotype and survival also occurred at relatively higher salinity treatments indicating the deleterious influence of prolonged exposure to decreases in salinity relative to the ambient (~34 ppt). Our results indicate that COTS larvae tolerate to salinity levels commonly experienced in a typical GBR coastal runoff plume, but not in extreme near-shore levels. These responses and the comparatively stress tolerant nature of the larvae may contribute to their abilities to seed outbreaks.

The phosphate overplus response in *Chlamydomonas reinhardtii* and *Chlorella vulgaris*: Different strategies and the influence of environmental factors on polyphosphate accumulation.

<u>Alex Cliff</u>¹, Dr Maxence Plouviez¹, Dr Nicola Brown¹, Prof Benoit Guiyesse¹ ¹School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand

Biography:

Alex comes from a diverse academic background, most recently being awarded a Bachelor's Degree in Chemical and Bioprocess Engineering from Massey University. Alex is currently undertaking his PhD studies at Massey University under the supervision of Prof Benoit Guieysse, Dr Maxence Plouviez, and Dr Nicola Brown. His current work with microalgae, focusing on phosphate sequestration, resonates with his interest in environmental engineering and its applications in bioremediation.

The ability to sequester phosphorus during periods of high availability, for later use during nutrient scarcity, is believed to be an important trait driving seasonal species dominance among freshwater algae. This phenomenon, known as the 'overplus response', is observable as the accumulation of granules of inorganic polyphosphate (polyP) within algal vacuoles known as acidocalcisomes. While this phenomenon has been described in many species of algae, there has to date been no rigorous comparison of the dynamics of luxury uptake across species. The two Chlorophyte species, Chlamydomonas reinhardtii and Chlorella vulgaris, were examined using a repeatable assay triggering an overplus response. A two-level full factorial experimental design was implemented, with light intensity, temperature, initial pH, concentration of phosphate dose, and time since last phosphate replenishment being the factors being tested. Responses were assessed by optical density, extracellular phosphate concentration, deviations in pH, in situ semiquantitative measurement of polyP, and ranked abundance of polyP granules (observed microscopically). Measurements were taken before giving the trigger dose and one, six, and twenty-four hours following the trigger. Analysis of the response variables indicated that these two species have markedly different capacities for phosphate uptake but, in both cases, uptake was initially higher for cultures which had experienced a longer duration since last being 'fed'. This latter observation supports evidence of an increased metabolic readiness to import phosphate revealed in previous studies. This knowledge will help to better understand how polyP accumulation may vary between species, and the implications this variation has for species dominance in differing ecological niches.

Histopathological assessment of *Haliotis iris* populations exhibiting divergent growth performance: A baseline study

<u>Miss Joanna Copedo^{1,2}</u>, Prof Andrea C Alfaro¹, Dr Norman LC Ragg², Dr Steve Webb² ¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ²Cawthron Institute, Nelson, New Zealand

Biography:

Joanna Copedo is an Aquaculture technician and researcher who has recently started a PhD candidature within the Aquaculture Biotechnology Research Group at the Auckland University of Technology. Her research focuses on using histopathology to understand the complex host-pathogen-environment relationship in marine molluscs. Joanna's research interests include environmental stressors, particularly the effect of climate change and pathogens on the host, mainly in aquaculture species that are commercially valuable for New Zealand.

The New Zealand Pāua, *Haliotis iris*, is a unique and valuable species to New Zealand with cultural importance for Māori. Pāua are marine gastropods that can display a high level of morphological variation, including slow-growing variants. They inhabit shallow coastal regions (4 – 20m), which are represented by variable habitat structures from bare rocky shores to kelp forests, and fluctuating environmental conditions, making Pāua vulnerable to these changes. In this study, histopathological tools were used to identify factors, such as digestive tract condition and food quality, that are associated with slow growth in populations with low access to food and habitats with higher levels of sedimentation, compared with fast growing populations in areas with better food access. General histological surveillance was conducted on gills, gonad, digestive tubules, gut (epithelium and content), kidney, buccal tissue and oesophageal pouch. Image analysis was conducted on the digestive tubules by using imaging software to quantify the relative area of tubule retraction and the change in interstitial space. The food quality in the gut was scored from 'old' to 'fresh' based on the macroalgal fragment structure and amount of debris. Preliminary results show that there is a high inter-individual variation within populations, (slow and fast growing). There also appears to be a relationship between the interstitial space between the digestive tubules and quality of algae in the gut. This may suggest that food, abundance and quality, influences the digestive tubule structure.

POSTER

Thermal stress in mussels: a histopathological perspective

<u>Miss Joanna Copedo^{1,2}</u>, Prof Andrea C Alfaro¹, Dr Norman LC Ragg², Dr Steve Webb² ¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ²Cawthron Institute, Nelson, New Zealand

Biography:

Joanna Copedo is an Aquaculture technician and researcher who has recently started a PhD candidature within the Aquaculture Biotechnology Research Group at the Auckland University of Technology. Her research focuses on using histopathology to understand the complex host-pathogen-environment relationship in marine molluscs. Joanna's research interests include environmental stressors, particularly the effect of climate change and pathogens on the host, mainly in aquaculture species that are commercially valuable for New Zealand.

The New Zealand green-lipped mussel, Perna canaliculus, is a keystone species in the rocky reef environment and is the country's most important aquaculture species. Although remarkably robust, there are limits to its thermal tolerance range. Atmospheric climate change is driving corresponding, and frequently amplifying, changes in the marine environment; mean seawater temperatures and frequency of heatwave events are projected to rise over the next century. Globally, marine heatwaves have already resulted in a number of mass mortality events. As these climate stressors increasingly impact upon marine poikilotherms, their performance and ability to respond to additional stressors, including pathogen presence, may become impaired. Laboratory thermal challenge trial have accordingly demonstrated that this mussel is susceptible to thermal stress, which appears to compromise its immune defences. In this study, histopathological techniques were used to compare the condition of tissues (e.g., mantle, gonad, muscle, digestive tubules and gills) in mussels exposed to three different temperatures, "benign" (17°C) and "elevated" (21 and 24°C), for 16 months. To track the evolution of tissue-level changes, monthly samples of eight mussels per treatment were shucked and a 5mm section was taken for histology (H&E stained). Preliminary results showed that gonadal development was particularly affected by elevated temperatures and in some cases the level of atresia and follicle atrophy increased. An interesting association between experimental temperature, acclimation time and pathogen occurrence were apparent. For example, mussels exposed for extended periods to the 24°C treatment were observed to have reduced gonadal development but had increases in pathogen prevalence.

"Got your nose!" Understanding the novel collagen composition from nasal cartilage of hoki

Dr Mathew Cumming¹

¹Plant And Food Research, Nelson, New Zealand

Biography:

I lead the Marine Biopolymer Sciences team at Plant and Food Research, Nelson. My research focus is the scientific understanding of the structural characteristics of marine-sourced collagen and associated molecules (such as glycosaminoglycans and hydroxyapatite) and how these characteristics influence the functional properties of native tissues and the collagen as an extracted, fabricated biomaterial. Through this knowledge, my research involves the development of efficient collagen extraction and isolation methods from a wide range of marine species and tissues such as fish skin, bones, cartilage, sea cucumbers, and shellfish. I continue to expand collagen-specific analytical methods including the use of vibrational spectroscopy and circular dichroism spectroscopy. This research has led to the development of high-value collagen-based products for cosmetic and nutraceutical applications. My particular interest and expertise is in the chemical functionalisation of collagen and fabrication of stabilised biomaterials that use biomimetic design principles for regenerative medicine applications.

Cartilage trauma, osteoarthritis and other cartilage-related illnesses are often caused by damage or degradation of cartilage-related collagens. The numbers of people affected by mobility issues is increasing alongside our aging population so there is an increasing demand for cartilage replacement scaffolds, new nutraceuticals and a better understanding of the types and properties of cartilage collagens.

To date there has been no research on understanding the composition and characteristics of collagens within the cartilage of fish yet they present potential for high-volume cartilage extracts without many of the religious and health issues associated with use of mammalian tissue.

Study identifies and characterises collagens in hoki noses that could lead to the development of treatments for cartilage related illness such as osteoarthritis, and for cartilage reconstructive surgery.

No-one has studied teleost fish cartilage collagens before. We have extracted, isolated and characterized both the major and minor collagen types from hoki nose cartilage. Our study showed that the composition and characteristics of nose collagen is similar to that of human cartilage. Hoki noses contained the expected cartilage collagens (including type II, type XI and type IX) as well as a highly glycosylated collagen that has not been identified in any other species. This study has significantly enhanced our understanding of collagen of teleost cartilage.

Our next step, is to investigate the role that hoki cartilage-derived collagens could play as the ingredients for biomaterials and treatments of cartilage-related diseases.

Leveraging an 8000+ Marine Invertebrate Collection for Biodiscovery – the NatureBank Approach

A/Prof. Rohan Davis¹

¹Griffith Institute for Drug Discovery, Brisbane, Australia

Biography:

A/Prof. Rohan Davis received a BSc (Hons) from the University of Melbourne (1992) and a PhD from Griffith University (2001); both degrees focussed on marine chemistry. Rohan subsequently worked for 2 years at University of Utah on an anti-cancer drug discovery project before being recruited back to Griffith in 2003. His research involves all aspects of biodiscovery including the design and synthesis of unique screening libraries based on natural product scaffolds. Rohan has authored 157 publications, holds 2 patents, and for the past 5 years he has been part of the Aussie-Kiwi team that publishes the world-renowned and highly-cited annual marine natural products review in Natural Product Reports. He has led both industry and academic projects that have resulted in the identification of >1000 natural products from plant, marine invertebrate and microbial sources. A/Prof. Davis is the Academic Lead of NatureBank, a unique Australian biodiscovery resource located at the Griffith.

Australia is one of 17 countries described as megadiverse. The concept of megadiversity is based on the total number of species in a country and the degree of endemism at this taxonomic level. While these 17 megadiverse nations cover <10% of the global surface, they collectively support >70% of the Earth's biodiversity. Biodiversity typically equates to chemical diversity, an important element for those undertaking biodiscovery, thus the use of Australian-sourced biota for natural products drug discovery and chemical biology research has a strategic advantage, since this unique resource has only been superficially explored for new pharmaceutical agents and chemical probes. This presentation will: (i) give a brief overview of NatureBank - a unique biodiscovery platform based on natural product extracts and fractions derived from Australian plants, fungi and marine invertebrates; (ii) describe the identification and biology associated with several new natural products that have recently been discovered by the Davis group and their potential impact to the field of biodiscovery; and (iii) detail the design and synthesis of several drug-like screening libraries that were created using isolated natural product scaffolds and semi-synthetic methodology.

Understanding the dynamic of POMS infection in New Zealand and the role of microbiota composition in the survival of Pacific oysters, *C. gigas*.

Dr Lizenn Delisle¹

¹Cawthron, New Zealand

Biography:

I'm shellfish physiologist in the Aquaculture Group at Cawthron. I'm working on diseases in aquaculture and physiology of marine organisms. My research aims to identify pathogens associated with severe mortality episodes occurring on aquaculture species in New Zealand and their mechanisms of infection through the analyses of field samples, experimental infection in laboratory and in vitro propagation. I'm focusing on mechanisms of host response to infection, by using large scale molecular approaches like transcriptomic, metabarcoding or proteomic and targeted analyses (PCR, qPCR) to improve understanding of shellfish resilience to pathogens which will support the aquaculture industry.

Pacific Oyster Mortality Syndrome (POMS) massively affects *Crassostrea gigas* oysters worldwide causing important economic losses during summer. The dynamic of the disease was recently characterized in France starting by an immune-compromised state in oysters induced by the Ostreid herpesvirus (OsHV-1), evolving towards bacteremia by opportunistic pathogenic bacteria leading to oyster death. Since 2010, mass mortality episodes associated with OsHV-1 have been recorded in juvenile *C. gigas* in New Zealand, but the kinetics of the disease and the role of the oyster microbiota have still not been well established. Using an experimental infection of ten oyster families with contrasted virus susceptibilities, combined with molecular analyses (qPCR) and 16S rRNA gene-metabarcoding, we showed that: 1) occurrence of mortality and viral replication occur later after infection in oysters compared to European observations (18h vs 96h), 2) the microbiota diversity is reduced in oysters presenting high survival rate 3) relative abundance of the bacterial taxa Mycoplasmataceae, Nitrincolaceae and Rhodobacteraceae in tissues appear to be associated with oysters resilient to the POMS.

Keywords: POMS, OsHV-1, Crassostrea gigas, microbiome, metabarcoding, mortality risk.

Effect of emersion and relative humidity on the stress response and recovery dynamics in juvenile *Perna canaliculus*

<u>Dr Natali Delorme¹</u>, Dr David Burritt², Dr Norman Ragg¹, Dr Paul South¹ ¹Cawthron Institute, Nelson, New Zealand, ²University of Otago, Dunedin, New Zealand

Biography:

Natalí is a scientist in the Aquaculture Group at the Cawthron Institute in Nelson, New Zealand. She is a passionate marine scientist working on the physiology of marine invertebrates. Currently, her research aims to understand stress, health and nutrition of different shellfish species through the development of immunological assays and quantifying oxidative damage and antioxidant biomarkers.

The early juvenile stages of intertidal mytilid mussels, face both direct and indirect existential environmental threats. Stressors may directly influence physiological status and, ultimately, survival or indirectly promote migration via byssal drifting with an associated increased risk of predation. A detailed understanding of the nature of stress experienced is critical to inform conservation and aquaculture efforts. Here we studied oxidative stress dynamics in the juvenile green-lipped mussel, Perna canaliculus, in relation to emersion duration (1-20h) and relative humidity (RH, 29-98%) by quantifying oxidative damage markers (protein carbonyls, lipid hydroperoxides, 8-hydroxydeoxyguanosine) and enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase). Mussels held in low RH during emersion experienced severe water loss (>70%), high apparent mortality (>80%) and increased oxidative damage (35-45% increase compared to control conditions), while mussels held at high RH were not impacted, even after 20h air exposure. Following re-immersion, reoxygenation stress resulted in further increases in damage markers in mussels that had experienced dryer emersion conditions; protective action of enzymatic antioxidant increased steadily during the 10h re-immersion period, apparently supporting a reduction in damage markers after 1-5h of immersion. Clearly, conditions during emersion, as well as duration, substantially influence physiological performance and recovery of juvenile mussels. Successful recruitment to intertidal beds or survival in commercial aquaculture operations may be substantially mediated by the nature of emersion stress experienced by these vulnerable juveniles.

Effects of thermal acclimation on heart mitochondrial energy balance in the Australian rainbow trout *Oncorhynchus mykiss*.

Dr Jules Devaux¹, Dr Tony Hickey¹, Dr Tony Farrell²

¹University Of Auckland, Auckland, New Zealand, ²University of British Columbia, Vancouver, Canada

Biography:

My research and general interest are peaked by the mitochondrial function and dysfunction. Currently, my research fellowship focusses on the effect of temperature on the mitochondrial function in fish heart.

Temperature has a significant influence over bioenergetic processes, and thermodynamic changes are particularly critical in ectotherms. Survival is rapidly compromised as temperature rises, and for many animals, the heart appears to be the first organ to fail when exposed to heat stress. Heart failure also appears to be mediated by a loss in mitochondrial integrity, the organelle failing to synthesize adequate ATP to meet increasing demands to beat. Acclimation to high temperatures alters mitochondrial function, yet the divergence in mitochondrial plasticity and shift in thermal-tolerance appears to be species specific. While rainbow trout (*Oncorhynchus mykiss*) are typically sensitive to elevated temperatures and inhabits cool water, some Californian populations experience considerable thermal ranges (circa. 17.5-24.5°C). Lineages in the Southern Hemisphere have originated from California and then populated cold freshwater systems.

Here we explore the acclimation capacity of cardiac mitochondria in a hatchery-raised population of rainbow trout previously selected for upper thermal tolerance. In animals acclimated to 19, 21 and 25°C, we measured oxygen consumption rate, mitochondrial membrane potential and ATP dynamics in permeabilised cardiac fibres at 20, 25, 30 and 35°C. While mitochondrial integrity is lost above 30°C, acclimation to higher temperatures improved mitochondrial coupling. This likely translates in a greater capacity to produce ATP below 25°C. We confirmed that more robust mitochondria can provide rainbow trout with a greater thermal-tolerance. In effect, this population may reflect a return to conditions in warm Californian rivers.

Cellular Systems for Fish Health

Dr Georgina Dowd¹

¹The New Zealand Institute for Plant and Food Research Ltd, Nelson, New Zealand

Biography:

Georgina is a cell and molecular biologist working at Plant and Food Research. He current work focuses on establishing cell lines from New Zealand finfish for applications in maintaining fish heath in open ocean aquaculture settings.

Extracting cells from animals or plants with subsequent growth in artificial environments is a method that has been used extensively to model responses of the whole organism. Applied primarily to mammalian systems, culture of cells from marine organisms remains relatively unexplored and, to date, there are no reported cell lines from finfish species endemic to New Zealand. Historically, fish cell line research has been driven mainly by a need to understand and control infectious disease agents, particularly in high density aquaculture. While this need remains, the application of fish cell lines is being extended to cellular agriculture, toxicology, whole animal modelling, immunological studies, vaccine development, and animal biobanking through gamete cryopreservation.

At Plant & Food Research, we are establishing a platform of cell lines from New Zealand-relevant teleosts, which will support our finfish by informing on optimal growth, resilience, health and welfare as part of an integrated health management strategy. We will describe a number of cell lines established from *Chrysophrys auratus* (tamure, Australasian snapper) and *Oncorhynchus tshawytscha* (Chinook salmon). These include gill-derived epithelial cells which can be employed to study gill functionality and gill disease. They are also ideal sentinel models for aquatic toxicology. We discuss hormone secreting cells of the pituitary gland and their implications in animal husbandry. We also present data on the culture of fish muscle in vitro.

Metabolomic analysis of prolonged aerial exposure in green lipped mussels (*Perna canaliculus*)

Angela Tagaloa¹, <u>Norman Ragg²</u>, Louis Ranjard¹, <u>Brendon Dunphy¹</u> ¹The University of Auckland, ²Cawthron Institute

Marine intertidal bivalves experience predictable bouts of aerial exposure during low tides. Whilst the short-term switch to anaerobic metabolism that bivalves make is well established the mechanisms underpinning prolonged (> 48 h) aerial exposure are less well known. To investigate this, we aimed to identify metabolomic biomarkers of aerial tolerance between two selectively bred mussel families known to have differing emersion capacity i.e. average survival of 8.5 (High tolerant family) or 6.8 (Low tolerant family) days respectively. Metabolite profiles were compared at Days 0, 4, and 8 of an emersion trial with the goal of seeing whether certain metabolites were associated with ability to cope with prolonged emersion.

Quadratic discriminant function analysis (Q-DFA) of metabolite profiles obtained from GC-MS analyses revealed three key metabolites (Succinic acid, Aspartic acid, and 'Unknown 127') to classify family and day of aerial exposure. Moreover, by days 4 and 8 succinic, and aspartic acid had upregulated at a comparatively rapid rate in mussels from the low tolerant family. Whereas, high tolerant mussels initially down regulated succinic acid before levels climbed by Day 8; with Aspartic acid showing no change.

Lastly, secondary enrichment of metabolite data using the 'Reactome' platform identified six key biochemical pathways that were up/down regulated in response to prolonged aerial exposure. Most notable was the Sodium-coupled sulphate, di- and tri-carboxylate transporter pathway which plays a key role in regulating mitochondrial function. Taken together together, these results provide a high-resolution insight into the changes mussels make as stresses mount.

CRISPR/Cas13 potential as a next-generation molecular biomonitoring tool of harmful algal blooms

Benjamín Durán-Vinet^{1,6}, Dr. Karla Araya-Castro^{1,2}, Dr. Susanna A. Wood³, Dr. Anastasija Zaiko^{3,4}, Dr. Kirsty Smith³, Dr. Tzu-Chiao Chao⁵, Dr. Jacquelinne J. Acuña^{1,6}, Dr. Milko Jorquera^{1,6}, Dr. Michel Abanto¹ ¹Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Av. Francisco Salazar, 1145, Temuco, Chile., ²Innovation and Entrepreneurship Institute (iDEAUFRO), Universidad de La Frontera, Av. Francisco Salazar, 1145, Temuco, Chile., ³Coastal and Freshwater Group, Cawthron Institute, 98 Halifax Street East, Nelson 7010, New Zealand., ⁴Institute of Marine Science, University of Auckland, Private Bag 92019, Auckland, New Zealand., ⁵Institute of Environmental Change & Society, Department of Biology, University of Regina, Wascana Parkway, 3737, Regina, Canada., ⁶Laboratorio de Ecología Microbiana Aplicada (EMALAB), Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Av. Francisco Salazar, 1145, Temuco, Chile.

Biography:

I am a Bachelor of Biotechnology (Honours) highly interested in marine and freshwater biosecurity, committed to generating quality research and scientific production, focusing on applied research to generate potential knowledge, technological transfers and solutions for the aquaculture industry. In this context, my applied research has focused on the CRISPR technologies and their great potential as diagnosis tools, especially over harmful algal blooms biomonitoring from their environmental nucleic acids. I am currently leading a project funded by the Chilean National Agency of Research and Development (ANID) to establish a biomonitoring tool based on CRISPR/Cas technologies and assess their overall efficiency to deliver valuable decision-making data for stakeholders.

Efforts to monitor harmful algal and cyanobacterial blooms (HABs/CHABs) have recently risen significantly due to the dramatic increase in their distribution, frequency and intensity. HABs/CHABs endanger public health, ecosystem sustainability, and aquaculture industries. Molecular approaches have enabled detection and quantification of cryptic species with enhanced efficiency regarding runtimes, sample throughput and sample costs. However, challenges do exist, including dependence of complex instrumentation, lack of portability and uncertainties regarding the viability of the detected microorganisms. Thus, environmental RNA (eRNA) has recently been proposed as a valuable biomarker capable of providing spatio-temporal detection, with resolution able to estimate the viable portion of a microbial community. Recent studies have shown that Clustered Regularly Interspaced Short Palindromic Repeats/Cas proteins (CRISPR/Cas)based approaches, specifically CRISPR/LwaCas13a that only targets RNA, hold great potential to be used as an eRNA-based biomonitoring tool with features including rapid, inexpensive, highly portable, ultrasensitive and robust detection in environmental samples. To assess the potential of CRISPR/LwaCas13a as a biomonitoring tool, we used two toxic microalga and their mRNA sequences of genes that initiate toxin biosynthesis (Alexandrium catenella and the sxtA4 gene for saxitoxin and Microcystis aeruginosa and the mcyA gene for microcystin), as in silico mRNA models to define conserved region zones for CRISPR RNA (crRNA) design. Conserved regions were analyzed by Cas13design v0.2 to model crRNA efficacies using matrix scores based on RNA secondary structure predictions, obtaining 52 and 235 potential crRNAs sequences complementary to sxtA4 and mcyA, respectively. Lastly, using BLASTn to discard off-targets and spacer restrictions filters, we selected two final crRNAs for each model organism. Results indicate a high average in silico detection efficacy above 70% on obtained crRNAs, showcasing the potential of a promising CRISPR/LwaCas13-based biomonitoring tool of HABs/CHABs. Subsequently, the proof-of-concept of LwaCas13-based biomonitoring is going to be run on synthetic and spiked samples. Acknowledgements: Cas13-biomonitoring FONDEF-VIU20P0030; Our lakes' health; past, present and future CO5X1707; SATREPS-MACH JPMJSA1705.

Sensitivity to oil dispersants: Effects on the valve movements of the blue mussel *Mytilus edulis* and the giant scallop *Placopecten magellanicus*, in sub-arctic conditions

<u>Guillaume Durier¹</u>, Jean-Bruno Nadalini^{1,4}, Richard Saint-Louis², Bertrand Genard³, Luc A. Comeau⁴, Réjean Tremblay¹

¹ISMER/UQAR, Rimouski, Canada, ²Departement de biologie, chimie et geographie, UQAR, Rimouski , Canada, ³Les laboratoires Iso-BioKem Inc., Rimouski , Canada, ⁴Fisheries and Oceans Canada Gulf Region , Moncton, Canada

Biography:

PhD student at ISMER (Rimouski, Québec) working on the use of valvometry (study of valve movements) to detect toxins in Environment. Previously I did a Master at University of Bordeaux and studied the growth and the behavior of blue mussel and their annual behavioral rythmes in extreme high Arctic.

In response to accidental oil spills at sea, chemical oil dispersants are utilized to limit negative impacts on nearby littoral zones. However, current evidence suggests that such dispersants may be toxic to aquatic organisms. Blue mussels (Mytilus edulis) and giant scallops (Placopecten magellanicus) were exposed to different environmentally relevant concentrations of oil dispersant and their behavioural responses were closely monitored using high frequency (10Hz) valvometry. Behavioural valve responses included rapid closures when oil dispersant was added to the experimental tanks. At higher concentrations, the mussels remained closed throughout the exposure period. The giant scallop displayed escape behaviours (clapping) prior to mortality, suggesting toxicity of the oil dispersant. Relationships between different behavioural indicators and oil dispersant concentrations were observed for both species, but with different trends. While scallops demonstrated positive correlations between gaping behaviours and dispersant concentration, mussels exhibited a concentration threshold beyond which the gaping behaviour was characteristic of longer closure periods. This study highlights behavioural response differences consistent with bivalve-specific biological traits: the continuous valve closure of an intertidal species, M. edulis, firmly attached to the substrate, and the escapement behaviours of a semi-mobile subtidal species, P. magellanicus. From these observations, it appears that valvometry could be used as a tool for environmental assessments.

Marine Biodiscovery and functional genomics

Suhelen Egan¹

¹UNSW Sydney, Australia

Biography:

Suhelen is an Associate Professor at the University of New South Wales in Sydney Australia, where she has held a teaching and research position since 2003. Her research focuses on understanding the diversity and mechanisms of microbial symbioses (including both beneficial and detrimental interactions) in important marine host organisms. In addition to addressing fundamental scientific questions, she applies her knowledge of these symbiotic systems towards the discovery and development of new marine biotechnologies. Suhelen has been a member of ANZMBS committee since 2016 and is currently vice president of the society.

Drug resistance among parasitic nematodes has resulted in an urgent need for the development of new therapies. Marine epiphytic bacteria are hypothesised to produce natural compounds as a defence against bacterivorous predators including nematodes, thus representing a promising, yet underexplored source for antinematode drug discovery. The marine epiphytic bacterium Pseudoalteromonas tunicata is known to produce a number of bioactive compounds. Screening genomic libraries of P. tunicata against the nematode Caenorhabditis elegans has identified a novel Nematode killing protein-1 (Nkp-1). The Nkp-1 toxicity appears to be nematode-specific, with the protein being highly toxic to nematode larvae but having no impact on nematode eggs. A putative carbohydrate binding module was identified at the N-terminus of Nkp-1 protein sequence which is suggested to bind to a yet unknown nematode glycoconjugate receptor. We further provide the first insights into the mode of action of Nkp-1 and the nematode response towards its toxicity. Recombinant E. coli producing Nkp-1 actively colonise C. elegans intestine and exposure to protein extracts from these strains results in physical damage and necrosis. Moreover, we reveal that the presence of a defined gut microbiota can significantly impact the sensitivity of *C. elegans* to Nkp-1, highlighting the importance of future work to carefully consider the efficacy of potential nematicidal drugs under both standard laboratory conditions and conditions that resemble natural settings. The outcome of these studies will contribute to development of Nkp-1 as a future antinematode drug but reaffirm the potential for marine epiphytic bacteria as a new source of novel antinematode drugs.

Greenshell[™] mussel health and stress in a warming ocean – characterisation of thermotolerance and implications for summer mortality

<u>Dr Jessica Ericson</u>¹, Dr Leonie Venter², Dr Natali Delorme¹, Ms Joanna Copedo^{1,2}, Professor Andrea Alfaro², Dr Norman Ragg¹

¹Cawthron Institute, Nelson, New Zealand, ²Auckland University of Technology, Auckland, New Zealand

Biography:

My research investigates how marine invertebrates respond to a changing ocean (e.g. ocean warming and acidification, marine heatwaves, seasonal changes). I study a range of aspects relating to animal physiology and biochemistry including respiration, growth, survival, condition, haemocyte immune responses, gaping behaviour, lipid and fatty acid composition). My work focuses on ecologically and economically important temperate and polar marine invertebrates. I work with a range of stakeholders including marine farmers, iwi, fishermen, scientists and the public.

The Greenshell[™] mussel (*Perna canaliculus*; GSM) is an ecologically and economically important bivalve endemic to New Zealand. It is intensively farmed in New Zealand's coastal waters, and mussel aquaculture contributes hundreds of millions of dollars each year to the country's economy. Marine farmers in New Zealand and around the world are facing numerous challenges as anthropogenic climate change alters ocean temperature and chemistry.

Summer mortality events have been reported in spat and adult GSM, but the causes of summer mortality are often a mystery. To ensure the success of the GSM aquaculture industry into the future, we require more knowledge on the role of pathogens, seawater temperature and food availability on GSM summer mortality, and the implementation of mitigation strategies (e.g. reducing carbon emissions, changes to onfarm practices, selective breeding, open ocean aquaculture) to better manage these summer mortality events. Results from a range of laboratory trials examining the effects of increased seawater temperature and exposure to vibrio bacteria will be presented. In addition to mortality, sublethal stress responses (e.g. haemocyte immune responses, antioxidant capacity, animal condition, feeding behaviour, growth, gaping behaviour, respiration rate) were measured. Measurements of sublethal stress allow us to detect physiological changes in mussels before death occurs (by which time it is too late). Many measurements were focused on the use of portable tools that are also being optimised and validated for use in the field.

Assessing embryo fate development as a means of selective climate adaptation in marine invertebrate

Dr Emily Frost¹, Prof Andrea C Alfaro¹

¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand

Biography:

Emily is a post-doctoral research fellow in the Aquaculture Biotechnology Research Group at the Auckland University of Technology. She has been researching in marine biology and environmental/comparative physiology and molecular biology for 11 years. Specifically, she is working in a wide array of projects, focusing on molecular biology, transcriptomics, and embryonic cell specification. In addition, Emily is an active member of the broader scientific community, including, as a scientific policy author for the United Nations Environmental Programme (UNEP) editorials under the direction of Dr Richard Munang. A member of the New Zealand Ocean Acidification Research Group, the Otago Institute for Arts and Science (a branch of The Royal Society of New Zealand), International Graduate Scholar for the Fifth International Conference on Climate Change, and the International Council for Science: Auckland, 2014 – General Assembly. In addition, she also attended the 2017 United Nations Oceans Conference as Head Delegate.

Ocean warming and acidification significantly alter the phenotype of developing larvae in a number of important aquaculture species, including the New Zealand sea urchin (*Evechinus chloroticus*) and the greenshell[™] mussel (*Perna canaliculus*). However, a critical outstanding research question is: what are the particular mechanisms that cause the significant increases in abnormal organism development due to short-term changes in ocean warming and acidification? One leading theory suggests that there is a disruption in cell specification through altering the specific asymmetric distribution of "patterning molecules" throughout the embryo. This can be either through how cells "communicate" with each other, referred to as cell-cell communication or the input of maternal signalling molecules, which starts the specification process. Establishing the mechanism causing abnormal embryonic development will serve as a means of selecting climate-change resilient families for future aquacultural ventures. This presentation introduces new research aiming to assess the impact of near future ocean warming and ocean acidification on the embryonic cell specification in the developmental model species, the New Zealand Sea Urchin *E. chloroticus*.

POSTER

MagPix, a high-throughput hero in gene quantification

Dr Emily Frost¹, Prof Andrea C Alfaro¹

¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand

Biography:

Emily is a post-doctoral research fellow in the Aquaculture Biotechnology Research Group at the Auckland University of Technology. She has been researching in marine biology and environmental/comparative physiology and molecular biology for 11 years. Specifically, she is working in a wide array of projects, focusing on molecular biology, transcriptomics, and embryonic cell specification. In addition, Emily is an active member of the broader scientific community, including, as a scientific policy author for the United Nations Environmental Programme (UNEP) editorials under the direction of Dr Richard Munang. A member of the New Zealand Ocean Acidification Research Group, the Otago Institute for Arts and Science (a branch of The Royal Society of New Zealand), International Graduate Scholar for the Fifth International Conference on Climate Change, and the International Council for Science: Auckland, 2014 – General Assembly. In addition, she also attended the 2017 United Nations Oceans Conference as Head Delegate.

The current techniques for analysis of gene expression can be divided into three classes. The first class is hybridization-based techniques such as Northern blotting, subtraction cloning, and DNA microarrays. The second class is PCR-based techniques, including differential display and qPCR. The third is sequence based techniques including SAGE and mass-spectrometry sequencing. However, few of them are suitable for detection of moderate numbers of genes in thousands of samples with high speed and low cost. Of which, there is a great demand for such method for use in environmental physiology, diagnostics and screening. This is where the Magpix (Luminex) comes in. The ligation-dependent amplification multiplex assays, such as that employed by the Magpix, allow the quantitative analysis of thousands of gene expression changes simultaneously in a single experiment. This is orders of magnitude more so than that can be achieved by any other technologies involved in quantitative gene expression. The use of Magpix is crucial for the advancement of environmental physiology, molecular biology and aquaculture biotechnology in New Zealand. This poster talk will introduce and outline the advancements Magpix technology offers and the applications for use currently being developed at Auckland University of Technology

Sensitivity of gametes and fertilization of the Pacific oyster, *Crassostrea gigas*, exposed to *Dinophysis* and pectenotoxin 2

Dr. Sylvain Gaillard¹, Nelly Le Goïc², Florent Malo¹, Dr. Myrina Boulais², Dr. Caroline Fabioux², Lucas Zaccagnini², Liliane Carpentier¹, Manoella Sibat¹, Dr. Damien Réveillon¹, Dr. Véronique Séchet¹, Dr. Philipp Hess¹, Dr. Hélène Hégaret²

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Harmful algal blooms (HABs) of toxic species of the dinoflagellate genus Dinophysis are a threat to human health as they are mainly responsible for diarrheic shellfish poisoning (DSP) in the consumers of contaminated shellfish. The direct effects of numerous HAB species have been demonstrated on adult bivalves, whereas the effects on critical early life stages remain relatively unexplored. We aimed to determine the effects of either cultivated strains of D. cf. sacculus and D. acuminata isolated from French Atlantic coast or their associated polycyclic ether toxins (i.e. okadaic acid (OA) and pectenotoxin 2 (PTX2)) on the quality of gametes and fertilization success of the Pacific oyster Crassostrea gigas. Using flow cytometry and microscopic observations, we assessed the ROS production, the viability of the gametes and the fertilization success. Oocytes were more affected than spermatozoa and their mortality and ROS production increased in the presence of D. cf. sacculus and PTX2, respectively. A decrease in fertilization success was observed at concentrations as low as 0.5 cell mL-1 of Dinophysis and 5 nM of PTX2, and the decrease was higher when both gamete types were concomitantly exposed compared to separate exposures, suggesting a synergistic effect. However, no effect of OA could be observed. Our results suggest that the effects could be due to cell contact between Dinophysis and gametes, to the macrocyclic structure of the PTX2 and/or to other bioactive compounds. We highlight for the first time a potential effect of Dinophysis and PTX2 on reproduction and recruitment of the Pacific oyster.

Immunohistochemistry as a tool to decipher the mechanisms involved in the accumulation and long retention of the phycotoxin domoic acid in the king scallop *Pecten maximus*

<u>Dr José Luis García-Corona¹</u>, Dr Helene Hegaret¹, Dr Malwenn Lassudrie¹, Dr Amelie Derrien¹, Dr Elodie Fleury¹, Dr Caroline Fabioux¹

¹Institut Universitaire Européen de la Mer, Brest, France

Biography:

José Corona is a marine biologist who graduated with honors from the Autonomous University of Baja California Sur. He completed his Master's degree in Aquaculture Biotechnology at the Biological Research Center of the Northwest. He was the advisor of the first-place winning project in the area of Biology at ExpoSciences Internacional, Argentina 2016. He has more than 25 participations in international conferences in countries like Mexico, the United States, Argentina, and France, as well as several book chapters and scientific publications. In 2017 he received the Youth Award in Baja California Sur, and the Medal for Innovation in Science and Technology, for his brilliant and outstanding career in favor of science. In 2019 he obtained funding to develop postgraduate studies abroad, so currently he is a doctoral researcher in the area of Ecotoxicology and Functional Physiology of Marine Invertebrates, at the European Institute of the Sea, in Brittany, France.

Domoic acid (DA), the phycotoxin responsible for amnesic shellfish poisoning (ASP), is an extremely dangerous excitatory amino acid naturally produced by at least twenty-six species of the bloom-forming marine diatoms Pseudo-nitzschia spp. Filter feeders, such as bivalve mollusks, can accumulate and lengthy retain high amounts of DA in their tissues, threatening human health and leading to extensive-prolonged fishery and aquaculture closures, and severe economic losses. This is particularly problematic for the king scallop Pecten maximus, which is the third most important fishery species in France for a total of 75 million euros in 2016. This species retains high burdens of DA from months to years compared to other fastdepurator bivalves. The cellular processes at the origin of this retention are still unknown. Recently, we successfully developed an immunohistochemical technique based on the use of an anti-DA antibody for the in situ specific DA-detection in bivalve tissues at a subcellular level. Our results show that in P. maximus naturally contaminated (200 – 800 mg DA kg-1 of DG; HPLC-UV) during Pseudo-nitzschia spp outbreaks, DA is visualized in the mucus of gills and digestive tract, in mucus-producing cells as well as and in small inclusion bodies ($^{1}\mu$ m) in the digestive gland. The intensity of the staining appeared directly correlated to the toxin load measured by HPLC. Moreover, we aim to analyze the evolution of DA distribution in scallop tissues during contamination and decontamination event. These results are essential to provide a better understanding of the fate of DA in P. maximus and the mechanisms involved in the high accumulation and long retention of this toxin.

Snail egg toxin effects on gut. A possible antipredation defense mechanism

Ph.D. Matías Giglio^{1,2}, Prof. Heras Horacio¹

¹CONICET - National Uiversity of La Plata, La Plata, Argentina, ²University of Utah, Salt Lake City, USA

Biography:

Matias Giglio is a Postdoctoral Fellow at the Department of Biology, University of Utah, USA. He is currently working on the charaterization of Conus conotoxins.

Apple snails from the *Pomacea* genus are freshwater amphibious snails characterized by aerial oviposition, an unusual reproductive strategy that exposes the eggs to harsh conditions and terrestrial predators. However, these eggs are ignored by most cohabitant fauna, which is explained by the presence of a complex defense system including aposematic coloration and a suite of defensive proteins currently under research. Among these, *Pomacea* snail eggs have evolved clade-specific toxic lectins. One such lectin is the perivitellin-2 (PV2) a pore-forming AB toxin that combines a lectin attached to a membrane attack protein from the MACPF family. This extends the presence of bacteria and plant AB-toxins to animals. Moreover, it is the first animal enterotoxin. It withstands gastrointestinal environment, including pH and enzymatic proteolysis. PV2 promotes morphological changes in intestinal cells and induced cell death by a lytic mechanism. Further, after oral administration to mice PmPV2 attached to enterocytes and induced large morphological changes on their small intestine mucosa, reducing the absorptive surface. We also observed membrane cholesterol levels strongly affect both, PV2 affinity and PV2 capacity to form pores triggering toxicity in a dose-dependent manner. These defensive toxins may contribute to apple snail egg protection against predators.

Impact of pile driving noise on *Pecten maximus* larval ecology

<u>Mathilde Gigot¹</u>, Dr Julien Bonnel², Dr Laurent Chauvaud¹, Dr Tarik Meziane³, Dr Frédéric Olivier³ ¹Laboratoire des Sciences de l'Environnement Marin (LEMAR) UMR 6539 UBO/CNRS/IRD/Ifremer, Plouzané, France, ²Applied Ocean Physics & Engineering, Woods Hole Oceanographic Institution (WHOI), Woods Hole, USA, ³Biologie des Organismes et Ecosystèmes Aquatiques (BOREA) UMR 7208 MNHM/SU/UNICAEN/UA/CNRS/IRD, Paris, France

Biography:

Mathilde Gigot completed a Master's degree in Integrative Biology and Physiology with a specialization in Marine Organisms at Sorbonne Sciences University (Paris 6, France). She is now working on a PhD funded by the CNRS and the Université de Bretagne Occidentale, in the BeBEST laboratory at LEMAR. She develops her subject « Characterization of physiological and behavioural responses to acoustic impact on marine invertebrates » under the supervision of Laurent Chauvaud (LEMAR), Julien Bonnel (WHOI) and Frédéric Olivier (BOREA) since October 2019. She is particularly interested in bivalve's physiology, applied science, acoustics, environmental changes and anthropogenic impact.

Human's marine activities produce high level sounds at low frequency. Among all, offshore installations like wind turbine introduce high powerful noise in the environment during the building phase. Whereas the impacts of anthropophony on invertebrates aren't well assessed, especially for larval phases, we investigated in this study the potential effects of pile driving noise on the Great Scallop *Pecten maximus* at different life stages.

We exposed adult animals to three levels of sound during gametogenesis and induced spawning to test the potential impact of pile driving noise on reproduction. Then, we exposed the resulting larvae to different levels of such noise, and assessed the impacts on survival, growth, settlement and metamorphosis. We also conducted fatty acid analyses to determine their lipid reserves both in quality and quantity. Our results reveal that pile driving noise reduces the settlement rate up to 28% and enhances metamorphosis of settlers by 32% for larvae directly exposed to sound. Also the sound treatment applied to parents impact their descendants with a 31% reduced settlement rate, a 15% decrease in lipid content, a 7% increase of the mortality and strong effects on metamorphosis and growth.

We provide evidence of the offshore noise consequences during building on great scallop larvae with potential repercussions on their recruitment and so population renewal. With the numerous wind farm construction planned in Europe, noise pollution is about to raise seriously. The Great Scallop has an economical value, an important ecological role and their natural stocks are already supplied by aquaculture spat production. Those acoustic impacts have to be considered to improve regulatory decisions and protect coastal environments.

Does high-frequency temperature fluctuations at depth increase the thermal resilience of local populations? Comparison between antipatharians and scleractinians from Mo'orea, French Polynesia

Miss Mathilde Godefroid¹, Professor Philippe Dubois¹, Doctor Laetitia Hédouin²

¹Université Libre de Bruxelles, Bruxelles, Belgium, ²Centre de Recherches Insulaires et Observatoire de l'Environnement CRIOBE USR3278, Mo'orea, French Polynesia

Biography:

My name is Mathilde Godefroid and I am a PhD candidate at the University of Brussels (ULB, Belgium), under the supervision of Pr Philippe Dubois (ULB, Belgium) and Dr Laetitia Hédouin (CRIOBE, French Polynesia). The aim of my thesis is to better understand the thermal resilience of antipatharians. To answer this question, I am using a comparative approach by studying different species that are adapted to different oceanic conditions. I have previously worked on the buoyancy of deep-sea sharks (with Pr Jérôme Mallefet, UCL, Belgium) and on the effects of ocean acidification on tropical scleractinians (with Dr Laetitia Hédouin).

Based on the deep reef refugia hypothesis, mesophotic coral ecosystems may provide a refugia for shallower coral reefs in the current context of ocean warming. In Mo'orea (French Polynesia), the discontinuous upwelling of cold and nutrient rich waters up the reef slope generates high frequency fluctuations in temperature at mesophotic depths, ranging from 1.5 to 3°C in amplitude. While it is likely that these fluctuations affect physiological processes over short-term exposure, they may also buffer the effects of warming at depths as compared to surface waters and results in some acclimation/adaptation of organisms. To assess the influence of these fluctuations on the thermal resilience of local organisms, we investigated the responses of the antipatharian (black coral) Stichopathes sp. to short-term (16 days) increases in temperature (up to +3.5°C). Several endpoints were assessed: respiration rates, healing capacities and tissue necrosis. Results showed that this species lives below its thermal optimum (28.3°C) during the coldest months of the year. Physiological responses of this antipatharian to acute thermal stress (time scale of a few hours) were also compared with those of the scleractinian corals Pachyseris speciosa from the same site (85 m depth) and from shallower depths (25 m), by performing a series of ramping assays covering the entire annual thermal range of both populations (23 to 32°C). This will allow to assess the role of local acclimation/adaptation and will give an insight into the validity of the deep reef refugia hypothesis in the local context of the island of Mo'orea.

Oxidative and Nitrosative Responses of the Mussel *Mytilus edulis platensis* During Seasonality and Harmful Algae Blooms

Dr. Paula Mariela González^{1,2}, Dr. Susana Puntarulo^{1,2}

¹Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Fisicoquímica, Buenos Aires, CP 1113, Argentina , CABA, Argentina, ²CONICET-Universidad de Buenos Aires. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Buenos Aires, CP 1113, Argentina, CABA, Argentina

Biography:

Licentiate in Biological Sciences (marine biology orientation) and Doctor from Buenos Aires University (UBA), Argentina. Researcher from National Scientific and Technical Research Council (CONICET) at the Institute of Biochemistry and Molecular Medicine (IBIMOL). Publications: 23 papers, 3 web articles, 9 book chapters, 23 abstracts. First Teaching Assistant, Physicochemistry and postgraduate courses in the School of Pharmacy and Biochemistry, UBA. Current project is 'Oxidative and nitrosative responses against natural and anthropogenic stressors in marine organisms and other systems'.

The objective of the work was to study the oxidative and nitrosative metabolism of the bivalve Mytilus edulis platensis after exposure to biotoxins during harmful algae blooms (HAB) in spring. Digestive glands (DG) and hemocytes were isolated from mussels of the Argentinean Sea during summer, winter and spring. In the DG, the 2',7'dichlorofluorescein diacetate (DCFH-DA) oxidation rate, ascorbyl radical/ascorbate and lipid radical $(LR \bullet)/\alpha$ -tocopherol content ratios were significantly higher between samples collected during the HAB season with those of summer and winter. The antioxidant enzyme activities showed different responses between seasons, and nitric oxide (NO) content was 8.5- and 2.7-fold higher in samples isolated from winter and spring than values obtained in summer mollusks, respectively. Even more, hemocytes' DCFH-DA oxidation rate was increased by 2.5- and 8.3-fold during spring and summer, respectively, as compared to winter samples. Antioxidant enzyme activities in spring cells were significantly higher as compared to summer. LR• content in spring was 140% and 50% higher as compared to winter and summer cells, respectively. NO and nitro-tyrosine content were significantly higher in hemocytes from spring as compared to values obtained in winter and summer. These data suggests that under natural seasonality conditions the overall network of oxidants/antioxidants seems to be adequately controlled to keep the organism far from damage. However, in the presence of toxins, the bivalves were facing oxidative and nitrosative stress.

Amphiphilic 2,5-Diketopiperazines As Novel, Eco-Friendly Marine Antifoulants

<u>Mr Thomas Grant</u>¹, Dr David Rennison¹, Dr Patrick Cahill², A.Prof Johan Svenson², D.Prof Margaret Brimble¹ ¹University of Auckland, Auckland, New Zealand, ²Cawthron Institute, Nelson, New Zealand

Biography:

Thomas is a 4th year PhD candidate who completed his BSc (Hons) in 2014 under the supervision of Prof. Brent Copp before commencing his PhD under the supervision of D.Prof Margaret Brimble. His main research interests include green chemistry techniques and bioactive antifouling peptides.

Marine biofouling is a problem that plagues all maritime industries at vast economic and environmental cost. Previous and current methods to inhibit biofouling have employed the use of toxic/highly persistent chemicals and heavy metals, including tin, copper, and zinc. These toxic methods are now coming under immense regulatory pressure, and an eco-friendly alternative is urgently required. Amphiphilic micropeptides have recently been shown to exhibit excellent antifouling activity, with a non-toxic mode of action. The present work has focused on incorporating the pharmacophore derived from these peptides into a 2,5-diketopiperazine scaffold. This privileged structure is present in a vast number of natural products, including natural product antifoulants. Compared to their linear peptide equivalents, they present an attractive target owing to their increased stability towards proteolytic degradation. Amphiphilic 2,5-diketopiperzines were subsequently demonstrated to be potent marine antifoulants displaying broad spectrum activity against a range of common marine fouling organisms, and this talk will showcase structure-activity relationships within this compound class.

Microalgae cultivation: reality versus expectations

Professor Benoit Guieysse¹

¹Massey University, New Zealand

Biography:

Professor Benoit Guieysse holds a Master of Science degree in Chemical Engineering (ENSIACET, France) and a PhD in Engineering (Lund University, Sweden). He is currently the Head of the Chemical and Bioprocess Engineering Department of Massey University, New Zealand. Dr. Guieysse research focuses on the development, modelling, and impact assessment of environmental biotechnologies with special emphasis on algae-based bioprocesses. He has contributed to the publication of more than 80 peer-reviewed articles that have been cited more than 8000 times. In his spare time, he founded NZ Algae Innovations Ltd, the only commercial spirulina farm in New Zealand (trading under Tahi Spirulina).

While the scientific literature consistently predicts that microalgae cultivation is economic and sustainable, especially when compared to current alternatives, only a few commercial microalgae-based facilities are currently in operation. Indeed, commercial reality often does not meet expectations because microalgae cultivation remains complex and costly to implement at scale. These high costs also mean that the minimum scale required to achieve commercial viability is often significant, which has critical implications in terms of the productivities practically achievable. Future assessments of costs and impacts of algae biotechnologies must therefore be based on 'real-life' productivity estimates and considered the hidden costs associated with poorly acknowledged constraints specific to microalgal production (e.g. logistics, manpower).

Gut microbiome and pathogenic eDNA investigations on New Zealand pāua (*Haliotis iris*)

Mr Jinchen (Martin) Guo¹, Prof Andrea C Alfaro¹, Dr Leonie Venter¹, Dr Thao Van Nguyen¹, Dr Emily Frost¹ ¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, Private Bag 92006, New Zealand

Biography:

Jinchen (Martin) Guo is a first-year doctoral student in the Aquaculture Biotechnology Research Group, School of Science at the Auckland University of Technology. Martin has nearly ten-year research experience in molecular biology and metagenetics focused in marine ecosystems. His current research projects include comparing abalone gut microbial composition across various treatments and detecting invasive species among coastal marine invertebrate communities under unique oceanographic or anthropogenic conditions via metabarcoding and Illumina MiSeq sequencing techniques.

Gut microbes in the black-footed abalone (*Haliotis iris*) are vital for the animal hosts to digest macroalgal and artificial diets. However, gut microflora of this commercially important mollusc has not been well studied in New Zealand. The proposed research project aims to compare the gut microbiome composition of wild and farmed pāua, both spatially and temporally as well as across various food options through metagenetic techniques. Genetically barcoded 16S ribosomal RNA (rRNA) amplicons collected from the animals' buccal cavity, stomach, digestive gland, and intestine in each group are sequenced via an Illumina MiSeq platform, and microbial diversity and multivariate analysis results are compared among factors/treatments. Additionally, genetic probes and markers for detecting and quantifying pathogenic microbes (e.g. *Perkinsus* spp., *Vibrio* spp., and *Candidatus Xenohaliotis californiensis*) of interest are being designed and tested using organismal genomic and environment DNA (eDNA) samples via qPCR assays. The proposed study is expected to be the first investigation on gut microfloral composition of *H. iris* with feeding experiments, and the abalone's gut microbiome profiles can be archived and referred to for similar studies in the future. The proposed metabarcoding pipeline plus the qPCR probes may be used for pathogenic or parasitic surveillance on local abalone farms as well as in coastal marine ecosystems.

Preliminary results of juvenile Californian white abalone (*Haliotis sorenseni*) microbiome composition under UV-light treatment

Mr Jinchen (Martin) Guo¹, Dr Laura Rogers-Bennett²

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Biography:

Jinchen (Martin) Guo is a first-year doctoral student in the Aquaculture Biotechnology Research Group, School of Science at the Auckland University of Technology. Martin has nearly ten-year research experience in molecular biology and metagenetics focused in marine ecosystems. His current research projects include comparing abalone gut microbial composition across various treatments and detecting invasive species among coastal marine invertebrate communities under unique oceanographic or anthropogenic conditions via metabarcoding and Illumina MiSeq sequencing techniques.

Californian white abalone (Haliotis sorenseni), the first federally announced endangered marine invertebrate species in the United States, have been negatively affected by a fatal bacterial disease (caused by Candidatus Xenohaliotis californiensis) called "Withering Syndrome" (WS) since the 2000s. The present study examined microbiome composition, particularly focused on pathogenic bacterial strains, of juvenile white abalone (~1 cm in shell length) that were reared in UV-light treatment. Juvenile white abalone were randomly assigned into four seawater/algae treatments: filtered ambient seawater treated with or without UV light and fed diatoms with or without encrusted coralline algae. Genomic DNA of the whole body of the animals from each group was genetically amplified with barcoded 16S ribosomal RNA markers, and the amplicons were sequenced through an Illumina MiSeq platform in 2019. A total of 64,434 operational taxonomic units (OTUs) at 97% sequence similarity or higher were observed, and 55,977 of which were assigned to prokaryotes. Microbiome composition, based on the assigned prokaryotic OTUs, was significantly different across the treatments (PERMANOVA test, Pseudo-F(1,31)=20.9, p(permutation)=0.001, 999 unique permutations). Top observed bacterial phyla included Proteobacteria, Cyanobacteria, Fusobacteria, Bacteroidetes, and Planctomycetes. Further microbial diversity, multivariate analysis, and detection of pathogenic microbes are still in progress. This should be the first investigation of microbiome composition on *H. sorenseni* at early juvenile stage under the UV-light treatment. Microfloral composition profiles for the white abalone across the treatments will be established for future reference, and the UV-light treatment on minimizing the pathogens will also be assessed for future aquacultural applications.

COVID-19: The Cure Within the Deep Sea

Sarah Hamade¹

¹Journal Of Marine Biology & Oceanography, Dearborn, United States

Biography:

Sarah Hamade, an aspiring marine biologist who created the cure for self-poisoning Corydoras Catfish last year was published in the Journal of Marine Biology and Oceanography. She graduated from Eastern Michigan University with psychology and biology background and is currently a behavioral therapist that works closely with autistic children. In 2017, Sarah created her own aquaponics experiment to demonstrate the damaging effect that radiation can have on aquatic life that was published earlier this year in the Journal of Agriculture and Forest Meteorology Research With two more articles to be published and an editorial board member of The American Journal of Life Science, she plans on leaving her mark on the marine biology community.

The COVID-19 pandemic has had an unprecedented impact on the population and healthcare system. Researchers worldwide are frantically searching for a cure or a vaccine to combat the virus. While this may be achieved via conventional methods, potential valuable resources in our fight may lie in our vast ocean ecosystems. Red Algae griffithisin, sea sponge lasonolide, sea lamprey lymphocytes, and cone snail ziconotide have all shown promising uses in the healthcare industry. The purpose of this research is to present and describe the aforementioned resources and how they can aid in our battle against COVID-19.

Mitochondrial Adaptions Involved in Brain Tolerance to Hypoxia and Reoxygenation in New Zealand Triplefin Fish (Tripterygiidae)

Mrs Alice Harford¹

¹University of Auckland, Auckland, New Zealand

Biography:

Alice Harford is a PhD student at the University of Auckland, her research examines the underlying structural and functional disruption to cardiac mitochondria which lead to thermally-induced heart failure in wrasse species at high temperature. This mechanistic approach will then be applied to wider conservation efforts in response to climatic change, to improve understanding on what influences species distribution and survival, and allow more accurate and predictive models. Alice's interests lie in the adaptive physiology of species inhabiting extreme and variable environments, as well as measuring the capacity and plasticity of these systems to compensate and maintain performance under changing environmental conditions. This is primarily focused at the level of the mitochondria, and examines their function, composition and stability under ecological stressors.

Oxygen is fundamental for eukaryotic life as it powers mitochondrial oxidative phosphorylation to supply ~90% of cellular ATP. Fluctuations in oxygen represent a major stressor, with hypoxia leading to a cascade of detrimental physiological changes that alter cell operations and ultimately induce death. Nonetheless, some species tolerate near anoxic environments, and have evolved an array of mechanisms to sustain function even during extended hypoxic periods. While species' physiological, biochemical and morphological adaptations have been described at the organism level, the role of mitochondria in hypoxia tolerance remains largely undefined. Given the vulnerability of brain tissue to hypoxia, mitochondrial function was tested in brain homogenates of three closely-related triplefin species with varying degrees of hypoxia tolerance (Forsterygion lapillum, Bellapiscis medius, and Forsterygion varium). High-resolution-respirometry coupled with fluorometric measurements of membrane potential and spectrometric analysis of cytochrome redox state permitted assessment of differences in mitochondrial function and integrity in response to intermittent hypoxia. Traditional steady-state measures of respiratory flux and membrane potential showed no differences among species. However, in the transition to hypoxia, tolerant species B. medius and F. lapillum maintained membrane potentials at oxygen pressures 7 and 4.4 fold lower than sensitive F. varium, respectively, and exhibited faster repolarisation upon reoxygenation. Cytochrome redox states also revealed different hypoxic responses that may reflect species' phylogeny and habitat. Notably, tolerant species appeared to re-establish redox equilibrium following reoxygenation, while F. varium remained compromised. These data indicate that dynamic oxic-hypoxic mitochondrial transitions underlie the hypoxia tolerance in intertidal triplefin fish.

Thermal sensitivity links to cellular cardiac decline in three spiny lobsters

A. Prof. Tony Hickey¹

¹University of Auckland, Auckland, New Zealand

Biography:

My research encompasses biomedical and ecophysiology problems. My interests are mitochondrial function in the contexts of metabolic demands on metabolism in unusual states that range from suppressed function of hibernation/aestivation to fast muscle contractions of insects. I have specific interests in extremes of temperature and oxygen. I have developed equipment and methods to measure reactive oxygen species, mitochondrial membrane potential and ATP synthesis.

Understanding mechanisms of thermal sensitivity is key to predict responses of marine organisms to changing temperatures. Sustaining heart function is critical for complex organisms to oxygenate tissues, particularly under temperature stress. Yet, specific mechanisms that define thermal sensitivity of cardiac function remain unclear. Here we investigated whole animal metabolism, cardiac performance and mitochondrial function in response to elevated temperatures for temperate, subtropical and tropical spiny lobster species. While oxygen demands increased with rising temperatures, heart function became limited or declined in all three species of lobsters. The decline in cardiac performance coincided with decreases in mitochondrial efficiency through increasing mitochondrial proton leakage, which predicts impaired compensation of ATP production. Species differences were marked by shifts in mitochondrial function, with the least thermal scope apparent for tropical lobsters. We conclude that acute temperature stress of spiny lobsters, irrespective of their climatic origin, is marked by declining cellular energetic function of the heart, contributing to an increasing loss of whole animal performance. Better understanding of physiological thermal stress cascades will help to improve forecasts of how changing environmental temperatures affect the fitness of these ecologically and commercially important species.

The impact of the exotic pathogen *Bonamia ostreae* on the NZ native flat oyster (*Ostrea chilensis*), and potential for selective breeding for resilience

<u>Dr Zoe Hilton</u>¹, Dr Anne Vignier¹, Ms Farhana Muznebin², Dr Steve Webb¹, Dr Andrew Fidler³, Prof Andrea Alfaro²

¹Cawthron Institute, Nelson, New Zealand, ²Auckland University of Technology, Auckland, New Zealand, ³AquaGeneNZ Ltd., Nelson 7010, New Zealand

Biography:

Dr Zoe Hilton has been a member of Cawthron Instutute's Aquaculture Group since 2010 and brings her experience in ecophysiology, biochemistry and evolutionary biology, to support research on selective breeding for improved performance and resilience in both shellfish and fish.

New Zealand's native flat oyster *Ostrea chilensis* forms the basis of an iconic wild fishery, and until 2017, a small but growing aquaculture industry. In 2015 the exotic and OIE notifiable haplosporidian parasite *Bonamia ostreae* was detected for the first time in NZ and the Southern Hemisphere. This triggered the closure of all NZ flat oyster farms to limit the spread of *B. ostreae* and protect uninfected wild populations. Our research turned towards understanding the effect of the novel pathogen on the native host. Samples from the last farms de-stocked in November 2017 were examined using histology and PCR. Very high infection intensity along with the unusually high levels of mortality observed on some farms at the time the parasite was first discovered, suggest that *B. ostreae* may be more virulent than its endemic congener *Bonamia exitiosa*. *B. ostreae* appears to exhibit similar pathology when infecting *O. chilensis* as it does when infecting *O. edulis* in Europe, which is different to that observed when B. exitiosa infects *O. chilensis*. Associations were also observed between infection with *B. ostreae* and other endemic pathogens such as *Bucephalus longicornutus* and Apicomplexan X but, conspicuously, not *B. exitiosa*, raising the possibility that *B. ostreae* may be competitively excluding the endemic *Bonamia*. Striking inter-individual variability in susceptibility to infection was also observed. Along with current molecular work, these results will help to inform future options for farming and potential selective breeding for resilience to *B. ostreae* in *O. chilensis*.

Effect of the extreme heatwave on mortality and hemocyte functions of intertidal mussel *Mytilisepta virgata* (Wiegmann, 1837) in indoor mesocosm experiments

Dr. Hyun-Ki Hong¹, Mr. Chang Wan Kim¹, Ms. Jeong-Hwa Kim¹, Dr. Kwang-Sik Choi¹

¹Department of Marine Life Science (BK21 FOUR) and Marine Science Institute, Jeju National University, Jeju, South Korea

Biography:

I'm a research professor in Department of Marine Life Science of Jeju National University, South Korea. I have been working on the immune parameters of marine bivalves and gastropods.

In the rocky intertidal environment, the frequency and duration of heatwaves have increased over the last decades, possibly due to global climate change. Heatwaves often result in lethal or sub-lethal disturbances of benthic animals by altering their metabolic activities. In this study, we investigated the impacts of extreme heatwave stress on hemocyte functions of Mytilispeta virgata and subsequent mortality to gain a better understanding of the potential causes and consequences of mass mortality events of the mussel in summer. Using flow cytometry, we first discriminated three types of hemocytes in the hemolymph, including granulocytes, hyalinocytes, and blast-like cells, based on the cell morphology and immunological functions. Flow cytometry also revealed that the granulocyte is the major hemocyte involved in cellular defensive activities such as phagocytosis and ROS production. For the study, the mussels were exposed to 40°C of air temperature for 12 h per day over a 5 day period at a laboratory condition, as we simulated a semi-diurnal tidal cycle. Mortality began to occur 66 h after the beginning of the experiment, and all mussels perished at the end of the experiment. Flow cytometry indicated that the mussels exposed to high air temperature produced significantly higher ROS levels compared to the control mussels within two days after the experimental onset, which may cause oxidative stress during this period. Such high ROS levels in the hemolymph increased hemocyte DNA damage after three days of exposure and decreased phagocytosis capacity of hemocytes four days after the experiment. The observed mortality and declined immune capacity suggested that the occurrence of extreme heat events in the rocky intertidal ecosystem during summer may exert sublethal to lethal impacts to the macrobenthic animals.

A blue mussel chromosome-scale assembly and genomic resources for aquaculture, marine ecology and evolution

Dr. Tiago Hori¹

¹Atlantic Aqua Farms, Charlottetown, Canada

Biography:

Director of Aquaculture Innovation - Atlantic Aqua Farms

The blue mussel is commonly described as the *Mytilus* species complex, encompassing at least three putative species: *M. edulis, M. galloprovincialis* and *M. trossolus*. These three species occur on both sides of the Atlantic and hybridize in nature, and both *M. edulis* and *M. galloprovincialis* are important aquaculture species. They are also invasive species in many parts of the world. This project aimed at assembling a high-quality genome for *M. edulis* and develop tools that can be used in breeding, molecular ecology and evolution to address questions of both commercial and environmental perspectives. We used a combination of PacBio sequencing and Dovetail's Omni-C technology to generate an assembly with 14 long scaffolds containing 94% of the predicted length of the *M. edulis* genome (1.6 out of 1.6 Gb). Assembly statistics were total length 1.65 Gb, N50 = 116 Mb, L50 = 7 and, L90 = 13. BUSCO analysis showed 90.59% complete eukaryote BUSCOs identified. AB-Initio annotation using RNA-seq from mantle, gills, muscle and foot predicted 41,319 genes. Using GBS and shotgun sequencing, we sequenced 3 North American populations of *Mytilus* to characterize single-nucleotide as well as structural variance. Population genetics analysis data will also be presented.

RNA-Seq analysis reveals downregulation of immune associated genes in *Delisea pulchra* (Rhodophyta) following exposure to an opportunistic pathogen

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¹Centre for Marine Science and Innovation, School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney, Australia, ²School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, Australia

Biography:

I am undertaking a PhD in the field of marine microbiology and symbiosis at the University of New South Wales. My interests and area of expertise is in understanding the molecular mechanisms driving hostpathogen interactions and characterising the virulence traits of opportunistic pathogens in the marine environment.

Host-microbe interactions are essential for the health of marine organisms but can be highly sensitive to changing environmental conditions. As such, increasing global seawater temperatures can promote host stress and lead to the emergence of opportunistic pathogens, resulting in a rise in the occurrence of diseases in marine organisms. Disease outbreaks affecting macroalgae are of particular concern due to their critical role as habitat forming organisms. However, there is a limited understanding of the cellular response strategies used by macroalgae to respond to opportunistic pathogens while subjected to thermal stress. In this study, we used mRNA-Seq analysis to investigate the response of the model macroalga Delisea pulchra (Rhodophyta), after exposure to a known opportunistic pathogen (Aquimarina sp. AD1) relative to a closely related benign strain (Aquimarina sp. AD10). Using de novo assembly methods, 27,586 genes belonging to D. pulchra were identified. The global gene expression profile of thermally stressed D. pulchra was comprised of transcripts predominantly involved in stress response and signal transduction processes which may assist in ameliorating the damaging effects of thermal stress. However, following pathogen exposure a clear downregulation of genes encoding for protein metabolism, stress response, energy generation and photosynthesis functions was observed, relative to the benign strain. This transcriptional repression of genes encoding for core cellular processes is predicted to ultimately interfere with the macroalgal pathogen response, leading to infection, tissue damage and bleaching symptoms. Overall, this study highlights the detrimental effect of pathogen exposure on thermally stressed algae and has implications for how we view host-pathogen interactions in a changing marine environment.

Sex-specific differences in the spawning biology of the crown-of-thorns starfish revealed by transcriptome analysis

<u>Mr Mathias Jonsson</u>¹, Miss Marie Morin¹, Professor Sandie Degnan¹, Professor Bernard Degnan¹ ¹The University of Queensland, St Lucia, Australia

Biography:

My name is Mathias Jonsson. I am currently studying the crown-of-thorns starfish at the University of Queensland, Australia as part of an Australian Research Council funded PhD project. The project aims to understand fundamental COTS biology with an emphasis on conspecific communication, to develop novel species-specific biocontrol methods for this marine pest. In my work, I use transcriptomics to understand how the COTS repertoire of G-Protein-Coupled Receptors differs between males and females, and how their expression is affected by season. I then use physiological and behavioural assays to experimentally validate the functions of these receptors.

Crown-of-thorns starfish (COTS) are highly fecund corallivores native to Indo-Pacific coral reefs. They are gonochoric broadcast spawners that rely on environmental cues and molecular mechanisms to synchronise spawning aggregation behaviour. The cues and conspecific signals that lead to the formation of spawning aggregations are currently unknown. Here, we (i) generated transcriptomes from 13 spawning-ready individuals, collected directly from the Great Barrier Reef, sampling 8 different tissues from both males and females, and (ii) used the unsupervised likelihood ratio test in DESeq2 to identify potential sexual dimorphism in the different COTS tissues. We found that 183 and 102 genes were significantly upregulated in female and male COTS, respectively, with the sensory tentacles having the most differentially expressed genes. Importantly, genes differentially expressed between males and females include five G-Protein-Coupled Receptors (GPCRs) and 36 secreted factors, many of which are involved in key reproductive pathways such as the relaxin-like gonad stimulating peptide, kisspeptin and the gonadotropin-releasing hormone. These findings expand our current understanding of spawning behaviours in echinoderms and may lead to new opportunities to create novel, species-specific biocontrol methods for this marine pest.

First report of levels and distribution of tetrodotoxin (TTX) in the bluelined octopus *Hapalochlaena fasciata* collected from Jeju Island of Korea

<u>Nobuhisa Kajino¹</u>, Dr. Hyun-Ki Hong¹, Dr. Hyuon Joong Kim¹, Dr. Kwang-Sik Choi¹ ¹Department of Marine Life Science (BK21 FOUR) and Marine Science Institute, Jeju National University, Jeju 63243, Republic of Korea, Jeju, South Korea

Biography:

I'm a doctoral course student majoring in marine life sciences at Jeju National University, and I study toxin detection analysis of marine organisms and monitoring changes in toxin distribution caused by the hybridization of marine toxin animals such as pufferfish.

Blue-lined octopus Hapalochlaena fasciata is one of four species of highly venomous blue-ringed octopuses. The predominant toxin in their tissue is tetrodotoxin (TTX), a sodium channel blocking neurotoxin, which causes dose-dependent muscle paralysis. The distribution of H. fasciata has been reported in shallow waters on rocky reefs in the intertidal and subtidal zones along the coast of Australia, Taiwan, and Japan. In Korea, recently, the appearance of *H. fasciata* on the southern coast has been increasing. In this study, we investigated for the first time the TTX levels and distribution in the bodies of four H. fasciata collected from Jeju Island off the south coast of Korea. The taxonomic status of the specimen was confirmed as H. fasciata based on mitochondrial 16S ribosomal RNA (16S rRNA) and cytochrome oxidase subunit 1 (CO1) genes. In phylogenetic analysis, H. fasciata identified in the present study were closely allied with H. fasciata previously reported from Japan, Taiwan, and Korea. TTX levels from the posterior salivary gland (PSG), arm muscle, and mantle skin of *H. fasciata* were analyzed using a TTX-specific competitive ELISA. The concentration of TTX varied among individuals and tissues. The highest concentration of TTX was found in the PSG in all specimens, with a range of 63,339 – 343,969 ng TTX/g tissue. The concentration of TTX in arm muscle and mantle skin ranged 525 – 5,959 ng TTX/g tissue and 967 – 4,284 ng TTX/g tissue, respectively. This result is the first report on the toxicity of TTX in the blue-lined octopus H. fasciata occurring in Korean waters, and the toxicity of this species is also significant from a food hygiene point of view.

Stretched Mussels: tracing the genetic basis of resilience to climate change and ocean acidification in cultured green-lipped mussels (kuku) from genome to embryo

Dr Nathan Kenny¹

¹Department of Biochemistry, University of Otago, Dunedin, New Zealand

Biography:

Nathan Kenny is an incoming Rutherford Discovery Fellow at the University of Otago, and a lecturer in the Biochemistry Department. He has recently moved back to New Zealand from the UK, and has studied a variety of topics in invertebrate molecular evolution and genomics. His RDF project will investigate the resilience of kuku, green-lipped mussels, in the face of climate change. More information on his work can be found at https://sites.google.com/view/marineinvertebrategenomics

Molluscs such as kuku (*Perna canaliculus*) are vital to our ecosystems, and taonga of cultural and economic importance. Climate changes, including temperature extremes and ocean acidification, threaten this species, but some populations are resilient to these problems. The source of this resilience is unknown, but differences in early development are strongly implicated.

In New Zealand, bivalves are of key economic interest, with the aquaculture industry growing in importance. Novel genetic and molecular tools have emerged which have the potential to be transformative in industry, while allowing significant scientific breakthroughs in mollusc growth, development and genetics. Some of these are already in development - genotyping by sequencing, for instance, has been applied to the kuku in the past. Other technologies, such as nanopore genome sequencing, RADseq, bulk and scATACseq and transient expression via lipofection/electroporation, are yet to be trialled, but will allow us to understand how some populations are resilient to climatic effects.

Using cutting-edge approaches, we will pinpoint the key differences exhibited by resilient kuku. These could be genetic signals provided by the mother, or those activated in initial stages of development. Knowing the source of this resilience will greatly assist conservation and aquaculture.

Algal lipids attenuate cytokine-mediated inflammatory signalling and tight junction barrier dysfunction in human keratinocyte models.

Ms Jamie Mei Lin Kok¹, Dr Jaydee Cabral², Dr Georgina Dowd³, Dr Lyn Wise¹

¹Department of Pharmacology and Toxicology, School of Biomedical Sciences, Dunedin, New Zealand, ²Department of Chemistry, Division of Sciences, Dunedin, New Zealand, ³Plant & Food Research, Nelson, Nelson, New Zealand

Biography:

I am a student at the University of Otago in the Pharmacology and Toxicology Department. After I completed my undergraduate degree in Biotechnology (Hons), I earned my master's degree in biomedical research on brown seaweed and its potential as a treatment for acne vulgaris. Prior to joining the PhD programme, I have worked as an educator in Malaysia for a few years. My current areas of research are investigating phytochemicals which come from brown algae and their possible medicinal properties.

Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects children and adults. Cytokines, such as tumour necrosis factor (TNF)- α and the interleukins (IL)-4 and IL-13, modulate epidermal inflammatory responses and barrier functionality which contribute to AD pathology. Lipids are used in topical emulsions to treat AD, but their therapeutic benefit and potential mechanism of action remains unclear. The aim of this study was to investigate the effects of algal lipids on cytokine- mediated inflammatory signalling and tight- junction barrier dysfunction in human keratinocyte models. Lipids extracted from a brown alga inhibited TNF-induced monocyte chemoattractant protein (MCP)-1 and interleukin (IL)- 8 at the mRNA and protein levels. The extract also reduced protein levels of TNF receptorassociated factor (TRAF) 2 which is a key intracellular signalling mediator. The algal lipids also prevented the cytokine- mediated reduction of elongase (ELOVL) 5 and ceramide synthase (CERS) 3 mRNA. These enzymes are involved in the synthesis of long chain fatty acids which are essential for barrier integrity. In a human skin model, algal lipids restored claudin-1 protein levels and localisation at the cell membrane tight junctions, and decreased trans- epidermal leakage following exposure to IL-4 and IL-13. In summary, algal lipids inhibited inflammation and barrier dysfunction mediated by cytokines in human keratinocyte models. These findings advance our knowledge of the mechanistic functions of lipids and their targets in inhibiting the inflammatory response in keratinocyte cells, potentially alleviating AD symptoms.

Transgenerational effects of parental exposure to marine heatwave conditions on larval performance of the green-lipped mussel, *Perna canaliculus*

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¹University of California Santa Barbara, Santa Barbara, United States, ²Cawthron Institute, Nelson, New Zealand, ³Shellfish Production and Technology New Zealand Ltd. (SPATnz), Nelson, New Zealand

Biography:

Logan Kozal is a PhD candidate at the University of California Santa Barbara in Dr. Gretchen Hofmann's lab. She studies the physiological mechanisms by which organisms can rapidly adapt to global change and the variability in environmental stressors. She focuses on transgenerational plasticity and the roles of maternal provisioning and epigenetic inheritance as potential avenues for resilience in early life stages. In New Zealand, she studies marine heat waves and thermal variation affecting mussel farms in the Marlborough Sounds and whether the thermal environment of bloodstock can influence the larval thermal tolerance of the greenshell mussel, Perna canaliculus.

The green-lipped mussel, *Perna canaliculus*, is critically important to New Zealand's aquaculture industry. However, the rise in marine heatwaves (MHWs) in recent years has driven large scale summertime mortality events, posing an acute threat to the industry. Therefore, we investigated the potential for parental exposure to MHW conditions during gametogenesis to improve P. canaliculus offspring performance under heat stress. Through a series of experiments, we exposed male and female broodstock to ambient or elevated temperatures prior to spawning. We then evaluated the influence of parental heat exposure on the successful development, size, and lethal thermal tolerance of veliger larvae when reared under different temperatures. We also quantified DNA methylation as a possible mechanism of epigenetically mediated transgenerational effects. We isolated maternally and paternally driven transgenerational effects and tested different exposure durations as well as the role of parental recovery in governing these effects. We observed variable effects of broodstock thermal exposure across our experiments. Paternal MHW exposure, over both 1 week and 1 month, had a negative impact on larval development at ambient and elevated temperatures, whereas maternal MHW exposure improved larval development at high temperatures when the mothers were allowed to recover prior to reproducing. These findings have important implications for both hatchery reared and wild spat supply as MHWs are forecasted to continue accelerating under both "business as usual" and reduced emissions scenarios.

Ichthyotoxicity of microalgae of the *Karlodinium* genus from the French coasts to bivalves assessed using bioassays on oyster hemocytes and reproductive outputs

Clémence Boucher¹, Dr Rima Beesoo¹, Nelly Le Goïc², Dr Hélène Hégaret², Dr Caroline Fabioux², Dr Damien Réveillon³, Fabienne Hervé³, Audrey Duval¹, Florent Malo³, <u>Dr Malwenn Lassudrie¹</u> ¹IFREMER LER-BO, Concarneau, France, ²LEMAR, UMR6539 UBO/CNRS/IFREMER/IRD, Plouzané, France, ³IFREMER PHYC, Nantes, France

Biography:

Malwenn Lassudrie is a researcher at IFREMER, France, since 2018. Her research projects focus on toxicity of microalgae to shellfish and fish. She investigates the microalgal species that can be ichthyotoxic, the factors that modulate this toxicity, and the toxic mechanisms involved.

Ichthyotoxic microalgal blooms have occasionally affected the French coasts during the last decades, causing marine fauna mortalities. A previous study by Nézan et al. (2014) in France highlighted the high diversity of Kareniaceae. This family of dinoflagellates includes several ichthyotoxic species, such as *Karlodinium* species, which are regularly detected on the French coasts. In other countries, blooms of *Karlodinium veneficum* and K. armiger were reported to affect the aquaculture industry due to fish and shellfish kills. However, in France, despite the socio-economic importance of shellfish farming (around 140 tons of oysters and mussels yearly, corresponding to a value of USD 600 000), the toxicity of French *Karlodinium* strains to shellfish remains unknown and needs to be expounded to determine the ichthyotoxic threat they could represent.

This study aims to assess the ichthyotoxic potential of French *Karlodinium* strains by comparing their cytotoxicity to *Karlodinium* strains from other regions worldwide, known to be ichthyotoxic. In vitro bioassays were used, targeting hemocytes and gametes of oyster *Crassostrea gigas*. These free cells, convenient to sample, are involved in important physiological functions, such as immunity and reproduction, making them ideal target model cells that can be used as indicator of toxicity to bivalves. Oyster hemocytes were exposed to different concentrations of cultured *Karlodinium* strains for 2 hrs and cell viability was measured by flow-cytometry. Gamete viability and fertilization rate are currently being examined by flow cytometry and light microscopy, respectively.

Results indicated that some French strains are as cytotoxic as foreign ichthyotoxic strains, with high intraspecific variability. In particular, some *K. veneficum* strains were non-cytotoxic whereas others induced up to 80% hemocyte mortality.

This in vitro study highlights that French *Karlodinium* strains have the potential to be harmful to shellfish, which has important implication for the management of algal bloom impacts by the aquaculture industry.

Allochthonous subsidies drive early recruitment of subtropical foundation species

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Biography:

Inês Leal is a marine ecologist finishing PhD studies at the Institute of Ocean Sciences of Rimouski (ISMER/UQAR), in the Laboratory of Ecophysiology and Aquaculture lead by Professor Réjean Tremblay. Using marine benthic invertebrates as study models, her research focuses on latitudinal patterns in (i) larval physiological quality, and (ii) early settlement and recruitment success. She uses lipidomics as a tool to understand very specific physiological processes, e.g., how larvae physiologically adjust to the environment around them by selectively retaining essential fatty acids, to broader effects, e.g., food sources entering benthic food webs. Passionate about rocky shore habitats, she is eager to share with you the wonders of barnacle larval physiology during her talk.

Terrestrial nutrients flushed to sea via coastal runoff may provide a trophic subsidy to filter-feeders, which might be particularly relevant in otherwise oligotrophic waters. In this study, we investigated how allochthonous subsidies might modulate the larval physiology, dispersal, and settlement of the mytilid Brachidontes solisianus by comparing wet and dry periods in Southeastern Brazil. Redundancy analyses linked nearshore fatty acid trophic markers of land origin with the frequency of primary settlers during the wet period, when the share of suspended terrestrial inputs was highest (13% of nearshore suspended matter). Conditions were thus favorable to localized recruitment through trophic settlement triggers. Further, lipid analyses of pediveligers revealed that during the dry period these competent larvae selectively incorporated the essential 22:6 ω 3 docosahexaenoic acid (DHA) into membrane phospholipids at the expense of reserve lipids, suggesting a dietary deficit. Secondary dispersal also prevailed (85% of settlers were drifters), indicating that the chance for relocation through secondary migrations during dry periods is likely higher. Overall, our results suggest that terrestrial allochthonous subsidies conveyed through coastal plumes may strongly modulate in situ settlement rate and dispersal optential in tropical-subtropical marine invertebrates. Pulsed energy transfers from land to the coastal ocean may thus be critical for the stability of benthic communities in regions of low marine primary production.

Bacterial controlled mitigation of dysbiosis in a seaweed disease

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Biography:

Jiasui Li is a third-year PhD student from the Centre for Marine Science and Innovation, The University of New South Wales, Australia. Her PhD project aims to identify disease-protective bacteria for marine macroalgae, and through assessing the interactions between probiotics and host-associated bacterial communities, investigating the mechanisms that bacteria contribute to the host disease resistance.

Disease in the marine environment is predicted to increase with anthropogenic stressors and already affects major habitat-formers, such as corals and seaweeds. Solutions to address this issue are urgently needed. The seaweed *Delisea pulchra* is prone to a bleaching disease, which is caused by opportunistic pathogens and involves bacterial dysbiosis. Bacteria that can inhibit these pathogens and/or counteract dysbiosis are therefore hypothesised to reduce disease. This study aimed to identify such disease-protective bacteria and investigate their protective action. One strain, *Phaeobacter* sp. BS52, isolated from healthy *D. pulchra*, was antagonistic towards bleaching pathogens and significantly increased the proportion of healthy individuals when applied before the pathogen challenge (pathogen-only vs. BS52+pathogen: 41% to 80%), and to a level similar to the control. However, no significant negative correlations between the relative abundances of pathogens and BS52 on *D. pulchra* were detected. Instead, inoculation of BS52 mitigated pathogen-induced changes in the epibacterial community. These observations suggest that the protective activity of BS52 was due to its ability to prevent dysbiosis, rather than direct pathogen inhibition. This study demonstrates the feasibility of manipulating bacterial communities in seaweeds to reduce disease and that mitigation of dysbiosis can have positive health outcomes.

An integrated omics approach to investigate summer mortality of New Zealand green-lipped mussels

<u>Mr Siming Li¹</u>, Prof Andrea C Alfaro¹, Dr Thao van Nguyen¹, Dr Tim Young^{1,2}, Mr Ronald Lulijwa¹ ¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ²Centre for Biomedical & Chemical Sciences, School of Science, Auckland University of Technolog, CBD, New Zealand

Biography:

SiMing Li is a PhD student with the Aquaculture Biotechnology Research Group (ABRG), at the Auckland University of Technology (AUT). Ming's research focusses on the application of omics and bioinformatics tools such as and metabolomics, proteomics, microbiomics, and omics integration to study Green-lipped mussels with poor health and unexplained mortalities.

Green-lipped mussels (*Perna canaliculus*) contribute > \$300 million to New Zealand's aquaculture exports. However, mortalities during summer months and potential pathogenic outbreaks threaten the industry. Thermal stress mechanisms and immunological responses to pathogen infections need to be understood to develop health assessment strategies and early warning systems. In this research we applied an integrative omics-based approach to explore biochemical associations with poor health. Mussels were collected during a mortality event on a farm in Firth of Thames and profiled via mass spectrometry. Protein and metabolite data were integrated via sparse multiple discriminative canonical correlation analysis. Pathway enrichment analysis was used to probe coordinated changes in functionally related metabolite sets. We identified numerous biochemical signatures which reveal metabolic perturbations and protein structural changes in the gill tissues of stressed *P. canaliculus*. This study provides new insight into metabolite and protein interactions associated with incidences of summer mortality in this species.

Mapping the mussel microbiome: Case studies from New Zealand

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Biography:

SiMing Li is a PhD student with the Aquaculture Biotechnology Research Group (ABRG), at the Auckland University of Technology (AUT). Ming's research focusses on the application of omics and bioinformatics tools such as and metabolomics, proteomics, microbiomics, and omics integration to study Green-lipped mussels with poor health and unexplained mortalities.

The New Zealand Green-lipped mussel (*Perna canaliculus*) is a commercially important species for aquaculture. However, poor health and unexplained mortality events pose a threat to this industry. To explore potential associations of poor health with host-associated microbiota and environmental factors, microbiomics profiling was performed on farm-collected mussels as part of my PhD research. Here, I will discuss findings-to-date regarding my pioneering survey of the mussel microbiome (marine bacteria and fungi), deliver some new insights, and provide an overview of our ongoing mussel microbiome project.

Haematological and metabolic profiles associated with age and sex in giant kokopu (*Galaxias argenteus*) broodstock

<u>Mr Ronald Lulijwa^{1,2}</u>, Prof Andrea C Alfaro¹, Dr Leonie Venter¹, Dr Tim Young^{1,3}, Mr Paul Decker⁴, Prof Fabrice Merien^{1,5}, Dr Jill Meyer⁵

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Biography:

Ronald Lulijwa is a PhD student with the Aquaculture Biotechnology Research Group (ABRG), Auckland University of Technology (AUT). Ronald's PhD research focusses on the application of immunological and metabolomics tools for health assessment of farmed fish under biological, physical, and chemical manipulations. Ronald has interests in understanding haemato-metabolic, and molecular biomarkers associated with farmed and wild fish stocks for health assessment.

The giant kokopu (Galaxias argenteus) is one of five galaxiids, whose juveniles contribute to a lucrative whitebait fishery in New Zealand. Due to high demands for whitebait, wild populations have declined. To supplement fishery and conservation, G. argenteus has been successfully bred in captivity and is considered an emerging aquaculture species. With anticipated growth in aquaculture, knowledge of its physiology is vital. This study characterised selected peripheral blood (PB) haematological parameters, liver, serum, and muscle metabolite profiles in 3- and 5-year-old male and female broodstock kept at 16°C. Sex and age did not affect PB total cell counts and haematocrit values. However, higher erythrocytes in 5-year-old fish, thrombocyte and lymphocytes in 3-year-old suggest age-specific cellular regulation. Higher thrombocyte counts in female fish suggest sex-specific regulation. Metabolically, liver abundance for long chain saturated fatty acids (FAs) were higher in males, while females had elevated levels of polyunsaturated FAs. Essential and non-essential amino acids (AAs) in liver and serum were also elevated in females compared to males. Results suggest differential allocation of FAs and AAs to reflect requirements for gonadal development and provisioning. Similarly, age significantly resulted in higher liver and serum abundances of some nonessential AAs in 3-year-old compared to 5-year-old fish, suggesting higher metabolism in younger fish. Overall, results enhance our understanding of sex- and age-based differences in fish haematology, muscle, liver, and serum metabolite profiles in healthy G. argenteus. Future studies should carefully consider potential age- and sex-specific differences in metabolic responses.

Polyinosinic: polycytidylic acid (poly I:C) in vivo enhances Chinook salmon immunity and alters its metabolome

<u>Mr Ronald Lulijwa^{1,2}</u>, Prof Andrea C Alfaro¹, Prof Fabrice Merien^{1,3}, Dr Mark Burdass⁴, Dr Jill Meyer³, Dr Leonie Venter¹, Dr Tim Young^{1,5}

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Biography:

Ronald Lulijwa is a PhD student with the Aquaculture Biotechnology Research Group (ABRG), Auckland University of Technology (AUT). Ronald's PhD research focusses on the application of immunological and metabolomics tools for health assessment of farmed fish under biological, physical, and chemical manipulations. Ronald has interests in understanding haemato-metabolic, and molecular biomarkers associated with farmed and wild fish stocks for health assessment.

Viruses produce double-stranded ribonucleic acid (dsRNA), which is recognized by the vertebrate immune system. Poly (I:C) is a synthetic dsRNA analogue, which orchestrates antiviral responses. In Chinook salmon (Oncorhynchus tshawytscha), the antiviral effects of poly (I:C) in vivo remain unknown. While viruses have decimated the salmon industry in many countries, New Zealand farming remains relatively free from diseases. However, increasing threats from rising sea surface temperatures, marine heat waves and summer mortalities necessitate the study of viral-associated physiological responses. This study investigated the effect of poly (I:C) in vivo on O. tshawytscha haematology, innate immunity, serum and liver metabolite profiles, and lymphoid tissue cytokine transcript expression 5-days post-stimulation. At the cellular level, poly (I:C) enhanced monocyte and neutrophil counts, and reactive oxygen species (ROS) production in peripheral blood mononuclear cells (PBMC). At the metabolic level, poly (I:C) up-regulated liver and serum metabolites involved in branched chain amino acid (BCAA) metabolism at day 1 and returned to baseline by day 5, while metabolites involved in glycolysis were persistently depleted from day 1 to 5. Metabolic results suggest that poly (I:C) induced response mechanisms similar to those observed in viral-infected fish, where the host metabolome is hijacked to favor viral replication. At the transcript level, poly (I:C) promoted antiviral ifny and Mx1 protein, and anti-inflammatory il-10 in fish lymphoid tissues, depict O. tshawytscha immune defense against infection. These findings may be useful for developing amelioration strategies against viral infections in finfish aquaculture.

Cyber-Physical Seafood Systems: The future of NZ marine products processing

Dr Susan Marshall¹

¹The New Zealand Institute for Plant and Food Research Ltd., Port Nelson, New Zealand

Biography:

Dr Susan Marshall is an industrial biochemist specialising in the development of new marine products and the efficient large-scale processes to make them. She is Science Group Leader for the Seafood Processing and Marine Products Group at Plant & Food Research (PFR). Sue is programme leader of MBIE Cyber-Marine; represents PFR on the board of MBIE Bioprocessing Research Alliance; and leads commercial product development projects in the seafood industry. Her research group has a wide focus covering seafood safety and shelf-life extension, through to development of high-value natural catalysts, cosmetic, nutraceutical and biomaterial products, and more traditional by-products processing such as rendering. Sue is responsible for ongoing development of the PFR Bioprocessing Pilot Plant (located in Nelson), an essential tool in transfer of science from the laboratory to commercial product development.

'Cyber-physical seafood systems: Intelligent and optimised green manufacturing for marine co-products' (usually known as Cyber-Marine) is a \$16.28m, 5 year NZ Government-funded Endeavour research programme that started in October 2020. It pulls together chemists, biochemists, engineers and computer scientists from Plant & Food Research, Callaghan Innovation, Victoria University of Wellington, University of Otago, and Deakin University in Australia, and includes staff and facilities from companies across the New Zealand seafood industry.

Bioactive and functional molecules from by-products and by-catch are a means to grow our seafood industry without affecting seafood availability or needing more fish to be caught. However, whilst the enormous diversity of harvested animals from NZ's aquaculture and wild-fisheries provides a great variety of seafood for consumers, it presents challenges for developing generally applicable processes for non-fillet resource utilisation.

To maximize value from our resource, we need flexible factories capable of optimally processing all raw materials, matching products to market demand and able to extract each component in the cascade without compromising the next. Right now, we can assess composition using traditional chemical testing, but this takes a long time. For a responsive factory, we need analysis and interpretation of extremely complex data in real time. In Cyber-Marine we are working to develop new Al-integrated sensor systems able to tell us immediately what's in any fish or shellfish raw material. The information will then be used to direct factory operations. In addition to new sensor systems, we're working on development of a suite of integrated switchable, low-energy extraction technologies for our flexible factory, using the differences in properties of molecules to sequentially separate the components.

Encapsulation technology for delivery of bioactives in abalone aquaculture

<u>Dr Seyedehsara Masoomi Dezfooli^{1,2}</u>, Dr Noemi Gutierrez-Maddox³, Prof Andrea C Alfaro², Associate Prof Ali Seyfoddin¹

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Biography:

Dr Sara Masoomi is a research fellow at Auckland University of Technology (AUT). She received her PhD in August 2020 in Applied Science and has been working as a research fellow at AUT since then. Sara is a scientist with multidisciplinary background. Her research experience spans from recombinant protein expression systems to development of controlled release systems and bioactive discovery from marine animals. Sara is currently working on various encapsulation projects to develop micro- and macro-capsules for delivery of bioactives to farmed aquatic species.

The aquaculture industry is a fast-growing food sector. Intensified aquaculture systems are designed and implemented to increase productivity and profitability. However, when animals grow in proximity, pathogens, feed wastage and contaminants can compromise their health and nutrition resulting in economic losses. Probiotics, nutrient supplements, and chemical additives are used to enhance the health and nutrition of animals in high density aquaculture settings. However, traditional delivery methods of these additives not only increase the organic load of the culturing water and decrease the water quality, but also have been shown to be inefficient, costly and time consuming. Targeted and controlled delivery of bioactives to aquatic species using encapsulation technology provides a potential strategy to ensure efficiency in terms of production as well as sustainability. Encapsulation is the entrapment of compounds inside a dispersed material which can be used to retain viability of probiotics, activity of bioactives and minimise nutrient leaching in the culturing water. This presentation will highlight the potential use of encapsulation techniques for aquaculture applications and discuss case studies to illustrate this approach. Different encapsulation techniques, including emulsion and simple extrusion will be discussed to produce capsules for delivery of probiotics and nutrients to New Zealand black footed abalone (Haliotis iris). The case studies propose new methods to improve abalone farming by enhancing health and growth performance of animals, reducing feed cost and increasing production using encapsulated bioactives.

Encapsulated bioactives for increased growth of farmed Paua

Dr Seyedehsara Masoomi Dezfooli^{1,2}, Dr Noemi Gutierrez-Maddox³, Prof Andrea C Alfaro², Prof Ali Seyfoddin¹ ¹Drug Delivery Research Group, School of Science, Auckland University of Technology, CBD, New Zealand, ²Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ³School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, CBD, New Zealand

Biography:

Dr Sara Masoomi is a research fellow at Auckland University of Technology (AUT). She received her PhD in August 2020 in Applied Science and has been working as a research fellow at AUT since then. Sara is a scientist with multidisciplinary background. Her research experience spans from recombinant protein expression systems to development of controlled release systems and bioactive discovery from marine animals. Sara is currently working on various encapsulation projects to develop micro- and macro-capsules for delivery of bioactives to farmed aquatic species.

The slow growth rate of New Zealand abalone (*Haliotis iris*) is one of the major industrial bottlenecks in New Zealand abalone farming. Probiotic supplements can be a great strategy to enhance growth rate and reduce mortalities of farmed abalone. However, conventional methods of delivering probiotics to aquaculture are inefficient and may lead to environmental contamination. In this study, a novel carrier to deliver probiotics to farmed abalone was developed using natural polymers. The model carrier was optimised to obtain a carrier with desired morphology, polymer concentration, size, sinking rate, matrix rigidity, stability in seawater. The developed carrier was subsequently used in a two-month feeding trial with a total number of 90 Juvenile abalone. It was shown that the spherical feed capsules (2.9 to 3.6 mm) could withstand disintegration in seawater whilst maintaining the activity of the entrapped probiotics. The percentage of feed wastage were 11.7±1.5 and 30.1±2.3 for encapsulated and non-encapsulated feed, respectively. Evaluation of bacterial load after feeding abalones with encapsulated probiotics demonstrated a significantly higher bacterial content compared to control animals. A significant improvement in growth was achieved with the encapsulated feed that included 27.95% wet weight gain and 87.61% increase in shell length compared to animals treated with non-encapsulated feed. This implies the efficiency and capability of the developed bioactive carrier to deliver probiotics to farmed abalone.

A predator-prey behavioural interaction between intertidal seastars and the influence of ocean acidification

Mrs Emily Mclaren¹, Professor Maria Byrne¹

¹The University of Sydney, Sydney, Australia

Biography:

I am a PhD candidate with Prof. Maria Byrne working on the tropicalization of sea urchin assemblages and implications for species processes on the east coast of Australia.

A predator-prey interaction occurs between the asterinid seastars *Meridiastra calcar* and *Parvulastra exigua*, which are sympatric on the shores of southeast Australia. Ocean acidification (OA) driven by sea water uptake of anthropogenic atmospheric CO2 has broad deleterious effects on marine species including modified behavioural interactions such as between predators and prey. *Parvulastra exigua* exhibits a fleeing response when they encounter *M. calcar*. We investigated this response to determine the cues that elicit this behaviour and the effect of elevated CO2 on this escape response. Physical contact with *M. calcar* resulted in an 8.6-fold increase in velocity of *P. exigua* compared to basal locomotion. Water conditioned by *M. calcar* (chemosensory cue only) also elicited an increase in velocity, but this was lower, a 3.5-fold increase. The escape trajectory of *P. exigua* was most linear, 180° away from the point of touch when contacted with *M. calcar*. The escape response of *P. exigua* with respect to velocity and escape trajectory was investigated after both species were acclimated in OA conditions. At pHT 7.6 and 7.8 velocity and escape trajectory of the fleeing response of *P. exigua* did not differ from that seen in the ambient treatment. However, there was a delay in the time that *P. exigua* started to flee with the initiation time being 2.8 times slower (10 vs 28 sec) at pHT 7.6. This delay may increase the vulnerability of *P. exigua* to predation and have ecological effects with respect to the role of this species as an algal grazer.

Captive crown-of-thorns starfish upregulate stress and immune genes, and downregulate cell signalling genes

<u>Marie Morin</u>¹, Mathias Jonsson¹, Sandie Degnan¹, Bernard Degnan¹ ¹University Of Queensland, St Lucia, Australia

Biography:

Marie Morin is a Ph.D. candidate at the School of Biological Sciences at the University of Queensland, Australia. She holds a Bachelor of Science with Honours in the field of marine biology from the same university. Her current project seeks to characterise the secretome of the crown-of-thorns starfish (COTS) using transcriptomes generated from wild animals. This allows for the potential identification of secreted proteins as candidates for development of COTS-specific attractants.

Insights from the study of marine animals in captivity are often extrapolated to their wild counterparts. This could be misleading if the physiology and behaviour of captive animals is significantly affected by the artificial environment. Here we use RNA-Seq to investigate how gene expression of a coral reef pest, the crown-of-thorns starfish (COTS), changes in captivity. We first compared transcriptomes from three external tissue types – skin, spines and tube feet – of wild and captive COTS. Samples were obtained from (i) wild COTS within 2 hours of collection from the Great Barrier Reef (GBR), and (ii) captive COTS transported from the GBR and maintained in aquaria for at least one week. We found more than 3000 genes upregulated in the captive COTS, including genes involved in DNA repair and stress response. Extending this analysis to coelomocytes - cells in internal coelomic fluid with known physiological and immunological roles - we found over 1500 genes significantly upregulated in captive starfish, with enrichment of genes involved in immunity. Moreover, we find that captivity induced quick and sustained changes in all transcriptomes. Overall, genes commonly upregulated in captivity across all four tissues are involved in stress and immune pathways, whereas genes commonly downregulated are involved in cell signalling. We conclude that captivity has an extensive influence on COTS gene expression, potentially reflecting changes in physiology, behaviour and health. The effect of captivity on animals should be accounted for when designing studies seeking to understand wild animals.

Bacteria associated with larvae of *Argopecten purpuratus* displaying less susceptibility to the pathogen *Vibrio bivalvicida*: a source of potential probiotics?

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Biography:

Biochermistry (Pontificia Universidad Católica de Valparaíso, Chile) and Doctor candidate in Biotechnology (joint program PUCV/USM). My broad scientific interests are the immunology and microbiology applied to the aquaculture of shellfish. My research is focused on understanding the immune responses and the interaction of host-microbiota of exposure to pathogens. Specifically, study how the host-microbiota interaction is affected by the presence of a pathogen, evaluating changes in immune markers with the composition of the probiotic bacterial microbiota of the scallop. I look for this answer at different levels of biological organization, from main effect genes, the whole cell, tissues, and the whole animal to obtain an interdisciplinary approach, which has been possible thanks to a great working group and the collaboration of other researchers.

The culture of the scallop Argopecten purpuratus has a significant impact in Chilean local economy. Worryingly, seed availability has decreased in part due to massive larval mortalities in hatcheries, caused by bacterial pathogens from the Vibrio genus. In bivalves, evidence suggest that disease resistance is a heritable trait, thus can be improve by genetic selection. Also, changes in the composition of the bacterial microbiota associated with A. purpuratus has been shown during scallop immune response, suggesting that scallop immunocompetence could be related to differences in the bacterial microbiota composition. In this context, the present study aimed to identify bacterial strains from scallop larvae less susceptible to the pathogenic Vibrio bivalvicida VPAP30. For this, 18 full-sibling (FS) families at the mature stage were produced following a paternal half-sibling nested design. Then, bacteria associated from each FS family were obtained using three different agar media. Single colonies were isolated and stored. In parallel, mature veliger larvae from each FS family were exposed to a lethal concentration of the pathogen for 24 hours and then affected larvae were determined by visual observation. This approach allowed the detection of FS families with highly contrasted phenotypes of susceptibility larvae after pathogen exposure (low and high susceptibility with respectively 15% and 80% mortality). Bacterial isolates associated with FS families showing higher resistance were classified by their morphological differences and were identified by PCR amplification and 16S rRNA gene sequencing. Now, functional approaches on the probiotic effect of the bacterial strains associated with the least susceptible FS families are being perform. FONDECYT 1200129.

Occurrence of *Perkinsus olseni* and other parasites in New Zealand black-footed abalone (*Haliotis iris*)

Miss Farhana Muznebin^{1,2}, Prof Andrea C Alfaro¹, Dr Steve C. Webb³

¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ²Department of Zoology, Faculty of Life and Earth Science, Jagannath University, Dhaka, Bangladesh, ³Cawthron Institute, Nelson, New Zealand

Biography:

Farhana Muznebin is a PhD student within the Aquaculture Biotechnology Research Group (ABRG) at the Auckland University of Technology (AUT) working on "Shellfish Pathology and Immunology". Farhana's research focuses on histopathology, haematology, enzyme chemistry and immunology of cultivated shellfish species, such as mussels, abalone and oysters. The aims of her project are to assess the health condition of New Zealand aquaculture species and to investigate their defence functions and disease susceptibility/resistance. She is interested in understanding the patterns of disease progression that may help elucidate host-parasite interactions and transmission of infections.

The endemic New Zealand pāua, or black-footed abalone (*Haliotis iris*), supports a small, but growing, aquaculture industry which is potentially threatened by disease agents. A histopathological study was undertaken of healthy- and unhealthy-looking pāua from a land-based aquaculture farm. Pathogens and parasites detected in histological sections of pāua samples were identified and characterised, with an assessment of prevalence. These included *Scyphidia*-like ciliates (56%), *Sphenophrya*-like ciliates (55%), unidentified disintegrated ciliates (26%), intracellular microcolonies of bacteria (IMCs) (9%), unidentified protozoan cells (1%), bacteria (2%) and *Perkinsus olseni* (5% prevalence). Subsequent in situ hybridization confirmed the identity of *P. olseni*, as a first report for *H. iris*.

Conditions seen in the histopathology preparations included deformed gills, the presence of ceroid material and haemocytosis. The presence of these was correlated with pathogen presence/absence and with the initial gross classification of the pāua as healthy or unhealthy. *P. olseni* infections were significantly associated with unhealthy gross appearance and presence of disintegrated ciliates. There was also a significant association between *P. olseni* and intracellular microcolonies of bacteria, and between *P. olseni* and swollen gills with ceroid material and haemocytosis. These findings may provide important insights for health assessment of *H. iris* in wild and farmed populations.

Metabolic responses to temperature of co-occurring post-settlement mussels, *Perna perna* and *Mytilus galloprovincialis*, during immersion do not explain adult distributional patterns

Dr Aldi Nel¹

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Biography:

The complex dynamics of abiotic factors that impact animal stress and nutrition, coupled with animal tolerance levels and physiological compensatory mechanisms forms one of my main interests. I have a background in marine mollusc aquaculture and biology. During my PhD I worked on the digestive physiology of abalone in response to bioactive dietary ingredients, and I have worked on ontogenetic and thermal biology aspects of both oysters and mussels.

For marine animals with biphasic life histories, different environmental conditions are experienced by different life stages so that physiological constraints on early ontogenetic stages could explain adult distributions and life history traits. Mussel populations on the coast of South Africa comprise mostly the indigenous *Perna perna* and the invasive *Mytilus galloprovincialis*, which approaches the eastern limits of its biogeographic distribution on the south coast. The two have different biogeographic distributions, but overlap on the south coast, and exhibit different metabolic responses to temperature as adults. We compared the acute metabolic response to temperature of post-settlement individuals of the two species. Respiration rates of recently settled recruits were measured monthly for five months under three thermal regimes set at 5 °C intervals around the ambient field seawater temperature at the time of collection. The size dependence of metabolic responses to temperature differed little between the species. Hence, early post-settlement metabolic responses to temperature differed little between the species. Hence, early post-settlement metabolic responses to temperature did not explain differences in adult distribution. Our data did, however, provide evidence of unexpectedly high plasticity in response to recent thermal history for both species.

Triploidy in *Mytilus edulis* allows better attachment which can result in higher production yields

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Biography:

I am a PhD student at the University of Quebec at Rimouski. I have a background in Biochemistry and in medical biology. Currently, I am working on the impacts of triploidy in Mytilus edulis, and more specifically on how it affects their attachment strength and reproductive investment.

During the last decades, intensive work has been put into the production of triploid bivalves, as triploidy can lead to reduced investment in fertility and faster growth. In mussel farming, the seasonal weakening of their byssal attachment can cause fall-off from mussel socks. In this study, we investigated the production and mechanical properties of byssal threads of diploid and triploid mussels related to their physiological performance by measuring the scope for growth. Finally, the seasonal gametes' production of juvenile and adult stage were estimated through histological observations. Production of byssal threads was induced with the use of a hydrodynamic flume to standardize the abiotic factors, including current and turbulence conditions. The triploid mussels produced 40% more threads than diploids characterized by different mechanical properties, particularly a higher Young's modulus. Scope for growth was calculated through measurement of clearance rates, respiration rates and excretion rates and triploid mussels showed higher scope for growth, as well as higher clearance rates. Preliminary results of the histological measurement tend to show a reduced investment or a delay in triploid gametogenesis. Indeed, primary observations, performed mid-May 2021 just before the beginning of spawning events demonstrated more advanced development stage in diploid gametes. Overall, this study showed that triploid mussels can be of great interest in mussel farming as they seem to have better attachment, better growth potential, and reduced or delay gametogenesis investment.

Effects of natural substances in the metamorphosis of pullet carpet shell *Venerupis corrugata* (Gmelin, 1791) larvae

<u>Dr M.Luz Pérez-Parallé</u>¹, Dr Vitor Vasconcelos², Mariana Reis², Dr Antonio J. Pazos¹, Dr José L. Sánchez¹ ¹Instituto De Acuicultura. Universidad De Santiago De Compostela, Spain, ²Centro Interdisciplinar de Investigação Marinha e Ambiental. Universidade do Porto, Portugal

Biography:

Luz Pérez-Parallé has a permanent position as Professor of the Department of Biochemistry and Molecular Biology at the University of Santiago de Compostela, Spain. She is a member of the Group of Molecular Biology and Development in Aquaculture (Institute of Aquaculture). Her interest in research is related to the improvement of the production of different species of marine bivalve molluscs and to the mitigation of the effects of toxic tides: elimination of biotoxins in bivalve molluscs and `omic' technologies applied to aquaculture. She is serving as secretary of the Institute of Aquaculture of the USC since 2015.

Metamorphosis and settlement are two closely associated processes in marine invertebrates. These processes involve rapid and irreversible changes in both morphology and habitat of the larvae. In order to metamorphose, bivalve larvae must achieve a stage of competence to be able to respond to appropriate environmental, chemical and/or physical cues. Competent larvae from different molluscan species have been induced to settle and metamorphose using not only natural chemical cues but also functional analogues of the natural inducers. In fact, we have previously reported the effect of several neurotransmitters on the metamorphosis of bivalve larvae such as Ostrea edulis, Mytilus edulis, Ruditapes philippinarum, Ruditapes decussatus and Mimachlamys varia. Furthermore, several authors have reported that bacterial films are involved in inducing settlement of both O. edulis and Crassostrea gigas larvae. In this research we investigate the potential effect of bioactive natural substances from several Cyanobacteria spp. as inducers on the larval metamorphosis of bivalves. Larvae were treated with ten cyanobacteria extracts at two different concentrations: 10 µg/ml and 20 µg/ml. Experiments were performed in triplicate in 90mmglass Petri dishes for each one of the concentrations in a total volume of 20 mL. A control with filtered seawater and without potential inductor was set in each experiment. Results will be discussed, especially their implications in the survival of the larvae to the metamorphosis process, an important bottleneck in bivalve culture.

This research was funded by the Interreg Program V Spain-Portugal (POCTEP) 2014-2020 co-financed with ERDF funds (0474_BLUEBIOLAB_1_E).

Expression of two *Mytilus galloprovincialis* ABC transporter proteins under a toxic tide of *Dinophysis acuminata*

<u>Dr M. Luz Pérez-Parallé</u>¹, Dr. Antonio J. Pazos¹, Dr. Roi Martínez-Escauriaza¹, Dr. José L. Sánchez¹ ¹Instituto de Acuicultura. Universidad de Santiago de Compostela, Santiago de Compostela, Spain

Biography:

Luz Pérez-Parallé has a permanent position as Professor of the Department of Biochemistry and Molecular Biology at the University of Santiago de Compostela, Spain. She is a member of the Group of Molecular Biology and Development in Aquaculture (Institute of Aquaculture). Her interest in research is related to the improvement of the production of different species of marine bivalve molluscs and to the mitigation of the effects of toxic tides: elimination of biotoxins in bivalve molluscs and `omic' technologies applied to aquaculture. She is serving as secretary of the Institute of Aquaculture of the USC since 2015.

The Mediterranean mussel Mytilus galloprovincialis is one of the most important aquaculture species in Europe. Its production is mainly concentrated in Galicia (NW Spain) with an average yield of 200,000 tonnes per year. The main problem for mussel production is the accumulation of toxins, especially DSP toxins (ex. okadaic acid), during microalgae blooms which are threatening mussel production and prevents their commercialization. The multixenobiotic resistance mechanism (MXR) acts by pumping out endogenous compounds and xenobiotics from cells preventing their accumulation and toxicity. This mechanism is mediated through ABC transporters such as MDR/P-glycoprotein and MRP proteins. In this work, we studied the possible role of P-glycoprotein and MRP proteins in the okadaic acid detoxification process. The expression of Mgmdr1 and Mgmrp2 genes was monitored in gills, digestive gland and mantle during a cycle (79 days) of accumulation-elimination of okadaic acid in mussels under a toxic tide of Dinophysis acuminata. The mdr1 gene significantly increased its expression in the digestive gland and gills (4 to 5-fold) where okadaic acid accumulated in the mussels, supporting the idea of an important role of the MDR1 protein in okadaic acid efflux out of cells in these tissues. As in the case of mdr1, there is a significant induction (7-8 fold) in the expression of the mrp2 gene in the digestive gland as the content of okadaic acid increases in this tissue. We speculated that P-glycoprotein and MRP may constitute a functional defence network against xenobiotics and might be involved in the resistance mechanisms to DSP toxins.

This research was funded by the Interreg Program V Spain-Portugal (POCTEP) 2014-2020 co-financed with ERDF funds (0474_BLUEBIOLAB_1_E).

Histology and biochemistry reveal the factors compromising the differential growth rate and physiological performances of the mussel *Mytilus galloprovincialis*

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Biography:

Maitane Pérez-Cebrecos is a second year PhD candidate at University of Basque Country. Her main research interest centres on how the environmental and endogenous variables affect the viability, growth and reproductive capacity of bivalves. The research in her PhD project has a multidisciplinary approach encompassing technical procedures from energetic physiology, histology and biochemistry. Maitane holds a B.S. in Biology, where she graduated with honours. Prior to starting her doctorate, she completed a master's degree in Environmental Contamination and Toxicology, in which she qualified as the best academic record. Her M.S. Thesis was an opportunity for her to delve into the field of energetic physiology and it was the spark to ignite her desire to venture in the science career. When she is not absorbed by science, she loves hiking, playing the piano, working her way through every recipe in the family cookbook and indulging her love for meeting new cultures

The inter-individual growth rate differences in bivalves may respond to endogenous factors affecting the capacities for either acquiring, digesting or absorbing food, as well as allocating metabolic energy. The aim of this study was to determine the physiological, histological and biochemical traits characterising individuals with different growth rates. Uniformly sized juveniles of Mytilus galloprovincialis were maintained under continuous feeding at the laboratory until size differentiation was evident. Selected fast-, slow- and intermediate-growers were used to perform physiological energetic experiments. The three growing conditions showed a similar regulatory response to particle concentration increase through clearance rate reduction. However, subsequent histological analysis showed that fast-growing individuals potentially have a greater digestive capacity and a higher intracellular digestive activity, thanks to a larger digestive-tissue proportion, and larger and thicker digestive diverticula. Also, fast growers showed a higher level of investment in storage in the form of adipogranular tissue. Previous studies showed that size-related intra-specific differences in clearance rate values may be explained by corresponding differences in gill surface areas. In good correspondence, our histological data has revealed that gills of slow growers possess a lower cilia density and a disorganized structure of the epithelium overall. Significant differences in catalase, glutathione-S-transferase and cytochrome oxidase activities revealed that slow-growers may cope with a higher oxidative stress in the gills but having a lower mitochondrial capacity. Hence, this study suggests that, whether or not the differences between the growing groups are genuine, slow growers do not have the histological or biochemical support for a greater growth.

Studying the structure of the microalgal vacuolar transport chaperone complex: a critical step to understand polyphosphate accumulation in microalgae.

Dr. Maxence Plouviez¹, Prof. Eric Dubreucq², Prof. Benoit Guieysse¹

¹School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand, ²Research Unit on Agropolymer Engineering and Emerging Technologies, Montpellier Supagro, Montpellier, France

Biography:

Initially from France, Maxence Plouviez obtained his M.Eng in agricultural sciences from Montpellier Supagro. In 2013, Dr Plouviez moved to New Zealand to undertake his PhD studies at Massey University. Dr. Plouviez's research focuses on developing fundamental knowledge of key pathways of microalgae metabolism in order to improve water management and microalgae biotechnology. In particular, his early research has identified the first pathway of N2O synthesis in microalgae and quantified the environmental significance of this poorly known metabolism. Now, he is studying poly-phosphate biosynthesis in C. reinhardtii in order to investigate the evolutionary, ecological, and industrial implications of this mechanism.

Microalgae are known to synthesize polyphosphate (polyP) when phosphorus (P) abounds or when cells are experiencing period of feast/famine. In *Chlamydomonas reinhardtii*, polyP synthesis involves the Vacuolar Transport Chaperone (VTC) protein complex, which synthesizes and stores polyP in vacuoles. To date, homologs of *C. reinhardtii* VTCs are found in at least 30 other microalgae species, suggesting a common polyP pathway in these microorganisms. However, data from our laboratory showed that microalgae have different kinetics and cellular accumulation efficiencies following P replete conditions. This finding suggest that species may differ in their regulation of polyP synthesis and/or their VTCs structure. The VTC protein complex comprises several subunits, which are themselves organised into subdomains. For example, VTC4 possesses a SPX domain suggested to be involved in P sensing and signalling, and a catalytic domain with polyP polymerase activity. Sequences and 3D models (deduced by homology modelling) of several VTC subunits from *C. reinhardtii*, Chlorella vulgaris and *Scenedesmus* sp. were compared to evaluate if the difference in polyP accumulation observed experimentally could be linked to differences in the VTC structure of the selected microalgae, whether in the binding site of the regulatory SPX domain or in the catalytic core of the polyP polymerase. This work aims at gaining knowledge on how the microalgal VTC complex operates, a critical step to improve our current understanding of P metabolism in microalgae.

Does Location Matter to the Giant Kelp?

A comparison of nutritional composition and water quality of the giant kelp (*Macrocystis pyrifera*) when sampled off the coast of Australia and New Zealand

<u>Dr. Diane Purcell-meyerink</u>¹, Dr. Michael Packer, Dr. Thomas Wheeler ¹Cawthron Institute, Nelson, New Zealand

Biography:

Dr. Diane Purcell-Meyerink is a Marie Skłodowska Curie Fellow, working with the Cawthron Institute in Nez Zealand. She has over 20 years experience working in Phycology, and has been fortunate to have worked on algae in several environments from Tibet, to Yellowstone National Park, and now the marine environment.

The giant kelp (Macrocystis pyrifera) is one of the fastest growing seaweeds globally, at up to 60 cm per day and plants reaching 50m. It is a positively buoyant species which grows up from the Ocean floor, enabling the creation of ocean kelp forests. It is a native species to both New Zealand and Australia, is it also found on the west coast of the US, South America, and South Africa. In the context of commercially grown seaweeds the giant kelp is not utilised extensively in comparison to another brown seaweed species Laminaria digitata. Previously reported Macrocystis pyrifera protein content values range from 9-17% depending on location and environmental factors including the season of harvest. Protein sourced from seaweed can contain all essential amino acids, and the protein profile of seaweeds is similar to egg protein. Initial nutritional analysis using AOAC standard protocols of wild harvested fronds of Macrocystis pyrifera from Tory Channel, South Island, New Zealand, found 12.2% dry weight (DW) crude protein, in comparison only 6.5% DW from Tower Bay, Tasmania, Australia. Total fat values were similar at both sites with 1.9% DW from New Zealand, and 1.2% and 1.4% DW from Australia. Macrocystis pyrifera usually has <2% fat content year-round therefore these values are in the expected range. Interestingly, total polysaccharides varied between sites with the New Zealand values at 21.6 mg / 100 g and the Australian samples at 21.8 mg / 100 g, and 35.3 mg / 100 g. Sampling was done at the same time of year in both locations, previous studies have found higher growth rates for Macrocystis pyrifera on wave exposed sites potentially improving nutrient uptake. This data sheds light on the how critical the location of aquaculture-based seaweed farms are when creating optimal conditions for seaweed production and quality.

The potential for genetic diversity and transgenerational plasticity to promote increased resilience to marine heatwaves in the subtidal mussel *Perna canaliculus*

<u>Dr Norman Ragg¹</u>, Nick King¹, Brendon Dunphy², Ellie Watts¹, Zoë Hilton¹, Bridget Finnie¹, Caitlin Fielder¹, Natalí Delorme¹, Carol Peychers¹, Leonardo Zamora¹, Jessica Ericson¹ ¹Cawthron Institute, Nelson, New Zealand, ²University of Auckland, , New Zealand

Biography:

Norman Ragg is a senior research scientist at the Cawthron Institute, leading work relating to environmental stress and climate change adaptation in cultured shellfish species.

Recent New Zealand summers have been accompanied by unprecedented marine heatwaves (MHW). Mean coastal sea surface temperature anomalies reached +3.7°C in 2018, with main driving factors attributed to a combination of stable anticyclonic conditions, positive Southern Annular Mode and weak La Niña. The Greenshell[™] mussel, Perna canaliculus, is found in shallow seas along the entire latitudinal range of New Zealand's coast (34 - 48°S) and supports a substantial aquaculture industry, based largely within MHWaffected areas. With the emergence of a 'summer mortality' syndrome in cultured P. canaliculus, there is now considerable interest in methods to capitalize upon biological resilience mechanisms. Genetic potential was considered by characterizing acute (3h) thermotolerance in 43 genetically diverse full-sibling families from a commercial selective breeding programme. The mean 50% mortality temperature (LT50) varied significantly from 28.9 to 31.8°C between Susceptible and Resilient families. The offspring of Resilient parents were more tolerant of chronically elevated temperatures (21 - 23°C for 14 months). Exposing thermotolerant parents to elevated temperatures during sexual maturation also appeared to shift the thermal window of offspring larvae upwards, but these transgenerational effects showed complex interactions with parental genotype and duration of heat stress. The combined data suggest that there is significant evolutionary capacity within standing stocks of P. canaliculus to respond to rapidly rising mean summer temperatures, while transgenerational plasticity may act synergistically to enhance thermotolerance of vulnerable pelagic life stages.

Biosynthesis of long chain polyunsaturated fatty acids in the razor clam *Sinonovacula constricta* (Lamarck 1818): Characterization of fatty acyl desaturases and elongases of very long chain fatty acid

Doctor Zhaoshou Ran¹, Pro. Jilin Xu¹ ¹Ningbo University, China

Biography:

Name: Zhaoshou Ran Age: 30 Email address: ranzhaoshou@nbu.edu.cn Major: shellfish aquaculture and lipid nutrition Affiliation: Key Laboratory of Applied Marine Biotechnology, Ningbo University, China

As an unusual economically important aquaculture species along the coasts of West Pacific Ocean, *Sinonovacula constricta* possesses high levels of long chain polyunsaturated fatty acids (LC-PUFA). To investigate the endogenous LC-PUFA biosynthetic ability in *S. constricta*, fatty acyl desaturases (Fad) and elongases of a very long chain fatty acid (Elovl) of this bivalve, namely, $\Delta 5$ Fad_a, $\Delta 5$ Fad_b and $\Delta 6$ Fad, as well as Elovl2/5, Elovl4_a, Elovl4_b and Elovl_c were cloned and functionally characterized. Therefore, *S. constricta* is the first marine mollusc demonstrated to possess all Fad and Elovl activities required for LC-PUFA biosynthesis via the 'Sprecher pathway'. This finding greatly increases our understanding of LC-PUFA biosynthesis in marine molluscs. The results also indicate that marine bivalves could be a promising source of sustainable natural LC-PUFA.

Physiological Markers in Early Life Stages of the Green-lipped mussel, Perna canaliculus, Quantified Using Semi-Automated Bioimaging Analysis.

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¹Hugh Green Cytometry Centre, Malaghan Institute of Medical Research, Wellington, Kelburn, New Zealand, ²Cawthron Institute, Nelson, Nelson, New Zealand

Biography:

Senior staff scientist – bioimaging specialist, who manages the histology and bioimaging core within the Hugh Green Cytometry Centre.

The green-lipped mussel, Perna canaliculus, is an economically, culturally, and ecologically important marine species in New Zealand. Currently, this species is the most significant aquaculture crop, supporting a multi-million dollar industry that is permanently striving to improve its practices and to find new and more efficient ways of adding value to the New Zealand economy. Accurate and precise identification of physiological parameters across different life stages of shellfish can be informative not only as a research tool to understand the physiological response, but also to improve husbandry conditions of commercially valuable species. Using P. canaliculus as a model species, we have developed two fluorescent image-based analysis procedures using state-of-the-art Laser Scanning Confocal Microscopy (LSCM) and a semiautomated image analysis approach to visualise and quantify selected energetic and oxidative stress markers in mussel larvae and juveniles, respectively. Neutral lipids were quantified in early veliger-stage larvae using Nile Red or ORO (a fluorescent energetic lipid dye) by determining oil droplet count, size distribution and volume. While a fluorescent oxidative stress dye (CellROX[™] Green Reagent, Invitrogen[™]) was applied to post-settlement mussel juveniles to quantify the area within the juvenile generating reactive oxygen species. Both markers were successfully applied following experimental challenges that involved varying temperature and aerial exposure for larvae and juveniles, respectively. These emerging imaging methodologies enable the development of precise analysis protocols that could be applied as research tools both in the laboratory and the field, as well as under commercial settings as health assessment screening tools.

Effects of temperature on early development of the New Zealand Geoduck *Panopea zelandica* (Quoy & Gaimard, 1835)

<u>Mr Shaneel Sharma</u>¹, Prof Andrea C Alfaro¹, Dr Norman LC Ragg², Dr Leonardo N Zamora² ¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of

Technology, CBD, New Zealand, ²Cawthron Institute, Nelson, New Zealand

Biography:

Shaneel Sharma is a PhD student in the Aquaculture Research and Biotechnology Group, Auckland University of Technology. His current research is focused on closing the life cycle of the New Zealand geoduck (Panopea zelandica) in hatchery settings. This in turn is part of a larger project to introduce P. zelandica into the aquaculture industry. Current topics of interest include broodstock conditioning, optimizing spawning and larval rearing practices.

Ambient seawater temperature is an important factor during the early life stages of marine invertebrates. Temperature is often manipulated in hatcheries to shorten the incubation period before the larval rearing phase. In this study, the effect of temperature on the early development of the geoduck *Panopea zelandica* was investigated over a 48-hr period to identify the optimum temperature for fertilization and development in a controlled environment. Eggs and sperm collected from broodstock were exposed to ten temperatures ranging between 11.8 and 23.7°C. Fertilization and subsequent development were monitored over 48 hr. Highest percentages of fertilization were achieved at 23.7°C, which was the highest temperature tested in this study. However, the development of *P. zelandica* embryos was greatly hindered at temperatures >18.5°C due to a range of abnormalities arising from uneven cell division and cellular blebbing. All larvae died at the highest temperature of 23.7°C within 48 hr of exposure. The combined fertilization success and embryo development data indicate that 18.5°C is the optimal temperature for incubating *P. zelandica* embryos under hatchery conditions.

Physiological responses of juvenile New Zealand geoduck (*Panopea zelandica*) post emersion and recovery

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Biography:

Shaneel Sharma is a PhD student in the Aquaculture Research and Biotechnology Group, Auckland University of Technology. His current research is focused on closing the life cycle of the New Zealand geoduck (Panopea zelandica) in hatchery settings. This in turn is part of a larger project to introduce P. zelandica into the aquaculture industry. Current topics of interest include broodstock conditioning, optimizing spawning and larval rearing practices.

The New Zealand geoduck clam is a unique seafood delicacy, with animals selling for up to \$USD 220-330/kg. Stress accumulated during transport of juveniles to grow-out sites represent a bottleneck in the aquaculture process. In this study, the physiological responses of juvenile geoducks following emersion (3and 8-hours), and recovery (1- and 5-days) were investigated. An integrated approach of flow cytometry, osmolality and gas-chromatography mass spectrometry metabolomics measures, along with behavioural assessments was used. Cellular and chemical haemolymph parameters and metabolite profiles were recorded for P. zelandica juveniles and are reported herein for the first time. An increase in haemolymph osmolality was experienced with an increase in emersion period, with significant differences seen between the 3- and 8-hour emersion groups after 5 days of recovery. Haemocyte viability results varied marginally between experimental groups, creating baseline ranges for juvenile geoduck. The proportion of haemocytes undergoing respiratory burst activity did not appear to be affected by emersion and re-immersion. Haemocyte mitochondrial membrane potential was highest following 1-day of recovery, likely linked to metabolic readjustment taking place following emersion. Metabolomics analyses suggest that protein, lipid and carbohydrate metabolite classes assist with energy production in geoducks. Activation of anaerobic metabolic pathways were prominent in the 8-hour exposure group, with metabolic recovery still taking place following 5-days of immersion, mainly due to proteins restoring energy reserves. Elucidating the physiological responses of juvenile geoduck subjected to transport stress can aid cultivation methods already underway to develop a novel, high value aquaculture industry.

Larval fish in a warming ocean: A bioenergetic study of growth of California grunion

Callyn Shelley¹

¹California State University, Long Beach, Long Beach, United States

Biography:

Callyn Shelley is a MS Candidate in Marine Biology at California State University, Long Beach. She is an experienced coastal research biologist and has studied the physiology and behavior of fish and other organisms in response to environmental change. Her recent work focuses on the bioenergetic response of larval fish to ocean warming. As an engaged citizen scientist, Shelley is involved in beach monitoring and population assessment surveys in partnership with the Grunion Greeters program and NOAA marine fisheries. She is currently open to work in California or New Zealand and is interested in marine research and wildlife biologist positions.

Rising ocean temperatures may have important consequences for growth of marine organisms but observed relationships between temperature and growth vary widely across species and studies. The key to understanding such variation is knowing how temperature affects the amount of energy available for growth. I examined the relationships between temperature, energy intake and expenditure, and growth of larvae of California grunion (Leuresthes tenuis). In the process of this larger study, I was able to make inferences about assimilation efficiency. In addition, I tested experimentally whether food availability could cause a qualitative shift in the relationship between temperature and growth. L. tenuis larvae were reared at temperatures ranging from 16 to 28°C. At each temperature, energy intake was measured from feeding rate and energy expenditure was measured from respiration rate and excretion rate. Both feeding and expenditure increased with temperature but feeding increased more rapidly, suggesting at least the capacity for faster growth at higher temperatures. An independent experiment confirmed that growth increased with temperature when food was plentiful, but also revealed that under moderate food limitation, growth rates declined with temperature. Comparing energy budgets with observed growth suggests that a larva's ability to assimilate food may become much less efficient at high temperatures, possibly because of the reduction in gut passage times when feeding rates are elevated. Overall, my study suggests that a detailed consideration of the bioenergetics underlying growth will be useful for understanding how growth rates are likely to change in a warming ocean.

A phlorotannin isolated from *Ecklonia radiata*, Dibenzodioxinfucodiphloroethol, inhibits neurotoxicity and aggregation of β-amyloid

<u>Mr Srijan Shrestha</u>¹, Dr Martin R. Johnston², Dr. Wei Zhang³, Dr Scott Smid⁴

¹The University of Adelaide, Adelaide, Australia, ²Flinders University, Adelaide, Australia, ³Flinders University, Adelaide, Australia, ⁴The University of Adelaide, Adelaide, Australia

Biography:

I am a Ph.D. student at The University of Adelaide. I completed my Master's degree from Pukyong National University (Busan, South Korea) in 2018 focusing on the various small molecules that have the potential to become future therapeutics for diabetes and Alzheimer's disease.

My current research focuses to explore and characterize the potential role of natural products (marine seaweeds) as neuroprotective and anti-inflammatory agents. It involves extraction and isolation of various components present in the natural product via HPCPC, HPLC, MS, and NMR spectroscopy and identification of the active compounds that are responsible for neuroprotection. We use various neuronal cell lines to mimic the neurotoxicity and try to rescue the cells with our extracts and pure compounds. Further, we seek answers from molecular docking and dynamic studies for the possible mechanism of action and the binding site information.

The polyphenolic phlorotannins derived from brown macroalgae consist of complex polymers of phloroglucinol residues with diverse structures ascribed pleiotropic bioactivity. This study used high performance counter-current chromatography (HPCCC) combined with size exclusion chromatography to isolate and purify a rare phlorotannin from the marine brown algae Ecklonia radiata. Nuclear magnetic resonance (NMR) including 1H and 13C and 2D COSY, HSQC, HMBC (broad and band selective), and NOESY along with mass spectroscopy enabled us to identify the phlorotannin as dibenzodioxin-fucodiphloroethol (DFD). The compound was subsequently investigated for protective bioactivity against the neurotoxic β amyloid protein A β 1-42. DFD was nontoxic up to 50 μ M in the neuronal PC-12 cell line and significantly prevented loss of cell viability in response to $A\beta 1-42$ (0-1.5 μ M). Furthermore, it significantly reduced the aggregation of A β 1–42 as evidenced by transmission electron microscopy and molecular docking studies revealed DFD binding to key Aβ1-42 residues associated with fibrillisation propensity. Additionally, DFD demonstrated moderate cholinesterase inhibitory activity with an IC50 value of 41.09 μ M and shared similar interacting binding residues to donepezil in the crystallized structure. Collectively, our present protocol was found to be simple and effective for the isolation and purification of a novel and complex neuroprotective phlorotannin. Similar approaches facilitating large-scale purification of novel phlorotannins will enable further bioactivity characterizations to be developed from brown algae for potential future therapies.

Is environmental adaptation in the invasive species, *Didemnum vexillum*, due to epigenetic mechanisms?

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Biological invasions provide a unique model to investigate adaptation and evolution within short timescales, as the introduced populations must rapidly adapt to new habitats. Didemnum vexillum is a highly invasive colonial ascidian that has become established in temperate and sub-tropical waters worldwide. Phylogenetic analyses have revealed two distinct clades: clade A is now found in temperate coastal areas around the world, while clade B is apparently restricted to its native region. The distribution of clade B may reflect a lower thermotolerance than clade A or difference in adaptation. The capacity of populations to adaptively evolve has generally been explained by existing heritable genetic variation or DNA nucleotide variation. However, there is increasing acceptance that epigenetic modifications can affect ecologically important traits. Environmentally induced epigenetic changes have been proposed as one process underlying the rapid adaption of invasive species. Global DNA methylation in introduced populations of clade A show distinct patterns and are significantly different to native populations. Introduced populations also show a significant reduction in global methylation levels, which has been suggested as a mechanism for increasing phenotypic plasticity. Using manipulative experiments, we found that exposure to elevated temperature resulted in significant changes in DNA methylation decreased growth rates over short time periods. The results of this study provide insights into potential role of DNA methylation as a rapid mechanism of adaptation in a globally invasive species. Epigenetic variation may act as a buffer against rapid environmental changes and compensate for low levels of DNA sequence-based variation.

Embryo-larval effects of early exposure to environmentally realistic pesticides mixture in the pacific oyster, *Crassostrea gigas*

<u>Thomas Sol Dourdin</u>¹, Killian Guyomard², Manuella Rabiller², Yoann Godfrin¹, Thomas Lemaitre², Guillaume Rivière³, Thierry Burgeot¹, Rossana Sussarellu⁴

¹Ifremer, BE, Laboratoire d'ecotoxicologie, Nantes, France, ²Ifremer, Plateforme Mollusques Marins Bouin, Bouin, France, ³Biologie des Organismes et Ecosystèmes Aquatiques (BOREA), FRE2030, Muséum National d'Histoire Naturelle (MNHN), Centre National de la Recherche Scientifique (CNRS), Institut de Recherche et Développement (IRD), Sorbonne Université (SU), Université de Caen Normandie (UCN), Université des Antilles (UA), 75231 Paris CEDEX, France, ⁴Ifremer, PBA, Laboratoire physiologie et biotechnologie des algues, Nantes, France

Biography:

Thomas Sol Dourdin has a master's degree (2018-2020) from La Rochelle University in environmental management and coastline ecology. As part of it, he did two internships. During the former, at the LIENSs laboratory in La Rochelle, he studied trace elements contamination in the Sine Saloum lagoon by monitoring heavy metals concentration in oysters and clams. The second internship, at Ifremer, focused on the population structure of Lamna nasus in the Northeast Atlantic. Thus, he developed skills in ecotoxicology and molecular biology that allowed him to start a phD thesis at Ifremer on the intergenerational effects of an early exposure to environmentaly relealistic pesticides mixture in the pacific oyster, Crassostrea gigas. He is currently in the first year of the project.

Early life stages are crucial for organism's development, in particular for external fertilization organisms, because embryos face environmental factors such as physico-chemical changes or xenobiotics. The pacific oyster, Crassostra gigas, is considered as a model species in ecotoxicology and monitoring because of its ecological characteristics (benthic, sessile, filter feeding). The impact of chemical exposure has been studied in this species, revealing embryotoxic, genotoxic and epigenetic effects of xenobiotics, sometimes at the multi-generation scale. However, so far studies focused on single molecules only while coastal waters concentrate many anthropogenic chemicals such as pesticides, and little is known about how those cocktails impact organisms and their progeny. Thus, our study aims to understand what are the physiological alterations induced in the oyster life cycle by such contaminations, when exposing them during embryolarval stage. To do so, we compared the development of larvae exposed to eighteen pesticides, mainly herbicides, at environmental concentration (the sum of their concentration was 2.85 µg.L-1 nominal concentration) for two days after fertilization (0h-48h), to larvae non-exposed. Physiological parameters such as size, feeding, metamorphosis rate or weight were monitored and samples were collected in order to make transcriptomic (6hpf, 24hpf, pediveliger stages) and epigenetic (pedivelieger stage) analysis. The first results about physiological data reveal no larval abnormalities at the D larvae stage, and little effects on larvae size between treatments.

Linking physiology and behaviour of juvenile, green-lipped mussels during the aquaculture seeding process

<u>**Dr Paul South¹**</u>, Dr David Burritt, Dr Norman Ragg, Dr Natalí Delorme ¹*Cawthron Institute, , New Zealand*

Biography:

Paul is a marine ecologist working in the Aquaculture group at Cawthron Institute. Much of his work is focused on the the fascinating behaviour of juvenile mussels and the processes that determine their recruitment success.

Seeding mussels into aquaculture is highly inefficient with most mussels being lost in the early stages of the production cycle. The seeding process involves removing juvenile mussels from the sea for extended periods of time to transfer them great distances from their site of capture or culture, before seeding them back into the sea onto a coastal nursery farm. Seeding is a critical event for the success of mussel aquaculture, not only must the mussels survive, but they must also remain attached to, or at least reattach to the grow-out structures on the nursery farm. Yet, seeding is a highly stressful process for the young mussels, especially given that they can experience enormous variations in stressors such as emersion duration and relative humidity (RH) as they are transferred to a nursery farm, and oxidative stress when they resume aerobic activity upon immersion in the sea. In this presentation, we will use experimental data to investigate the links between transfer- and seeding-associated stressors and the physiological (reactive oxygen species, survival) and behavioural (resettlement success) responses of juvenile, green-lipped mussels (*Perna canaliculus*), the most important aquaculture species in New Zealand.

Big Defensin ApBD1 from the scallop *Argopecten purpuratus* is an antimicrobial peptide which entraps bacteria through nanonets formation

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Biography:

I am Felipe Stambuk Opazo, Biochemist, student of Master of Microbiological Science of the PUCV, Chile. Part of Grupo de Marcadores Inmunológicos de Organismos Acuáticos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Chile. I have worked in multiple investigation projects as Technician

(FIE 201708070149, FONDECYT 1170118, 2018-2021; FONDECYT 1200129, 2021-current), purifying and using secondary antibodies against innate immune effectors (Big Defensin, Lysozyme, BPI) of the scallop Argopecten purpuratus in Western blot and Laser confocal microscopy techniques, detecting them in hemocytes and tissues of non-stimulated and challenged scallop with Vibrio sp. bacteria.

Currently performing experiments to discover new antimicrobial peptides (AMPs) using a combination of RNA-seq and bioinformatic tools to characterize them.

I am very energetic person, capable of lead and work in multidisciplinary teams, and able to develop new projects related to the use of AMPs against pathogenic bacteria in aquaculture.

Antimicrobial peptides (AMPs) are efficient innate immune components. Big defensins is a family of AMPs found in a restricted number of animal phyla, in particular mollusks. Big defensins are composed of a hydrophobic N-terminal region and a C-terminal β -defensin-like region, stabilized by three disulfide bridges. They have been shown to be active against Gram-positive, Gram-negative bacteria and fungi. Antimicrobial aggregates called nanonets entrapping bacteria have been recently described as the mechanism of action of the Cg-BigDef1 from the oyster Crassostrea gigas. Specifically, the N-terminal domain of Cg-BigDef1 was responsible of nanonet formation. To determine whether nanonets are specific to oyster Cg-BigDef1 or common to other big defensins, we assessed the potential entrapping of bacteria through nanonets of the big defensin from the scallop Argopecten purpuratus, namely ApBD1. Recombinant ApBD1 was produced as a fusion polypeptide with a N-terminal His6 tag, a thrombin cleavage site before the mature peptide sequence and a C-terminal domain with the last Cys mutated to Arg. Results showed that rApBD1 is microbicidal against the gram-positive bacteria Staphylococcus aureus SG511. Moreover, rApBD1 inhibited S. aureus growth and strong immune staining of rApBD1 in numerous areas surrounding bacteria was observed by confocal microscopy. Overall, results showed that rApBD1 entrap bacteria in peptide aggregates like Cg-BigDef1 and suggest the conservation of nanonet formation across big defensins. These results are a contribution to the understanding of the immune physiology of the scallop and provide new basis for application of AMPs to boost immunity of species in culture. FONDECYT 1200129.

In silico identification and functional characterization of ApGKR, a new antimicrobial peptide expressed by hemocytes from the scallop *Argopecten purpuratus*

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Biography:

I am Felipe Stambuk Opazo, Biochemist, student of Master of Microbiological Science of the PUCV, Chile. Part of Grupo de Marcadores Inmunológicos de Organismos Acuáticos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Chile. I have worked in multiple investigation projects as Technician

(FIE 201708070149, FONDECYT 1170118, 2018-2021; FONDECYT 1200129, 2021-current), purifying and using secondary antibodies against innate immune effectors (Big Defensin, Lysozyme, BPI) of the scallop Argopecten purpuratus in Western blot and Laser confocal microscopy techniques, detecting them in hemocytes and tissues of non-stimulated and challenged scallop with Vibrio sp. bacteria.

Currently performing experiments to discover new antimicrobial peptides (AMPs) using a combination of RNA-seq and bioinformatic tools to characterize them.

I am very energetic person, capable of lead and work in multidisciplinary teams, and able to develop new projects related to the use of AMPs against pathogenic bacteria in aquaculture.

Antimicrobial peptides (AMPs) are effector molecules present in all multicellular organisms. AMPs play a critical role in defense against pathogens and great efforts have been made to understand their role in mollusk physiology and immune capacity. The scallop Argopecten purpuratus is an important reared species in Chile which is affected by pathogenic infections, but only one AMP has been described so far. Thus, we aimed to identify new A. purpuratus AMPs by in silico approaches. For this, contigs obtained from a de novo assembly of the hemocyte transcriptome were analyzed searching for common physicochemical features for AMPs such as: the presence of a signal peptide, a molecular weight below 10 kDa, a positive net charge > 4, an isoelectric point between 8 and 12, and hydrophobicity > 30%. Next, selected sequences were examined using three AMP databases to predict putative AMPs (CAMPR3-APD3-ADAM and dbAMP). Out of five putative AMPs obtained by this approach, one candidate, named ApGKR, (for glycine/lysine rich peptide) was chosen to be chemically synthesized by Fmoc strategy. ApGKR is a peptide of 4.52 kDa which displays a net charge of +12. It adopts an alpha-helix secondary structure, as determined by circular dichroism and 3D modeling. ApGKR is active against both Gram-positive and Gram-negative bacteria, showing 100% bactericidal effect against 1x105 c.f.u. of Staphyloccocus aureus SG-511 and Escherichia coli ML-35 when tested at 5 μ M. Results support the notion that in silico approaches based on transcriptomic data mining for physicochemical features shared by effector molecules is an effective strategy to identify AMPs. FONDECYT 1150009 and 1200129.

Fast and Slow Growing Teleost Species Show Differences in Muscle Fibre Growth and Connective Tissue Content.

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¹Department of Pharmacology and Toxicology, University of Otago, Dunedin, New Zealand, ²The New Zealand Institute for Plant and Food Research, Nelson, New Zealand

Biography:

I completed my undergraduate degree at the University of Otago in Neuroscience and Pharmacology and then my honours degree in Pharmacology focusing on spinal cord injury. Now I am in my second year of my PhD studying the growth and intramuscular connective tissue of fish skeletal muscle. Though the topic of my PhD research differs from my other degrees, the cellular and tissue physiology and the techniques I have learnt are very translatable. The research I am doing for my PhD is more aligned with my interests and priorities and I feel it has useful applications.

Fish demonstrate both hyperplastic and hypertrophic mechanisms of skeletal muscle growth. Studies across a range of species have highlighted differences in patterns of muscle growth, however, these patterns have not been directly compared between fast- and slow-growing fish species. In addition, the role intramuscular connective tissue has in fish muscle growth is not understood. This study aimed to investigate patterns of muscle growth, and changes in the extracellular matrix of two species relevant to New Zealand fisheries and aquaculture: Australasian snapper (Chrysophyrus auratus) and Chinook salmon (Oncorhynchus tshawytscha). Snapper and salmon were studied as slow- and fast-growing species, respectively. The prevalence of hyperplastic and hypertrophic growth was assessed across different age groups using histological analysis. The abundance and localisation of collagen and proteoglycans were also examined using histology. To validate histological findings the expression of specific myogenic regulatory factors and connective tissue components are being assessed using quantitative PCR. Snapper showed a shift from hyperplasia to hypertrophy with age while salmon demonstrated maintained hyperplasia. Furthermore, increases in both collagen and proteoglycans with age were observed in snapper. Salmon demonstrated an increase in collagen with age though proteoglycans were seen to peak at an earlier age, then decrease and were maintained at lower levels. This study demonstrates differences in extracellular matrix components along with distinct patterns of muscle growth when comparing fast- and slow-growing fish species. Characterisation of skeletal muscle growth mechanisms can aid the optimisation of aquaculture practices and provide useful information about the natural growth of these fish species.

Physiological, behavioural and metabolomic response of Chinook salmon (*Oncorhynchus tshawytscha*) to tryptophan supplementation

Mr David Donald Stephens^{1,2}, Dr Mark Burdass², Dr Tim Young^{1,3}, Prof Andrea C Alfaro¹

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Biography:

David Stephens is a master's student in the School of Science, at the Auckland University of Technology. His research interests focus on sustainable aquaculture, aquaculture nutrition, invasive marine species and marine ecosystem restoration.

Increased allostatic load due to repeated or chronic stress in fish is known to have negative physiological responses, including reduced development, growth, disease resistance, reproductive capacity, and changes in behaviour. The use of feed additives within aquaculture diets can be beneficial in controlling physiological stress responses to handling processes, thus improving fish welfare. Tryptophan is an essential amino acid significantly affecting the regulation of teleost species' response to stress. As yet, behavioural and physiological effects of supplementing tryptophan levels within diets for Chinook Salmon, *Oncorhynchus tshawytscha*, subjected to stressful procedures are unascertained. It is hypothesised that a functional diet containing raised tryptophan levels will mitigate adverse behavioural and physiological effects associated with these procedures. Research is currently being conducted to determine whether increasing levels of tryptophan in *O. tshawytscha* diet reduces stress-related anorexia and size heterogeneity, and improves growth. We are also interested in knowing whether this modifies and improves fish husbandry outcomes, and in understanding the biochemical mechanisms of action.

Development of Bio-composite Materials from Seaweed Polysaccharides for Environmentally-friendly Food Packaging Product Innovations

<u>Mr Peng Su¹</u>, Mrs Niki Sperou¹, Dr Reinu Abraham¹, Ms Wanling Cai¹, Professor Yonghong Tang¹, Dr Wenjin Xing¹, Professor Wei Zhang¹

¹Flinders University, Health Sciences Building, Registry Road, Bedford Park, Australia

Biography:

In my current position, I am responsible for international and domestic project management and development, as well as staff and student supervision in the Flinders Centre for Marine Bioproducts Development (CMBD). With years of experience in end-to-end project management and program coordination my specialties are focused on high-level communication skills with proven ability to influence and bringing all parties, internally and externally, together to achieve successful outcomes; stakeholder engagement with a demonstrated capacity to engage a variety of diverse groups; acute attention to detail, strong time management, planning and organization skills. I am specialized in algal (microalgae and macroalgae) bioprocessing optimization, with a focus on advanced extraction technologies and high-value bioproducts development.

By 2050, plastic waste is expected to increase toward 12 Gt. Of current plastic waste (6.3 Gt), only 21% is recycled or incinerated, whereas the remaining 79% enters landfills or the environment. Many plastics take over 100 years to fully degrade, highlighting the need to reduce or replace them with degradable bioplastics. Despite the urgency for sustainable materials, the current use of biopolymers is limited. Seaweed shows great potential for renewable biopolymer production. A wide range of polymers derived from seaweeds such as alginates, carrageenan, and agar are proposed for product applications. Other useful polymers remain underutilized (i.e. fucoidan, ulvan). Seaweed biopolymers have diverse applications due to their unique film-forming ability and excellent mechanical properties. Southern Australia is a hotspot for seaweeds and has pathways available for sustainable commercial production. This region contains amongst the highest level of recorded macroalgal diversity and endemism in the world, with approximately 1,200 described species, 62 % of which are considered endemic. The seaweed industry of Southern Australia is largely limited to beach-cast harvest, utilized for lower-value commodities such as fertilizer and animal feed. This study focused upon novel seaweed biopolymer bio-composite materials for environmentally friendly food packaging products. Various novel bio-composite biofilm materials were developed from seaweed polysaccharide matrices with a glycerol plasticizer. Materials were tested via SEM, density, thickness, water solubility, and tensile, analysis methods. The results demonstrate the feasibility of developing innovative and environmental-friendly packaging using seaweed polysaccharide-derived bio-composite materials. These products demonstrate the potential to grow the future seaweed industry in Australia.

Keywords: Seaweed, Alginate, Agar, Carraganeen, Bioplastic, Bio-film, Packaging

Understanding N_2O synthesis in *Chlamydomonas reinhardtii* to evaluate the microalgal contribution to N_2O emissions from eutrophic lakes.

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Biography:

Laura obtained her Diploma in Agronomic Engineering (master degree equivalent) specialized in Green Chemistry and Bioprocess at Montpellier SupAgro (France). During her master studies, she went on an Erasmus exchange at the University College of Cork (Ireland) and took a Gap Year during which she got the opportunity to do an internship at the School of Engineering and Advanced Technology at Massey University under the supervision of Dr Maxence Plouviez and Prof. Benoit Guieysse. After this enriching experience, she decided to pursue a PhD in New Zealand with the same supervisors to study nitrous oxide emissions from eutrophic lakes.

The ability of microalgae to synthesize nitrous oxide (N₂O) has broad ecological implications. Critically, unravelling the pathways and the conditions triggering N_2O production by microalgae is needed to improve the methodology used to investigate N₂O emissions from microalgae-rich ecosystems (e.g. eutrophic lakes). Several synthesis pathways have been discussed in the literature. Recently, flavodiiron proteins (FLV), which are associated to the photoprotection of the photosystem I (PSI) during light fluctuation, and the nitric oxide reductase (NOR) CYP55, which catalyses the reduction of nitric oxide to N₂O and is similar to the fungal gene CYP55 involved in denitrification, were proposed as N₂O sources in the microalgae Chlamydomonas reinhardtii. However, the physiological relevance of N₂O synthesis and the environmental conditions that promote its synthesis are barely known. During this work, batch assays using wild type axenic cultures of C. reinhardtii supplied with nitrite (NO₂⁻) under aerobic conditions have been carried out under constant illumination and temperature to investigate the implication of different environmental factors that could affect N₂O synthesis by C. reinhardtii. Further inhibition assays were carried out using DCMU to block the electron flow from the photosystem II. The results showed that the wildtype was producing up to $31.5 \pm$ 10.7 µmole N₂O·gDCW⁻¹ over 24 hours, and that this production was slowed when the electron flow from the photosystem II was blocked. These preliminary results suggest that the electron transport chain is involved in N_2O production in *Chlamydomonas*. However, there is a high variability in the observed N_2O productions, and it is necessary to understand the cause of this variability to propose accurate methodologies for computing N₂O emissions from aquatic ecosystems

Transcriptomic and behavioural responses of a cephalopod to elevated CO2

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Biography:

Jodi is a PhD candidate at James Cook University, Australia, supervised by Prof. Philip Munday and Dr. Sue-Ann Watson. She is investigating the neurobiological mechanisms through which elevated CO2 affects marine invertebrate behaviours, focusing on squid. Jodi completed her Bachelor of Science (Honours) in Neuroscience at the University of Otago, New Zealand. Her honours project, and following work as a research assistant, focused on the neuroendocrine regulatory and molecular mechanisms underlying female-to-male sex change in sequentially hermaphroditic fish. Jodi is interested in the intersection of neuroscience and zoology, and the role of the brain in behaviour and phenotypic plasticity.

Projected future carbon dioxide (CO₂) levels in the ocean can alter marine animal behaviour. Most mechanistic studies exploring CO2-induced behavioural alterations have occurred in fish and focused on disrupted GABA-A receptor functioning. Recent research suggests the GABA hypothesis also applies to marine invertebrates, although CO_2 could induce behavioural alterations through multiple mechanisms. Here, we exposed two-toned pygmy squid (Idiosepius pygmaeus) to ambient (~450 µatm) or elevated $(\sim 1,000 \,\mu atm) \, CO_2$ levels for seven days followed by behavioural measurements upon mirror exposure. Conspecific-directed attraction and aggression, and activity levels, were increased at elevated compared to ambient CO₂. RNA-sequencing data from the central nervous system (CNS) and eyes was mapped against a de novo transcriptome, assembled from PacBio long-read ISO-seq data. A small number of genes were differentially expressed between CO₂ levels, and some genes were differentially expressed in both tissues. Gene set enrichment analysis using gene ontology (GO) terms identified upregulation of multiple translation-related GO terms in both tissues, and multiple mRNA splicing-related GO terms in the CNS, at elevated CO₂. Thus, despite only a small number of differentially expressed genes, squid may respond to elevated CO₂ with altered protein expression. Multiple ion channel-related GO terms were significantly downregulated in the CNS but upregulated in the eyes, adding to the growing evidence for the GABA hypothesis in marine invertebrates. This finding also agrees with our previous pharmacological research that supports the GABA hypothesis, and suggests altered functioning of similar ligand-gated ion channels may also underlie CO₂-induced behavioural changes, in the two-toned pygmy squid.

Failure of bivalve foundation species recruitment in a context of extreme heat wave event

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Biography:

Réjean Tremblay is professor in aquaculture at the Institut des sciences de la mer (Université du Québec à Rimouski) since 1999. He is involved in several research projects in ecophysiology and shellfish aquaculture and is director of the French canadian research network on aquatic species exploitation, RAQ.

Bivalves are important regulator of coastal lagoons providing a wide range of ecosystem services, but these environments are very sensitive to climate change. Here, we present the ecological cascade of an extreme heat wave until the recruitment failure of a bivalve foundation species, the oyster *Crassostrea gigas*. Results show evidence that high salinity and temperature modified largely the planktonic community with a shift on small-sized taxa. These trophic changes had no impact on food accumulation by oyster larvae, but act on the metamorphosis process where the development of gills by young juveniles could not be adapted to these small particles. The result is a recruitment failure of oysters and the stimulation of annelid development, a trophic and space competitor, more adapted for the ingestion of small particles. This new knowledge prove that the ecological limits of oyster larvae are narrower than the physiological limits in this context of marine heat waves.

Heat stress response in abalone

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Biography:

Dr. Thomas (Thao) Nguyen is a marine scientist with inter-disciplinary research interests in marine shellfish and aquaculture. His research focuses on understanding the complex interactions between marine shellfish, pathogens and their environment using metabolomics approaches integrated with molecular biological tools. He also has interests in natural antimicrobial metabolites and metabolite biomarkers for stress and disease assessment in shellfish.

Temperature is one of the most important factors that affect the physiology of aquatic animals. Elevated temperatures during summer months have been associated with high mortalities (summer mortality events) in many shellfish species worldwide, including abalone. Despite this, our understanding of the molecular mechanisms of thermal stress and heat shock proteins (HSPs) in thermal stress regulation in abalone remain limited. This study aims to use an integrated approach that combines multiple metabolomics platforms and molecular tools to characterize the metabolic responses of abalone to thermal stress and expression of HSPs in abalone. We observed alterations of many metabolites (e.g., amino acids, fatty acids, organic compounds) and different expressions of many HSP genes in abalone tissues between different temperature conditions. These changes at both metabolic and gene expression levels revealed the correlation between HSP genes and associated metabolites. These findings provide many new insights into heat stress responses in abalone which may be valuable information for development of heat resilient abalone to mitigate global warning threats to wild and cultured populations.

Metabolic differences between stunted and non-stunted abalone in the Chatham Islands, New Zealand

<u>Dr Thao Nguyen Van¹</u>, Prof Andrea C Alfaro¹, Dr Leonie Venter¹, Dr Jessica A Ericson², Dr Norman L.C Ragg², Dr Craig Mundy³

¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ²Cawthron Institute, Nelson, New Zealand, ³IMAS Fisheries and Aquaculture Centre, College of Science and Engineering, University of Tasmania, Taroona, Australia

Biography:

Dr. Thomas Nguyen is a marine scientist with inter-disciplinary research interests in marine shellfish and aquaculture. His research focuses on understanding the complex interactions between marine shellfish, pathogens and their environment using metabolomics approaches integrated with molecular biological tools. He also has interests in natural antimicrobial metabolites and metabolite biomarkers for stress and disease assessment in shellfish.

Abalone fisheries in the Chatham Islands account for 34% of the total wild-caught production in New Zealand, but these stocks have experienced declining biomass over the past 30 years, resulting in significant financial consequences for the local economy. Stunning of abalone, or reduced growth, is thought to be one of the reasons for this decline. Despite this, not much information is known about this phenomenon at the molecular level. This study was conducted to identify metabolite profiles indicative of physiological processes associated with stunting in New Zealand abalone populations. The metabolite profiles of abalone haemolymph and muscle tissues revealed significant differences in many metabolites between stunted and non-stunted adult abalone. However, no significant differences were observed between the two populations in juveniles. For adults, most of the differences occurred in hemolymph, which mainly include the higher levels of many amino acids, fatty acids and other organic acids in non-stunted abalone compared to that of stunted individuals. These differences may be due to differences in food and environmental conditions between these two sites. The higher levels of citric acid cycle intermediates and lactic acids in non-stunted abalone suggest the enhancement of both aerobic and anaerobic metabolism, which was hypothesized to be a consequence of high energy demands for faster growth in non-stunted abalone compared to stunted abalone. It is envisaged that the findings from this study can inform efforts to rebuild abalone biomass and aid fishery management strategies in Chatham Islands and other areas in New Zealand, and potentially overseas.

POSTER Establishing sampling confidence parameters for Greenshell[™] mussels (*Perna canaliculus*)

Dr Leonie Venter¹, Dr Tim Young^{1,2}, Prof Andrea C Alfaro¹, Prof Jeremie Zander Lindeque³

¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ²Centre for Biomedical & Chemical Sciences, School of Science, Auckland University of Technology, CBD, New Zealand, ³Human Metabolomics, North West University, Potchefstroom Campus, Pothefstroom, South Africa

Biography:

I am a post-doctoral research fellow working in the weird and wonderful world of aquaculture, striving to serve and advance the New Zealand's growing Aquaculture industry. My current research involves the application of multi-omics tools and physiological assessments to investigate aquatic animals like, mussels, pāua, salmon, geoducks and whitebait. The application of metabolomics to research questions concerning energy-driven metabolism processes remains one of my special interests. I am also fascinated by methodical processes aimed at solving analytical and technical problems, and believe that laboratory-based tools should be applied to other industries.

Information regarding the variability of mussel metabolite levels and haemocyte quality over time is required to estimate the reproducibility of methods commonly used to investigate shellfish health. Also, metabolism-quenching is a crucial step to ensure reflection of adequate metabolome composition at the time of sample collection. Considering the status of Perna canaliculus as a lucrative aquaculture product and as a research model, the effects of different transportation conditions and quenching methods on the haemolymph metabolome was investigated using gas chromatography-mass spectrometry analyses. Haemocyte viability was also measured as a consequence of transportation stress via flow cytometry. Mussels submersed in water during transport showed smaller changes in haemocyte numbers compared to mussels transported in aerated boxes, which showed a significant decrease in haemocyte viability over 24hours of transport. Significant changes in energy metabolism were inferred following transport in both holding systems over time, highlighting a shift towards anaerobic energy production. The metabolism quenching of haemolymph samples devoted to metabolomics analysis were assessed following freezing in liquid nitrogen, dry-ice-ethanol-slurry, dry ice and standard ice. Sampling on-site followed by metabolite quenching with liquid nitrogen should be considered as the optimal quenching method. However, the use of dry-ice-ethanol-slurry or dry ice alone still ensures reliable samples for metabolomics analysis and provides an alternative method, particularly when sampling in the field where liquid nitrogen may be inaccessible. Resultantly, it is suggested to minimise transport time of mussels, while using water as a transport medium where possible, and quenching samples based on metabolites of interest.

Exploring the temperature-stress metabolome of Perna canaliculus

<u>Dr Leonie Venter</u>¹, Dr Jessica A. Ericson², Dr Natalí J. Delorme², Dr Norman LC Ragg², Prof Andrea C Alfaro¹ ¹Aquaculture Biotechnology Research Group, Department of Applied Ecology, School of Science, Auckland University of Technology, CBD, New Zealand, ²Cawthron Institute, Nelson, New Zealand

Biography:

I am a post-doctoral research fellow working in the weird and wonderful world of aquaculture, striving to serve and advance the New Zealand's growing Aquaculture industry. My current research involves the application of multi-omics tools and physiological assessments to investigate aquatic animals like, mussels, pāua, salmon, geoducks and whitebait. The application of metabolomics to research questions concerning energy-driven metabolism processes remains one of my special interests. I am also fascinated by methodical processes aimed at solving analytical and technical problems and believe that laboratory-based tools should be applied to other industries.

New Zealand aquaculture has grown into a significant primary industry, with the Greenshell[™] mussel (Perna canaliculus) sector being the largest in terms of value and production quantity. Extreme events such as heat waves have increased in frequency over the last decades and are responsible for mass mortalities in mussels, threatening economic and ecological security. Understanding the effects of temperature on mussel physiology can be complex, but it is critical to understand the mechanisms that drive such events. In particular metabolome studies targeting small molecules, identify unique and dynamic metabolic changes within an organism in response to a certain stimulus. By exploring the response of the mussel metabolome to temperature stress, we sought to identify metabolic pathways activated during these events. In the first experiment, mussels were exposed to acute heat stress for 60 min at 30°C. In the second experiment, a chronic exposure was implemented by housing mussels at 17, 21 and 24°C for 14 months. Using gas chromatography mass spectrometry, both haemolymph and gill samples were analysed with main energy producing metabolic pathways mapped out and assessed. In broad terms, glycolysis, the Krebs cycle, the electron transport chain, the urea cycle, beta-oxidation and the methionine cycle were altered in the severely heat stressed mussels. In addition, lipids and fatty acids were largely affected following long-term housing at increased temperatures. From this research, various hypotheses concerning pathways associated with thermal stress in mussels were generated, and they form the foundation for future work aimed at host metabolic responses.

Dietary exposure of Pacific oyster (*Crassostrea gigas*) larvae to compromised microalgae results in impaired fitness and microbiome shift.

<u>Julien Vignier</u>¹, Olivier Laroche¹, Anne Rolton¹, Pandora Wadsworth, Karthiga Kumanan¹, Xavier Pochon¹, Nick King¹

¹Cawthron Institute, Nelson, New Zealand

Biography:

I am a shellfish biologist and hatchery specialist. My research focuses on the ecophysiology of bivalve larvae as a whole. More specifically, my work looks at improving the reliability of hatchery spat production using selective breeding, disease-resilient stock, polyploidy, or cryopreservation. I am also interested in integrating early life stages of bivalve as a model to evaluate environmental stressors.

The Pacific oyster *Crassostrea gigas* is the world's most cultivated oyster and seed supply is heavily reliant on hatchery production where recurring mass mortality events are a major constraint. Outbreaks of bacterial infection via microalgal feed are frequently implicated in these mortalities. This study assessed the effects of feeding compromised microalgae to developing oyster larvae. Intentionally 'stressed' (high pH) or non-stressed microalgae were fed to umbo oyster larvae at two feeding rations for 96h, followed by a recovery period. Biological endpoints of larval fitness were measured following the 96h exposure and subsequent recovery. Bacterial communities associated with the microalgae feed, rearing seawater, and the oyster larvae, were characterized and correlated with effects on oyster fitness. Feeding stressed algae to oyster larvae for 96h increased the occurrence of deformities, reduced feeding and swimming ability, and slowed development. Following the recovery period, fewer larvae reached pediveliger stage (2.7% vs 36% in control) and became spat (1.5% vs 6.6% in control). The quantity of stressed algae supplied to larvae also influenced overall larval fitness, with high feeding rations generally causing greater impairment than low rations. Bacterial profiling using 16S rRNA showed that most bacterial families characterized in larval tissue were also present in larval rearing seawater and in the microalgae (98%). The rearing seawater showed the highest bacterial richness compared to the larval and the microalgal compartments, regardless of feeding regime. In larval tissue, bacterial richness was highest in stressed and high-feed treatments, and negatively correlated with larval fitness. These results suggest significant dysbiosis induced by compromised feed and/or increased feed ration. Several bacterial genera were strongly associated with impaired larval fitness while the presence of genera in larvae including Vibrio was closely associated with overfeeding. This is the first time metabarcoding has been used to identify microbiota features associated with larval fitness.

Green shell summer mortality (GSM SM)

Dr Stephen Webb¹

¹Cawthron Institute, New Zealand

In recent years, farm stocks of Perna canaliculus, the New Zealand green-lipped mussel, or Greenshell™ mussel (GSM) have suffered unexplained mortalities in Marlborough and Coromandel growing areas. A review of the national and international literature on bivalve mortality revealed many assertions of multifactorial causation, including bacteria such as Vibrio spp. accompanied by environmental components such as elevated temperature and physiological components such as spawning stress. Here in NZ, the problem is poorly understood - research on New Zealand GSM summer mortality (GSM SM) has begun in earnest only recently. The literature provided a range of standard diagnostic histopathological features and conditions for investigating bivalves. These included condition criteria for cells, organs, and tissues such as digestive tubule thickness and presence of ceroid. Histopathology of Marlborough spat/juveniles revealed no disease organisms associated with GSM SM, and pathology was obscure. Further investigation showed that GSM SM was associated with changes in the relative proportions of haemocytes, storage cells and fibrous connective tissue cells in the mantle. These, and other features, helped distinguish healthy mussels from moribund and also those affected with GSM SM. Strategies, suggested, from the literature to manage/mitigate GSM SM may include use of probiotics, selective breeding, hardening by prior environmental exposure, precautionary movement of stock, new surveillance methods and exploitation of bacterial quorum disruption.

Physiological and behavioural responses of green lipped mussels (*Perna canaliculus*) to intermittent aerial exposure

<u>Miss Mena Welford^{1,3}</u>, Dr Natalí Delorme¹, Dr Norman Ragg¹, Dr Brendon Dunphy³, Dr Nicholas Tuckey², Dr Leonardo Zamora¹

¹Cawthron Institute, , New Zealand, ²The New Zealand Institute for Plant & Food Research Ltd, , New Zealand, ³School of Biological Sciences, University of Auckland, , New Zealand

Biography:

Rooted in countless childhood adventures along the beach, is a life-long love for our oceans and its exquisite diversity. Presently it comes as little surprise then, my broad research interests encompass climate change, ecological physiology, and sustainable aquaculture. Pursuing a MSc at the University of Auckland in collaboration with Cawthron Institute, my thesis work explores the thermotolerance of selectively bred green lipped mussels (Perna canaliculus) and Pacific oysters (Crassostrea gigas) - heavyweights of the New Zealand aquaculture industry. Particularly intrigued by how integrated analyses of stress, conducted across discrete levels of biological organisation can assist refinement of stress tolerance mechanisms; through this experience I intend on equally developing and challenging my existing perceptions to maximise potential contributions to this field.

Endemic to New Zealand coasts, green-lipped mussels (Perna canaliculus) are ecologically and economically significant. Distributed both sub- and inter-tidally, a broad suite of physiological tolerances are observed, purposed to combat a multitude of abiotic stressors. Understanding survival mechanisms under such perturbations are therefore crucial not only to inform species-specific responses, but in harnessing resilience for commercial application. Particularly, mapping consequences of aerial exposure on postharvest individuals to improve distant live-transport procedures have become a strong focus for industry. Thus, we investigated behavioural and physiological responses of P. canaliculus during 10 days of prolonged (dry) and intermittent (wet) aerial exposure; the former simulated in complete absence of seawater and the latter divided into four treatments, automated to submerge mussels in re-circulating seawater for 3, 5, 8, and 10 minutes at hourly intervals. Gaping behaviour was checked daily, while haemolymph stress makers of oxygen content, osmolality, pH, and total antioxidant capacity were quantified at trial conclusion. Overall, intermittent aerial exposure, especially with longer submersion periods, seemed to beneficially alter mussel responses, extending survival. Notably through delayed onset of gaping behaviour, modified metabolic activity, and preservation of antioxidant defences. While previous work often assesses behavioural and physiological stress responses in isolation, here we intentionally characterise potential implications of intermittent mussel emersion through integrated analyses, demonstrating the utility of multi-disciplinary insights in refining stress tolerance mechanisms in bivalves.

Shifting dynamics: The energy balance in brain of New Zealand Triplefin fish (Tripterygiidae) under heat stress

<u>Mr Jaime Willis</u>¹, Associate Professor Anthony Hickey¹, Dr Jules Devaux¹ ¹University Of Auckland, Auckland, New Zealand

Biography:

Completed my Masters in 2018 at the University of Auckland focusing on the impacts of acute thermal stress on mitochondrial energetics of triplefin brains.

Continuing on to a PhD with Associate Professor Tony Hickey, looking at the metabolism of several Decapod crustaceans during live transport and the potential for inducing metabolic depression.

Temperature impacts all levels of organization. Shifts of only a few degrees above thermal optima detrimentally impact growth, reproduction and survival. Poikilothermic species are especially prone to temperature shifts as their body temperature closely matches that of the environment. Metabolic rates are sensitive to temperature and for organisms exposed to high acute temperatures, in particular for intertidal ectotherms. Previous investigations looking at acute heat stress implicate mitochondrial dysfunction in thermal tolerance and it is argued that the heart sets the upper thermal limits for species. The brain is, by weight, one of the most metabolically active and arguably also a very temperature sensitive organ. It is highly aerobic and as a result almost entirely reliant on oxidative phosphorylation (OXPHOS) to meet energetic demands. As temperature rises mitochondria become less efficient at synthesising adequate amounts of ATP to meet the ever-increasing demands leading to an energetic crisis. Using brain homogenates of three closely related triplefin species (Bellapiscis medius, Forsterygion lapillum and Forsterygion varium) respiration and ATP dynamics were assessed at three temperatures (15, 25 and 30°C). It was found that at 30°C all species showed significant decreases in mitochondrial function. While only the intertidal *B. medius* maintained efficient OXPHOS at high temperatures. The balance of energetic supply and demand was disrupted in all three species at 30°C and only B. medius maintained a high P:O ratio at 30°C compared with F. lapillum and F. varium. These results showed that brain mitochondria become less efficient at temperatures below their respective CTmax and may play a significant role in thermal tolerance limits.

A whole new body in only 8 days: Whole-body regeneration in the ascidian, *Botrylloides diegensis*

Ms Rebecca Clarke¹, Dr Michael Meier¹, Prof Miles Lamare¹, <u>Dr Megan Wilson¹</u> ¹University of Otago, Dunedin, New Zealand

Biography:

Megan completed her PhD in the Biochemistry dept (Otago), characterising the structure and function of a sigma factor involved in infection and virulence in the pathogenic bacteria Pseudomonas, before moving to the Institute for Molecular Biosciences, University of Queensland in 2001, working on the molecular genetics of mammalian sex and gonad development. Megan returned to Otago in 2005 as a Research Fellow, studying evolution and development at the University of Otago, using both honeybee and Drosophila models. Since late 2011, she has been a lecturer in the Department of Anatomy at Otago where she established her own research group, the Developmental Biology Laboratory, using both vertebrate (mouse) and invertebrate (ascidian) models to explore how gene expression is regulated during embryonic development and evolution of these pathways. For further information: http://wilsonlab.otago.ac.nz/

Ascidians are filter-feeding marine invertebrates that compose the closest phylogenetic group to the vertebrates. One striking ability is whole-body regeneration (WBR), the restoration of an entire adult body from a small collection of cells. We study this process using the colonial ascidian, *Botrylloides diegensis*. This ascidian lives in colonies of adults (zooids) that share a vascular system. *B. diegensis* can regenerate from only a few hundred blood cells, to form a fully functioning zooid within 8 days. However, if even a zooid fragment remains, the vascular tissue heals but WBR is not initiated. It is currently unknown what triggers regeneration versus a healing-only state.

We have used transcriptome and differential gene expression analysis at early stages post-injury to determine the genes and pathways unique to wound healing and regeneration. A common feature across all the WBR time points was calcium-binding. Intracellular Ca2+ levels coordinate a range of physiological responses such as stem cell differentiation and tissue remodelling. Experiments inhibiting Ca2+ signalling, in both regenerating and wound healing colonies, indicate that it is essential to trigger the regeneration process in tissue fragments. In contrast, an injured non-regenerative colony is still able to heal and continue to grow and asexually reproduce. In addition to gene expression studies, we have developed a method for expression reporter genes to help characterise the cellular origin and molecular processes of WBR.

In summary, a better understanding of the physiological triggers and the molecular differences between regeneration and wound healing will not only give insight into ascidian regeneration but also as to why certain species have lost the ability to regenerate.

Larval Transition from Pelagic to Benthic is Much More than Settlement and Metamorphosis

Dr Robert Paul Wolf¹

¹Cawthron, Nelson, New Zealand, ²Paua education and Recovery project Kaikoura, Kaikoura, New Zealand

Biography:

2012 - Master in Biology (@ University of Rostock, Germany)
Thesis title:
A Taxonomic Investigation into the
Scoloplos armiger (Müller, 1776) Species complex of the White Sea and the North Sea"
2020 - PhD in Marine Biology (@Victoria University of Wellington, New Zealand)
Thesis title:
Ecology and Development of Spirobranchus cariniferus (Gray 1843).
2020 - consultant & technician for abalone hatchery project to recover paua population in Kaikoura
2021 - Assistant taxonomist at Cawthron institute

The shift from a pelagic life stage to a benthic juvenile is a crucial point in the life of many marine organisms. For most marine invertebrates, settlement is closely followed by a metamorphosis when the individual transforms from a pelagic larva to a juvenile with features adapted to benthic life. To better understand these processes, it is necessary to recognise and observe larval development and behaviour until the juvenile individual is formed. However, these stages in the larval ontogeny are not always simple because marine taxa have a variety of primary and secondary larval stages with various specific metamorphosis and settlement processes. For many sessile species has been observed how secondary larvae explore the surface and temporarily attach to the substrate.

Particular for immobile species like *Spirobranchus cariniferus* (Serpulid, Polychaeta) is the process of settlement and metamorphosis complex. Because of the variability, observations made in different artificial environments and through chemically induced settlements, as well as the terminology, are inconsistent, which leads to misinterpretation and false assumptions about settlement and recruitment. The resulting concepts affect our understanding of the spread of invasive species and the management of native sessile invertebrates. Clearer differentiation between metamorphosis and settlement stages is required for the understanding of recruitment. Here I explore the relationship between settlement and larval size and the utility of distinguishing between competence to metamorphose, the metamorphosis from one form to another, and settlement on the example of a New Zealand endemic sessile polychaetae *S. cariniferus*.

What lives in and on the shell of Pāua? Infestation and epibionts on *Haliotis iris* from Kaikoura

Dr Paul Wolf¹

¹Pau3 Industry Association Incorporated, Kaikoura, New Zealand

Biography:

(2013) Master's in Biology by thesis: A taxonomic investigation into the Scoloplos armiger (O.F. Muller, 1776) species complex of the White Sea and Northern Sea" - Rostock University (Germany) (2019) PhD in Marine Biology: "Ecology and Development of Spirobranchus cariniferus (Gray, 1843)

The endemic New Zealand blackfoot abalone, *Haliotis iris* (Gmelin 1791), has high cultural and economic importance, being considered a treasured species by Māori and supporting significant commercial and recreational fisheries. Pāua are a slow-growing species, taking three or more years to reach maturity, and five to seven years to reach legal harvest size, and experience high predation and mortality at smaller sizes. Infestation by polychaetes, sponges and other invertebrates as well epibionts on the shell can negatively affect growth, survival and ultimately the market value of pāua. Understanding the role that shell borer and epibionts play in pāua survival and development is essential for the management of this valuable species and fishery.

The 2016 Kaikōura earthquake presented additional challenges for the fishery, resulting in mass-mortality of pāua and an ongoing closure to all commercial and recreational harvest. In this context, it is particularly important to assess survival, reproduction and growth of the recovering populations. With the here presented primary morphological and taxonomical studies we assess the extent and diversity of epibiont infestation in pāua along the affected coastline. We examined 100 shells of >110 mm shell length for all epibiota and potential parasites. The identification of species living in and on

H. iris shells is a first step in evaluating the impact of shell infestation on growth, survival and reproduction. From these preliminary studies, we can continue to explore the effect of particular species on ontogeny and growth of pāua. Our findings will likely be relevant to other commercial New Zealand shellfish, such as oysters, and to other international abalone fisheries.

Passive Migration of Abalone Offspring Through Utilisation of Water Turbulences by Juveniles of *Haliotis iris* (Gmelin, 1791)

Dr Paul Wolf¹

¹Pau3 Industry Association Incorporated, Kaikoura, New Zealand

Biography:

(2013) Master's in Biology by thesis:

A taxonomic investigation into the Scoloplos armiger (O.F. Muller, 1776) species complex of the White Sea and Northern Sea" - Rostock University (Germany)

(2019) PhD in Marine Biology: "Ecology and Development of Spirobranchus cariniferus (Gray, 1843)

The settlement of larvae determines the survival, growth and, later on, the reproductive success of juvenile and succeeding adult organisms. This fact is particularly evident for sessile or slow-moving marine invertebrates as they are more or less unable to actively change their substratum post settlement. Therefore, planktotrophic larvae are often able to extend their pelagic stage until a suitable settlement substratum is found.

On the other hand, lecitrophic larvae have only limited time before they have to move into the benthos and settle. Therefore, for slow moving invertebrates like *Haliotis iris* (Gmelin, 1791) seems the fate to be sealed if they settle on an unfavorable substratum. At our facility in Kaikoura (New Zealand), we were able to observe and study passive distribution of *H. iris* juveniles through water turbulences. As these observations only occur at night once the juveniles are active, it is assumed that the offspring of *H. iris* intentionally moves towards a source of perturbation like air bubbles or water currents and "rides" on those. For various mussel species, a secondary migration has been observed in response to trophic cues. We assume similar behavior for *H. iris* and other abalone juveniles in response to unfavorable conditions. As Haliotidae larvae cannot extend their pelagic stage to find suitable substratum, is the ability for secondary migration vital for survival.

For our observations, we used a setup of two tanks. Each tank was divided into a bigger and a smaller section. In the smaller compartments we inserted up to 50 juveniles of H. iris and in the larger placed a cue. In one of the tanks, we directed water turbation beneath the smaller section moving upwards, whereas the other tank had been used as control.

New insights into the sponge microbiomes for untapped marine microbial resource mining

Dr Qi Yang¹, Prof Chris Franco¹, Dr Yue-Fan Song², Prof Wei Zhang¹

¹Centre for Marine Bioproducts Development, College of Medicine and Public Health, Flinders University, , Australia, ²College of Food Science and Engineering, Key Laboratory of Aquatic Product Processing and Utilization of Liaoning Province, Dalian Ocean University, Dalian, China

Biography:

Dr Qi Yang completed PhD in Medical Biotechnology from Flinders University, studying marine sponge microbiome. She mainly focused on developing and validating the methodology to respond the various limitations in the study of sponges, particularly their identification and microbial diversity. In 2017, she continually worked on a Postdoc research project with international collaborators in ETH Zurich, Switzerland and Shanghai Jiao Tong University, China. She uses her expertise in molecular biology and marine biotechnology, with next generation sequencing technology (amplicon-based and metagenomic sequencing) to investigate the microbiomes of different types of sponge cells. Her research focus is to improve the fundamental understanding of sponge extracellular and intracellular symbionts to explore untapped microbial resources and their potential functionalities.

Sponges (phylum Porifera) are enriched by associated microbes that make up to 60% of the tissue volume. An impressive number of novel metabolites have been discovered from the associated microbes, and indeed became the largest sources of marine natural products. However, most of the microbial members have not been explored. The microbiome specificity has also seldom been characterized above the host species or down to the cellular level. Therefore, our study established an advanced microbiome analysis approach and revealed a significantly increased microbial taxa coverage by selecting and validating a combination of region-specific primer sets for 16S rRNA gene. Applying this approach, 33 representative sponge species belonging to 19 families in five orders were analysed, which for the first time demonstrated that the diversity and the structure of sponge microbiomes were highly specific to the phylogeny of the hosts at both order and family levels. 70-87% of the unique class-level OTUs for a particular sponge family or order were unaffiliated and represent a vast resource of untapped microbiota. Moreover, the intracellular microbiomes were uncovered using this reinforced approach in conjunction with a tailored sponge cell purification protocol. It was found that the microbial community of the sponge cell fractions hosted three unique phyla not found in the extracellular and bulk tissue microbiomes. These three phyla counted for 43% out of all the known genera revealed from the sponge bulk tissue, extracellular bacteriaenriched fraction, and sponge cell-enriched fractions. The multi-primer approach developed and validated in our study can be conveniently applied and represents a fundamental change from conventional single primer set amplicon-based microbiome studies to achieve a more comprehensive microbial profile. The superior capacity on uncovering the unaffiliated microbial OTUs allows for a greater potential to discover the taxonomic 'blind spots' within the largely unknown microbial world.

Mapping the metabolome of King salmon: Searching for signals associated with feed efficiency

<u>**Tim Young**</u>, Jane Symonds², Seumas Walker², Matt Miller², Paula Casanovas², Andrea Alfaro¹ ¹Auckland University of Technology, Auckland, New Zealand, ²Cawthron Institute, Nelson, New Zealand

Biography:

Research Fellow interested in developing new tools for assessing fish and shellfish health, and applying integrative omics approaches to understand mechanisms of disease and resilience to adverse environmental conditions. A certain soft spot for invertebrate larval biology.

Mechanisms underpinning certain traits in fish are poorly understood. In this talk I discuss our integrative approach aiming to better understand feed efficiency in a salmonid model (*Oncorhynchus tshawytscha*). Using multi-platform metabolomics, we have established baseline biochemical data and are exploring different trait relationships.

Ocean warming impacts on growth performance, health, and physiology of salmon

Tim Young¹, Ronald Lulijwa^{1,2}, Jane Symonds³, Seumas Walker³, Natali Delorme³, Andrea Alfaro¹ ¹Auckland University of Technology, Auckland, New Zealand, ²National Agricultural Research Organisation, Fort Portal, Uganda, ³Cawthron Institute, Nelson, New Zealand

Biography:

Research Fellow interested in developing new tools for assessing fish and shellfish health, and applying integrative omics approaches to understand mechanisms of disease and resilience to adverse environmental conditions.

Ocean warming and extreme sea surface temperature anomalies are threatening wild and domesticated fish stocks in various regions. Understanding mechanisms for thermotolerance and processes associated with divergent growth performance is key to the future success of aquaculture and fisheries management. Herein, we exposed Chinook salmon (*Oncorhynchus tshawytscha*) to environmentally-relevant water temperatures (19–20°C) approaching their upper physiological limit for three months and sought to identify blood biomarkers associated with thermal stress and resilience. In parallel, blood biochemical associations with growth performance were also investigated. Temperature stress activated leucocyte apoptosis, induced a minor immune response, and influenced blood ion profiles indicative of osmoregulatory perturbation, regardless of how well fish grew. Conversely, fish displaying poor growth performance irrespective of temperature exhibited numerous biomarker shifts including cellular haematology indices and blood clinical chemistries associated with malnutrition and disturbances in energy metabolism, endocrine functioning, immunocompetence, redox status, and osmoregulation. Findings provide insight into mechanisms of stress tolerance and compromised growth potential. Biochemical phenotypes associated with growth performance can be used for marker assisted selection to improve breeding strategies.

Chromatin and gene expression analyses reveal rapid activation of signaling pathways at onset of metamorphosis in the demosponge *Amphimedon queenslandica*

<u>Miss Huifang Yuan¹</u>, Professor Bernard Degnan¹, Professor Sandie Degnan¹ ¹The University of Queensland, Brisbane, Australia

Biography:

I work in the Degnan Marine Genomic Lab. I am studying the molecule mechanism at marine biphasic animals metamorphosis process, and my study system is the demosponge Amphimedon queenslandica. I explored the molecular regulation of metamorphosis in the sponge by combining ATAC-Seq with CEL-Seq2. My study suggests that the connection of cell states at the onset of metamorphosis and the chromatin state that activate metazoan signalling pathways.

A wide range of marine invertebrates have a pelagobenthic life cycle that includes both planktonic larval and benthic juvenile/adult forms. The often rapid transition between these distinct morphological and ecological forms, known as metamorphosis, requires a dramatic change in transcriptional and cell states that is tightly regulated and environmentally induced. Here, we explore the molecular regulation of metamorphosis in the sponge Amphimedon queenslandica by combining transposase-accessible chromatin sequencing (ATAC-Seq) with a sensitive RNA-Seq method, CEL-Seq2. We obtained ATAC- and CEL-Seq data from dissociated cells of individual swimming larvae and settled postlarvae just commencing metamorphosis. As ATAC-Seq identifies open chromatin where transcription factors bind with regulatory promoter and enhancer DNA sequences, this approach allowed us to relate chromatin state to gene expression in individual larvae and postlarvae, and to trace dynamic changes through early metamorphosis. ATAC- and CEL-Seq profiles were highly consistent with each other, and revealed the very dynamic nature of chromatin state and gene expression associated with global cell state transitions occurring at metamorphosis. Specifically, we identified an immediate activation of multiple metazoan signalling pathway genes right at the onset of metamorphosis, including Wnt, FoxO, mTOR, and TGF-β pathways. In contrast, chromatin state and gene expression before and after this transition are more reflective of larval and postlarval homeostasis, characterized by active metabolic pathways. This study suggests that the rapid cell states changes that occur at the onset of metamorphosis in this sponge are regulated by changes in chromatin state that immediately activate metazoan signaling pathways.

Enhancing recovery from aerial exposure: kinetics of oxidative stress biomarkers in the green-lipped mussel (*Perna canaliculus*) exposed to air under different conditions

<u>Dr Leonardo N. Zamora¹</u>, Dr David J. Burritt², Dr Norman L.C. Ragg¹, Dr Natali J. Delorme¹ ¹Cawthron Institute, Nelson, New Zealand, ²University of Otago, Dunedin, New Zealand

Biography:

Marine Biologist, with a background on applied biology and ecology of marine invertebrates (e.g., mollusks and echinoderms). Currently working as a researcher in the aquaculture group at the Cawthron Institute (New Zealand), focused on enabling the development of better husbandry practices and understanding environmental interactions of commercially valuable marine species.

Aerial exposure in marine invertebrates is one of the most stressful conditions, leading to their prompt death depending on the level of stress and their ability to recover from it. The severity of aerial exposure depends on the conditions and the duration of the event, as well as how the individuals respond to it, either behaviourally or physiologically. In this study we evaluated the potential to enhance recovery in seawater after aerial exposure in the green-lipped mussel (*Perna canaliculus*). This was done by restricting gaping behaviour during aerial exposure and quantifying the effect on several oxidative stress biomarkers (lipid peroxidation, protein carbonyls, DNA damage) and enzymatic antioxidants (CAT, SOD, GPOx) at different times during aerial exposure and recovery. Our results suggest that gaping restriction has a positive effect in terms of survival after aerial exposure, however the benefits are time constrained and decreased as aerial exposure time increased before recovery. This was reflected in the changes observed in the physiological biomarkers, with mussels spending seven days out of the water before recovery being unable to cope with the reoxygenation stress, and dying within ten days of recovery regardless of the treatment. This provides further information to understand the mechanisms driving aerial exposure survival and potential for recovery when re-immersed in seawater.

Effects of thermal stress on the mesophotic arborescent antipatharian Antipathella subpinnata (Ellis & Solander, 1786) in the Mediterranean Sea

<u>Tom Zeimes¹</u>, Mathilde Godefroid¹, Dr Charlène Guillaumot¹, Margherita Thoma², Dr Marzia Bo², Dr Lorenzo Bramanti³, Pr Philippe Dubois¹

¹Marine Biology Laboratory, Université Libre de Bruxelles, Brussels, Belgium, ²Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università degli Studi di Genova, Genova, Italy, ³CNRS-Sorbonne Université, Laboratoire d'Ecogéochimie des Environnements Benthiques, LECOB, Observatoire Sur-Mer, France

Biography:

Tom Zeimes is a Master's student at the Marine Biology Laboratory at the Université Libre de Bruxelles under the supervision of Pr. Philippe Dubois. His MSc thesis is dedicated to the effects of warming on the black coral species Antipathella subpinnata.

While many studies on the effects of thermal stress on scleractinian corals can be found in the literature, no studies focussing on antipatharians (black corals) are currently available. However, antipatharians often play an important ecological role as they can form high density aggregations hosting a high diversity of species. The aim of this study was to assess the effects of rising ocean temperatures on the mesophotic arborescent antipatharian *Antipathella subpinnata* from the Mediterranean Sea by using two complementary approaches.

First, a Species Distribution Model (SDM) based on presence-only records was performed to predict the distribution of *A. subpinnata* across the Mediterranean Sea and to assess the environmental drivers of its distribution, including temperature.

Then, the physiological effects of increased temperature were assessed experimentally. Fragments of colonies were maintained under six different temperature treatments ranging from current in situ values to temperatures projected for 2100 under RCP8.5 scenario. Tissue necrosis and total fragment mortality were quantified daily allowing to draw survival plots for each treatment. After 15 days of exposure, oxygen consumption rate was measured by respirometry, and the total antioxidant capacity of the fragments was assessed using a colorimetric technique. For both oxygen consumption rate and total antioxidant capacity, data analysis by non-linear regression should allow to identify physiological tipping points of *A. subpinnata*.

Biorefinery production of bioactive ingredients from under-utilized marine bio-resources for functional foods and nutraceuticals

Prof Wei Zhang¹

¹Centre for Marine Bioproducts Development, College of Medicine and Public Health, Flinders University, Adelaide/Bedford Park, Australia

Biography:

Professor Wei Zhang is a world-leading expert in marine bioproducts engineering, industrial and pharmaceutical biotechnology. He has secured over A\$50 million grants in the last 10 years, and recently as the Bid lead secured a total of \$270m Marine Bioproducts Cooperative Research Centre (MBCRC) grant cofunded by Australian government and 68 research and industry partners. He has published over 280 refereed Journal papers. He is an editorial board member of five International Journals, Marine Biotechnology Specialty Chief Editor in Frontiers in Marine Science, and the Editor of Book Series "Advances in Marine Bioprocesses and Bioproducts" published by Springer-Nature. He has won several prestigious awards including South Australia Young Tall Poppy Science Awards. Presently, he is the Director of Centre for Marine Bioproducts Development at Flinders University, the Inaugural President of Australia New Zealand Marine Biotechnology Society, Research Director of MBCRC, Board member and incoming President of Asia-Pacific Marine Biotechnology Society.

The estimated global nutraceuticals market valued at US\$385 billion by 2020 demands the development and supply of potent bioactive ingredients. Underutilized marine bio-resources such as micro-algae, macroalgae and seafood processing by-products are abundant and readily available, which could be used as inexpensive feedstock for the production of many functional food and nutraceutical products. In this presentation, we draw a few case studies conducted in our laboratory to demonstrate that these marine bio-resources are rich in valuable nutritional and bioactive ingredients such as polysaccharides, oligosaccharides, polyphenols, proteins and peptides, PUFAs, and carotenoids. These ingredients have a wide range of applications in multiple products. We have further presented the advances in biorefinery technology for comprehensive utilization of these resources with zero wastes using clean technology solutions in the context of Circular Economy. All these technology advances will not only create economic and environmental benefits for the industry, but also promote the industrial sustainability. However, the majority of these technologies focus on primary processing, advanced secondary and tertiary processing will be critical to modify the primary ingredients to improve functionalities, bioactivities, bioavailability, and stabilities in the future.