7.

Innovation Fund Research Proposal Template

A. PROJECT TITLE
Quantifying marine biodiversity using environmental DNA

B. PROJECT TEAM

Project Leader:	Investigators:
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C. ABSTRACT

A key to efficient, ecosystem---based management of marine resources is the availability of suitable tools to measure patterns in biodiversity. Current methods are costly, labour intensive and rely on surveys of a limited number of indicator species and sites to provide an estimate of biodiversity and ecosystem health. Consequently, their capacity for resolving the complexity of marine communities at an ecosystem level is highly compromised.

We will establish and test an innovative, high---throughput and cost efficient strategy for quantifying marine biodiversity using environmental DNA (eDNA) extracted from marine water samples. We will determine the spatial and temporal resolution of marine eDNA, the required sampling density and frequency, and the effects of external factors (e.g. weather and sea conditions), on the results obtained. We will ground---truth our approach by comparing biodiversity metrics obtained through eDNA with those achieved via traditional monitoring.

Working closely with DOC, MPI and kaitiaki, we will develop simple, web---based tools to explore our eDNA data so that both scientists and non---scientists can readily use this information to undertake their own research and inform management decisions. Our project will establish a new standard for quantifying marine biodiversity, empowering long---term ecosystem---based management of New Zealand's marine resources.

D. RELEVANCE TO CHALLENGE OBJECTIVE

- --- Enhancing utilisation of our marine resources within environmental and biological constraints requires new approaches to benchmark and measure the impact of resource utilisation/management overtime.
- -- These tools must be adaptable to all marine systems, accurate, reproducible, cheap and scalable, for ecosystem---based management of marine resources to be achievable.
- --- eDNA is a promising technology that may meet these requirements, but needs robust testing.

--- Once established eDNA sampling protocols coupled with simple software, will empower ongoing evaluation of the effects of marine resource utilisation and marine management measures by government agencies, industry, communities, and kaitiaki of customary protection areas.

E. INTRODUCTION

The National Science Challenge "Sustainable Seas" identifies ecosystem---based management (EBM) as a key tool to achieve the objective of "Enhancing utilisation of our marine resources within environmental and biological constraints". A key to efficient, ecosystem---based management of marine resources is the availability of suitable tools to measure patterns in biodiversity accurately over space and time. Current methods are costly and labour intensive. They often require significant taxonomic expertise and rely on surveys of a limited number of indicator species and sites to provide an estimate of biodiversity and ecosystem health. Thus, their capacity for resolving the complexity of marine communities at an ecosystem level is highly compromised.

Environmental DNA (eDNA), i.e. DNA extracted from environmental samples such as seawater, may help to overcome these limitations. Environmental DNA technology can be applied to a large range of habitats and species at all life stages, without requiring specialized taxonomic skill [1, 2]. It allows us to identify marine organisms present in an area non---invasively from the trace amounts of DNA they shed into their environment [3]. Recent studies have shown the potential of eDNA to detect even rare, highly mobile, marine organisms [4, 5]. The technology therefore has the potential to provide measures of biodiversity and ecosystem health at an ecosystem level from easy to obtain water samples. Water samples can be collected by government agencies (e.g. MPI, DOC), industry, NGOs, communities, and kaitiaki of customary protection areas and analysed in a standardized and cost efficient way, providing ecosystem information from around New Zealand to support management decisions.

We will establish and test a new, rapid and cost efficient strategy for quantifying marine biodiversity using eDNA extracted from marine water samples. The key challenge for our project is the translation of existing proof---of---concept studies [4, 5] to a standardized marine monitoring strategy that produces comparable outcomes across NZ and beyond. Major questions that will be addressed include the spatial and temporal resolution of marine eDNA, the required sample density and frequency and the influence of sampling season on the results obtained.

We will benchmark our new approach by comparing the biodiversity metrics obtained through eDNA with those achieved via traditional monitoring at two well---researched study areas (Milford Sound, East Otago Taiāpure).

Working closely with DOC, MPI and kaitiaki, we will develop simple, web---based tools to explore our eDNA data so that both scientists and non---scientists can readily use this information to undertake their own research and inform management decisions they may be making. Our project will establish a new benchmark for quantifying marine biodiversity and empower long---term ecosystem---based management of New Zealand's marine resources, while also creating new opportunities for community engagement, education and outreach.

Our world---class team includes leading geneticists, ecologists and experts in marine kaitiakitanga (guardianship) and is ideally placed to successfully complete the proposed project. The project will contribute significantly to the "Sustainable Seas" National Science Challenge (NSC) and also align with the "Te Tiaki Mahinga Kai" project (www.mahingakai.org.nz), which monitors customary fisheries areas.

F. AIMS

This research aims to lower the cost and increase efficacy of marine biodiversity determination by developing and testing a state---of---the---art strategy for quantifying marine biodiversity based on eDNA technology.

Specific aims include:

--- Determine spatial resolution of eDNA data. Studies have shown the high sensitivity of eDNA technology to detecting species presence, but what is the power of the technology to distinguish different communities in close spatial proximity?

--- Determine seasonal variation of eDNA data. Is there a time of higher or lower DNA content in seawater?

--- Benchmark eDNA biodiversity data against existing data from traditional monitoring. This will help with ground---truthing the new technology and provide insights into how to combine traditional surveying strategies and eDNA technology most efficiently.

--- Integrate all results to propose a standardized strategy for incorporating eDNA data into biodiversity surveying.

--- Create new opportunities for community engagement in marine resource management, and contribute to education and outreach activities.

G. PROPOSED RESEARCH

Study location

The proposed project will study eDNA samples from two locations: The East Otago Taiāpure (EOT) and Milford Sound.

East Otago Taiāpure: The EOT is a customary protection area established in 1999. It covers a 25 km^2 stretch of coastal waters between Ohineameo (Cornish Head) near Kāritane in the North and Waiweke (Potato Point) near Purakāunui in the South. The Taiāpure covers areas identified as very significant for the local hapu Kāti Huirapa ki Puketeraki Rūnaka including important mahinga kai sites and places of cultural and spiritual significance (Wahi Tapu sites). The Taiāpure area has sustained Kāti Huirapa for more than 500 years and the area provides a nationally significant example of kaitiakitanga in practice. The Taiāpure is managed by a local fisheries management committee [6], of which Associate Investigator (AI) Dr Christopher Hepburn is a ministerially gazetted member.

Scientific monitoring of the EOT started in 2005, lead by Dr Hepburn and since then more than 20 papers have been published and 30 postgraduate students (including PhD, Masters and Honours) have completed their degrees within the EOT. As a result there are detailed environmental, habitat and biodiversity records as well as established monitoring and ongoing transects [7–9] against which our eDNA data can be benchmarked.

Most of our project work will be conducted in the EOT because of the extensive reference data available and because the easy access from the University of Otago will facilitate regular sampling under any specific weather and sea conditions desired for our experiments (see also section K. Aligned and co---funding).

Milford Sound: Milford Sound is a narrow, 16km long and up to 450 meter deep fjord in Fiordland National Park. Part of the fjord is a marine reserve which was established in 1993 and which is administered by the Department of Conservation. The reserve covers 690 hectares. The steep and sheltered fjord habitat with large freshwater run-off provides a stark contrast to the open and shallower EOT.

Extensive studies of marine biodiversity in Milford Sound have provided a detailed picture of species presence and absence [10–13]. Our sampling will align with 22 sites sampled by Inglis et al. 2008 [10] for a survey of non-indigenous and indigenous marine species.

Environmental DNA studies of Milford Sound will be used to further ground-truth our sampling and analyses strategies developed in the EOT. Once a standardized strategy is developed from EOT results, this strategy will be applied in Milford Sound and results will be benchmarked against the most up-to-date biodiversity surveys of the fjord to evaluate transferability of our strategy across very different environments.

Sampling

Samples will be collected from the surface, by diver or, for deep water samples, using Niskin bottles attached to a CTD to measure temperature, salinity and depth. Sampling will take place at high tide in calm conditions unless otherwise stated (see below).

EOT:
Sampling will focus on benthic communities around the Huriawa Peninsula in Kāritane for which the most detailed biodiversity records exist. The area provides a patchwork of distinct communities in close proximity, including rocky shoreline, kelp forest, and estuary habitats. It is therefore an ideal setting to test the spatial resolution of eDNA data in complex environments.

We will establish a grid of 30 sampling sites in six different environments (Fig. 1) including five sites in the estuary (surface sampling), fives sites above *Haliotis iris* (Pāua) beds (2 meters deep), five sites inside kelp forest (2 meters deep), five sites in the kelp canopy (surface sampling), five sites in the deep shallows (10 meters deep) and five reference sites along an open water transect for which AI Dr. Baltar has conducted eDNA analyses over the past three years [14]. The distance between each of the different environments with the exception of the open water sites is between 100 meters and 500 meters. Each sample site will be logged with GPS coordinates to allow easy resampling at locales across time.

All sampling will take place at the same 30 sites to evaluate reproducibility of our data. Samples containing two litres of seawater each will be taken three times during year 1: In November, coinciding with the time of highest ecosystem productivity, in March, coinciding with declining productivity, and in August, coinciding with lowest productivity. 40% of samples will be replicated, i.e a total of 42 samples will be collected per sampling session.

During the November sampling we will establish an initial workflow. During the March sampling, we will sample each site at high tide, mid tide, low tide and mid tide to evaluate differences in eDNA throughout a tidal cycle.

Sampling will be conducted as part of routine monitoring excursions within the "Te Tiaki Mahinga Kai" project and NIWA's CARIM project.

Fig. 1: Sample environments in the East Otago Taiāpure. 1: Open water site; 2. Estuary; 3. Pāua beds; 4. Kelp forest; 5. Kelp canopy; 6. Deep shallows. Locations approximate. (Graphic modified from EOT information prospectus by the East Otago Taiāpure management committee.)

Milford Sound:

Sampling in Milford Sound will be conducted in year two of our project during a South Island sampling expedition as part of the Dynamic Seas NSC project 4.1.1 (Ecosystem Connectivity: Tracking biochemical fluxes to inform Ecosystem Based Management). 66 samples will be collected from the same 22 sites used for the 2008 baseline survey by Inglis *et al*. [10]. The sites are distributed throughout Milford Sound within and outside the marine reserve. Three samples will be collected from each site.

DNA extraction

Environmental DNA will be extracted from two litres of seawater for each sample. The optimal extraction strategy is currently being tested by PhD candidate Gert-‐Jan Jeunen, supervised by Gemmell, Knapp and others. To avoid contamination of the water samples, which may contain only trace amounts of DNA, with amplified DNA present in large amounts in any post-‐PCR molecular biology laboratory, DNA extraction and manipulation will be conducted in a physically isolated, PCR free laboratory following cleanroom protocols as outlined in Knapp et al. (2012) [15].

DNA sequencing

The two most common approaches for obtaining eDNA sequence data are shotgun sequencing and metabarcoding [3]. Shotgun sequencing refers to a technique in which all DNA extracted from an environmental sample gets sequenced, usually on a high-‐throughput sequencing platform. The advantage of the approach is that it provides an unbiased overview of all DNA present in a given sample. For our seawater analyses, the approach has a few major disadvantages, though. First, depending on the sample, there could be a relatively high content of DNA from microorganism present in the seawater sample. The microorganism DNA could completely overwhelm any traces of DNA from higher organism, which are also important for our studies. Second, the DNA fragments sequenced using this approach are randomly distributed across the genomes of all organisms

contained in the sample. This poses a significant limitation to the identification of the taxa in the sample, as only very few of these taxa will have had their genomes sequenced. Thus, most DNA fragments will have never been sequenced before and will therefore be unassignable to any species.

Because of these limitations we will be using a metabarcoding approach [16]. In this approach a set of universal primers is used to amplify specific genomic regions known to be suitable to identify taxa, often to species level. As these genomic "barcoding" regions are commonly used, extensive reference data exist for a very broad range of taxa. Barcoding protocols for marine eDNA metabarcoding are currently being developed by Gert---Jan Jeunen at Otago and by the lab of AI Professor Michael Bunce (Curtin University). At the start of the project Dr Knapp will visit Prof Bunce's lab at Curtin University to discuss the best analyses strategy based on results from both labs. We will target the commonly used barcoding markers 16S, 18S, 23S, COI, and trnL. Together these markers cover a broad range of plant and animal taxa and have already yielded high resolution data from Western Australian marine samples (Bunce, unpublished results).

Barcoding primers will be designed to have a target specific part as well as an illumina sequencing adapter tail, that covers half of the standard sequencing adapter used for the illumina MiSeq high---throughput sequencing platforms. Adapters will be extended to full length by amplification of the amplicons with extension primers carrying sample specific 8 base pair (bp) "Tags", that can be used to differentiate sequencing reads from different samples pooled on a single high---throughput sequencing run. We will sequence 50---90 samples (five barcoding markers each) on each MiSeq sequencing run. DNA sequence data will be produced on a total of six MiSeq sequencing runs.

DNA sequence data analyses

DNA sequence data will be evaluated against GenBank records to identify species present in the sample. Software for this process is constantly improving and the most up---to---date software at the time will be used. Currently the standard software for this part of the analyses is "MEGAN5" [17] and Kraken [18].

Evaluation of results

Both EOT and Milford Sound have been subject to intensive monitoring for many years and as a result, the biodiversity of these areas is well known. Such intensive surveying would be too costly and labour intensive for an ecosystem level study of marine resources throughout New Zealand's Exclusive Economic Zone. The overall aim of this project is therefore to develop a rapid and cost efficient strategy for quantifying marine biodiversity based on eDNA technology.

In order to evaluate the suitability of our approach for large---scale ecosystem level studies of biodiversity, we will compare our data to existing species and abundance lists for the EOT and Milford Sound (Hepburn, Wing). We will also evaluate how well the approach distinguishes between neighbouring, distinct habitats in the EOT and the effects of seasonality on our results. Furthermore we will evaluate the quality of information provided by each of our five barcoding markers (16S, 18S, 23S, COI, and trnL). From these data we will propose a standardized marine eDNA sampling and analyses strategy that can be used on a national and international scale to study biodiversity in marine ecosystems.

Databases and web interfaces

A key output from our study will be the establishment of a simple database tool that can be rapidly interrogated by scientists and lay people to understand the biological diversity of a given site, how

that compares to other sites and how this is changing overtime. It can be further refined to examine trends overtime for specific species, that are of interest to key commercial, regulatory, recreational and cultural groups interested in management of our marine resources. Such tools have been developed previously for related projects by e.g. the Allan Wilson Centre's New Zealand Genomic Observatory Data Warehouse (http://data.modelecosystem.org.nz/) and iMicrobe a database that documents microbial diversity across the world's oceans (http://imicrobe.us/) and significant components of these can and will be adapted for our purpose.

H. RESEARCH ROLES

Researcher	Organisation	Contribution
Dr Michael Knapp	University of Otago	Overall coordination of project, data analyses
Prof Neil Gemmell	University of Otago	Coordination of data production, data analyses
Dr Christopher Hepburn	University of Otago	Reference data, experimental design for sampling, link to communities
Dr Federico Baltar	University of Otago	Sampling design, experimental coordination with Sustainable Seas Project 4.1.1, interpretation of data
Prof Michael Bunce	Curtin University	Experimental design for laboratory experiments

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I. LINKAGES AND DEPENDENCIES

The project links directly to Sustainable Seas project 4.1.1 Ecosystem Connectivity: Tracking biochemical fluxes to inform Ecosystem Based Management (in the Dynamic Seas Programme, Wing et al.) and it will make use of synergies in field sampling and planned activities in the programme. Dr Federico Baltar is an Associate Investigator within Sustainable Seas project 4.1.1 and will coordinate complementary research across both projects. Shared research will include common sampling as well as, where suitable, incorporation of eDNA data into the analyses of food webs and ecosystem connectivity conducted within the framework of project 4.1.1.

J. RISK AND MITIGATION

The project will use existing infrastructure and link into ongoing fieldwork. Risks to the success of the project are therefore limited. Potential deviations from the proposed plan may arise in year two if the best sampling time identified in our studies does not coincide with the project 4.1.1 sampling expedition to the Milford Sound area. In this case separate sampling for eDNA analyses can be conducted in Milford Sound at similar costs.

Throughout the project, we will use existing laboratory infrastructure at the University of Otago, which is already being used for eDNA studies of seawater (Gemmell, Knapp, Baltar). This includes a PCR free DNA extraction facility as well as post---PCR laboratories. High---throughput sequencing will be conducted by New Zealand Genomics Limited (NZGL), and we will work closely with the sequencing team to identify optimal sequencing settings.

K. ALIGNED FUNDING AND CO---FUNDING

The proposed project will benefit from alignment to ongoing projects of team members.

Sampling in the EOT will be conducted within the framework of established and ongoing sampling for the "Te Tiaki Mahinga Kai" (TMK) project led by Dr Hepburn. Coordination of our eDNA sampling with TMK sampling trips will reduce sampling cost from NZ$6,000 to NZ$2,000 for each of the three sampling trips in the EOT (plus NZ$3,000 for extended sampling of tidal cycle), corresponding to a total aligned funding value of NZ$12,000. Marine eDNA extraction protocols are currently being tested as part of ongoing University of Otago PhD research supervised by Prof Gemmell and Dr Knapp and funded predominantly via a NZ$97,000 University of Otago PhD scholarship and an Allan Wilson Centre legacy grant. Our proposed molecular markers and sequencing strategy were developed as part of Prof Bunce's eDNA research in Western Australia which is supported by Chevron and BMT Oceania grants to a total of AU$260,000. Masters scholarship opportunities to provide a stipend for an associated Masters student will be provided by the University of Otago.

L. VISION MĀTAURANGA (VM)

The project will be developed in close collaboration with kaitiaki (guardians) as part of the "Te Tiaki Mahinga Kai" project, which monitors customary fisheries areas. We will explore existing indigenous knowledge and provide outcomes directly addressing customary fisheries management challenges.

Our experiments complement those conducted within the framework of the "Te Tiaki Mahinga Kai" project, and results will be disseminated to the kaitiaki of the studied areas, specifically the EOT, through the Mahinga Kai network. The tools produced will be of significant value to kaitiaki in their roles in managing and restoring coastal fisheries and the environment. The tools will be engaged in Ngāi Tahu's monitoring programme of Customary Protection Areas if found to be effective.

Our key stakeholders include iwi and a variety of NGOs such as Forest and Bird. Ngāi Tahu (through Mahinga Kai Principal Advisor Dr Dan Pritchard and customary fisheries expert Nigel Scott) have already confirmed their interest in and support for our research.

One of the key outcomes from our work will be a better framework of knowledge and understanding of our marine biodiversity, knowledge that will add to and support the traditional environmental knowledge (tāonga tuku iho, mātauranga o te taiao) and knowledge of fishing (kai moana) that are critically important to Māori. We hope to trial the utility of this resource via the Mahinga Kai network as part of this project.

M. CONSENTS AND APPROVAL

This work has been approved by the Ngāi Tahu research committee at the University of Otago. The project was developed and will be conducted in partnership with kaitiaki at the East Otago Taiāpure and fisheries and environmental advisors and scientists within Ngāi Tahu.

Consent is required for sampling within the Milford Sound marine reserve. An application for consent will be lodged with the Department of Conservation once a sampling strategy has been finalized from the results of our method development experiments in the EOT. Processing of the application will likely take 40 working days and the cost of NZ$1771 has been included in our budget for "sampling" in year two.

N. DATA MANAGEMENT

The data collected during the course of this research will be maintained using large flat file structures, but we use standard relational database tools wherever practicable to neatly summarize

the results from these analyses. The full data set will be publicly available on completion of the project and open access so that new data might be added to this overtime. Ideally this data would be curated by a major genetic database entity such as Genbank and we will explore that opportunity, but it may well be that the data need to be hosted locally via e.g. NeSI. In the interests of communicating our findings to the greatest possible audience we will seek to publish all our key results in the international peer reviewed literature.

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