Final Report

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Final Report

Provide a description of the learnings and outcomes of the Project.

The project had seven Technical Objectives (each with several milestones) that created outcomes and learnings. The outcomes that map directly to the Technical Objectives are provided in Table 1, and an overview of the all the project outcomes and learnings are described below.

Table 1. Brief synopsis of project Technical Objectives and directly relevant outputs

Technical Objective	Relevant Outputs
1. Generate DNA sequences for protected marine fishes (9 taxa), and co-occurring congeners (6 taxa)	DNA sequences for seven protected marine fishes and five congeners collated (12 taxa)
2. Generate DNA sequences for priority marine fishes found in estuarine (15 taxa)/coastal habitats (417 taxa)	DNA sequences for 10 fish taxa exclusively found in estuarine habitats and 289 fish taxa found in coastal habitats collated
3. Generate DNA sequences for freshwater insect taxa used in the Macroinvertebrate Community Index (MCI)	DNA sequences for 89 insect taxa relevant to the MCI generated
4. Generate DNA sequences for freshwater insect taxa of conservation interest	DNA sequences for 22 insect taxa of conservation interest generated
5. 850 DNA sequenced fish and freshwater invertebrate specimens	DNA sequences for 1,222 specimens of freshwater insect and fish taxa collated, including relevant metadata, into the DNA reference library in the Genomic Observatories Metadatabase (GEOME; https://geome-db.org/workbench/project-overview) available for use in environmental DNA (eDNA) studies
6. Formalise case-study for generating DNA sequences for use in eDNA studies	We have provided an overview of our project approach in presentations and webinars (details below), as a project video (https://vimeo.com/991879770/8c282670b0), and in an open access publication (in preparation)
7. Hosting of a webinar and project video launch for regional council staff	Regional council staff involved in the Project (including: Waikato Regional Council, Hawke's Bay Regional Council, Bay of Plenty Regional Council, Environment Southland, Greater Wellington, Marlborough District Council), those in the Surface Water Integrated Management Special Interest Group, Coastal Special Interest Group, those who regularly attend the national "Freshwater eDNA working group", and any other interested parties were invited to attend a webinar on 25 May 2024 (see: https://vimeo.com/950596913/86fb378f84?share=copy)

Outcomes

Outcome 1. DNA reference sequences for freshwater insects and marine fishes

This project has generated DNA sequences for priority New Zealand (NZ) freshwater insects and fishes to enable their detection using environmental DNA (eDNA). Specifically, we have generated DNA sequences for the CO1, 12S and 16S gene regions that are typically used in eDNA approaches to detect and discriminate taxa. Our sequencing effort focused on taxa of high interest to councils and other end-users, including: freshwater insects prioritising taxa of conservation interest (i.e. those listed as threatened, at risk, and data deficient in the NZ Threat Classification System) and taxa relevant to the Macroinvertebrate Community Index (MCI); protected marine fishes (and their potentially co-occurring closely related species hereafter "congeners"); fish taxa that are exclusive to estuarine habitats and fish taxa reliant on coastal habitats

(as defined by project team members, Carl Struthers, Te Papa Tongarewa, and Thomas Trnski, Auckland Museum).

We sequenced the CO1 gene region for 111 freshwater insect taxa (including 146 specimens), 110 of which are taxa relevant to the MCI, and 22 that are of conservation interest (Table 2; note that all taxa of conservation interest were also relevant to the MCI, except one caddisfly taxon, *Atrachorema mangu*). The sequenced CO1 gene region for every taxon was unique and able to be used to confidently distinguish among taxa, except in the case of two caddisflies, *Pycnocentrodes aeris* and *Pycnocentrodes aureoles*, that shared the same DNA sequence. Overall, the freshwater taxa proved more challenging to sequence than the marine fishes for reasons related to specimen acquisition, access and resourcing of appropriate taxonomic expertise, specimen preservation, and primer specificity (see "Learnings" for more information).

For the marine fishes, we were able to collate sequences from at least one of the gene regions for: seven protected marine fishes and five of their congeners (12 taxa including 14 specimens); taxa that are exclusive to estuarine habitats (10 taxa including 15 specimens); many fish taxa found in coastal habitats (289 taxa including 509 specimens); as well as freshwater (3 taxa including 3 specimens) and offshore (324 taxa including 535 specimens) taxa. Across these fish taxa, we found that CO1 was able to discriminate among all of the sequenced taxa, except for a few closely related species (~2% of pairwise taxon comparisons). However, we found the very short 12S and 16S gene regions were less discriminatory across taxa, with the DNA sequence matching exactly for several species (~15% and 6% of pairwise comparisons, respectively) within the same genera, and sometimes within the same family.

Table 2. Summary of the DNA reference sequences generated in the project. For marine fishes, sequences were generated for the COI, 12S, and 16S gene regions for protected marine fishes and their congenerics (Protected and congener), fishes exclusive to estuarine habitats, fishes reliant on coastal habitats, and others found in offshore and freshwater habitats. For freshwater insects, sequences were generated for the COI gene region for taxa listed as threatened, at risk, and data deficient in the NZ Threat Classification System (conservation interest) and taxa relevant to the Macroinvertebrate Community Index (MCI). For some taxa, several specimens were sequenced, and many of these taxa were not previously represented in the existing genetic databases (i.e. No. taxa not in Genbank).

Taxa + gene region	No. specimens sequenced	No. taxa sequenced	No. taxa not in Genbank
Fishes COI			
Protected and congener	11	11	1
Estuarine	14	10	5
Coastal	436	286	160
Offshore	440	304	272
Freshwater	3	3	2
12S			
Protected and congener	10	10	1
Estuarine	8	8	3
Coastal	240	206	111
Offshore	91	91	72
16S			
Protected and congener	12	12	0
Estuarine	8	8	3
Coastal	228	194	72
Offshore	80	80	59
Freshwater insects COI			
Conservation interest	25	22	6
MCI	121	89	10

Outcome 2. Curated DNA reference library available for searching and use

The molecular library hosted in the Genomic Observatories Metadatabase (GEOME, https://geome-db.org/) consists of 1,787 DNA reference sequences from 1,222 expertly identified specimens and is curated, retaining links to voucher specimens, metadata (such as the institution that holds the specimen and date of collection), and acknowledging the potential for Māori interests in the specimens and derived DNA sequences. The DNA reference library is held as a project in GEOME called "DNA Reference Libraries of Aotearoa New Zealand" (https://geome-db.org/workbench/project-overview; Figure 1). Through this web user interface the DNA reference library is easily queryable – for instance, to

quickly address whether a particular taxon is represented – but it can also be programmatically accessed via the R package geomedb for inclusion in automated, high-throughput taxonomic assignment in eDNA workflows.

The GEOME infrastructure supports research to follow established standards in the biodiversity and genomic sciences, and with respect to the FAIR Guiding Principles for Scientific Data and CARE Guiding Principles for Indigenous Data Governance. The specimens used to generate DNA sequences in the project have been collected over decades, and in many cases the cultural context in which they were collected is unknown or there is not an existing relationship with mana whenua. Contextualizing access to the DNA reference library through the project landing page in GEOME helps provide this important context and disclosure that there is incomplete provenance and attribution information with regard to the specimens, and to invite and acknowledge the right of Māori to connect with these collections.

In this way, our molecular library has been developed according to best-practice guidelines for genetic and biodiversity research and provides a proof-of-concept approach and infrastructure for other DNA reference libraries.

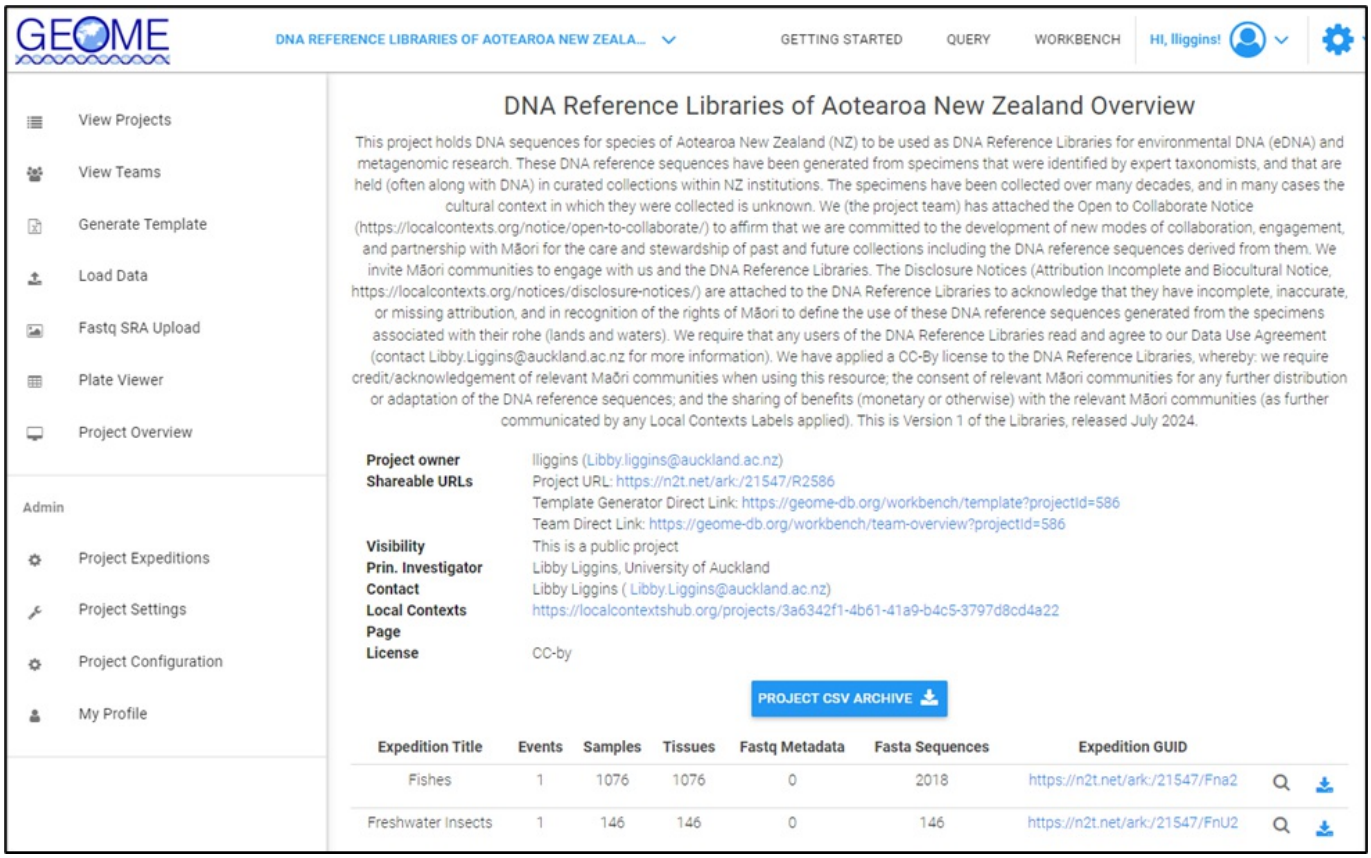

Figure 1. Screenshot of project landing page for the DNA reference library in the Genomic Observatories Metadatabase, GEOME. Note that the number of "Fasta Sequences" for Fishes includes sequences for all three gene regions, including the two partial gene regions of 16S and 12S targeted by the sequencing.

Outcome 3. Invitation and acknowledgement of Māori interests in the DNA reference library

We have used the Local Contexts system (https://localcontexts.org/) to disclose and to safeguard potential Māori interests in the DNA reference library. Specifically, we have created a Local Contexts Project, "DNA Reference Libraries of Aotearoa New Zealand" (https://localcontextshub.org/projects/3a6342f1-4b61-41a9-b4c5-3797d8cd4a22) that interoperates with the project and data in GEOME. This interoperation ensures that Engagement and Disclosure Notices from the Local Contexts Project are visible within GEOME and any sample/DNA sequence information downloaded from the GEOME project page (Figure 2). The Engagement Notice, "Open to Collaborate", affirms that we are committed to the development of new modes of collaboration, engagement, and partnership with Māori for the care and stewardship of past and future collections including the DNA reference sequences derived from them. The Disclosure Notices ("Attribution Incomplete" and "Biocultural Notice") are attached to the DNA reference library to acknowledge that it has incomplete, inaccurate, or missing attribution, and in recognition of the rights of Māori to define the use of these DNA reference sequences generated from the specimens associated with their rohe. Through the Project's use of the Disclosure Notices, Māori communities are invited to apply Biocultural Labels to further describe the cultural context of the specimen/s or data and expectations for future use of the genetic resource.

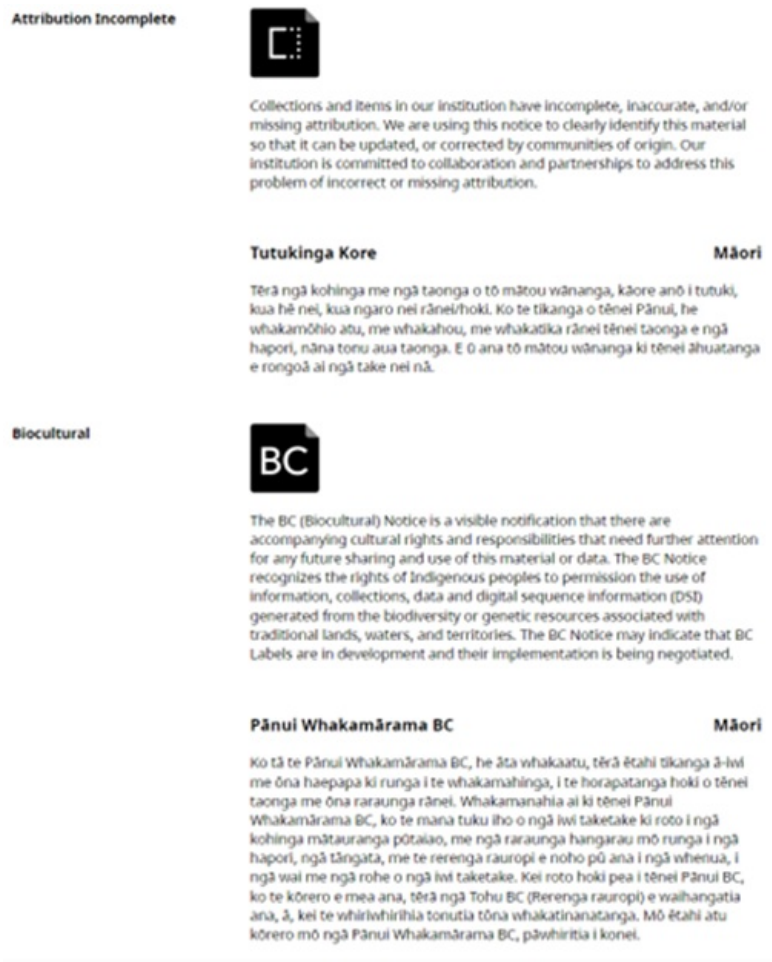

Figure 2. Screenshot of the Disclosure Notices provided by the Local Contexts system that interoperates with GEOME. These Notices are applied to the GEOME project "DNA Reference Libraries of Aotearoa New Zealand".

To further encourage the ethical use of the DNA reference library and constituent sequences, a Data Use Agreement is provided on the GEOME project landing page. The Data Use Agreement is based on a template designed to promote alignment with the CARE Principles of Indigenous Data Governance and encourages use for the purposes of supporting biodiversity research, conservation, and collective benefit. Users of the DNA reference library are asked to read and agree to the Data Use Agreement.

Outcome 4. Consolidated specimen and DNA collection curated by the Auckland Museum

All specimens from which DNA has been extracted and sequenced in this project, have been expertly identified by a trained taxonomist. Where possible, voucher specimens for the DNA reference sequences are also retained in national collections held at the Auckland War Memorial Museum Tāmaki Paenga Hira or Te Papa Tongarewa. In addition to the physical specimens, any leftover DNA extractions from the sequenced insects and fishes are retained in a newly formed DNA extraction collection held at the Auckland Museum. The voucher specimens enable the taxonomy of the sequenced specimens to be revised according to any nomenclature changes or systematic revision within the taxonomic groups. Furthermore, the DNA extractions are available for any further generation of DNA reference sequences for these specimens and taxa.

Outcome 5. GitHub repository providing the pipeline used to quality-control DNA reference sequences

The project team developed a series of semi-automated transparent, standardised, and rigorous quality control steps to take the "raw" DNA sequences generated by the sequencing platforms (Sanger sequencing at University of Otago and Illumina sequencing at Wilderlab) and provide quality-controlled DNA reference sequences. The code for this bioinformatic pipeline (in the R statistical language) will be made publicly available on GitHub (a web-based platform that allows users to create, store, manage and share their code). This GitHub repository describes the steps taken to ensure the DNA reference sequences are of good quality, and provides the code for others to use the methodology themselves, in the generation of their own DNA reference sequences.

Outcome 6. Regular ongoing communications among project team, council, and interest groups

In the course of the project, the project team and other interested groups formed a widely communicating network. The core project team (including council) held fortnightly meetings for the duration of the project and email updates to the wider project team (including: Waikato Regional Council, Hawke's Bay Regional Council, Bay of Plenty Regional Council, Environment Southland, Greater Wellington, Marlborough District Council) and interest groups (including: Department of Conservation, Ministry for the Environment, NIWA) regarding all samples processed, project activity, and priorities were provided: after the first 12 months, and then every quarter thereafter. In addition, several presentations were given and webinars were hosted to encourage dialogue across the network, and to ensure the project was responsive to the input and needs of council as well as other end users, as follows:

- October 2022: We hosted a webinar "eDNA webinar: use cases, progress, and considerations" (https://www.youtube.com/watch?v=KeEAGzaGSzs) to which we invited the full project team (including from Waikato Regional Council, Bay of Plenty Regional Council, Environment Southland, Greater Wellington Regional Council, Hawkes Bay Regional Council, Marlborough District Council, MfE, NIWA, DOC, two universities, two museums, and private consultancies) as well as others working on similar projects nationally (including Cawthron, the National Science Challenge [NSC] "Biological Heritage") or are potential end-users.

- November 2022: Shaun Wilkinson (project team member, Wilderlab) presented "Identifying and prioritising gaps in Aotearoa's DNA reference libraries" at "Waitī Waitā" the New Zealand Marine Sciences Society and New Zealand Freshwater Sciences Society Joint Conference (https://www.waitiwaita.co.nz/) communicating our rationale within the project for prioritizing the generation of DNA reference sequences for certain groups.

- May 2023: We held a webinar for the project team and other interested parties to provide an update on our progress, Envirolink Tools project update Stocking Aotearoa NZs molecular library for eDNA monitoring_11May2023.mp4 (presented by project team member, Libby Liggins, University of Auckland).

- July 2023: Council lead for the project, Michael Pingram (Waikato Regional Council) organised for Aimee van der Reis (project team member, Massey University) to deliver a presentation to the Surface Water Integrated Management (SWIM) Special Interest Group. The presentation covered the general background and motivation for the Envirolink Tools project as well as progress to date.

- May 2024: We held a webinar for the project team and other interested parties as part of the "Freshwater eDNA methods group" meetings coordinated by Wilderlab providing an overview of the project outcomes and learnings, https://vimeo.com/950596913/86fb378f84?share=copy (presented by Libby Liggins and Aimee van der Reis).

Following the project end, members of the project team will continue to stay in communication, ensuring the DNA reference library is used and it's utility communicated through forums such as SWIM.

Outcome 7. Overview video of project approach and outcomes

The project team has contributed to a video (https://vimeo.com/991879770/8c282670b0) that provides an overview of the project's purpose and motivation, and the project approach. Our goal in the video was to highlight the role of council in motivating the research, and the broadly collaborative team and network of experts that were established to ensure the project's success. The video is intended to provide council, and other interest groups, with a quick introduction to what the DNA reference library is, and the infrastructure and tools that support it, to encourage uptake and use of the generated resource.

Outcome 8. Contribution to several across-project wānanga, webinars, reports and educational materials relevant to DNA reference libraries and eDNA-based research

Our project team has corresponded extensively with researchers involved in other complementary research and projects within NZ. Specifically, project team members Aimee van der Reis, Libby Liggins, Andy Hicks (MfE), and Shaun Wilkinson have worked with the Institute of Environmental Science and Research (ESR), the National Institute of Water and Atmospheric Research (NIWA), and the Te Kotahi Research Institute to support the project "Molecular Library of Groundwater Fauna" funded by MfE. This project aimed to generate DNA reference sequences for stygofauna and the production of resources to support education and encourage kōrero about eDNA technologies, including the generation of DNA reference libraries. As part of this work, Aimee van der Reis and Libby Liggins both presented at the "Whāki Webinar Series" on "eDNA: Exploring Māori Data Governance and Sovereignty" (https://www.waikato.ac.nz/research/institutes-centres-entities/institutes/tkri/resources/webinars/whaki-webinar-series/) on in September 2023. This webinar on Māori data science, Māori data governance and Māori data sovereignty issues in relation to the current DNA reference libraries being developed, and included an interactive discussion component for community engagement.

Our team members have also been attending and contributing to the "Te Tiriti-guided national DNA reference library wānanga series" (https://www.landcareresearch.co.nz/events/national-dna-database-webinar-series/) and the wider project lead by Manaaki Whenua. Libby Liggins attended a hui in Wellington (hosted by Manaaki Whenua, February 2023) aimed at kicking off this work programme funded by the NSC "Biological Heritage". Subsequent to this, Michael Pingrim contributed a presentation as part of the their webinar on "End use-cases of reference databases" in May 2023 highlighting the opportunity of eDNA-based monitoring for councils and their needs from the research community, and Libby Liggins contributed as a speaker and panellist for their webinar "Cross Project Fertilisation of best-practice" in September 2023 where she overviewed the approach of this Envirolink Tools project, and the aligned MfE project highlighting where these are both incorporating best practice learnt from past projects and infrastructures established in NZ and internationally.

Within the project we also organised an "Aligning Synergies hui" across all these groups in July 2023, to discuss issues raised about DNA reference libraries, and in particular, best practice regarding Indigenous Data Sovereignty. There were productive discussions which helped align the approaches of the various projects. In general, working across these projects ensured that our Envirolink Tools project has been in keeping with best-practice as it has been established nationally.

Learnings

Learning 1. Using existing curated collections can lead to immediate gains in generated DNA reference libraries

Our project benefited from decades of past collection and taxonomic efforts that have culminated in the personal collections of project team member Steve Pohe (Pohe Environmental Ltd.), and the collections of the Auckland Museum and Te Papa. Extracting DNA from these existing voucher specimens enabled us to quickly and economically generate DNA reference sequences, and avoided unnecessary collections of wild organisms, some of which are rare. Several other persons, and institutions, hold similarly valuable collections for other taxonomic groups that could also be used for the rapid generation of DNA reference libraries.

Learning 2. Some existing collections are not appropriately preserved for DNA-based research

Despite the many benefits of using existing collections, in many cases (and particularly the freshwater insects) the voucher specimens were either too old, or had not been treated or preserved in a manner that and successful DNA extraction and/or sequencing. We could not identify any specific indicators for whether we would have success with a specific specimen or not, but certainly the probability of gaining a suitable DNA reference sequence decreased with the age of the specimen.

Learning 3. Certain taxonomic groups require molecular and bioinformatic research to design primers or explore appropriate gene regions

For some taxonomic groupings within the freshwater insects, good DNA extractions were gained from specimens, but the amplification of the target gene region using a suite of universal primers, in different combinations, did not result in adequate amplification to enable DNA sequencing. In these cases, it is likely that the DNA sequence at the primer binding site is too diverged from the sequence of the primers we have used. If this is the case, appropriate primers may need to be designed or alternative gene regions explored as potential DNA reference sequences. Alternatively, the DNA extraction protocol from these specimens may co-extract other interfering chemicals from the tissue. Both these issues would benefit from time invested in molecular and bioinformatic research on these taxonomic groupings.

Learning 4. Significant gains in the DNA reference library for freshwater insects could be achieved through retrospective data-mining and curation of existing DNA sequence collections

In the course of communicating about the project, we were alerted to the previous work of other researchers and institutions who had generated DNA reference sequences for expertly identified specimens, but these had never been published, or the published sequences had not retained their association with the voucher specimen. For the marine fishes, we were often able to collate these DNA sequences into the DNA reference library through their association with specimens held at the Auckland Museum or Te Papa. However, to do this adequately for freshwater insects would require more funded time for a taxonomic expert who is familiar with the collections and researchers in the field.

Learning 5. Further targeted field collections by expert taxonomists are required to enhance the DNA reference library for freshwater insects

Despite freshwater insect taxa of conservation interest being a priority for DNA sequencing, many of these taxa are naturally rare, are very range restricted, or are limited to specific habitats, making the acquisition of specimens difficult. Several of these taxa were not available in the collections we accessed in the project, and their inclusion in the DNA reference library would require targeted field collections by expert taxonomists. In particular, field collections over the summer period when many of these taxa are mature and easily identifiable, and in the South Island, would provide valuable specimens to extend the DNA reference library.

Learning 6. The COI gene region will provide higher precision than 12S and 16S for marine fishes

In some cases, although a 12S or 16S DNA sequence was successfully generated for a fish taxon, we were unable to verify whether it was a truly representative sequence, or contamination, as it matched the DNA sequence of several other species. In these circumstances, we have elected not to include the DNA sequence in the DNA reference library, until a time we can be more confident that it is an appropriate representative sequence for that taxon. These 'pending' sequences include 12S sequences for: 1 protected species/congener (from one specimen), 60 coastal taxa (from 64 specimens), and 23 offshore taxa (from 23 specimens); and 16S sequences for: 1 protected species/congener (from one specimen), 50 coastal taxa (from 54 specimens), and 18 offshore taxa (from 18 specimens). In most cases, DNA sequences for these taxa, and closely related taxa, are not available in Genbank or other public databases making it difficult to surmise whether the level of conservatism at these gene regions is to be expected. Nonetheless, even if these sequences are truly representative of the focal specimen/taxon, they will be of limited use in eDNA studies that endeavour to infer biodiversity patterns at the species-level.

Based on this experience in working with 12S and 16S for marine fishes, we would recommend any further efforts to generate DNA reference sequences for marine fishes either focus on different gene regions, or in gaining longer sequences for 12S and 16S.

Learning 7. Further gains in the DNA reference library for marine fishes will need to be opportunistic

The marine fish taxa for which voucher specimens need to be collected and DNA reference sequence generated are relatively few, but it would be difficult and largely unfruitful to invest in a targeted collection effort. For example, they include: protected species; large bodied species, for which holding a voucher specimen would be too resource intensive; and very rare, vagrant species that do not yet breed in NZ. Nonetheless, a list of "species-of-interest" could be collated and circulated to recreational and commercial fishers, and others with an interest and aptitude in observing fishes, through existing citizen science projects, such as "What's That Fish NZ?" (https://www.facebook.com/WhatsThatFishNZ/). Auckland Museum has previously equipped Fisheries Observers with collection kits and protocols to aid them in recording specimen information and taking tissue samples suitable for later genetic analysis.

Learning 8. Researchers and institutions need to be supported in a whole-science-system approach to reconnecting mana whenua with historical collections

Cultural sensitivities (as stipulated in WAI262 and the Nagoya Protocol) regarding the attribution and sharing of information and data pertaining to NZ's biodiversity, including DNA sequences, have been an important consideration for the project. In general, short DNA sequences of the mitochondrial genome, such as those targeted in this project, are of use for species identification only, and have a low risk of misuse. Furthermore, benefits arising from the generation of these data will be shared among all NZers, present and future. Nonetheless, we acknowledge and support the interests and rights of mana whenua in reconnecting with biological collections and related data that originates from their lands and waters. In this project we elected to use the Local Contexts Notices in support of the CARE Principles of Indigenous Data Sovereignty to invite Iwi and Hapū to connect with the project and generated DNA sequence data, to rightfully attach provenance information or stipulate particular interests. However more work needs to be done to re-connect mana whenua with these biological collections.

In general, NZ's museums have made much better progress in re-connecting their collections with mana whenua than most other research institutions. Our project was fortunate to have relationships with some Iwi and Hapū through the involved museums, but the establishment of these connections and notification of these Māori communities of the existing collections, and on-going research from these collections, is far from complete. This is particularly the case for historical collections dating from decades or even centuries ago. A comprehensive effort to proactively contact relevant mana whenua for these collections was beyond the scope of this project. However, now that most museums have well digitized and georeferenced collections, it is possible to identify the provenance of large numbers of specimens in an efficient and accurate way. Such an exercise of sleuthing relevant biological collections within museum holdings, and reaching out to mana whenua would be hugely valuable in restoring trust between Māori and researchers, fostering communication and collaboration, and providing educational and capacity building opportunities, including for eDNA relevant research.

Provide a description of whether the tool developed is more or less successful than expected.

Help:

Please explain, giving consideration to the extent the tool will enhance environmental management by regional councils.

Provide a description of whether the tool developed is more or less successful than expected

The project was more successful than expected in terms of the total number of specimens for which DNA reference sequences were generated. This success was due to the larger number of marine fish specimens that were available to the project than anticipated. However, the project was less successful in generating DNA reference sequences for freshwater insects for reasons such as specimen accessibility, the required taxonomic expertise being limited in NZ, and substandard DNA preservation of DNA in specimens (see "Learnings").

The fully functional DNA reference library has been made available to councils as expected, and it is anticipated that this will immediately improve the taxonomic assignment of eDNA sequence reads for past eDNA datasets, as well as future eDNA monitoring. The intimate involvement of councils (and the Ministry for the Environment, MfE) in the project, and their interest in eDNA monitoring advances more generally, assures the project team that the resource developed is key to improving the implementation of eDNA monitoring. More specifically – through the generation of reference DNA sequences for many taxa of interest in NZ, and establishing a comprehensive and publicly available DNA reference library, including for co-occurring and closely related species – a major limitation for eDNA monitoring has been addressed. The increased precision and comprehensiveness of the DNA reference libraries now available through the project are expected to significantly improve inferences from eDNA-based monitoring and targeted biodiversity assessments undertaken by council in freshwater, estuarine and marine ecosystems.

Are the councils satisfied with the tool development/adaptation?

Yes, the project has met the objectives that were developed with councils at its inception. This project has generated the resource required to meet the needs of regional councils to better take advantage of the potential offered by eDNA for efficient and holistic environmental monitoring.

Was the work completed within the original estimated timeframes and budget?

Yes, the planned project was completed within the original estimated timeframe and budget. Fortuitously, however, the broad communication and socialisation of the project approach and progress attracted co-funding from the MfE (from February – July 2024) to complement and enhance the project objectives. For instance, co-funding enabled fieldwork collaboration with Ngāti kūri, and collection of an additional 162 freshwater insect specimens, their identification, and attempted DNA extraction and sequencing. From these efforts, an additional 15 specimens of conservation interest (11 taxa) and 85 specimens relevant to the MCI (51 taxa) will soon be added to the DNA reference library (and to the totals provided in Table 2). Furthermore, the co-funding has incentivised the synthesis of the project learnings and next steps for future work. As a result of this later collection-to-sequencing effort, and larger complementary written synthesis, the submission of our own project publication to an open access journal has been delayed to include these related results and activities.

Did the tool development require new research or did it adapt previous research for use in each region?

The tool development adapted previous research methodologies and infrastructures to be fit for purpose. The novelty of this work programme was that we brought together a diverse project team (including council, museums, universities, CRIs, private research institutions, and commercial laboratories), with diverse expertise (in field ecology and biodiversity studies, taxonomy, molecular biology, bioinformatics, and data management). The work programme benefitted from some established collaborations, but also the identification and inclusion of several project members who are the appropriate expert/s in their field nationally. The project sought and used appropriate infrastructure and tools that enables the DNA reference library to be made available to users, but in a way that is culturally responsible and responsible to communication and interactions with interested Māori communities. Our comprehensive and cohesive approach to generating a DNA reference library has established a new level of "best-practice".

Will the tool be of use to organisations other than regional councils?

Yes, the tool will be of use to organisations and stakeholders other than regional councils. Any group that has undertaken, are currently undertaking, planning to undertake, or likely to undertake, eDNA (or metagenomic) research or monitoring in the freshwater or marine environment of NZ will find the DNA reference library immediately useful. Most regional councils and the Department of Conservation (DOC) are already implementing eDNA monitoring, several hapū and iwi are exploring the use of eDNA based research in their rohe, and there is a growing application within the citizen science community due to the Environmental Protection Agency's Wai Tuwhera o te Taiao – Open Waters Aotearoa programme.

The DNA reference library is available for council and any other group to use, provided that they read and agree to the terms of the data use

agreement. The data use agreement is general, requiring that users acknowledge the Indigenous (Māori) provenance of DNA sequences generated from the biodiversity of Aotearoa and stipulating that the use of the DNA sequences should be for the purposes of biodiversity, conservation, and environmental research, monitoring, or education, where the intent is not commercial gain.

The DNA reference library is hosted on GEOME as a project. Through the web user interface the specimens and taxa (and their gene regions) included in the library can be explored in a map or a table. The DNA reference sequences can be downloaded from here to be used for the taxonomic assignment of eDNA or metagenomic sequences, or queried via the geomedb R package.

Will the tool require regular updating to remain current?

Although the DNA reference library will be available in GEOME in perpetuity without maintenance, it's future value would be assured by on-going curation. This maintenance would ensure that any changes to the taxonomy of specimens in the DNA reference database are updated, and that newly generated DNA reference sequences can be added, further enhancing the resource. The interoperation of the Local Contexts Hub project and GEOME project is automated, so that any updates from Māori communities regarding the application of Biocultural Labels to the specimens or DNA sequences will be automatically synced with the project in GEOME. The Auckland Museum (and Te Papa Tongarewa) can continue to hold the tissues and DNA extracts from the specimens, and in support of the DNA reference library, as part of their core service provided to the scientific, and broader community.

Have councils indicated that they will fund this activity?

No councils have communicated an intent to fund any ongoing curation or enhancement of the DNA reference library, but there is a willingness to support the inclusion of further taxa through opportunistic collection of specimens and direction as to what taxa would be of next priority to include. It has been indicated that this project will likely foster an increase in the use of eDNA tools by regional councils more generally, and as such, there will be wider interest in the improvement and continued advancement of the DNA reference library.

Are the councils satisfied with the tool development/adaption?

	Mark with X
Yes	X
No	

Was the work completed within the original estimated timeframes and budget?

	Mark with X
Yes	X
No	

Did the tool development require new research or did it adapt previous reserach for use in each region?

	Mark with X
New	
Adapt	X

Will the tool be of use to organisations other than regional councils?

	Mark with X
Yes	X
No	

Confirm your plans for the next quarterly reporting period.

Project has now been completed.

Will the tool require regular updating to remain current?

	Mark with X
Yes	X
No	

Have councils indicated that they will fund this activity?

	Mark with X
Yes	
No	X