A. TITLE OF PROJECT

4.1.1 Ecosystem Connectivity: Tracking biochemical fluxes to inform Ecosystem Based Management

B. IDENTIFICATION

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C. ABSTRACT

A fundamental requirement of Ecosystem-Based Management is to understand the critical connections within ecosystems that drive responses to environmental change. Resolving connectivity involves quantifying physical transport (as in *Stressor Footprints* - 4.2.2) and describing functional relationships between organisms and their environment (as in *Tipping Points* - 4.2.1) but equally important, is how food, detritus and contaminants are processed and channelled through food webs. This project will use advanced forensic chemistry techniques to describe food web 'connectivity', and focus on how these connections are altered by human activities. The project has two major work streams: 1) tracing organic matter, nutrients, metals and contaminants through marine food webs and (2) identifying how marine developments alter the number and magnitude of connections in food webs, which can shape ecological function and the provision of goods and services. Specifically, we will trace the fate of land-sourced materials from water and sediments through coastal food webs, evaluate energetic subsidies between coastal and deep sea habitats, and resolve effects of key resource uses on food web connectivity. Outputs include visualisation tools that will aid stakeholders in evaluating these biologically connected domains, minimise negative impacts of contaminants and help ensure maintenance of ecosystem function and the goods and services we receive from our coastal seas.

D. INTRODUCTION

Effective ecosystem-based management (EBM) necessitates understanding how ecosystems function and respond to human disturbances. Central to this is an understanding of the role of critical species that ecologically connect ecosystems and influence the flow and fate of organic matter and nutrients in food webs, including how they process, and are affected by, contaminants sourced from land or marine activities. In this context the structure and function of ecosystems are typically described in terms of connectivity and dynamics, the flow of energy and materials through the component parts resulting in interaction chains, as well as changes to biodiversity. Changes in ecosystem complexity (e.g., species richness, trophic structure), for example, can affect nutrient cycling which underpins patterns in primary production. Furthermore, ecosystem properties such as resilience, or the tendency for the system to return to a former state following a perturbation, are fundamentally linked to chains of interactions within food webs [1]. Accordingly, understanding biological influences on the production and transport of organic matter, nutrients and contaminants in marine ecosystems and how they interact with food web architecture has led to fresh insights into the dynamics of these processes that define ecosystem function [e.g. 2, 3-6] and underpin effective EBM.

Changes in food webs and the loss or decline of key species can greatly alter biochemical fluxes, leading to changes that may ripple through the ecosystem[7, 8]. For example, in many coastal systems, filter-feeding bivalves are key to linking vast reserves of organic matter in the water column with the benthos. Where bivalve populations have been removed, effects can be seen through the highest levels of coastal food webs [e.g. 9]. Alternatively because different bivalve species accumulate toxic metals and organic pollutants in very different ways, changes in the composition of bivalve communities can greatly influence the chemical and ecological fate of contaminants[10]. Similarly, removal of some organisms in high trophic levels can alter the connectedness and stability of food webs [11]. In addition, "biological vectors", organisms that transport organic matter, nutrients and contaminants among habitats, form a vital component of ecosystem connectivity and can link habitats on broad geographic scales [12-15]. This project will focus on biochemical fluxes from the base of the food web (microbes and primary producers) through communities differentially impacted by human-sourced disturbances, including land-based sediments and contaminants, and those from marine activities. We will use field experiments and sampling to contrast the consequences of differences in food web architecture for biochemical fluxes, and then use targeted lab-based experiments to test and understand critical underlying physiological and ecological mechanisms that influence biochemical fluxes and the chemical fate of contaminants. Biokinetic models of contaminant accumulation for key species[10, 16] will be developed to support chemical fate modelling.

The mission of *Sustainable Seas* is to enhance the use of marine resources within biological constraints. Given the complexity of natural systems, these constraints can be challenging to define, especially with the numerous connected flows through ecosystems. *Tracking biochemical fluxes* provides an avenue to understand ecosystem connectivity using chemical signatures residing in and linking all organisms. Collaboration with *Tipping Points* (4.2.2) and *Stressor Footprints* (4.2.1) strengthens the understanding of spatial and temporal dynamics of materials coursing through ecosystems. It will aid in understanding processes before and after a tipping point has been reached, and allows identification of sources of materials (i.e., from point sources into transport plumes) and their flow through the recipient ecosystem. Our team has a firm commitment to collaborations with other programmes in the Challenge to fulfil its mission.

E. AIM OF THE RESEARCH AND RELEVANCE TO OBJECTIVE

The aim of this project is to aid stakeholders, iwi, investors and managers in making wiser choices for coordinating marine activities and developing a sustainable marine economy. We will achieve this by investigating the ways in which human-sourced disturbances and marine activities affect the structure and functioning of food webs. We will conduct a series of biochemical analyses that provide the spatial and temporal domain of fluxes of materials, including chemical and biological pollutants, from the nearshore zone to shelf waters. We will identify connectedness of food webs within systems with reference to impacted and less affected areas and differing food web architectures to provide insights into how food webs, their complexity and diversity interact with stressors. In this context we will consider both marine-based disturbances and those arising from sedimentation and nutrient pollution from land. We will develop flux and chemical fate models to underpin decision-making about management strategies, both to minimise impacts of chemical and biological pollutants and help ensure maintenance of ecosystem function and services.

F. PROPOSED RESEARCH

Our work is focussed around **two major work streams**. These are not 'business as usual' because they are aimed to be integrative, to unite several themes on connectivity and process across *Dynamic Seas* and meaningfully feed into the other Challenge programmes. The results of this project can then be considered in the context of coastal resource management [17, 18] relating to multiple uses (e.g., various types of aquaculture compared to natural systems), the influence of biological vectors on connectivity [19], and the flow and fate of nutrients and contaminants from point sources such as industry or land-based runoff through food webs [20, 21]. Together, these studies will parameterise food web models to test the fates and flows of materials in response to changing food web architecture and their relationships with marine activities. Our comprehensive approach, linking chemical processes with food web structure and dynamics to improve sustainable development in the context of EBM, will be the first of its kind. Our studies will be of direct use to effective EBM and the realisation of a sustainable blue economy.

Work Stream 1: Tracing organic matter, nutrients, contaminants and metals through marine food webs.

This theme addresses the question: *How are the flows and fates of organic matter [9, 22], nutrients [23, 24], metals [25-27] and organic contaminants (i.e. biochemical fluxes) influenced by the architecture of marine food webs in differentially impacted areas [20, 28]?*

Here we will take a comparative approach by sampling food webs with distinctly different architectures. By comparing different case study areas, it allows us to address how the loss or modification of abundance of key species can influence biochemical fluxes and fates in the marine ecosystem. In this context, our selection of sites includes considerations of land-based influences from coastal catchments as well as composition of marine communities that make up the food web. In key case study areas within the focal region (e.g., Tasman Bay, Marlborough Sounds) we will examine how biochemical flows have been altered via modifications of the food web architecture. Tasman Bay is an area of initial focus, where the composition of the food web, particularly the bivalve community, has been heavily impacted by both direct exploitation and land-based activities (sedimentation and nutrient pollution). Similarly, the Marlborough Sounds has areas where food web structure has been significantly altered by human-based activities. We will make direct comparisons between biochemical fluxes in these systems, specifically the number and magnitude of connections among biological components.

Carrying out sampling along stress gradients (i.e., from point sources of pollutants) will help resolve the spatial and biological extent of stressors from particular activities. Importantly, this work will have strong interactions with the other projects in *Dynamic Seas*: with *Stressor Footprints* in co-experiments in Tasman Bay so that biological footprints of contaminants can be mapped to their physical transport (plume experiments), and with *Tipping Points* as the consequences of changes in food web architecture to critical thresholds are explored in terms of biochemical fluxes.

We will map biochemical fluxes from sampling of the water column or from point source inputs (e.g., land-based runoff) to microbial communities and primary producers (phytoplankton and macroalgae) through marine food webs. By chemically tracking these primary organic matter source pools and the metals and contaminants that move with them through food webs we will be able to assess the consequences of differences in food web architecture for ecosystem function. For example, by comparing different case study areas, we will be able to address how the loss or modification of abundance of key species can influence biochemical fluxes and fates in marine ecosystems.

We will have a particular focus on land-sea and cross-shelf connections. Stakeholders and iwi have repeatedly told us that land-sourced contaminants and nutrients arising from sediments and runoff are a major problem for aquaculture, kai moana, and the general health ('mauri') of the coastal zone. Because of food web interactions, physical transport and the movement of mobile organisms, the fate of nearshore inputs can extend many kilometres out into shelf waters and habitats, which can be seen in the chemical signatures of resident organisms and those that migrate among habitats. Accordingly within different food webs we will consider biochemical fluxes in important sessile and mobile species that are sensitive to nearshore stressors.

For example, filter-feeding bivalves provide essential ecosystem services because they buffer nutrient inputs in coastal systems, filter and condition the water and provide habitat in the form of both substratum and stabilisation of sediments. Bivalves also bioaccumulate toxic metals and organic contaminants [10, 16] and can incorporate an intricate record of physical and chemical conditions into their calcium carbonate shells [29, 30], including exposure to some contaminants. Unlocking these data offers important information on the past and present conditions of our marine ecosystem, and how changes in the composition of marine communities influence biochemical fluxes and fates.

Coastal fishes are a key mobile group supporting ecosystem services and connectivity among habitats. Here we will consider the consequences of changes in the structure and composition of coastal fish communities for biochemical fluxes across the coastal and shelf food webs. Information from biochemical analysis can be used to understand how some fishes connect habitats from the coast to shelf communities. In this context fish can be employed as bioindicators for accumulation and movement of toxic metals (e.g. Hg, Cd, As) and organic pollutants and represent potentially important biological vectors among habitats[19, 31]. The first components of this project lead to a very important second question in the context of chemical fate of contaminants.

How do differences in bioaccumulation of metals and organic pollutants among key species influence the chemical fate of pollutants?

The sampling above will provide the basis for assessing consequences of community change for the chemical fate of materials. Here we develop a series of orthogonal exposure experiments to resolve passive and active uptake of toxic metals and organic contaminants. The results will provide the basis for developing biokinetic chemical fate models for key species[10, 16], and for incorporating these models with information on changes in species composition and community structure. Target species include those important in each local food web, including mussels (*Perna canaliculus*, *Mytilus edulis galloprovincialis*)[30, 32], horse mussels (*Atrina zelandica*), oysters (*Tiostrea chilensis lutaria*)[25], cockles (*Austrovenus stutchburyi*)[33, 34], Pipis (*Paphies australis*) [35] and scallops (*Pecten novaezelandiae*) which likely differ in their use and processing of particulate organic matter[32], and bioaccumulation of metals and organic contaminants[10]. Key fish species, displaying a range of life history characteristics, to be sampled in this context are large omnivores such as blue cod (*Parapercis colias*)[36, 37] and sea perch (*Helicolenus percoides*)[38], smaller estuarine species such as common triplefins (e.g. *Forsterygion lapillum*)[39] and spotties (*Notolabrus celidotus*)[40, 41] and highly mobile species such as hapuku (*Polyprion oxygeneios*) and trumpeter (*Latris lineata*). Accordingly, results from both our sampling and chemical fate experiments will have high relevance to *Tangaroa* (e.g., potential contaminants in kai moana), *Our Seas* and other programmes in *Sustainable Seas* as models of direct use to stakeholders and managers are developed.

Work Stream 2: Identifying how marine activities can alter biochemical fluxes

Aquaculture is a major economic activity in New Zealand, poised to expand dramatically in the next decade. In this context the value of New Zealand's aquaculture products are dependent on branding linked to high quality of food safety and ecological integrity. Here, development of high input aquaculture farming of salmon and other finfish creates significant challenges and opportunities in the context of EBM. First, large inputs of feeds to farms and the production of organic and nutrient wastes increase the ecological source and sink footprints of farms. The potential for introduction of contaminants, pathogens and invasive species from far afield of the farming practice have been of increasing concern to stakeholders, iwi and managers and are important issues for maintaining ecological integrity. Many of these potential problems may be mitigated by strategic management of the recipient ecosystem, development of polyculture systems or managing the activity to make the most efficient use of the by-products of intensification. Here we investigate the biochemical consequences of different configurations of aquaculture by understanding the chemical fate of its by-products within different recipient food webs. Furthermore, we will use advanced forensic techniques to identify the potential sources of contaminants to passive (bivalve farming) and intensive (finfish farming) aquaculture, thereby defining the ecological source-sink footprints of farms. These issues lead to the research question:

How do the by-products of aquaculture modify biochemical fluxes within the recipient food web?

Here we focus on how different configurations of aquaculture can alter biochemical fluxes within natural food webs among contrasting sites. The field work will be primarily done around the Marlborough Sounds, where a range of aquaculture systems are in place. We will resolve differences in the biological uptake, cycling and fate of nutrients, organic matter and contaminants arising from high and low input farming practices. This initial focus will lead to important information on how biochemical dynamics vary among different aquaculture management systems, and recipient food webs. A corollary is that effective management of ecologically sustainable practice in this context relies on an understanding of the specific mechanisms for bioaccumulation and chemical cycling of aquaculture by-products in recipient systems. Accordingly, field data will be augmented with focused laboratory experiments on the physiological and ecological bases for differences in biochemical fluxes among key species of the recipient ecosystem. Here we focus on the specific question:

What are the mechanisms for processing of by-products of aquaculture within the recipient food web?

To address this issue we will develop laboratory studies on bioaccumulation of contaminants within key species and on microbial processing of contaminants, organic matter and nutrients associated with aquaculture. We will explicitly test how trophic connections of key detritivores (e.g., *Australstichopus mollis*) and suspension feeders (e.g., *Perna canaliculus*) with the microbial community influence the chemical fate of aquaculture by-products (see *microbial community functional responses*). This research programme creates the opportunity to develop information on the "chemical fingerprint" of clean, ecologically sustainable practice. In this work stream we will develop the ecological basis for defining the ecological footprints for NZ aquaculture within IAEA food traceability guidelines (see Methods below).

Methods for Quantifying Biochemical Fluxes in Marine Food Webs

Facilities and analytical approaches: In both work streams we will employ approaches that integrate ecological studies and process with forensic tracking of materials through ecosystems. This will allow precise measurements of the primary currencies of ecosystem function: organic matter and nutrients, and some of the pollutants that are carried along with these currencies. World-class analytical facilities and expertise available from the research team within the NIWA/University of Otago Research Centre for Oceanography, the University of Otago's Department of Chemistry, and the Otago Centre for Trace Element Analysis provide a comprehensive and cost-effective platform to support the project. The Chemistry Department is currently becoming a collaborating centre with the International Atomic Energy Agency. Our formal collaboration is specifically with the joint FAO-IAEA Division of Nuclear Applications through the Food and Environmental Protection Laboratory. The formal link will be an important vehicle for the international acceptance of results.

Stable isotope analysis to track organic matter in food webs: Resolution of biochemical processes within food webs requires precise chemical techniques and facilities. The University of Otago Department of Chemistry's stable isotope lab (Isotrace) hosts 7 mass spectrometers, capable of a full suite of analyses including compound specific (fatty acids, n-alkanes, amino acids) and bulk isotope approaches. Isotrace has a long history of application of novel isotope techniques for environmental and forensic studies including using fatty acid isotope measurement (C and H) for source apportionment of contaminants [42-47]. Otago's Isotrace is currently the only lab in NZ set up for the very challenging amino acid ^{15}N analyses required in this research. These analyses will be supported by advances in mass balance modelling of isotopes in ecological systems, and experimental approaches to understanding temporal, spatial and trophic discrimination of isotopic signatures in the food webs of interest.

Organic contaminants: Quantification of persistent organic pollutants (POPs), current-use pesticides, flame retardants, petroleum products (i.e., polycyclic aromatic hydrocarbons, PAHs), and antibiotics will be determined by gas and/or liquid chromatography coupled to mass spectrometry. In addition, we will use focussed experiments and chemical fate modelling to resolve differences in bioaccumulation of organic contaminants in key species (bivalves and fishes).

Essential and toxic metals: Though often solely regarded as toxins, many metals are required as nutrients for the growth of all organisms. They form the reactive centres of enzymes, enabling these to perform biochemical functions, such as oxygen-transport or photosynthesis. However, elevated concentrations of essential or non-essential metals in the ocean, mainly from anthropogenic activities, can lead to metal toxicity [48, 49]. High resolution sampling of essential metals (Mn, Fe, Co, Ni, Cu, Zn) and non-essential (toxic) metals (As, Cd, Sn, Hg, Pb) in the water column and in organismal tissues will be accomplished by combining advanced sample preparation and extraction techniques, coupled with ICP-MS [27, 50]. Laser ablation ICP-MS can resolve times series of metal concentrations laid down in bivalve shells that represent the ambient environmental concentrations at time of formation [51]. This offers an effective method to develop time series from single sampling events. We will apply each of these techniques to obtain insight into the chemical fate of metals and transport within biological vectors.

Microbial community functional responses: This project involves sampling microbial communities, which reprocess nutrients and contaminants, and link closely with food web configurations along environmental gradients or among different management systems. Bacterial abundance will be quantified by flow cytometry [52], bacterial production will be estimated from the incorporation of ^{3}H-leucine by bacteria [52, 53], bacterioplankton carbon substrate utilization profiles determined with BIOLOG Ecoplates will be used as a proxy of bacterioplankton community metabolic capacity [54], enzymatic activities (i.e., glucosidase, aminopeptidase, phosphatase) will be analysed with fluorescently-labelled substrates analogues [55, 56], and respiration via the analysis of the respiratory activity of the electron transport system [57]. These analyses will clarify how biochemical fluxes are modified by bacterial communities, the role of bacteria as sinks of contaminants and how stressors and environmental conditions modify the function of the bacterial community in the coastal marine ecosystem.

Field work: Field station, logistic support, vessels and ships of opportunity for sampling will be provided by UoO Otago and partners of *Sustainable Seas*. Data and samples will be stored and archived within the NIWA/Otago Research Centre for Oceanography and the University of Otago Department of Chemistry using established best practice and laboratory standard and to meet the requirements of the *Sustainable Seas* challenge (section 1.7 of PROP-38764-NSC2-NIW).

G. LINKAGES AND DEPENDENCIES

The project will not depend on but will interact actively with 4.2.1 "*Tipping points*" and with 4.2.2 "*Stressor footprints and dynamics*". We plan to collaborate on microbial and biochemical aspects of several planned experiments on *Tipping points* where manipulations result in alternate communities. We will also interact closely with project 4.2.2, particularly the physical modelling of materials from point sources where we can add information about the biological processing and fate of metals and contaminants in plumes, and in this context we will seek to integrate information from our programme with outputs of the "Connectivity Tool".

H. COLLABORATIONS

Professor James Leichter (Center for Integrative Oceanography, Scripps Institution of Oceanography) will provide expert advice on nutrient dynamics in coastal ecosystems. Professor Professor Gerhard J. Herndl (Department of Marine Biology, University of Vienna, Austria) will provide advice on the link between microbial oceanography and marine biogeochemistry. Professor Eddy Y. Zeng (School of Environment, Jinan University, Guangzhou, China) will provide expert advice on tracing by-products and contaminants in aquaculture systems. Dr Len Wassenaar (Laboratory Head Hydrology, International Atomic Energy Agency) will provide advice on research design and data interpretation in the context of IAEA food traceability guidelines. Professor Dave Hutchins (University of Southern California, Head of Biological Sciences) will advise on work that supplements experimental fieldwork with laboratory culture manipulations. Dr Carl Lamborg (Institute of Marine Sciences, University of California at Santa Cruz) will provide technical expertise for developing high-resolution detection of mercury in New Zealand.

I. INTERNATIONAL LINKAGES

Our team has a large collection of International collaborations and programme connections including GEOTRACES, IAEA and key connections to international laboratories (see attached CVs and key collaborators above). The current project is not explicitly dependent on these collaborations but will benefit from support they offer by providing international standards for the analytical work.

J. ALIGNED FUNDING AND CO-FUNDING

This project does not explicitly rely on aligned funding but will benefit from alignment with NIWA's Coasts and Oceans Programme, and the other projects within Dynamic Seas. Significant facilities support (i.e., in-kind funding) will be provided by the NIWA/Otago Research Centre for Oceanography, by the Departments of Marine Science and Chemistry and through the Otago Centre for Trace Element Analysis. PhD scholarship opportunities to support stipends for the associated PhD students will be provided by the UoO, Canterbury University and Victoria University Wellington. A MBIE NZ-China Research Alliance proposal, if successful, will provide expertise from overseas (PI Hageman). A collaboration (Middag) with Dr Hartland and Prof Battershill from the University of Waikato on metal contamination from the sunken 'Rena' will, if funded, add an aligned case study.

K. VISION MATAURANGA

Vision Mātauranga is seeking to unlock the innovative potential of Māori knowledge, resources and people to assist New Zealander's to create a better future. One of the four themes in the Vision Mātauranga (VM) policy framework is hauora/oranga, which is aimed at improving the health and social wellbeing of Māori. The health and productivity of New Zealand's coast and its connectivity to the marine and ocean environment, has a close association to the health and social wellbeing of Maori as tāngata whenua and as kaitiaki.

By aligning itself with this project, the VM programme considers that there is potential to recognise the sophistication of traditional Maori healing and medicinal use of various plant species that inhabit New Zealand's coastlines. Internationally, the use of natural products in medicines is growing and the healing attributes of New Zealand's indigenous flora are acknowledged1.

The intent of VM will be to work with the project leader and team in an observer capacity to identify opportunities for Māori knowledge, resource and people, as it relates to rongoa Maori, to be explored further either within the Challenge, where it aligns with its objective, vision and mission, or to support the implementation of the VM policy framework in another National Science Challenge.

L. ENGAGEMENT AND CAPACITY BUILDING

Our project will actively incorporate stakeholder, community and government engagement within workshops of the Challenge and as a part of targeted projects focussed on resolving biochemical flux and fate within regions of aquaculture development and expansion, and at potential point sources for chemical and biological pollutants. Here we will seek community engagement for several aspects of the science, from choices of sites to accessible vehicles for dissemination of results. Within the Challenge we will actively engage with programmes within *Our Seas, Valuable Seas, Tangaroa, and Managed Seas* to achieve best use of our results. Our work will have direct application for management decision making concerned with mitigating the impacts of industrial development such as aquaculture and resource extraction, and we will engage with managers, stakeholders and policy makers in this context. Though university and community networks our team will mentor and develop young scientists (PhD students) with the skills to bring the mission of the Challenge to fruition and we will actively engage with the international science community to provide exposure for the Challenge. We will also use the communication and outreach facilities of both the Challenge and our Universities to fully engage with the public to highlight the gains possible through Sustainable Seas.

M. ROLES, RESOURCES

To achieve our objectives, we will consult stakeholders to select study sites within natural, heavily impacted and aquaculture-based systems. We have a team that is strongly committed to this process and has considerable expertise with community engagement and Ecosystem Based Management (e.g. Fiordland [59]). Stephen Wing will oversee the project, focussing particularly on food web dynamics, and will lead investigations on nutrient and organic material cycling. Russell Frew will use isotopes to trace the origins of the influxes and destinations of outfluxes, including bioaccumulation within food webs. Jeff Shima will focus on resolving movement, and life history patterns of coastal fishes to provide information on ecological connectivity among habitats. David Schiel will collaborate on resolving regional patterns in productivity and benthic-pelagic coupling with land-based influences. Rob Middag will lead investigations on essential and toxic metal cycling. Kimberly Hageman will lead investigations on organic contaminant cycling and impacts. Federico Baltar will study the influence of the bacterial community on biochemical fluxes, and interactions with stressors as well as their role as sinks for contaminants. This comprehensive approach, linking a

[1] Ko Aotearoa Tenei 7.10

number of chemical processes with food web dynamics, specifically for the purpose of improving economic development in the context of EBM, will be the first of its kind. This group represents the best team in New Zealand in terms of both expertise and facilities to accomplish the proposed work (see attached CVs and References below). Our team consists of well-established, as well as some of New Zealand's best emerging, researchers in biochemical cycling and food web dynamics.

N. RISKS AND MITIGATION.

The PI (SRW) has primary responsibility for organising risk management and mitigation. Here each of the partner institutions (University of Otago, University of Canterbury, Victoria University Wellington and NIWA) have extensive health and safety and risk assessment guidelines, best practice codes for field and laboratory work. Best practice will be followed throughout the project in adherence to these guidelines. We will follow ethical guidelines from Otago University's Animal Ethics Committee in all sampling and experimental work.