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eDNA Standards to Improve NZ's Biosecurity System

Prioritisation Workshop Proceedings

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Contents

Executive Summary

This report summarizes the findings of a November 13, 2025, workshop in Wellington aimed at establishing national environmental DNA (eDNA) standards for Aotearoa New Zealand. Funded by an Envirolink grant, the purposes of the workshop were to:

- Assess guidelines for sampling effort, collection methods, and interpretation thresholds
- Design case studies for fit-for-purpose generation and interpretation of eDNA data in the context of detection and sensitivity thresholds.
- Progress toward implementation in an eDNA dashboard that is fit-for-purpose for biosecurity decision-making, via a subsequent Envirolink grant.

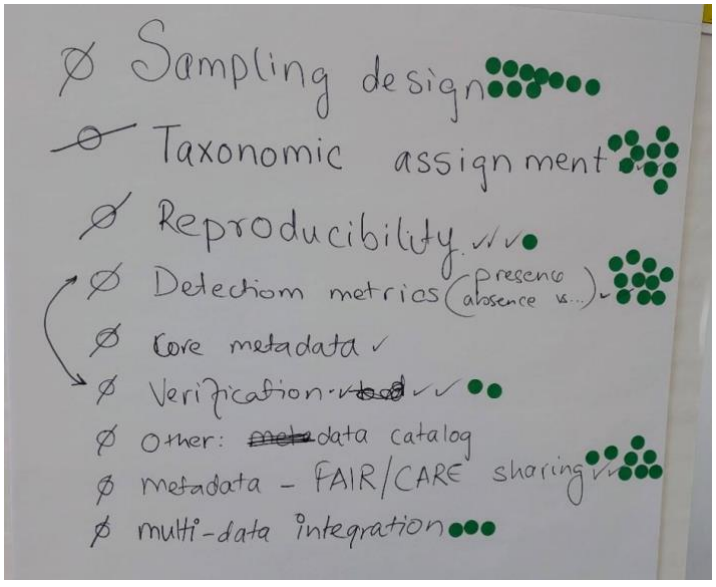
The Critical Need for Standardization

Currently, the utility of eDNA as an operational asset is hindered by inconsistent use of standards, making it difficult to integrate data across regional and national levels. Establishing national standards is essential to ensure that biosecurity detections are reliable, allowing agencies to act decisively without wasting resources on false alarms.

Priority Gaps and Technical Findings

Based on expert feedback and workshop discussions, four priority gaps were identified as the highest needs for future research and development:

1. Sampling Design
2. Taxonomic Assignment
3. Detection Metrics and Verification
4. Metadata and FAIR/CARE Sharing.



To refine these standards, the workshop proposed several case studies targeting different aspects of the biosecurity invasion curve.

Immediately following the workshop, the research team circulated a proposed sampling plan for case studies that would addresses some of the priority gaps in the standards. Sampling focused on three key freshwater invasive species in New Zealand's North Island: brown bullhead catfish, koi carp, and gold clam. A total of 86 eDNA filters (plus 12 controls) were collected to address three main research questions:

1. How gold clam eDNA detection relates to its population density gradient in the Waikato River?
2. How the co-occurrence of all three species influences eDNA detection in Lake Karapiro?
3. How eDNA detection differs across sites with high, medium, and low population densities for each of the three invasive species?

Upon completion of these case studies, the data collected will be recorded in a brief report of findings. These data will be parsed through the Dashboards to assess how the differences in sampling strategies can influence confidence thresholds for decision makers utilising the different biosecurity dashboards. These analytical aspects will be covered under an Envirolink Tools Grant, which follows this Envirolink Advice grant.

Ultimately, the workshop confirmed that standardization is a strategic necessity to transform eDNA from a scientific tool into a trusted, actionable biosecurity asset.

Background and Context

A workshop was held November 13, 2025, in Wellington to review existing environmental DNA (eDNA) standards and prioritise improvements and the development of new standards. The workshop was part of a larger research programme to implement standardised workflows for biosecurity reporting and decision-making for New Zealand's central and regional government agencies.

The workshop was funded by an Envirolink grant and endorsed by Hawkes Bay Regional Council and the Biosecurity Working Group collective of regional councils. Appendix 1 is a list of workshop participants.

This event built on a February 2025 workshop, also funded by an Envirolink grant, to explore development of a framework for an eDNA biosecurity dashboard that would improve data sharing, increase the likelihood of early detection and reduce response times.

The Need for Standards

Establishing national eDNA standards is critical to transform environmental DNA from a promising scientific tool into a reliable operational asset for New Zealand's biosecurity system. Currently, the utility of eDNA is limited by a lack of standards, or inconsistent use of existing standards, which undermines its use for biosecurity detection and response.

The February 2025 workshop explicitly identified this lack of standardization as a critical gap. Participants found that while various eDNA biosecurity dashboards exist, they rely on metadata using different standards, which makes joined-up biosecurity responses difficult. Post-workshop reviews confirmed that the main limitation associated with application of any biosecurity dashboard remains the lack of clear and standardised guidelines. Without these protocols, data remains fragmented and difficult to integrate across regional and national levels.

Standards are essential for instilling confidence in datasets, which directly impacts decision-making. There is currently a lack of confidence in eDNA data in certain cases, stemming from variable sampling efforts and potential misidentifications. This crisis of confidence can delay or paralyse biosecurity responses. In contrast, by implementing a standardised framework, agencies can gain confidence in biosecurity detections that enable appropriate responses, ensuring resources are not wasted on false alarms or withheld during critical incursions.

Workshop Structure

Prior to the November workshop, eDNA experts in New Zealand and Australia, across marine, aquatic and terrestrial ecosystems, as well as end-users and regulators commented on a summary of existing eDNA standards. The draft document suggested guidelines and recommendations for sampling effort, validated collection and laboratory protocols, and data analysis, metadata management and result validation. The analysis emphasised early detection, reproducibility and ecological relevance across aquatic and terrestrial environments. Identified gaps highlighted the need for standardisation in terrestrial sampling, metadata frameworks, and confidence scoring to support robust biosecurity decision making.

The draft document that was circulated for comment is attached as Appendix 2.

Feedback was collected primarily through a Google form; the responses are summarised in Appendix 3.

Prioritisation of eDNA Gaps

Using feedback received on the draft standards review, workshop participants discussed and prioritised the gaps that should be addressed.

Sampling design

Participants criticised current eDNA sampling designs and site selection as being overly reliant on biodiversity sampling methods rather than being tailored for specific biosecurity needs. There was strong support for a "purpose-driven" approach, where practitioners define the purpose of the sampling first and work backwards to determine the appropriate strategy.

The demands of biosecurity argue for moving toward a risk-based sampling scheme. Instead of uniform sampling, participants suggested quantifying risk to adjust sampling frequency and replication -- for example, distinguishing between high-risk ship imports and low-risk remote locations. This approach could be supported by decision trees and occupancy modelling.

Another theme was confidence and environmental context. Participants noted that while freshwater river sampling is reasonably well established, there is a lack of confidence and separate knowledge regarding still waters (such as ponds and wetlands) and marine environments. In addition, there is much less knowledge about sampling terrestrial substrates. Overall, there is a need to clearly define what constitutes "high confidence" in terms of the number of samples or studies required, regardless of the environment.

Collection considerations

The comments on eDNA collection considerations focus heavily on balancing scientific rigour with practical realities. A recurring theme was the impact of operational and logistical constraints. Cost

and time pressures are making field blanks[1] less feasible, though some collection methods may render them unnecessary. To navigate these challenges, participants suggested using decision trees to guide collection based on specific operational constraints and species requirements.

Risk management and consistency were also central themes. For biosecurity applications, participants emphasised starting collection upstream to prevent within-site contamination or the spread of the target species. Additionally, they noted that preservation methods must be consistent to avoid introducing biases that could skew results for certain taxa or substrates.

Finally, the role of community or citizen science emerged as a key theme. Because there is less control over samples collected by the public, increased education is necessary. Participants viewed this "informal sampling" as a valuable "first red flag," suggesting that community detections should serve as early warnings that trigger more rigorous follow-up sampling by professional end-users.

Laboratory Methods

A primary concern was the lack of consistency and transparency among service providers. Participants noted that different providers use varying amplicons and methods, citing specifically that data from Hill Laboratories and Wilderlab differ and are not comparable.

Another theme focused on the need for optimised and specific technical tools. Participants suggested that "biosecurity primer panels" would be valuable for determining marker sensitivity relative to the specific question being asked, and that longer DNA regions would improve targeted species detection.

The discussion highlighted the technical complexity and context-dependence of these methods. Participants observed that DNA extraction techniques must be tailored to the substrate type and the presence of inhibitors, often requiring specific expertise.

Metadata

The discussion on metadata centred on the tension between aligning with global standards and addressing local, practical needs. A primary theme was interoperability and standardization, with participants emphasizing that national standards must be interoperable with international ones. This includes alignment with genomics standards and SeDNA[2], while also incorporating indigenous considerations. The group suggested creating tools, such as a metadata spreadsheet, which combine international standard fields with those specifically relevant to the Aotearoa context.

Data sovereignty and ethics also were a recurring theme. Participants argued for adding FAIR and CARE principles to data management, ensuring data is treated according to specific "flags" regarding its origin, such as private landowner or mana whenua collection sites.

[1] Field blanks in eDNA sampling are control samples of pure substrate (e.g., distilled water, sterilised soil) treated exactly like real environmental samples (opened, exposed, filtered, preserved) but without collecting the actual substrate, to detect contamination from equipment, handling, or the field environment, ensuring results accurately reflect the target organism's DNA, not background DNA from the sampling process itself.
[2] The Southern environmental DNA Society (SeDNA) is an Australian and New Zealand society of environmental DNA researchers and end users that promotes best practices and supports the adoption of methods across sectors.

Image source: Global Indigenous Data Alliance

Participants noted that while in many cases the information for standards mostly exists, the critical step is to mandate its use. To improve compliance and ease of use, suggestions included developing apps or integrating information management systems directly into dashboards with clear optional and mandatory fields. The need to distinguish between field metadata and lab metadata also was noted.

Data analysis and interpretation

Comments centred on the need for standardization and accreditation. Participants were concerned that data interpretation is currently not accredited. They suggested implementing standard pipelines (such as Galaxy) or looking to international infrastructures like Australian BioCommons. There was also a call for establishing recommended biosecurity-specific PCR primer sets tailored to specific sample types and taxa.

A second theme involved the nuance in interpreting results. While presence/absence is a fundamental question, participants noted that a positive detection does not necessarily require immediate action, as it is unclear if the organism is alive or still present in the detection area. The impact of gaps in reference databases on these determinations was also raised as an ongoing concern.

Regarding data workflows and commercial models, a tension exists regarding what data should feed into dashboards: while accessing raw sequencing data would allow for greater downstream consistency and bias avoidance, it conflicts with current models where users pay providers specifically to handle the taxonomic bioinformatics.

Results validation

The importance of contextual "sanity checks" before operational action is taken was strongly emphasised, also the need for a feedback loop that integrates local knowledge and expertise. Results should be validated against historical ranges, seasonality, methods of introduction and expected taxa.

Participants wondered whether a physical sample is required as a "bare minimum" for validation. Beyond initial confirmation, the discussion highlighted the need to determine the stage of incursion

or establishment to inform the next set of decisions. To manage this process efficiently, participants suggested utilizing technical aids such as positive controls and automated systems for flagging anomalies and sending notifications.

Proposed Case Studies

The workshop explored several case studies that could target multiple species or specific sampling/risk contexts, utilizing existing studies where possible and aiming to represent several aspects of the invasion curve.

Case Study Candidates[3]

- *Corbicula* (Asian Clam): This was proposed as a priority pest case study because it has a targeted assay, is highly verifiable (visual detection is possible), and has established engagement from iwi. The core questions would revolve around determining the best sampling protocol for detection, including whether littoral sampling is sufficient or if depth sampling is needed in stratified lakes. Experimental designs could test individual versus pooled samples, compare high- and low-density lakes, and contrast large versus small lakes.
- Koi Carp Biomass: A study in the lower Waikato lakes could address taxonomic assignment questions, potentially data-mining previous eDNA data to quantify read counts across time and locations.
- Hornwort Northland: This species is of interest to Te Arawa Lakes Trust and could test eradication success or presence/absence.
- Range Delimitation of *Gambusia*: To assess if eradication is feasible.
- Wallabies or Mammalian Pests: Could involve cross-water and air sampling, combined with camera traps or detection in water near wallaby collars.
- Predator Free Initiatives: Linking with Predator Free fencing projects (Zealandia, ZIP) to investigate air DNA or focusing on the interface of terrestrial and aquatic environments.
- Gobies in Auckland: Assessing eDNA effectiveness in high rainfall and dynamic environments.

Sampling Environments Discussed

While water is currently the main focus for eDNA applications, other uses were noted, including air (active or passive filters), and specific applications like scat sampling for individual deer ID or testing pest control success in rats and mice. Sampling for terrestrial weeds is possible but challenging, with less well-developed tools and often higher costs.

Next Steps

The workshop concluded with several proposals for moving forward, based on the identified priorities:

- **Iwi Engagement:** Identify and contact potential iwi partners.
- **Case Study Design:** Develop experimental designs for the chosen case studies, with guidance from attendees.

[3] Ultimately, case study sites were chosen to target koi carp, catfish, and gold clam (*Corbicula fluminea*).

- **Decision Tree:** Create a decision tree/triage protocol to guide responses when a positive eDNA detection is made.
- **Metadata Development:** Develop the proposed metadata spreadsheet. The Ministry for Primary Industries has an internal Standard Operating Procedure (SOP) for metadata reporting that may be useful.
- **Tools Grant:** The next stage of the proposed Envirolink tools grant will be developed and shared.
- **Further Collaboration:** Involve other relevant parties, including the former National Institute of Water and Atmospheric Research (now part of Earth Sciences New Zealand) and Te Tira Whakamātaki, the Māori Biosecurity Network, and other interested regional councils.

-ENDS-

Appendix 1 – Workshop attendees

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Appendix 2 – Brief pre-workshop draft of eDNA Standards Gaps

Draft standards governing eDNA methods for reporting biosecurity outcomes

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This document summarises existing standards for environmental DNA (eDNA) methods aimed at enhancing biosecurity outcomes through reliable detection and reporting of target taxa. It provides guidelines and recommendations for sampling effort, validated collection and laboratory protocols, and data analysis, metadata management, and result validation. Emphasis is placed on early detection, reproducibility, and ecological relevance across aquatic and terrestrial environments. Identified gaps highlight the need for standardisation in terrestrial sampling, metadata frameworks, and confidence scoring to support robust biosecurity decision making.

Table 1. eDNA processes and confidence scores – summary table.

Category	Process	Environment	Sources	Confidence	Metadata	Notes
Sampling design	Site selection	All	1, 3, 6, 7, 8, 9, 13, 14, 15	High	Partial	Ecological relevance, hydrology, species distribution, biosecurity pathways
	How to sample	Aquatic	1, 4, 8, 14, 15	High	Partial	Downstream sampling, riffle sections, contamination control
	Standard vs composite sampling	Aquatic	4, 8, 14, 15	High	Partial	Composite sampling improves detection in remote or low-density sites
	Weather considerations	All	1, 2, 4–9, 14, 15	High	Partial	Avoid extremes; align with seasonal stability and species activity
	Replication	All	1, 2, 4, 6–9, 11, 14, 15	High	Partial	3–5 replicates recommended; more for rare taxa or pilot assays
	Examples	Aquatic	15	High	Partial	Lake fish: n > 10; Riverine: 6–11 replicates for 86–95% richness

	Gaps	Terrestrial	13, 14, 15	Low	Incomplete	No terrestrial standards; limited seasonal and replication guidance
Collection considerations	Equipment and sterilisation	All	1, 2, 4, 6, 11, 14–16	High	Complete	Sterivex, open membrane filters, peristaltic pumps; air samplers detailed, bleach
	Volume to filter	Aquatic, Airborne	1, 2, 4–11, 14, 15	High	Partial	1–2 L standard; up to 5 L in low-density; air volume varies by sampler type
	Sample preservation	All	11, 14, 16	High	Partial	Lysis buffer, freezing, ethanol; RNA/DNA shield for integrity
	Field controls	All	1–11, 14–16	High	Partial	Field blanks, filtration blanks, sterile gear, bleach cleaning
	Gaps	Terrestrial	13, 14	Low	Incomplete	No protocols for terrestrial or inaccessible areas; drone use suggested
Laboratory methods	DNA extraction	All	1, 3–5, 7–9, 11, 15, 16	High	Complete	Qiagen, Takara, PowerSoil, Zymo; Wilderlab kits for NZ
	PCR / Primers	All	1–6, 10, 13–16	High	Complete	COI, 12S, 16S; species-specific primers for biosecurity
	Sequencing platform	All	3–6, 11, 13–16	High	Partial	MiSeq standard; NovaSeq, HiSeq for high-throughput; Nanopore emerging
	Gaps	Terrestrial	13, 14, 16	Low	Incomplete	No portable kits; incomplete

						reference libraries for non-aquatic taxa
Metadata	Core metadata	All	14, 16	High	Complete	GPS, time, weather, flow, turbidity, personnel, protocols
	Metadata capture tools	All	14, 16	High	Complete	Field datasheets, LIMS, GitHub, FASTQ headers, dashboards
	Metadata standards	All	13, 16	High	Complete	GBIF toolkit, TDWG standards, Darwin Core, MIxS
	FAIR principles	All	13, 16	High	Complete	Persistent identifiers, open-access repositories, protocol transparency
	Gaps	All	13, 16	Low	Incomplete	No national standard; limited Indigenous governance and automation
Data analysis and interpretation	Detection metrics	All	1–11, 13–16	High	Partial	Presence/absence most reliable; absence must be interpreted cautiously
	Bioinformatics	All	1, 3, 4, 6, 8, 9, 16	High	Complete	Cutadapt, DADA2, QIIME2, OBITools, BLAST; tool documentation needed
	Taxonomic assignment	All	1–9, 13, 14, 16	Medium	Partial	Use multiple databases; flag unclassified taxa; affects assay confidence
	Statistical tools	All	5, 11, 13, 14, 16	Medium	Partial	NMDS, PERMANOVA, occupancy models; link

						detection to validation level
	Gaps	All	13, 14, 16	Low	Incomplete	No unified pipeline; limited support for novel taxa and decision tools
Result validation	Literature review	All	13, 14	High	Complete	Ensures methods match application goals and targets
	Protocol optimisation	All	11, 13, 14	High	Complete	Validated for each target and sample type; quality controls required
	Consistency/Repeatability/Reproducibility	All	11, 15	High	Partial	SOPs and statistical tracking of variability recommended
	Verification	All	13, 14, 15	Medium	Partial	Compare results to known habitat, ecology, and distribution
	Gaps	All	14, 16	Low	Incomplete	No fully adapted framework for assigning confidence levels

[1]Confidence level:

Definition:
An assessment of how reliable and actionable the eDNA detection or standard is, based on sampling design, laboratory validation, and analytical robustness.

Key Dimensions:

Sampling Design: Replication, controls, and effort
Laboratory Validation: Sensitivity, specificity, reproducibility
Bioinformatics: Quality filtering, dereplication, taxonomic assignment
Interpretation Context: Known transport effects, viability uncertainty
Source Strength: Number and quality of supporting guidelines or studies

Scoring Example:

High: Strong replication, validated methods, multiple sources
Medium: Some uncertainty or limited validation
Low: Sparse evidence, unvalidated methods, or major gaps

[2]Metadata quality:

Definition:
The completeness, consistency, and interoperability of the metadata associated with each eDNA sampling event — including environmental, procedural, and contextual information.

Key Dimensions:

Completeness: Are all required fields (e.g., GPS, date, time, flow, turbidity, personnel, equipment) present?
Consistency: Are formats standardized (e.g., ISO date/time, coordinate systems)?
Transparency: Are protocols, pipelines, and data formats documented?

Interoperability: Is the metadata compatible with FAIR principles and dashboard systems?
Governance: Does it align with Māori data sovereignty principles (e.g., Te Mana Raraunga)?

Scoring Example:

Complete: All core fields present, standardized, and FAIR-compliant
Partial: Some fields missing or inconsistent; limited FAIR alignment
Incomplete: Major gaps; not interoperable or traceable

eDNA methodology categories for consideration

Sampling design

Context: Sampling eDNA for biosecurity applications will typically require concentration or aggregation of trace genetic material. The opportunities and barriers will depend on the specifics of the organism(s) of interest, environment being sampled, and feasibility of sampling to optimise detection sensitivity, efficiency, and reproducibility. Significant research has been performed within aquatic systems to support the development of eDNA detection guidelines, and below we present a minimum sampling design guide based on existing research – including non-water substrates where possible. We also identify gaps in research associated with stochasticity that may pertain to sampling in the aquatic environment, as well as when performing sampling using methods that are still being developed – particularly in terrestrial environments.

Site selection: Biosecurity screening prioritises early detection in high-risk areas, even if they overlap with sensitive habitats, while conservation sampling aims to protect vulnerable species and align with biodiversity goals. Still, biosecurity efforts should consider potential ecological impacts, including harm to rare taxa and disruption of culturally significant sites (12, 13). Site selection should consider ecological relevance, hydrology, and accessibility. Areas with high disturbance or contamination risk should be avoided (1, 14, 15). The selection should be based on:

- Biosecurity risk and pathways (6, 8, 13, 14, 15)
- The target taxa habitat and ecology (1, 3, 4, 5, 7, 11, 13, 14, 15) and species distribution (2, 6, 9, 13, 14, 15)
- Hydrological connectivity (1, 3, 7, 9, 14, 15)
- Environmental gradients (2, 6, 11, 13, 14, 15)
- The detection or monitoring objectives, feasibility, and history (2, 4, 5, 8, 9, 11)
- General and seasonal accessibility (1, 7) and land use (3).

How to sample: Often cited as the ‘gold standard’ for eDNA approaches, water sampling in the aquatic environment currently offers the most robust understanding of the movement, persistence, and decay of trace DNA. Based on these attributes, water sampling thresholds have been determined for fish, invertebrates, amphibians, and mammalian species.

- *Water:* Sampling of water should minimise contamination and maximise representativeness of the overall ecosystem:
    - Start sampling at the downstream end of the targeted site to avoid site disturbance and human contamination of the sample (15)
    - Sampling from the stream or lake bank is preferred to avoid entering the water (15) – if stream entry is necessary, sterilised waders are required
    - Collect samples from the flowing sections of the stream (the middle of a riffle section) rather than slower-moving or still areas (15) – this may not be possible in wide or high-velocity streams, in which case sampling from the stream margins is appropriate
    - The sampling context (e.g., flow conditions and water body access) should be documented.

- *Soil*: Soil cores are sampled at specific depths to capture vertical profiles; composite samples are used for large area coverage; consider dry vs wet soil for DNA preservation (6).
- *Air*: For aerial samples, filter-based sampling is most common; airflow control and storage is critical to prevent DNA degradation; passive sampling is good for long-term, low-cost monitoring (21)

Standard vs composite sampling: Replicates can be taken directly as single samples from the water body, but composite sampling is often preferred for remote sites and assay validation (4, 8, 14, 15). Species richness detection from standard and composite samples is similar for fish and macroinvertebrates (15) – for example, Smith et al. 2024 (18) compared standard (single samples) and 'boosted' samples (random pooling of paired samples from the same location) and found no significant difference in species richness between the eight replicate standard or boosted (16 replicates composited to eight pairs) for fish or macroinvertebrate detections. For low-density or biosecurity targets, composite sampling should pool samples from multiple microhabitats. Composite methods involve:

- Sub-sampling at a single site
- Pooling sub-samples into a single bucket
- Filtering the water in the sterilised bucket after a brief (~10 min) period to allow substrate settling.

Weather considerations: Weather and seasonality affect eDNA persistence and sampling reliability. Recommendations are to:

- Sample during stable conditions (2, 6)
- Avoid any extremes, storms, or high-flow events (during or after rain and snowmelt until flow, turbidity, and debris are back to normal) (1, 4-9, 14, 15)
- Apply seasonal consistency (2, 6, 14, 15).

Replication: Replication improves spatial coverage and detection confidence:

- Take a minimum of three replicates per site, but 3-5 are recommended to capture spatial heterogeneity (1, 4, 6, 7, 9, 14, 15)
- The number of replicates depends on available occupancy data (more for rare taxa) (2, 4, 6, 14, 15), taxa and habitat (8), and level of validation or state of the assay (pilot versus routine testing) (11, 14, 15).
- Examples:
    - ***Fish in still water:*** **Sampling threshold of n > 10 for 95% species coverage for fish (15)**. Based on Sellers et al 2024 (17), where 101 lakes were sampled to estimate sampling thresholds for fish communities. At n = 11, > 95% of lakes reached > 95% coverage of fish species. Randomly sub-sampling the metabarcoding data further confirmed that **fewer than two species remained undetected on average**, with a sample size of 10 randomly distributed samples. No relationship was identified between sampling thresholds and abiotic features of the lakes, including alkalinity and surface area. Other studies have found that species diversity can influence sampling thresholds, with **higher diversity requiring a higher sampling threshold to achieve > 95% coverage**, and lower species diversity lakes reaching optimum coverage at lower sampling thresholds.
    - ***Species in riverine/lotic systems****:* In 54 riverine/lotic systems, to reach 89.5% and 86% richness for fish and NEMS (National Environmental Monitoring Standards invertebrate taxa list of 197 MCI taxa) taxa respectively, six replicates were needed. A further two replicates were required to reach 89% richness for invertebrates. **To reach 90% and 95% confidence, 7 and 11 replicates, respectively, were required for fish, and 9 and 11 replicates, respectively, were required for NEMS taxa (15)**. See https://onlinelibrary.wiley.com/doi/10.1002/edn3.70017 for further details.

Gaps:

- No standardised effort for terrestrial substrates
- Limited guidance on sampling frequency for early detection, and rare or invasive species
- Limited seasonal guidance
- No link between sampling effort and assay validation/confidence of results (or between replication and detection of invasive species).

Collection considerations

Context: Collection of samples for eDNA must ensure sample integrity and minimise contamination. Aquatic sampling methods are well-supported, but terrestrial protocols are lacking. Standardised equipment, preservation techniques, and contamination controls are essential for reliable results.

Equipment and sterilisation: The use of sterile bottles with sterivex filters is standard, but open membrane filters (2, 4, 6, 15, 16) and peristaltic pumps are also used (1, 15, 16). The filter method (active or passive), brand, material, and pore size of the filter should be recorded (11, 14, 16). For air eDNA, passive samplers include dust traps (e.g., Big Spring Number Eight), marble-filled pan traps, filter and funnel sedimentation traps, sticky traps, and opportunistic methods like spiderweb collection, while active samplers include cyclonic air samplers and fan-powered filters (14). Field contamination during sampling should be avoided by sterilising equipment (such as bottles, funnels, bases, clamps, buckets, tweezers) in 10% household bleach (0.5-0.6% sodium hypochlorite) for at least 30 minutes, followed by thorough rinsing with sterile water to remove bleach residues that could degrade DNA, and sterile non-powdered gloves should be worn and changed frequently during sampling and processing (11, 19, 20).

Volume of water to filter: Filter volume should be adjusted based on site turbidity and target density:

- 1-2 L per replicate recommended (1, 2, 4-11, 15)
- Up to 5 L in low-density areas (8, 11, 15)
- For air eDNA, passive samplers may require longer deployment times (days to weeks) to accumulate sufficient DNA, while active samplers can sample larger air volumes over shorter periods (i.e., hours), improving temporal resolution and detection probability (14).

Sample preservation: Samples are usually preserved by using a lysis buffer, frozen, or submerged in ethanol. Any preservation methods should be optimised for the sample type (11). RNA or DNA shield can be added to the filter for increased DNA and RNA integrity (14, 16).

Field controls: Field controls should include negative controls, such as field blanks and filtration blanks (sterile water exposed to environment) (1-11, 14), at least one per sampling site or batch; collected before and after sampling (1,4). To confirm sterilisation of reusable sampling equipment, each equipment use session during sampling events should also include an equipment blank (2). Any potential contamination should be recorded (3, 14-16).

Gaps:

- No standardised protocols for terrestrial sampling
- Limited integration of automated or real-time samplers
- Lack of simplified kits and training for non-experts
- Lack of adaptations for non-wadable rivers and inaccessible areas, e.g., use of drones to collect samples.

Laboratory methods

Context: Laboratory processing of eDNA samples requires strict contamination control and validated workflows. DNA extraction, PCR, and sequencing must be optimised for the sample type and target taxa. Commercial kits and universal primers are widely used, but biosecurity applications demand higher sensitivity and specificity.

DNA extraction: Clean lab conditions and validated kits are essential. The eDNA lab should not also be used for handling potential contaminants (e.g., fish or fish DNA). Sample preparation, DNA extraction, PCR, and post-PCR processes should be physically separated as much as possible. For high assay validation levels, this should include unidirectional lab flow, dedicated pre-PCR and post-PCR areas, and contamination prevention protocols, such as UV sterilisation, flame sterilisation, and autoclaving (11, 19, 20). Each DNA extraction batch should include at least one blank (4). The use of commercial kits is standard for DNA extraction:

- Most aquatic eDNA workflows use Qiagen DNeasy PowerWater (1, 4, 5, 9, 11) or Takara NucleoSpin eDNA Water kits (7, 11) for filtration-based DNA recovery
- Soil and sediment samples require kits like DNeasy PowerSoil Kit and ZymoBIOMICS DNA Miniprep Kit due to inhibitors and complex matrices present in the soil (3, 8)
- Some service providers (e.g., Wilderlab NZ Ltd) provide eDNA kits for water samples and include both sampling and lab processing (2).

PCR / Primers: PCR protocols must be tailored to the target taxa and assay. Universal primers exist for most taxonomic groups. However, primers and PCR conditions need to be tested, optimised, and validated for the chosen target and assay (1-6, 10). Common primers/gene regions for taxonomic groups are:

- COI: Cytochrome oxidase I is widely used for invertebrates and general biodiversity (1, 3, 4, 6, 8, 9, 10)
- 12S / MiFish: Mitochondrial 12S is preferred for fish and vertebrates (1, 3, 4, 6, 7, 8, 9, 10)
- 16S is used for bacterial communities (4).

PCR conditions usually include 35 cycles, 50–65 °C annealing temperatures (depending on primer), and high-fidelity enzymes (e.g., Platinum SuperFi II Taq). PCR protocols need to be documented in detail.
For low-density and biosecurity applications, a highly sensitive and contamination-aware approach is recommended. Instead of universal primers, validated taxa- or species-specific primers may be used (2), the PCR protocol should pass criteria for sensitivity, specificity, and reproducibility, and relevant positive and negative controls should be included (11). Each PCR batch (96-well plate) should contain two to three PCR blanks (no-template controls) (4).

Sequencing platform: The sequencing platform choice depends on scale and resolution needs:

- Illumina MiSeq is the most widely recommended platform (all references) and is ideal for paired-end metabarcoding with moderate throughput
- Illumina HiSeq is used for high-throughput applications (3)
- Illumina NovaSeq is used in high-diversity, large-scale, or multi-target applications (e.g., marine parks, national biodiversity surveys) (4, 6)
- Oxford Nanopore is mentioned for long-read or portable assays, but not yet standard (11)
- Platform documentation is required in validation workflows (5, 11).

Gaps:

- No portable/field-based kits
- Incomplete reference libraries for non-aquatic taxa

Metadata

Context: Metadata are critical for reproducibility, validation, and regulatory compliance. FAIR principles (Findable, Accessible, Interoperable, Reusable) should guide metadata practices across sampling, lab, sequencing, and interpretation stages. There are several initiatives in development for integrating metabarcoding standards and eDNA data (e.g., with the Darwin Core structure and Biodiversity Information Standards).

Core metadata: These data are essential to link eDNA signals to environmental contexts and support data interpretation. They include collection GPS coordinates, date/time, weather, flow, turbidity, personnel, equipment, kits, protocols, and workflows.

Metadata capture tools: To capture consistent and reliable core metadata, the following tools are recommended:

- Sampling: field datasheets, mobile apps, and GPS loggers
- Lab: lab sheets, chain-of-custody forms, lab notebooks, LIMS system, protocol sheets, assay registry
- Sequencing: sequencing provider reports, FASTQ headers
- Bioinformatics/data analysis: GitHub repositories, workflow logs, bioinformatics output files and annotation logs
- Data interpretation: Dashboard logic, validation framework
- Regulatory: Submission templates, agency portals.

Metadata standards: GBIF is piloting a Metabarcoding Data Toolkit to simplify the process of publishing DNA metabarcoding data to GBIF using familiar templates that map data to standardised terms (16), and there is an opportunity to join a community call representing Oceania (https://www.gbif.org/metabarcoding). The TDWG Environmental Samples and eDNA Interest Group is developing a data standard and usage guidelines for sharing environmental sample and environmental DNA data; it integrates infrastructures such as GBIF, OBIS, and INSDC (16), and would also benefit from New Zealand participation (https://www.tdwg.org/community/gbwg/enviro/).

FAIR principles: To make data findable, accessible, interoperable, and reusable, the use of persistent identifiers (e.g., DOIs), standardised formats (e.g., Darwin Core, MIxS), and open-access repositories (e.g., GBIF, ENA) are recommended. For transparency, all protocols, pipelines, and data formats must be documented.

Gaps:

- No national metadata standard
- Bioinformatics metadata often missing or underspecified
- Interpretation metadata (e.g., detection thresholds, indicator strength) rarely formalised
- Regulatory metadata not standardised or linked to reporting formats
- No guidance on metadata automation or integration with dashboards
- Limited alignment with indigenous data governance principles

Opportunities to contribute and benefit from global metadata standard initiatives.

Data analysis and interpretation

Context: eDNA data analysis must account for detection limitations, ecological context, and bioinformatic rigor. Pipelines vary, but core steps include quality filtering, dereplication, and taxonomic assignment. Interpretation should consider assay validation level and ecological plausibility. Most references caution that eDNA detects presence, not viability, and that transport effects can mislead.

Detection metrics: The most reliable detection metrics are presence/absence (1-11, 13-16) and relative abundance (1). Recorded absence should be treated carefully as it is technically impossible to prove (14-16). The metrics should be aligned with ecological inference and other datasets, such as traditional monitoring.

Bioinformatics: Common pipelines include quality filtering, normalisation with negative controls (removing contamination), dereplication, read count filtering, and taxonomic assignment with tools, such as Cutadapt, DADA2, and BLAST/MEGAN (1, 4, 6, 8, 9, 16), OBITools (3, 4, 6, 16), QIIME2 (3, 8, 16), USEARCH and BLAST (7).

Taxonomic assignment: Reference databases, such as NCBI GenBank (1, 3-9), BOLD (3, 6), custom or local reference libraries (1, 2, 4-6, 8, 9), and MiFish DB (7) are used. Any reference libraries should be validated. To handle unclassified or less resolved taxa, raw data should be retained and records flagged during interpretation (1, 4, 7, 9). Unclassified taxa should be included in community metrics (at higher taxonomic level) (4, 6, 16), but excluded from species-level summaries (3, 9). As taxonomic resolution affects assay level, unclassified taxa lower the assay confidence (5, 11, 14). Using multiple reference databases, including local and custom, can help resolve taxa (7, 8, 16).

Statistical tools: Commonly used are NMDS, PERMANOVA, occupancy models, detection probability, species accumulation curves, and alpha diversity. The detection confidence should be linked to the level of assay validation, and scoring of false positives, false negatives, and potential misidentifications (5, 11, 14, 16).

Gaps:

- No unified pipeline across labs; limited guidance on confidence scoring; limited support for low-density and biosecurity taxa, including ecological plausibility and reporting thresholds for novel taxa
- Sparse guidance on database completeness or regional gaps, minimal reproducibility, and taxonomic confidence scoring
- No protocols for resolving ambiguous taxonomic assignments; no standards for reporting uncertainty; limited integration with decision-support tools.

Result validation

Context: Result validation ensures reliability and confidence. Validation should include literature reviews, protocol optimisation, reproducibility testing, and ecological verification. A fully structured validation framework is drafted – it uses 187 criteria across 17 thematic blocks, assigning a level 0 to 5 validation scale and providing interpretation guidance (11, 14). Consistent adaptation of this framework would support validation across projects, targets, and labs.

Literature review: Before starting a new eDNA project, ensure that methods and workflows are appropriate for the application and goals (e.g., correct primers for the target, the sample types, and the chosen habitat) (13,14).

Protocol optimisation: Protocols should be tested, optimised, and validated for each target and sample type, and appropriate quality controls should be used (11, 13, 14).

Consistency/Repeatability/Reproducibility: Set workflow (SOPs) should be used for repeated analyses within the same research context, and variability tracked with statistical evaluations. Any limitations, such as taxonomic coverage and resolution, potential non-target taxa, and possible contamination should be recorded (11, 15).

Verification: Compare results to existing data on target taxa habitat, ecology, distribution, or occupancy (13, 14, 15).

Gaps:

- No fully adapted validation framework with clear and consistent criteria for assigning validation/confidence levels.

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Appendix 3 – Summary of pre-workshop feedback[4]

I. Invasion Contexts

- Primary Contexts: Respondents are most frequently involved in the detection of known exclusion pests and detection of unknown pests.
- Secondary Contexts: The detection of containment pests is categorized as a moderate frequency activity.
- Least Frequent Context: Post-eradication confirmation of pest removal is the context least experienced by the organizations surveyed.

II. Sampling Design and Collection

- Primary Urgent Gaps: Site selection and replication is considered a high-priority gap, with six out of eight respondents marking it as "most urgent". How to sample (protocols) is also highly urgent for several respondents.
- Secondary Urgent Gaps: Standard vs. composite sampling and equipment, sterilization, volume considerations are generally seen as "somewhat urgent".
- Least Urgent Gaps: Weather considerations, preservation methods, and field/laboratory controls are consistently ranked as "least urgent".

III. Laboratory Methods and Other Aspects

- Primary Urgent Gaps: DNA extraction and PCR/primers show mixed urgency but are critical for organizations focused on biosecurity-specific panels. Under "Other aspects," respondents identified decision frameworks and sampling designs for standing water as urgent needs.
- Least Urgent Gaps: Sequencing platforms are considered the lowest priority in this category, with most respondents marking them as "least urgent" or "NA".

IV. Metadata and Data Analysis

- Primary Urgent Gaps: Taxonomic assignment and reference databases represent the most significant gap in this section, with seven respondents labelling it "most urgent". Metadata standards (e.g., GBIF, Darwin Core) and data sharing/FAIR principles are also high priorities.
- Secondary Urgent Gaps: Core metadata reporting, capture tools, detection metrics, and bioinformatics are widely viewed as "somewhat" to "most" urgent.
- Least Urgent Gaps: Statistical tools for analysis are generally ranked lower than the technical requirements of taxonomic assignment.

V. Result Validation and Decision-Making Barriers

- Primary Urgent Gaps: Verification and consistency, repeatability, and reproducibility are the top concerns for validating eDNA results.
- Secondary Urgent Gaps: Protocol optimization is viewed with moderate urgency.

[4] Text here includes refinements and clarifications made during the workshop.

- Least Urgent Gaps: Literature reviews are ranked as the least urgent validation step.

VI. Summary of Barriers to Success

The primary barriers identified by respondents centre on confidence and transparency. Specifically, the lack of standardized methods to navigate taxonomic uncertainty and the risk of false negatives in large or standing water bodies make it difficult for end-users to make informed biosecurity decisions. There is a clear demand for interoperable metadata and quality reference libraries to ensure that "red flags" raised by eDNA can be trusted and acted upon.

Summary Table of Urgency

Priority Level	Key Categories
Most Urgent	Taxonomic assignment, Site selection, Metadata standards, Verification.
Somewhat Urgent	Bioinformatics, Detection metrics, Core metadata reporting, DNA extraction.
Least Urgent	Sequencing platforms, Weather considerations, Preservation methods, Literature reviews.