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Isotopic analysis of dissolved and gaseous nitrogen and nitrous oxide: new capability for application to marine studies

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Over the last two years we have been developing methods to analyse nitrogen (N_2) and nitrous oxide (N_2O) in water and gas samples to support research investigating nitrogen cycle pathways. A GasBench II linked to a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Germany) enables us to measure gas species concentration, and isotope ratios of $^{15}N/^{14}N$ of N_2, plus $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of N_2O. These methods have been used at natural abundance levels, and on samples containing enriched isotope tracers. We have modified methods in the literature to produce optimal conditions for accurate and precise N_2 and N_2O measurements, and here present details of our analytical set-up and quality control reporting parameters. We also present a case study illustrating how we applied these measurements to investigations aimed at mitigating emissions of N_2O, a highly reactive greenhouse gas. Finally, we discuss how these analyses can be applied to better understand nitrogen processes in the marine environment.

Expanding New Zealand's capability of stable isotope analysis for terrestrial, marine and freshwater applications

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NIWA's recent multi-million dollar stable isotope facility upgrade provides water, gas and solid-matrix sample analyses, delivering highly accurate and precise results. The investment included the acquisition of a GasBench II and Thermo Chemical Elemental Analyser (TCEA) linked to Delta V Plus mass spectrometers. The GasBench II is an on-line preparation system, capable of high throughput analysis of carbon, nitrogen, oxygen and hydrogen isotopes in water and gas samples; whilst the TCEA enables us to measure O and H isotopes in organic, inorganic and water samples. Recent in-house methods developments now provide new applications to the isotope research community. The Gasbench II measures: 2H/H and 18O/16O in water, with applications relevant to climatic, hydrological, oceanic water mass, ecological and animal movement studies; 18O/16O and 13C/12C in carbonates for palaeoceanographic and climate studies; 13C/12C of dissolved inorganic carbon for carbon flux and palaeoclimate research; and 15N/14N of N2, plus 15N/14N and 18O/16O of N2O for nitrogen cycle studies. The TCEA analyses 2H/H and 18O/16O in solid organics, such as plants, soils, feathers, claws, tree-ring cellulose, and chironomid head capsules supporting terrestrial water dynamics, palaeoclimate, climatology, animal migration and ecological research. We will present some examples of these developments and applications.

Precision Farming Technologies for Aquaculture

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Emergence of essential technologies provides New Zealand with an opportunity to become a leading exporter of aquaculture intelligence systems, spanning advanced autonomous sensors, and data analytics, communication and visualisation technologies that can transform ocean farming worldwide. The Precision Farming Technologies for Aquaculture (PFTA) spearhead project, recently launched within the Science for Technological Innovation National Science Challenge, aims to catalyse sustainable growth in production and value of NZ's aquaculture industry. This will be achieved by developing technologies that transform NZ aquaculture from its traditional experience-based mode of operation to one that is high-tech and knowledge-based. The project focuses on three key areas: (1) sensing, recognising farmers cannot manage what they do not measure, (2) communicating, recognising they are unable to 'see' what is happening on their farms in the absence of remote connectivity with on-farm sensors, and (3) enabling, recognising data is only useful if provided through practical tools and accessible formats. The research integrates novel laser spectrometry and imaging sensors, computer vision, machine learning, and advancements in below and above water data communications that will lead to combined production and environmental gains through precision aquaculture.

HEALTH AND DEMOGRAPHICS OF THE NEW ZEALAND FUR SEAL (ARCTOCEPHALUS FORSTERI) IN THE WESTERN BAY OF PLENTY

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The New Zealand Fur Seal (Arctocephalus forsteri) is now recolonising areas of former habitat, with the population continuously rising. Current research indicates multiple new rookeries across the North Island. However, there is a lack of research on the rookeries and population demographics in the Western Bay of Plenty. Sightings since 2013 indicate seasonal variation in population numbers across the Western Bay of Plenty, with more sightings of individuals in colder months and fewer sightings in warmer months. This suggests that this region is more important for 'haul-out' than for breeding. In colder seasons, when population increases have been observed, there appears to be an increase in juvenile mortality rates; it is hypothesized that many of these individuals are dying from starvation.
The aim of this research is to determine seasonal abundance and distribution patterns of Fur Seals as well as examining the health of live juveniles and recently deceased individuals. Necropsies will be performed where possible, with a focus on stomach contents analysis to determine whether individuals are dying from starvation. Consequently, by analysing historic reports of sightings and juvenile mortality rates, combined with current demographic information and health observations, this research will improve the understanding of current populations. Conservation management strategies can then be knowledgeably formulated.

Improving benthic recovery at finfish farms

Olivia Johnston[1]

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Impacts to the seabed at many of New Zealand's finfish farm sites are managed by measuring and comparing enrichment stage (ES) against environmental quality standards. Heavily degraded seabed conditions can necessitate reduced feed inputs and production, and in extreme cases fallowing. Effective fallowing currently takes years resulting in significant production losses. The aim of this research is to develop a semi-commercial scale technique for removing enriched sediments from finfish farms, to enable faster seabed recovery. At a research scale, a previous project (Stage I), identified removal of the top 5-10 cm of enriched sediment at a finfish farm site as a viable solution to mitigate enrichment effects and accelerate seabed recovery. The accelerated seabed recovery was attributed to the physical removal of farm-derived organic matter (faeces and feed waste) from the seabed beneath the farm, promoting successional macrofaunal recolonization and increased bioturbation (resulting in a significantly lower benthic 'ES' score). We propose a larger semi-commercial scale enriched sediment removal study (Stage II), to confirm feasibility and to ensure environmental risks are fully evaluated. If effective, this technology will provide a valuable remediation tool for finfish farms worldwide.

GC-MS-based metabolomics insights into the immune responses of mussel haemocytes during Vibrio sp. infection: toward the identification of immune biomarkers

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Metabolomics, the study of metabolites in biological specimen, has been an emerging field in marine science for the last few years. Due to its high sensitivity, metabolomics is a powerful tool for understanding endogenous metabolic changes of an organism in response to pathogen infections or environmental perturbations, and identification of biomarkers of these conditions. In this study, we used a GC/MS-based metabolomics and flow cytometry approach to characterize metabolic and immunological responses in haemolymph of male and female mussels (Perna canaliculus) experimentally infected with Vibrio sp. Sex-based differences in immunological responses were identified, with male mussels displaying higher mortality, oxidative stress and apoptosis after pathogen exposure. However, central metabolic processes appeared to be similar between sexes at 24 h post-injection with Vibrio sp. DO1. Significant alterations in relative levels of 43 metabolites were detected between infected and non-infected mussels. These metabolites are involved in major perturbations on the host's innate immune system. In addition, there were alternations of seven metabolites in profiles of mussels sampled on the second day and mussels that survived seven days after exposure. Among these, itaconic acid could be considered as an important biomarker for Vibrio sp. DO1 infection. These findings provide new insights into the host-pathogen interactions, and identify some potential biomarkers for future investigations.

Gradients of taxon richness in latitude and depth of marine fish

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Latitudinal and depth distribution data of 5,631 marine fish were collected from the AquaMaps database (www.aquamaps.org/), and classified as pelagic (3,743 species) and demersal fish (1,888 species). For the latitudinal pattern of pelagic fish, the number of taxonomic Classes increased from high to low latitudes at both hemispheres and peaked at 65 N ~ 50 S (i.e. unimodal). The number of Orders also increased from high latitude to low latitudes but peaked at 21 N ~ 45 N, then gradually decreased from 20 N to 90 S, and thus was also unimodal. In contrast, latitudinal patterns in family, genus, species level of pelagic were similar, rising from high to low latitudes with a dip at equator (i.e. bimodal). For demersal fish, latitudinal patterns of class, order, family, genus and species level were similar, values increased from high to low latitudes with a dip at equator area (bimodal). Four layers of depth (euphotic, mesopelagic, bathypelagic, abyssal) were used to calculate the taxon richness with depth zones. The number of Classes of pelagic fish was higher in the mesopelagic and bathypelagic zones than other zones. In contrast, the number of orders, families, genera, and species of pelagic fish, and all taxon levels of demersal fish decreased from shallow to deep zones.

Assessing the biofouling risk of research vessels: a case study

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Up to 87% of non-indigenous marine species in New Zealand have been introduced via biofouling on international vessels; however, this pathway has historically been unmanaged, both by New Zealand and internationally. In May 2018, the Craft Risk Management Standard for Biofouling on Vessels Arriving in New Zealand (CRMS) was implemented, following a four year lead-in period, to manage the biosecurity risk associated with international biofouling. During the lead-in period, the Ministry for Primary Industries (MPI) risk-profiled international vessels, and took action on vessels that posed a severe biofouling risk. While MPI's risk profiling procedures were generally shown to be accurate for commercial, passenger, and fishing vessels, data on the risk presented by "one-off" vessels, such as research vessels, is insufficient for accurate risk profiling. These vessels are generally assessed on a case-by-case basis, and often pose a high biosecurity risk due to their variable operational profiles and itineraries within New Zealand, which are often long and include visits to ecologically sensitive areas. This presentation uses a case study of a severely fouled research vessel to illustrate the risks associated with this vessel type, and the options available for risk mitigation.

A new carrier to deliver probiotics to farmed paua

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For sustained growth of New Zealand paua industry novel approaches such as probiotics supplements are required to enhance growth rate and reduce mortalities of farmed abalone. 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 paua was formulated. The model carrier was made of natural polymers with sphere shape and size range between 2.9 to 3.6 mm in diameter. Optimisation studies was performed to obtain a carrier with desired morphology, polymer concentration, size, sinking rate, matrix rigidity and stability in seawater. Overall, it was shown that the optimum formulation could withstand disintegration in seawater whilst maintaining the activity of the entrapped probiotics and possessed a programmed release of probiotics into the intestine of paua. Evaluation of bacterial load after feeding abalones with encapsulated probiotics demonstrated a significantly higher bacterial content compared to control animals. This implies the efficiency and capability of the developed bioactive carrier to deliver probiotics into the animal's intestine in a controlled manner.

The Little App that Could: Growing protection for critically endangered Hector's dolphins

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Hector's and Māui dolphins are the smallest dolphins in the world, endemic to New Zealand and endangered. There are approximately 63 Māui dolphins left. A few Hector's dolphins are living in the Māui population. With Hector's dolphins at the top of the South Island being geographically closest to Māui, they may be the source of the species exchange but are currently unprotected and little is known about them. We designed an app to facilitate the collection of dolphin sightings to learn more about them.

The Hector's Dolphin Sightings App was recently launched where users can record locations, other data, and upload photos. The app community can see data and report all marine mammal sightings.

Data from the app is shared with DOC to inform management of the species. Data collected so far highlights the presence of these dolphins in several areas where protection is urgently needed. The project is an example of how NGOs, private enterprise, government and communities can work together in a citizen science framework to gather important information.

The app has now doubled official sighting records for the top of the South and has a continually growing user-base.

The effect of marine protection on New Zealand Bryozoa

Hannah Mello[1]

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Marine protected areas (MPAs) are necessary for the conservation and restoration of global marine habitats, as they provide important refugia for exploited species, protect sensitive habitat, and serve as hotspots of biodiversity. Little is known as to how marine protection affects benthic invertebrates, particularly bryozoans, a rich phylum capable of creating three-dimensional habitat that characterizes large areas of the New Zealand seafloor. Historically, these bryozoan beds have been greatly damaged by anthropogenic activities. Eliminating the use of destructive fishing practices through establishing non-extractive MPAs is thought to be an adequate mechanism of bryozoan recovery, as trawling was often cited as the reason for initial decline. While protecting previously damaged ecosystems is important for re-establishing dynamic marine habitats, the effect of marine protection on bryozoans in unknown, as well as whether current marine protection practices are sufficient for reestablishment of biogenic habitats. By comparing historic and modern benthic images, this study will determine whether there have been significant changes in bryozoan abundance, diversity, and growth form within three existing MPAs and one proposed MPA. Not only will these data show whether New Zealand's current marine management strategies, through the design and use of MPAs, are adequate for the maintenance and recovery of New Zealand bryozoans, but also provide a broad survey of modern bryozoan communities spanning New Zealand's three largest islands.

The use of large brown macroalgae in monitoring and as indicators of ecosystem change

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Human-induced modification of the coastal zone is clearly evident in New Zealand at a range of scales. There is need for evidence to evaluate the impact and extent of changes in order to develop appropriate interventions and management initiatives. Large brown canopy-forming species are vital for the ecosystem functions in coastal rocky shore environments globally. To retain healthy systems there is a need to protect and retain intact canopies and the associated communities. Currently there are important knowledge gaps that constrain sound decision making – preventing decision makers from anticipating, and where possible mitigating, changes in the composition of seaweed communities, and accompanying ecosystem-level effects. This project drew on a wide range of approaches: evaluating international and national developments in the field, carrying out culture studies on selected species, analysing monitoring in the East Otago Taiāpure, as well as testing new technologies such as machine learning and AI for analysis of underwater video, and the use of drones. New technologies have very significantly increased the options for more accessible and cost-effective approaches to monitoring. There is a need for a standardised approach to documentation of species distribution data and a national repository of data in agreed forms with associated levels of verification and confidence. Marine biodiversity data also needs to be accompanied with the collection of representative marine biodiversity specimens.

GC-MS based metabolomics and flow cytometric study of the toxic effects of copper on mussel (Perna canaliculus) haemolymph

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Copper is a common contaminant in aquatic environments which may cause immune dysfunction in marine organisms. However, the toxicity mechanisms of copper at a molecular level in marine bivalves is not fully understood. In this study, we applied an integrated approach that combines flow cytometry and Gas Chromatography–Mass Spectrometry (GC-MS)-based metabolomics to characterize cellular and molecular mechanisms of copper immunotoxicology in mussel (Perna canaliculus) haemolymph. Flow cytometric results showed significant increases in haemocyte mortality, production of reactive oxygen species and apoptosis (via alteration of caspase 3/7 and mitochondrial membrane potential) of haemocytes exposed to increasing high concentrations of Cu(II) (1.60, 3.20 and 4.80 µg/ml) compared to a low Cu(II) concentration (0.64 µg/ml) and control (0 µg/ml). These results reveal that Cu(II) induces oxidative stress and apoptosis in mussel haemocytes. In addition to flow cytometric data, our metabolomics results showed alterations of 25 metabolites within the metabolite profile of Cu(II)-exposed haemolymph (3.20 µg/ml) compared to those of control samples. Changes in levels of these metabolites may be considered important signatures of oxidative stress (e.g., glutathione) and apoptosis processes (e.g., alanine, glutamic acid). This study provides insights into the cellular and molecular mechanisms of oxidative stress and apoptosis in marine bivalves and highlights the applicability and reliability of metabolomic techniques for immunotoxicological studies in marine organisms.

The use of machine learning and artificial intelligence to analyse underwater videos and recognise canopy-forming brown algae

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Underwater videography is an effective method for mapping and ground truthing data, although video analysis is very time consuming, even simple tasks such as extracting georeferenced data printed on images is not straightforward, and analyses are not always very accurate (e.g., analysis at fixed intervals vs frames that are randomly selected). We investigated machine learning and computer vision techniques to identify the dominant macroalgal species from videos taken along the Wellington south coast. The trial of machine learning approaches produced very strong results and indicated great future potential, with Ecklonia radiata, Lessonia variegata and Carpophyllum spp., correctly identified from video streams with different levels of confidence. A representative training dataset can be created by labelling images evenly distributed throughout the total dataset, and more diverse training datasets have greater capability at classifying new datasets.
The results of experiments showed that it was possible to get a very good representation with a fraction of the labelled data if the training data was sparsely spread throughout the target dataset, i.e., if only 2% of data is labelled, it is far more useful if every 50th image is labelled as opposed to a 2% section of consecutive images.

The world's largest Marine Protected Area: new technology is needed to track change in the Ross Sea ecosystem

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Large high-seas Marine Protected Areas (MPA) are being established at an increasing rate worldwide, mainly in remote regions, yet many question whether they have any demonstrable benefit. The world's largest MPA was established in the Ross Sea region of Antarctica in 2017, and will only continue beyond 35 years if its conservation value can be demonstrated. This provides a opportunity to study the extent to which high seas MPAs (1) conserve representative ecological structure and function; (2) mitigate threats to ecosystems from fishing; and (3) provide a reference area to better gauge the effects of fishing and climate change.

At over 1.55 million km2 in size, tracking change and evaluating the conservation value of the Ross Sea MPA is a highly complex, technical and unprecedented scientific challenge. The region is enormous, remote, inhospitable, complex (both physically and biologically), spatially heterogeneous, and varies on time-scales of days to decades.

New and innovative sampling technology, advanced remote sensing, and informatics are needed to help address this challenge. But how can technological and big-data developments be applied to observing and understanding key components of the Ross Sea, including the physico-chemical environment, primary producers, microbes, zooplankton, fish, benthic habitats, seabirds and marine mammals?

How robust is data gathered by citizen scientists for monitoring intertidal habitats?

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Citizen scientists are volunteers which aid local resource managers, scientists and governments in the collection of data, often in the field. Information collected via citizen scientists has the potential to be used for making more informed management decisions. Despite the mutual benefits of citizen science, including scientific outreach, development of knowledge and science skills, promotion of guardianship and reduced project costs, there is concern regarding the quality of data collected by volunteers. To investigate the robustness of data collected by citizen scientists, a facilitated project monitoring the potential impacts of dredging on the intertidal area of Otago Harbour was used. Students aged 7-15 collected data on the number of species found (both flora and fauna) and the percentage of substrate cover using transect/quadrat methods at both mid and low tide heights across seven locations in Otago Harbour. Student collected data was then compared to scientist collected data to assess its quality. These comparisons found that students and scientists show similar ability to quantify species presence and abundance. This work has prompted further research into the application of practical science skills to different environmental issues as well as investigating the reliability of estimations of finer substrate material.

Modifications of kelp seasonality and invertebrate diversity where an invasive kelp co-occurs with native mussels

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Non-native species have invaded coastal systems worldwide, altering community structures and ecosystem functioning. One of the most widely distributed marine invaders, is the kelp Undaria pinnatifida. Despite being a large and abundant invader on rocky reefs and mussel farms no studies have examined whether Undaria co-occurs with mussels on rocky reefs or quantified ecological implications of Undaria-mussel interactions. Here, we tested whether Undaria and mussels co-occurred on rocky reefs at different temporal and spatial scales, and whether Undaria affects the diversity of mussel-associated small mobile invertebrates. Analyses of survey data showed that individuals of Undaria are often attached to, or interspersed around, mussel aggregations in the low intertidal zone where Undaria was found at comparable abundances in its typical winter growth and summer senescence seasons. We suggest that this pattern is caused by less synchronous overlapping generations coupled with longer growing seasons, triggered by localized higher wave action, rather than individual plants persisting for an entire year. Analyses of Undaria holdfasts and mimics of Undaria holdfasts showed that kelp holdfasts alter invertebrate communities and increase small-scale diversity where Undaria co-occurs with mussels. We conclude that Undaria has a longer temporal presence on wave exposed reefs where it co-occurs with mussels and that Undaria alters mussel-associated invertebrate communities.

The ultimate environmental gradient: Patterns in algal community structure across the intertidal/subtidal interface

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Predicting and understanding community patterns (e.g. trends in biomass and biodiversity) is a core component in ecology. Despite this, studies exploring how algal community structure responds across the intertidal/subtidal interface, perhaps the ultimate environmental transition, are rare. Standing algal biomass and richness were measure across five strata from the high intertidal (1.5 m above MLW) down to depths 10 m below MLW on six representative rocky reefs in southern New Zealand. This is one of the first studies to describe a unimodal pattern between algal richness and biomass across the span of the intertidal/subtidal interface, where maximum species richness occurred at intermediate levels of biomass. These results are consistent with terrestrial plant studies across strong environmental gradients. Biomass and richness patterns varied significantly between depths examined and likely to be consequences of available Photosynthetically Active Radiation (PAR) and contrasting stability of environmental conditions. In addition, one species accounted for more than 60% of total biomass across all depth strata examined. Although one species dominating biomass is common across a range of differing systems, very rarely is this proportion quantified. The strong environmental gradients over relatively small distances of coastal rock reefs provide excellent opportunities to to further advance our understanding of the mechanisms controlling these important, but often poorly described, patterns in ecological systems.

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