Australia's National
Science Agency

Integrating environmental DNA science into Australia's marine parks: a roadmap

Citation and authorship

De Brauwer M, Deagle B, Dunstan P & Berry O (2023). *Integrating environmental DNA science into Australia's marine parks: a roadmap*. CSIRO, Hobart.

ISBN (online): 978-1-4863-1917-6
ISBN (print): 978-1-4863-1918-3

Acknowledgements

CSIRO acknowledges the Traditional Owners of the land, sea and waters of the area that we live and work on across Australia. We acknowledge their continuing connection to their culture, and we pay our respects to their Elders past and present.

The roadmap was created in close consultation with Parks Australia; the authors would like to specifically thank Steffan Howe, Cath Samson, and Alex Tomlinson for their valuable input. The authors would also like to thank the many people who provided advice, information, and feedback: Neville Barret, Cindy Bessey, Danny Brock, Simon Bryars, Michael Bunce, Kerry Cameron, Kris Cooling, Pascal Craw, Vanessa Crowe, Joseph DiBattista, Jon Emmet, Francisco Encinas-Viso, Jenny Giles, Madeline Green, Erin Hahn, David Harasti, Jamie Hicks, Jessica Hoey, Tom Holmes, Josephine Hyde, Florian Leese, Rich Little, Craig Meakin, Nicole Middleton, Karen Moloney, Tom Mooney, Barbara Musso, Kristian Peters, Meaghan Rourke, Michael Sams, Chloe Schauble, Nicole Strehling, Alejandro Trujillo-González, Nicola Udy, Sven Uthicke, Jodie Van De Kamp, David Wachenfeld, Mark Wallace, Katrina West, Andrea Wild, Shaun Wilson, and Anastasija Zaiko.

Editing and design: Biotext Pty Ltd.

Important disclaimers

Contents

Executive summary 6

The roadmap: integrating eDNA science into marine parks in Australia 12

Short-term recommendations (present–2030) 14
Short-term – immediate recommendations (present–2025) 14
Short-term – near future recommendations (2025–2030) 16
Mid-term expectations (2030–2040) 17
Practical expectations 17
Analytical expectations 18
Technological expectations 18
Long-term projections (2040–2050) 18
Practical projections 19
Analytical projections 19
Technological projections 19

The context: understanding eDNA and marine parks in Australia 21

Understanding eDNA 21
Sample collection 22
Laboratory analysis 23
Bioinformatics 24
Data analysis 25
Applications 26
Australian eDNA research community 28
Understanding marine park management 28
Marine parks in Australia 28
Marine park monitoring priorities 36
Marine park monitoring strategies 39

How eDNA can be used in marine parks: applications, benefits, challenges and opportunities 42

How eDNA can be used in monitoring 42
Frequently asked questions about the applications of eDNA 43
Current uses of eDNA methods in Australia's marine environment 45
Benefits of integrating eDNA methods in marine parks 46
Scaling up 46
Accessing the inaccessible – health and safety 46
Cost 46
Ethical considerations 47
Stakeholder inclusion 47

Challenges to integration of eDNA methods in marine parks 47
Fragmented monitoring landscape 47
Cost 48
Expertise 48
Reference sequence libraries 49
Abundance 49
Organismal biology and health 50
Result interpretation 50
Spatial and temporal comparability of eDNA datasets 51
Existing and new opportunities 52
eDNA opportunities in existing monitoring programs 52
Environmental DNA opportunities beyond monitoring 56

Action plan for managers: practical steps to integrate eDNA in individual projects 60

Marine park objectives 60
eDNA opportunities 60
Pilot study 63
Monitoring 63
Budget considerations 63

Research priorities and future developments: supporting integration into monitoring programs 64

Research priorities 64
Infrastructure and logistics 64
Assay development and calibration 66
Applied research 67
Analytical improvements 68
Reporting 70
Long-term future developments 70
Abundance: single species 71
Abundance: species communities 71
Physiological condition 71
Population genetics 71
Cross-technological integration 72
Global perspectives and the inter-operability of eDNA data 72

Acronyms, initialisms and glossary 78

References 81

Executive summary

Environmental DNA technologies can contribute to the long-term monitoring and management of Australia's vast marine environment.

This roadmap is a guide for marine resource managers seeking to understand the value of environmental DNA (eDNA) for monitoring. It explains eDNA technologies and empowers resource managers to assess the feasibility of using eDNA to address their monitoring and research needs. It highlights eDNA technologies that need further development before they can benefit marine monitoring programs.

The roadmap is also a guide for eDNA researchers who work in Australian Marine Parks. It describes marine park monitoring requirements and provides background information on existing monitoring programs.

Monitoring Australia's marine environment

Australia has one of the world's largest marine estates, with a network of more than 160 marine parks exceeding 4 million km^2. The marine environment contributes an estimated $105 billion per year to Australia's GDP by providing food, mineral resources, recreation and cultural value, as well as supporting shipping and defence. However, the environment faces local and global threats that may have significant ecological and socioeconomic impacts.

Effective management is vital to addressing these threats and protecting the health of the marine environment. Many jurisdictions and stakeholders are involved in managing Australia's marine environment, and data are critical to support evidence-based decisions. But with its wide geographic extent, diverse habitats and remoteness, Australia's marine environment poses unique monitoring challenges. Effective monitoring of the Australian marine estate requires a broad toolset and expertise. The challenge now is to develop appropriate monitoring frameworks to assess impacts on marine environments and deliver information to support management and policymakers.

> Decision-makers, proponents and the community do not have access to the best available data, information and science. This results in sub-optimal decision-making, inefficiency and additional cost for business, and poor transparency for the community.
>
> *Independent review of the* Environment Protection and Biodiversity Conservation Act 1999*, 2020*

How eDNA can help

Environmental DNA refers to the traces of DNA present in environmental samples (soil, sediment, water, air and more), which can be detected without observing the original organism. Environmental DNA surveys offer a new and useful capability for marine monitoring. Environmental DNA technologies collect, sequence and match traces of DNA to the species present at a site. They are a powerful, non-destructive, cost-effective, and fast complement to traditional monitoring approaches such as visual surveys, video, multibeam sonar or fisheries catch records.

The rapid uptake of eDNA methods globally signals its huge potential in environmental monitoring (Box 1). There are opportunities to integrate eDNA into marine reporting and management to:

- detect pest species
- detect rare and threatened species
- provide data on multiple species to characterise ecosystems
- detect changes in environmental condition.

Once established, eDNA surveys are fast, cost-effective and non-lethal, with higher detection probabilities and fewer personal safety risks than many other methods (Beng & Corlett 2020, Richards et al. 2022). Australian researchers have been a part of the eDNA revolution. As a nation, Australia has strong expertise in eDNA research and increasing capacity to meet monitoring needs.

Purpose of the roadmap

This roadmap suggests pathways for integrating eDNA technologies into Australian monitoring programs to adaptively manage the sustainable use and conservation of the marine environment.

The roadmap describes how eDNA technologies with high technology readiness levels can be integrated into routine monitoring. It also looks ahead to emerging methods that will allow management bodies to anticipate future monitoring scenarios. While the roadmap focuses on monitoring programs for marine parks in Australia, its methods and timelines are likely to be transferrable to marine protected areas elsewhere.

Short-term roadmap recommendations

Adopt

eDNA technologies offer a powerful approach that is ready to address policy and management needs and improve monitoring in marine parks. The methods are being adopted globally and Australia is ready to begin implementation.

Scale

Increase eDNA capacity. Upskill staff and molecular researchers in eDNA use to support marine park management. Invest in the transition to integrate eDNA technologies in marine park monitoring programs to ensure continuity of high-quality data series. Test and deploy emerging eDNA approaches.

Standardise

Provide best practice standards and user guides for national marine monitoring using eDNA. Develop standard operating procedures. Include park-specific eDNA strategies. Formalise eDNA workflows.

Calibrate

Run parallel surveys. Use traditional monitoring methods to calibrate and ground truth new eDNA approaches and add value through creating additional data.

Include

Work with Traditional Owners, citizen scientists, and other stakeholders to deploy eDNA technologies across Australia's marine environment.

Embed

Scale up successful eDNA monitoring systems and coordinate between jurisdictions. Complete reference databases for matching eDNA to species. Develop automated eDNA collection, analysis, and reporting in marine parks.

Guide to this document

This document has 5 sections:

- The roadmap: integrating eDNA science into marine parks in Australia – identifies current and future pathways for large-scale integration of eDNA methods into Australian marine monitoring and applied research programs. This section is for those interested in current needs and opportunities to use eDNA methods in monitoring programs, as well as future technical developments relevant to marine park management.
- The context: understanding eDNA and marine parks in Australia provides background information about environmental DNA technologies to allow resource managers to assess the feasibility of using eDNA methods to address monitoring and science needs. It also provides information on monitoring programs in Australia's national marine reserve system to allow molecular scientists to understand the aims and needs of resource managers.
- How eDNA can be used in marine parks: applications, benefits, challenges and opportunities answers questions about the practical applications of eDNA, and discusses both the benefits and the challenges of eDNA technologies. It looks at how eDNA could be used in existing monitoring programs, and eDNA opportunities beyond monitoring.
- Action plan for managers: practical steps to integrate eDNA in individual projects presents a practical action plan for marine park managers who are considering using eDNA technologies. The framework can inform managers' decision-making about whether and how to incorporate eDNA methods into monitoring programs.
- Research priorities and future developments: supporting integration into monitoring programs considers the research activities that should be prioritised to improve the integration of eDNA technologies in marine monitoring programs, based on existing needs and priorities in Australian Marine Parks.

Box 1 A vision for the future

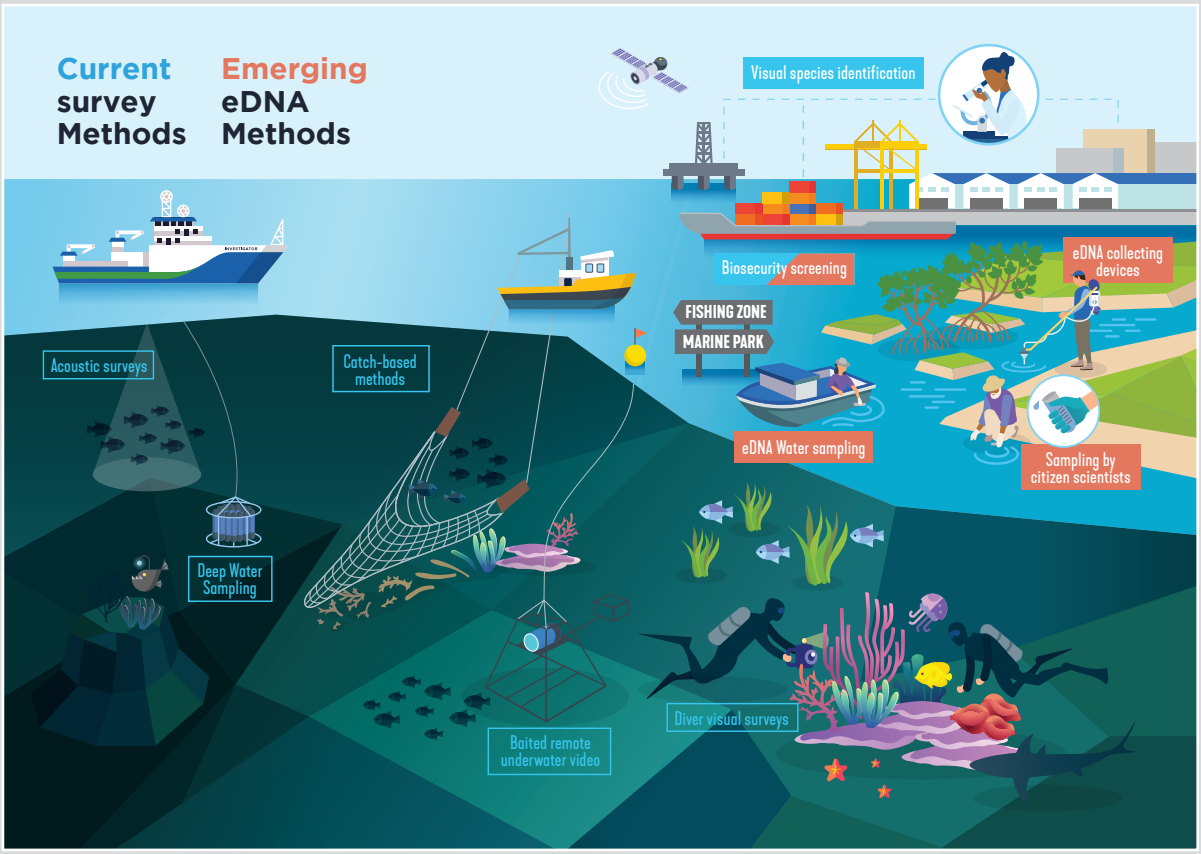
Imagine a fleet of automated samplers transmitting real-time eDNA data from the marine environment, supported by on-ground sampling teams where they are most needed. Automated systems integrate this eDNA data with physical and chemical data.

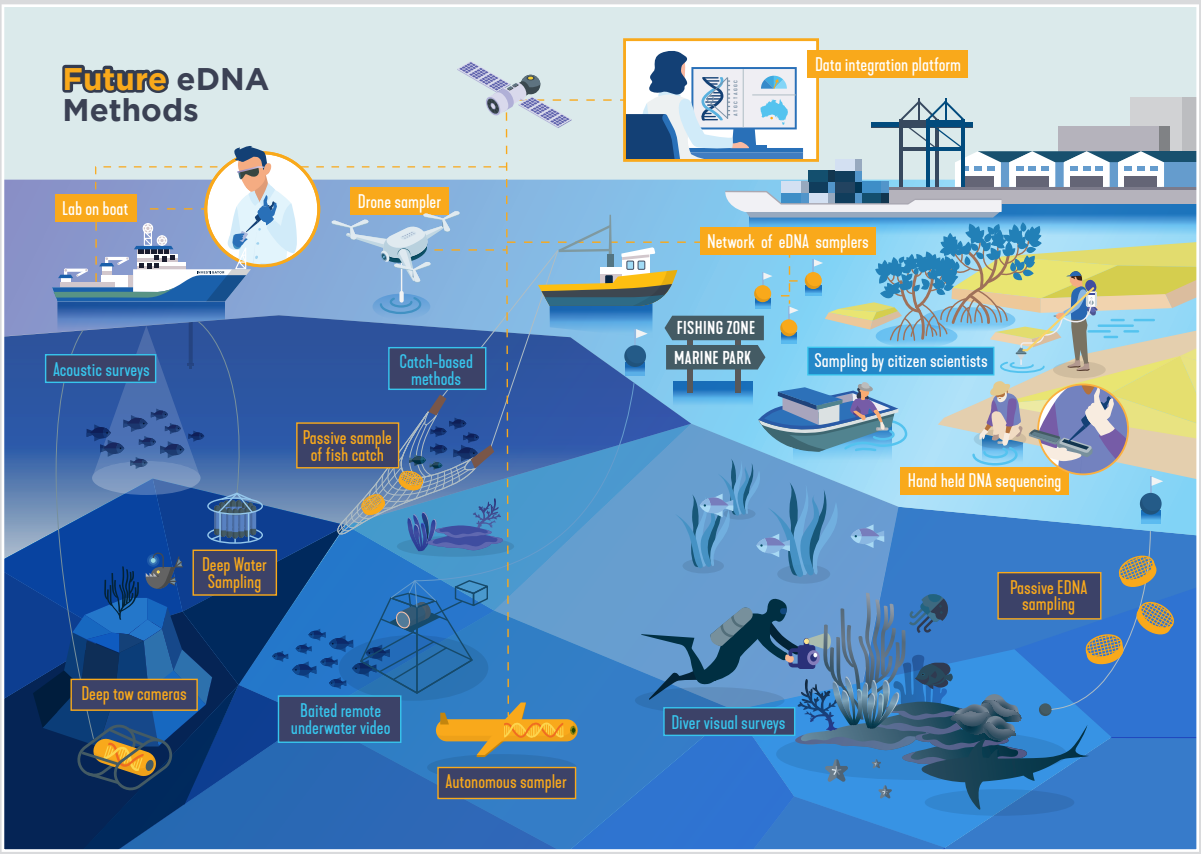
Analyses based on machine learning quickly detect changes in ecosystem health, pinpointing the cause and predicting the impacts on different species. Biosecurity threats are detected before critical population thresholds are reached, while detailed provenance and spatial information identifies the source of the incursion. Rangers and community groups feel more connected to marine management and contribute data from local samples to a shared national database.

The data feeds into an online platform where anyone, from marine scientists to holiday-makers, can check the condition of the ocean. The platform provides scientists and managers with data they can analyse or use to direct on-ground monitoring teams to collect targeted information using conventional methods. Simultaneously, the platform offers non-experts user-friendly information so that they can monitor the ocean in their area, just as they monitor the weather through weather forecasts.

continues

Box 1 *continued*





The roadmap: integrating eDNA science into marine parks in Australia

The rapid adoption of eDNA technologies in natural resource management means a national roadmap for their integration in the Australian context is timely and necessary. Environmental DNA and other molecular methods can complement and improve existing science and monitoring programs in marine parks (Stepien et al. 2022). The opportunities are far-ranging and likely to be transformative.

Effective governance and coordination are needed to take full advantage of eDNA technologies and to transform marine monitoring (Turrell 2018, Kelly et al. 2023). Globally, multiple jurisdictions are taking a strategic approach to the integration of eDNA into environmental monitoring (Kelly et al. 2023).

European roadmaps optimistically forecast that eDNA will become a standard monitoring method within 2 to 10 years (Norros et al. 2022). In the USA, the National Oceanic and Atmospheric Administration (NOAA) *Omics strategic plan* aims to accelerate the integration of eDNA and other transformational 'omics' tools by 2025 (Goodwin et al. 2020). Closer to home, New Zealand's Department of Conservation expects eDNA to have an important place in the long-term monitoring of the country's biodiversity (DOC & Toitū Te Whenua Land Information New Zealand 2023).

While these international plans are informative, Australia's unique marine park management context requires bespoke consideration. The next decade offers a window of opportunity for Australia to shape the future of eDNA science in monitoring: Australia has eDNA capability; eDNA technologies have reached high technology readiness levels; and global and national interest in genomic methods mean a higher chance of uptake from policy makers. The Australian eDNA roadmap aims to facilitate and streamline the integration of eDNA methods, maximising their benefits for marine park management.

The roadmap identifies short-, mid- and long-term integration opportunities tailored to the Australian marine park management context (Figure 1). For the short term (until 2030), the roadmap sets out recommendations for integrating eDNA methods. It then canvasses expectations for the development of eDNA methods over the medium term (2030 to 2040), and projections for the longer term (2040 to 2050).

The roadmap investigates how eDNA methods can address marine park monitoring goals based on the needs of marine resource managers. Australia's marine parks encompass a vast spectrum of environments; their science and monitoring programs therefore have varied requirements. Some of the roadmap's general recommendations will not be relevant to every marine park jurisdiction.

Figure 1 Projected timeline for integration of eDNA methods in monitoring programs

Short-term recommendations (present–2030)

The roadmap's short-term recommendations can be summarised as:

1. Adopt: Environmental DNA technologies offer a powerful approach that is ready to improve monitoring and address policy and management needs. The methods are being adopted globally and Australia is ready to begin implementation.
2. Scale: Increase eDNA capacity. Upskill staff and molecular researchers in eDNA use to support marine park management. Invest in the transition to integrate eDNA technologies in marine park monitoring programs to ensure continuity of high-quality data series. Test and deploy emerging eDNA approaches.
3. Standardise: Provide best practice standards and user guides for national marine monitoring using eDNA. Develop standard operating procedures (SOPs). Include park-specific eDNA strategies. Formalise eDNA workflows.
4. Calibrate: Run parallel surveys. Use conventional monitoring methods to calibrate and ground truth new eDNA approaches and add value by creating additional data.
5. Include: Work with Traditional Owners, citizen scientists and other stakeholders to deploy eDNA technologies across Australia's marine environment.
6. Embed: Scale up successful eDNA monitoring systems and coordinate between jurisdictions. Complete reference databases for matching eDNA to species. Develop automated eDNA collection, analysis and reporting in marine parks.

Short-term – immediate recommendations (present–2025)

In the immediate term, the recommendations are to:

- design park-specific strategies for eDNA integration
- develop national marine monitoring eDNA SOPs and metadata standards
- increase eDNA capacity
- begin eDNA monitoring projects.

Design park-specific strategies for eDNA integration

So that eDNA methods can be incorporated into monitoring programs efficiently, marine park managers should identify the most impactful integration opportunities. Analysis of management goals, stakeholder needs, department capacities and local environmental factors will help managers to direct resources and plan monitoring programs (see Action plan for managers). Parks should design their eDNA monitoring strategies in consultation with molecular experts to guarantee achievable goals at a realistic scale.

Strategic, coordinated policy action on governmental and departmental levels could expedite the integration of molecular survey methods (Lodge 2022). Regional management groups would benefit from a national vision of how eDNA fits within Parks Australia's strategy. Strategies should be aligned with international initiatives promoting inter-operable eDNA monitoring data (see Global perspectives and the inter-operability of eDNA data).

Develop national marine monitoring eDNA SOPs and metadata standards

Developing SOPs will ensure:

- data is findable, accessible, inter-operable and reusable (FAIR)
- metadata is comprehensive
- future inter-operability between regions and time series.

The National Marine Science Committee has recommended that national guidelines for data collection, management, sharing and delivery be developed (NMSC 2021). Although National Environmental Science Program (NESP) initiatives are working to standardise marine data collection methods, eDNA methods are not included. Future efforts in this area should include eDNA methods (Trujillo-González et al. 2021).

SOPs for the collection of eDNA samples should be designed as soon as possible. Sample collection is executed in the field, often by non-experts. Without quality standards, there is a high risk of generating unreliable data due to issues such as contamination and poor alignment between collection methods and project goals. In the absence of SOPs, eDNA projects should follow current best practice guidelines to ensure quality (De Brauwer et al. 2022a,b).

Increase eDNA capacity through targeted training and employment

The uptake of eDNA methods in monitoring will require capacity building (Kelly et al. 2023). Parks personnel will need to build their eDNA literacy, while molecular scientists must deepen their understanding of marine park management. Molecular methods are specialised and to integrate them successfully, parks will either need to employ staff with relevant expertise or consult with external experts.

Begin eDNA monitoring projects

The first eDNA projects will reflect local capacities and priorities. Generally, eDNA can be used immediately to monitor regions that are not easily accessible for divers, and to complement other data for a more holistic characterisation of the environment.

Comparing eDNA and conventional monitoring methods will help to calibrate methods and collect baseline data. To interpret temporal variation, method comparisons should ideally be conducted for at least 3 monitoring seasons, although duration could differ depending on local variability. Improving existing DNA reference libraries will be vital to establish clear baselines and allow for better comparisons between methods.

Some other applications of eDNA, such as detecting invasive or endangered species, are already well established at high technology readiness levels. Such projects would require limited investment beyond the development of species-specific assays (see Current uses of eDNA methods in Australia's marine environment).

A dedicated budget for eDNA integration would support the deployment of new methods and the continuity of high-quality data series. Funding for eDNA monitoring projects should focus on areas where eDNA results can meaningfully inform monitoring goals or improve the efficiency of future eDNA monitoring.

New eDNA projects should follow best practices. Experts in eDNA methods in the marine environment should be included at all stages of project design and analysis (De Brauwer et al. 2022a).

Short-term – near future recommendations (2025–2030)

In the near future, the recommendations are to:

- increase eDNA method uptake and wider deployment
- sustain investment and formalise workflows
- fine-tune existing applications
- create decision frameworks to guide management
- test and deploy new technologies.

Increase eDNA method uptake and deployment

The second half of this decade is likely to see increased use of eDNA methods and their wider integration into marine park monitoring. Timely establishment of eDNA survey protocols and preparing for anticipated scientific advances will ensure their integration in monitoring and research programs is optimised. Technological developments and method improvements – such as more complete DNA reference libraries, faster and inter-operable bioinformatics pipelines, and new analytical methods – will start to become operational. By this time, DNA reference databases should be complete for all Australian fishes and a significant proportion of invertebrates, improving the species identification of eDNA surveys (CSIRO 2023). The projected evolution in portable eDNA devices will enable rapid assessment of the presence of species in the field.

To allow for ecosystem-scale comparability, the large quantities of data generated by metabarcoding should be made accessible via platforms such as the Atlas of Living Australia, the Global Biodiversity Information Facility, the Ocean Biodiversity Information System, or future custom-made platforms designed for marine parks data. Key to the usability of such systems will be the ability to access eDNA survey data alongside other data types or layers, such as geographical information and temperature data.

Sustain investment and formalise workflows

Sustained investment in eDNA capability will be needed. Investments could focus on embedding molecular scientists within departments; developing programs to improve eDNA literacy among marine park science managers and major science partners; or designing structures to increase molecular scientists' understanding of policy drivers and marine park management needs.

Cost decreases, efficiency improvements and the growth of external service providers will make eDNA surveys more useful as a monitoring tool. Detailed cost–benefit analyses will help ensure the highest return on investment (Andres et al. 2022). For example, while some parks may benefit from investing in specific eDNA tools or infrastructure (e.g. sampler units, laboratory robots), for others it may be more cost-effective to outsource parts of the eDNA monitoring workflow that require the purchase of high-cost assets.

Formalising end-to-end sample processing workflows will further improve monitoring efficiency (Minamoto et al. 2021). Such workflows could incorporate sampling SOPs, preferred lab protocols and assays, standardised analysis methods, and centralised data repositories.

Fine-tune existing applications

This phase will need continued, focused calibration and fine-tuning of existing eDNA methods. To make monitoring programs more effective, it will be important to understand technical issues such as assay sensitivity and the limits of detection for specific single-species assays. Better knowledge of how metabarcoding assays compare with other methods will make eDNA more useful in informing management decisions.

Create decision frameworks to guide management

Improved analytical methods will inform clear management decision frameworks, unlocking the full potential of eDNA methods to answer monitoring questions and guide management decisions. Such frameworks and improved technologies are needed to act upon big eDNA datasets. They will also allow for increased integration and inter-operability of data from other survey methods in large, cross-stakeholder projects.

Test and deploy new technologies

Around this time, novel methods such as automated samplers and AI-assisted analyses will start to reach maturity, becoming ready for testing and, eventually, large-scale deployment. The shift to automated sampling methods could scale up sampling and reduce OH&S risks. This could happen through facilities such as Integrated Marine Observing System (IMOS) or the deployment of temporary local sampling units and autonomous underwater vehicles (AUVs). Automated samplers could be installed on 'ships of opportunity': vessels that repeat the same route could conduct routine sampling, while vessels that trace multiple routes could characterise species communities.

Developing and testing custom eDNA-based health indices for specific environments may allow detection and response to emerging ecosystem changes or anthropogenic stressors. Such indices can be based on microbial or metazoan communities or a combination of the two – or they can be taxonomy-free (van de Kamp et al. 2023, Wilkinson et al. 2023). Finally, relative abundance indices will also likely become ready for calibration and feasibility testing.

Mid-term expectations (2030–2040)

Over the next decade, better understanding of the possibilities and limitations of molecular surveys will yield benefits. In this phase, eDNA methods should become standard in monitoring programs, used by a wide range of stakeholders to monitor different facets of the marine environment.

In the mid-term, the expectations are:

- practical – large-scale cross-stakeholder integration, automated sampling, ecosystem health indices, single-species assays in full use, complete reference database for Australian fishes
- analytical – used in spatial planning, automated bioinformatics, novel modelling approaches, real-time data reporting
- technological – abundance indices, cross-method applications, organismal health methods, population genetics.

Practical expectations

Large-scale deployment will see automated eDNA sampling systems covering a broader spatial scale. The customised health indices developed previously will now be used to monitor changes in ecosystems.

Monitoring of single species will become more refined as the ecological interpretation of targeted assay results is fully understood. Resource managers will go beyond testing for the presence of Threatened, Endangered and Protected (TEP) or pest species, using validated assays and sampling designs to assess relative abundance and detailed spatial distribution. This information will then be used to design targeted warnings or responses to concerns such as toxic algal blooms, Irukandji swarms and biosecurity threats.

The resolution of DNA reference databases for habitat forming species and mobile invertebrates will continue to increase and improve the resolution and analytical power of metabarcoding surveys. On-site sequencing technologies should become more commonly available, making rapid detections of full species assemblages a reality.

Analytical expectations

Increased eDNA integration into multidisciplinary programs will generate big monitoring datasets, offering new opportunities and challenges. To process the wealth of data now available, investment in data analysis and bioinformatics capability will be necessary. Similarly, data storage and computing infrastructure will be needed to support efficient data processing. Efficiency will be further improved by the development of automated bioinformatics and data analyses pipelines customised to local monitoring needs.

Such measures will allow for improved ecosystem modelling. Better access to biodiversity information will benefit spatial planning processes in both the design of new parks and the management of existing ones. Improved analytical methods will enable the development of reporting pipelines that use near-real-time data stored in central repositories or sent to relevant management bodies in line with pre-determined triggers, which are set out in eDNA decision frameworks.

Technological expectations

While eDNA monitoring methods are likely to become integrated into day-to-day management, technological improvements and development of new methods should continue. In this phase, multi-species assays could start to deliver abundance metrics comparable to other methods.

Investigating the links between remote sensing and eDNA datasets will improve ocean-scale understanding of ecosystems. With this data incorporated into ecosystem models, it may be possible to predict how global climate change, regional weather or local anthropogenic impacts will affect marine ecosystems – similarly to how weather is forecast today.

We can also expect more research into applications that monitor not only presence or abundance, but also the physiological state of species. This would facilitate the monitoring of, for example, coral bleaching, reproductive cycles and the age of organisms.

Long-term projections (2040–2050)

Predicting long-term developments for rapidly developing molecular technologies is challenging. However, by considering general trends and the expectations of eDNA practitioners, we can form an idea of the possibilities that might exist in 30 years.

In the long term, the projections are:

- practical – increased scaling up of eDNA monitoring systems, complete reference databases, automated sample collection, capability to respond quickly to emerging trends on all levels of biodiversity
- analytical – automated analysis and reporting, long-term eDNA data used to model management intervention effects
- technological – continued development of new technologies, population genetics data available, applications to understand the physiological state of species.

Practical projections

Technology costs will reduce, making it more practical to scale up molecular monitoring applications. Fleets of automated testing buoys and ocean gliders could be uploading biodiversity data in real time. Implementation of large-scale eDNA monitoring will allow conventional methods to be deployed for targeted research, or in response to specific ecosystem trends that require more detailed data.

By now, DNA reference libraries should be complete for all Australian species and a large proportion of global species. Consequently, all fauna and flora in Australian Marine Parks can be detected and identified in a consistent way using eDNA methods.

Analytical projections

Automated data analyses linked to central data repositories could provide information on real-time trends in marine park indicators; the presence and abundance of TEP species; and the state of important habitat-forming organisms. Emerging issues first apparent in fast-responding microbial communities can be flagged, allowing a rapid management response. As long-term eDNA datasets across nationwide spatial scales are now available, modelling future scenarios and outcomes of policy and management interventions will become easier and more accurate.

Technological projections

New and improved molecular technologies will continue to develop in ways that are hard to predict. It is likely that at this stage, technological advances in other fields of science will open complementary possibilities with developments in eDNA methods. Advanced eDNA technologies for the study of intraspecific genetic diversity or population genetic structures may become readily available. Such tools could support sustainable use and conservation planning and allow for more targeted management actions (Bani et al. 2020). Alongside developments in metagenomics and transcriptomics, better understanding of new biomarkers could make it possible to assess target species' biological metrics and physiological states such as health, age, reproductive state and stress.

The context: understanding eDNA and marine parks in Australia

This section provides background information about environmental DNA technologies to allow resource managers to assess the feasibility of applying eDNA methods to monitoring and science needs. It also provides information on monitoring programs in Australia's national marine reserve system to allow molecular scientists to understand the aims and needs of resource managers.

Understanding eDNA

What is environmental DNA (eDNA)?

The term 'eDNA' refers to the DNA present in environmental samples (water, sediment, air, etc.). This DNA can include whole cells, parts of cells or extracellular DNA, which are shed by organisms through skin, mucous, faeces, etc.

eDNA is not a survey method. Rather, it is a **variable** that can be measured using an array of molecular methods, each of which has specific applications and limitations.

Environmental DNA (eDNA) science offers new and useful capabilities for marine monitoring. Environmental DNA was first defined as 'DNA that can be extracted from environmental samples (such as soil, water or air), without first isolating any target organisms' (Taberlet et al. 2012). Environmental DNA is a mixture of DNA molecules in the environment, which can be measured using a range of molecular methods. As such, eDNA is a measured variable, not a method. The different methods used to collect and analyse eDNA samples can be as distinct as the different visual methods used to measure marine biodiversity.

Present-day eDNA methods have their technical origins in microbiology and ancient DNA (Clark et al. 2018). However, the first use of eDNA to detect macro-organisms from water samples was just over a decade ago (Ficetola et al. 2008), while the first study to detect multiple marine taxa was conducted in 2012 (Thomsen et al. 2012).

Environmental DNA methods have progressed much since Ficetola et al. first used water samples to detect invasive bullfrogs in 2008. In less than 15 years, the field of eDNA science has matured well beyond the proof-of-concept state to its current integration into research and monitoring projects globally. There have been considerable improvements at each step of the eDNA workflow: from sample collection through to laboratory analysis, bioinformatics, data analysis and visualisation. These advancements have enabled eDNA methods to address a vast range of applications in all biomes.

Below is a brief overview of recent developments and the current technological state of eDNA methods which are summarised in Figure 2. For more information, see recent reviews by Mathieu et al. 2020, Gilbey et al. 2021, Rourke et al. 2021, and Takahashi et al. 2023.

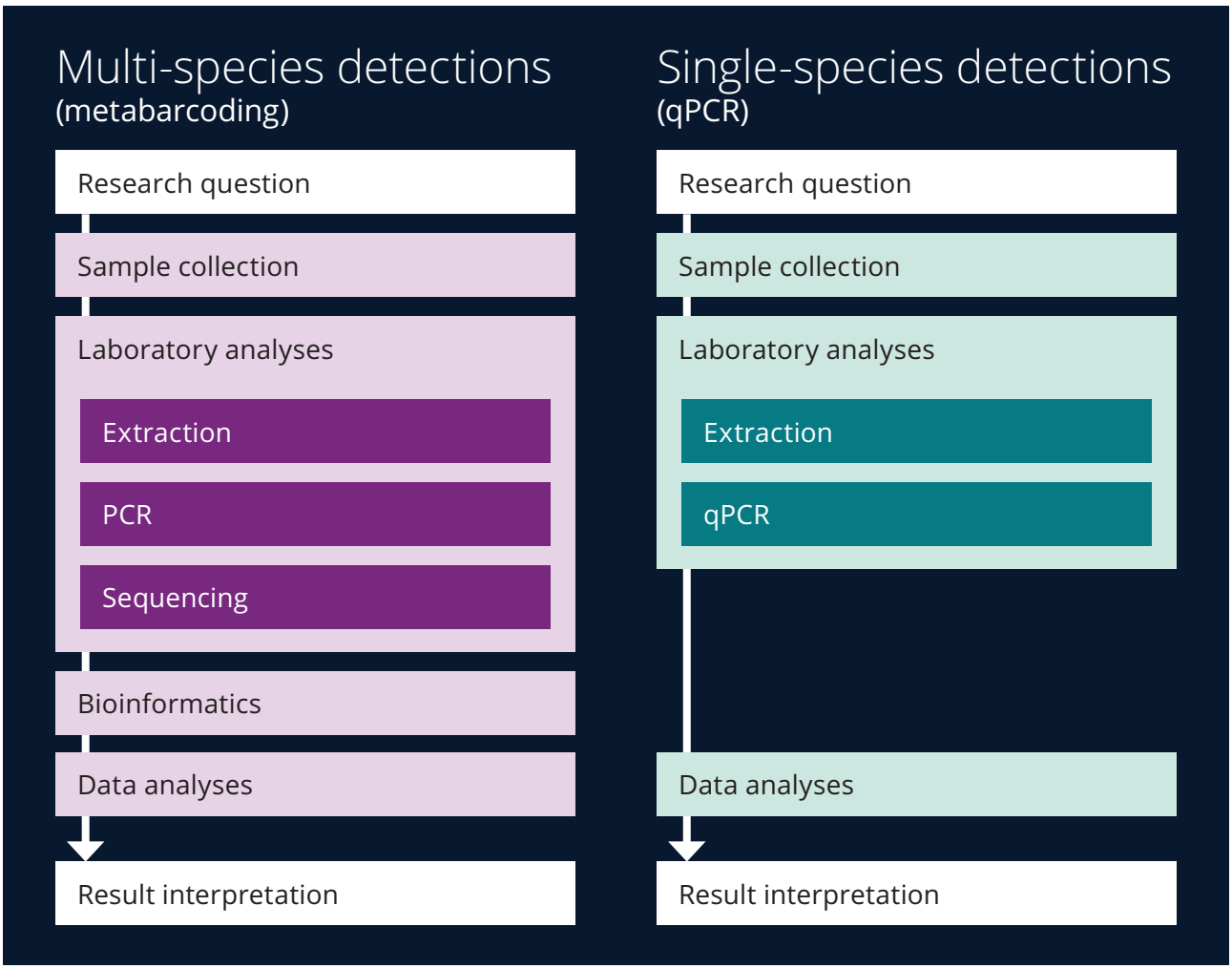


Figure 2 Environmental DNA workflow for (left column) multi-species detections (metabarcoding); and (right column) single-species detections (qPCR)

Sample collection

Environmental DNA samples from the marine environment can be collected from sources including water, sediment and scat.

Water is the most frequently used substrate and can be collected with a range of methods. The simplest method, surface water collected with a container, is quick, easy, cheap and widely used. Water can also be collected from different depths and environments by scuba divers, or using technologies such as Niskin bottles, rosette samplers or remotely operated underwater vehicles (ROVs). These approaches allow eDNA to be collected from inaccessible or remote ecosystems, such as remote seamounts, or where direct human sampling is unsafe.

Factors such as water turbidity or species richness can influence results; research is ongoing into the water volume and number of replicates that maximise sampling accuracy and precision. Higher water volumes and replicate numbers generally yield higher richness, but may require more effort and increase cost (Bessey et al. 2020, Takahashi et al. 2023).

Once collected, samples need to be processed or preserved as soon as possible to reduce degradation of DNA (Goldberg et al. 2016). While entire water samples can be preserved by freezing or adding stabilising buffers (Villacorta-Rath & Burrows 2021), it is more common to filter water samples soon after collection, preserving the resulting filters containing DNA. Filtration methods can vary according to type of pump, type of filter

material, or filter pore size. These can be adapted to fit survey aims, which should be considered during the design phase of any eDNA project (Takahashi et al. 2023).

DNA on the filters is still prone to degradation, and needs to be preserved to maintain its integrity. This can be done via freezing (ideally at −80° C), stabilising buffers or desiccation (De Brauwer et al. 2022a). Various commercially available sampling systems streamline the collection process, using a single device to collect, filter and preserve samples (e.g. Thomas et al. 2019).

Even simpler collection procedures are being trialled. One promising, low-cost avenue is passive sampling, where filters are directly submerged in water, eliminating the need to collect or filter water (Bessey et al. 2021, 2022). The development of autonomous and potentially mobile eDNA samplers is another highly active research topic. These methods greatly reduce workload and might offer a cost-effective solution for large-scale (temporal and geographical) monitoring projects. A range of autonomous samplers have been developed: from small stationary units that collect and filter water in situ, to integrated eDNA sampling units within ocean gliders, which sample transects at much larger scales (Flanigan et al. 2021, Truelove et al. 2022, Hendricks et al. 2023).

Other environmental substrates including sediment, stomach contents, scats, bulk plankton samples and scrapings of biofouling can also be valuable sources of eDNA. These substrates can be used to detect larger taxa or to address specific questions, such as species diet (Deagle et al. 2009, Berry et al. 2015, Koziol et al. 2019). The choice of sample substrate can significantly affect which taxa are detected; it is therefore essential to understand both the survey objective and the dynamics of eDNA substrates in order to design a suitable eDNA monitoring method (Koziol et al. 2019, Kawakami et al. 2023).

To optimise sample collection, it is essential to understand the factors that influence how much DNA is present in a sample and where it comes from. Often termed ‘the ecology of eDNA’, this active field of research studies the various factors affecting DNA dynamics in the environment (Barnes & Turner 2016, Scriver et al. 2023, Kawakami et al. 2023). Research is testing how long eDNA can persist in different environments and how far it can travel from its source, as well as how eDNA signal might differ across diurnal or seasonal timescales, and between species or life history stages (Harrison et al. 2019, Collins et al. 2022, Richards et al. 2022). This field is advancing rapidly and has already shown that the ecology of eDNA depends on local environmental factors.

Laboratory analysis

In the laboratory analysis phase, increased automatisation of methods is one of the biggest advances. Robotics platforms have allowed samples to be processed faster, more cheaply and more precisely, with less room for human error.

The first step of lab processing, the extraction of DNA from environmental samples, is routinely conducted using commercial kits, which offer comparability, quality assurance and reduced costs. However, in some cases, such as when working with samples from turbid environments or from materials like scats, custom extraction protocols might be needed to make eDNA extraction more efficient.

The subsequent processing of the extracted DNA can be broadly categorised by its purpose: detecting a single species (such as an invasive or threatened species) or detecting a community of multiple species (Nagarajan et al. 2022).

Targeted single-species detection can be conducted using a range of methods, most commonly quantitative polymerase chain reaction (qPCR) or digital droplet PCR (ddPCR).

Species-specific assays must be designed and tested before targeting single species with eDNA methods (Thalinger et al. 2021, De Brauwer et al. 2022b), but once these assays have been validated, they are faster and cheaper than multi-species (metabarcoding) approaches. In limited situations and if sufficiently ground truthed, they can also produce relative abundance estimates (Rourke et al. 2021). In some cases, rapid in situ species detection can be achieved with new portable methods such as lateral flow (dipstick) assays or Nanopore MinION technology (Thomas et al. 2019, Doyle & Uthicke 2021, Egeter et al. 2022).

Detection of species communities requires the use of metabarcoding methods. These have 2 steps:

1. PCR amplification of the extracted DNA using primers designed to target a particular group of species.
2. High-throughput sequencing (HTS) of PCR products.

The primers used in the PCR step determine what information can be retrieved from the DNA sample (i.e. what group of taxa will be recovered and the degree to which species can be distinguished from one another). The primers bind to specific regions in the DNA, allowing for targeted amplification of the taxonomic group of interest (e.g. eukaryotes, fish, corals, elasmobranchs). A wide range of primers have been developed (see Takahashi et al. 2023 for a detailed database). The design and testing of primers can be time-consuming, but is important, as primer performance can affect results and needs to be considered when interpreting data (Deiner et al. 2017). These primer biases also mean metabarcoding is less reliable than single-species (qPCR) methods for assessing abundance (Deiner et al. 2017) (see also Data analysis and Abundance).

During the subsequent HTS step, the DNA sequences amplified during the PCR phase are read and assembled ready for analyses. All the DNA sequences amplified by PCR are read separately, so the data produced is thousands of independent sequences, which can be matched back to their biological source.

Several HTS technologies have been used in eDNA studies, but most applications use Illumina sequencing, which is available at commercial laboratories and is ideal for the short DNA fragments typically found in eDNA. There are several new sequencing technologies that can produce longer sequences and, in some cases, use smaller sequencing machines (MinIon) that could be deployed in the field for specialised applications (Egeter et al. 2022).

Bioinformatics

The goal of bioinformatics is to transform the outputs of metabarcoding sequencing data (which often yields data from millions of DNA fragments) into data that is useful for biodiversity assessment. The millions of DNA sequences vary depending on which taxa they came from, so data processing involves cleaning data (including removing errors introduced during the laboratory steps) and assigning sequences to species by interrogating DNA sequence reference libraries (Mathon et al 2021).

During this stage, choices are made about quality control and the method used for taxonomic assignment. These decisions can impact the final dataset, but raw sequence data can be retained and reanalysed if required.

The bioinformatics process is run using pipelines of code. These can be custom-made for specific projects, but common, publicly available pipelines are widely used (Casas & Saborido-Rey 2022, Pauvert et al. 2019). Bioinformatics workflows are not needed when using single-species assays, because each assay targets a single taxon (De Brauwer et al. 2022b).

Complete DNA sequence reference libraries (also called reference databases) are crucial for the accurate assignment of eDNA sequences to taxonomic identities. Ideally, reference libraries

provide DNA sequences from authoritatively identified specimens of all relevant species. However, the completeness and authoritativeness of available DNA reference libraries varies substantially depending on the taxon of interest (Weigand et al. 2019). This is a globally recognised issue, which can be resolved by concerted efforts to improve reference libraries (Weigand et al. 2019, Yao et al. 2022). CSIRO and partners are engaged in a major effort to create reference sequences for all Australian species, the National Biodiversity DNA Library (NBDL).

An alternative to conventional taxonomic annotation is to use uniquely recurring DNA sequences as a proxy for diversity. These sequence variants can be clustered based on similarity and are referred to as operational taxonomic units (OTUs), molecular operational taxonomic units (mOTUs), zero-radius operational taxonomic units (zOTUs), or amplicon sequence variants (ASVs).

The use of different clustering approaches can affect result interpretation (Pauvert et al. 2019, Brandt et al. 2021). For non-experts, it is important to understand that while these non-taxonomic metrics can provide valuable insights on the diversity of a system, they are not an exact proxy for individual species, as intraspecific genetic variation can result in multiple OTUs per species and some recently separated species can share the same OTU (Blackman et al. 2023). Furthermore, it might not be possible to ascribe functional contributions to OTUs or ASVs.

Data analysis

The data analysis options for eDNA methods depend on whether qPCR (single-species, semi-quantitative) or metabarcoding (multi-species, rarely quantitative) approaches were used in the lab. Surveys using qPCR methods can result in presence/absence or semi-quantitative data, depending on the level of calibration of specific workflows (Thalinger et al. 2021). These data can then be analysed as conventional single-species data to directly inform management needs.

Metabarcoding data provides information on the number of DNA sequences recovered from each taxon in each sample, but data is almost always converted to presence/absence due to biases in sequence recovery. In some instances, however, where sampling effort was sufficiently extensive, inferences can be made about the abundance of species (e.g. common species tend to be present in more samples than rare species).

This is a developing field of research where a variety of analytical approaches are increasingly applied to provide more relevant ecological information (see Analytical improvements). Furthermore, presence/absence data is sufficient for some uses such as biotic indices (Beentjes et al. 2018, see Applied research). Examples of novel analytical approaches that use metabarcoding data include multi-species occupancy models (e.g. McClenaghan et al. 2020, Holmes et al. 2022) and network analysis methods (e.g. DiBattista et al. 2020, Djurhuus et al. 2020). These methods can make it possible to infer broad ecological trends without the need for abundance data, but often need higher replication levels and rigorous experimental design (Ficetola et al. 2015, Fukaya et al. 2022).

Metabarcoding data can be analysed using taxonomic information (i.e. comparing identities of species or other taxa), but this approach can be limiting when incomplete taxonomic reference databases yield poor resolution (i.e. a small percentage of sequences can be assigned to known taxa). Analysing recurring genetic sequences (e.g. ASVs, OTUs) as species-equivalent units maximises the use of available genetic information without the need to assign all sequences to a taxonomic level.

This approach has benefits when studying ecosystems as high-level ecological assemblages. Compared with conventional methods, it allows researchers to use much more biological data, such as for groups where taxonomy is less resolved (including microbial communities, small-bodied invertebrates and cryptic fish species). As it does not rely on taxonomic expertise or visual observations, this approach eliminates common biases towards charismatic and highly visible species, providing insights that might be missed with other methods. However, OTUs are not easily transferable between eDNA studies, nor easily combined with other observation data, which can limit spatial comparisons or long-term temporal studies.

Applications

Single-species assays

In the marine environment, eDNA-based methods have had a variety of monitoring and research applications (Gilbey et al. 2021). Single-species analyses have mainly been applied in biosecurity and the detection of TEP species. Globally and in Australia, there are well-developed surveillance programs for pest species, including invasive and native nuisance species (see Box 2; McDonald et al. 2019, Bolte et al. 2021, Uthicke et al. 2022). Indeed, the first published paper to use eDNA methods (Ficetola et al. 2008), aimed to detect invasive bullfrogs in Europe. Surveys using qPCR methods have been applied to the detection of TEP species including sharks, seahorses and cetaceans in Australia and across the world (Simpfendorfer et al. 2016, Nester et al. 2020, West et al. 2021).

Metabarcoding

Multi-species (metabarcoding) methods have a wider range of applications than single-species methods. The ability to survey a wide range of taxa using a single sample has given rise to so-called 'tree of life' metabarcoding, where marine taxa ranging from protozoans and plants to corals, fishes and crustaceans can be detected from a single sample (Stat et al. 2017). In monitoring, these methods allow the study of changes in species assemblages over time (Berry et al. 2019, Chrismas et al. 2023), between habitats, across large and small spatial scales (West et al. 2020, 2021, DiBattista et al. 2022) and across human impacts gradients (DiBattista et al. 2020), or to inform spatial planning decisions (Bani et al. 2020). A wide body of literature addresses how eDNA metabarcoding methods differ from and complement conventional monitoring methods. Reviews indicate that eDNA metabarcoding generally shows similar results, but detects slightly different aspects of species assemblages, with eDNA methods more likely to detect small, rare, or cryptic species than conventional methods (Richards et al. 2022). This suggests eDNA metabarcoding surveys can complement conventional surveys (Gilbey et al. 2021, Guri et al. 2023).

Indices of environmental health

There is growing interest in the development of biotic indices of ecosystem health (Pawlowski et al. 2022, DiBattista et al. 2020). Conventional biotic indices are based on visual observations and rely heavily on dwindling taxonomic expertise, and getting results can be time-consuming (Borja et al. 2000, Pawlowski et al. 2018). Incorporating eDNA methods into biotic indices allows for a wider range of taxa to be detected without the need for extensive morphological identification. If designed correctly, such molecular indices do not need abundance data to be accurate (Beentjes et al. 2018). Examples from New Zealand and Norway show that such eDNA indices are quicker, cheaper and more efficient in detecting anthropogenic pressures than morphometric approaches (Lanzén et al. 2021, Pochon et al. 2015). These indices are incorporated into routine monitoring, such as monitoring of the benthic impacts of salmon farming practices in New Zealand (Pochon et al. 2019).

Alternative molecular indices, such as taxonomy-free indices or indices developed through supervised machine learning, are being trialled globally. Promisingly, these indices can use a much broader range of available DNA sequences associated with differently impacted ecosystems (Cordier et al. 2017, Apothéloz-Perret-Gentil et al. 2017, Wilkinson et al. 2023).

Microbial communities

The Australian Microbiome project, a collaboration between Parks Australia, IMOS, Bioplatforms Australia and CSIRO, has developed standardised methods for the collection, processing and analysis of samples and baseline data on microbial communities across the Australian continent and surrounding oceans (Bissett et al. 2016, Brown et al. 2018). A core dataset within the Australian Microbiome project is the long-term timeseries data from Australia's IMOS, which has been using microbial eDNA technology since 2012 to deliver microbial community observations in the marine environment at national scale. These observations were used to assess marine microbial communities in the marine chapter of the *2021 State of the Environment Report* (Brown et al. 2018, Brown & Bodrossy, 2021). Combining data from the Australian Microbiome project and macro-organismal eDNA has the potential to provide valuable information about, for example, the base of the marine food chain. It could also be used to develop comprehensive environmental indices for marine parks (Berry et al. 2023).

Fisheries management applications

The practical applications of eDNA methods in fisheries management are becoming increasingly clear, and have received extensive attention in the literature (Jerde 2021, Gilbey et al. 2021). In particular, eDNA methods can be used where focal species are hard to survey using conventional methods (e.g. rare or invasive species), or where extractive methods are not desirable (e.g. threatened species) (Nester et al. 2023). Environmental DNA methods can also help managers understand important stages in the life cycle of target species by detecting spawning aggregations, or characterise feeding preferences (Takahashi et al. 2020, Holmes et al. 2022). There are promising applications under development in post-catch compliance, such as detecting catch composition, biosecurity threats and illegal catches in the live fish trade (Green et al. 2021, Maiello et al. 2022, Urban et al. 2022).

Metrics such as abundance, biomass and target species' condition are vital to fisheries management, but cannot yet be measured reliably with eDNA methods (see Abundance). Improving the understanding of how relative abundance metrics from eDNA surveys correlate with abundance from catch data is a highly active field of research and in some cases, estimates are strongly correlated (Rourke et al. 2021). In Australia, eDNA surveys are already being applied in freshwater fish monitoring (e.g. by the New South Wales Department of Primary Industries), but formal integration in the marine environment remains limited.

Historical environmental change

Environmental DNA methods, particularly metabarcoding, are also being used in paleogenomics. This field uses ancient DNA, often extracted from sediment cores, to study how past marine communities changed over large temporal scales (Capo et al. 2021). While it is possible to study communities as far back as 2 million years (Kjær et al. 2022), reconstructing more recent community baselines to study historical and current impacts on marine ecosystems has more direct relevance to resource managers (Shaw et al. 2019, Siano et al. 2021, Williams et al. 2023).

Australian eDNA research community

Australia has strong and increasing capacity to deliver eDNA research and monitoring activities. Australian researchers have been a central part of the eDNA revolution and Australia has a strong expertise in eDNA research. The national eDNA community continues to produce groundbreaking research and is at the leading edge of many developments in the field. In the past 10 years, publications such as Furlan et al. 2016, Stat et al. 2017, DiBattista et al. 2020 and many others have set the standards in the field for quality control, metabarcoding applications and ecological inference. At the time of writing, Australia has an estimated 45 laboratories and sequencing facilities active in the broader field of environmental genomics (marine and terrestrial).

The Southern eDNA Society (SeDNAS), the official eDNA society for Australia and New Zealand, was formally established in 2023. SeDNAS aims to promote science and industry collaboration, promote adoption and advance best practice eDNA methods. It will connect researchers and end users, lead the establishment of best practice, and be an important source of advice on future developments in the field.

The recently published *Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand* (De Brauwer et al. 2023) were created by SeDNAS and partner institutions, with the support of the Australian Government Department of Agriculture, Fisheries, and Forestry. The guidelines outline minimum standard considerations for eDNA surveys across the complete project workflow (De Brauwer et al. 2023).

Understanding marine park management

Australia's exclusive economic zone (EEZ) has more than 160 marine parks, which represent a wide diversity of ecosystems ranging from tropical coral reefs to sub-Antarctic sea canyons. Successful monitoring and management are vital to the conservation and sustainable use of the marine environment. Management of marine parks is a key component of management of Australia's marine estate.

Marine parks in Australia

Australia has one of the world's largest marine estates and a large network of marine parks. This network includes a national network of Commonwealth parks, known as Australian Marine Parks, and other marine park networks in different jurisdictions (Table 1). Combined, there are more than 160 marine parks in Australia's EEZ, covering a combined area of more than 4 million km^2. They are home to multiple global biodiversity hotspots and some of the world's highest marine biodiversity. Australia's marine environment is expected to contribute up to $105 billion per year to GDP by 2025 through ecosystem services ranging from food provisioning and resource extraction to recreation, cultural values, shipping and defence (AIMS 2023, NMSC 2015, 2021).

Table 1 Overview of marine parks in Australia

Component	Type of marine park			
	Australian Marine Parks	**Heard & McDonald Islands Marine Reserve**	**Great Barrier Reef Marine Park**	**State and territory marine parks**
Jurisdiction	Australian Government	Australian Government	Australian Government and Queensland Government	State and territory governments
Management agency	Parks Australia	Australian Antarctic Division	Great Barrier Reef Marine Park Authority	Different management agencies
Location	National: more than 3 nautical miles offshore	Heard & McDonald Islands	Queensland	Coastal waters: up to 3 nautical miles offshore

Australian Marine Parks

The Australian Marine Parks were first proclaimed under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) in 2007 and revised in 2018. The parks are generally established in waters 3 nautical miles (5.5 km) from the shore up to the end of Australia's EEZ, 200 nautical miles (370.4 km) from shore. There are currently 60 parks, which are organised in 5 regional networks (North, North-West, South-west, South-east, Temperate East), as well as the Coral Sea Marine Park and the Indian Ocean Territories (Figure 3). Combined, the Australian Marine Parks span more than 3.3 million km^2, making the network one of the largest marine park networks globally. The smallest park is 4 km^2 and the largest is 989,842 km^2. The parks stretch from the tropics to the sub-Antarctic and contain a variety of marine habitats ranging from coral reefs and shallow sand-cays to deep canyons and sea mounts more than 6 km deep.

The Australian Marine Parks are managed centrally by the Marine and Island Parks Branch within Parks Australia in the Department of Climate Change, Energy, the Environment and Water (DCCEEW). Management of the parks is based on 2 objectives:

- Protection and conservation of biodiversity and other natural, cultural and heritage values of marine parks.
- Ecologically sustainable use and enjoyment of the natural resources within marine parks, where this is consistent with first objective.

The parks are designed as multiple-use parks, with specific zonation allowing a range of activities such as resource extraction, research, shipping and recreation. Specific no-take zones are also included within this zonation design. Parks Australia uses an adaptive management and evidence-based approach. It works closely with a range of other regulators, partners and stakeholders, including the Australian Marine Park Advisory Committees. The Marine and Island Parks Branch does not have internal monitoring capability, and monitoring is therefore outsourced through a system of grants and collaborations with science partners (e.g. NESP) and marine parks staff in other jurisdictions.

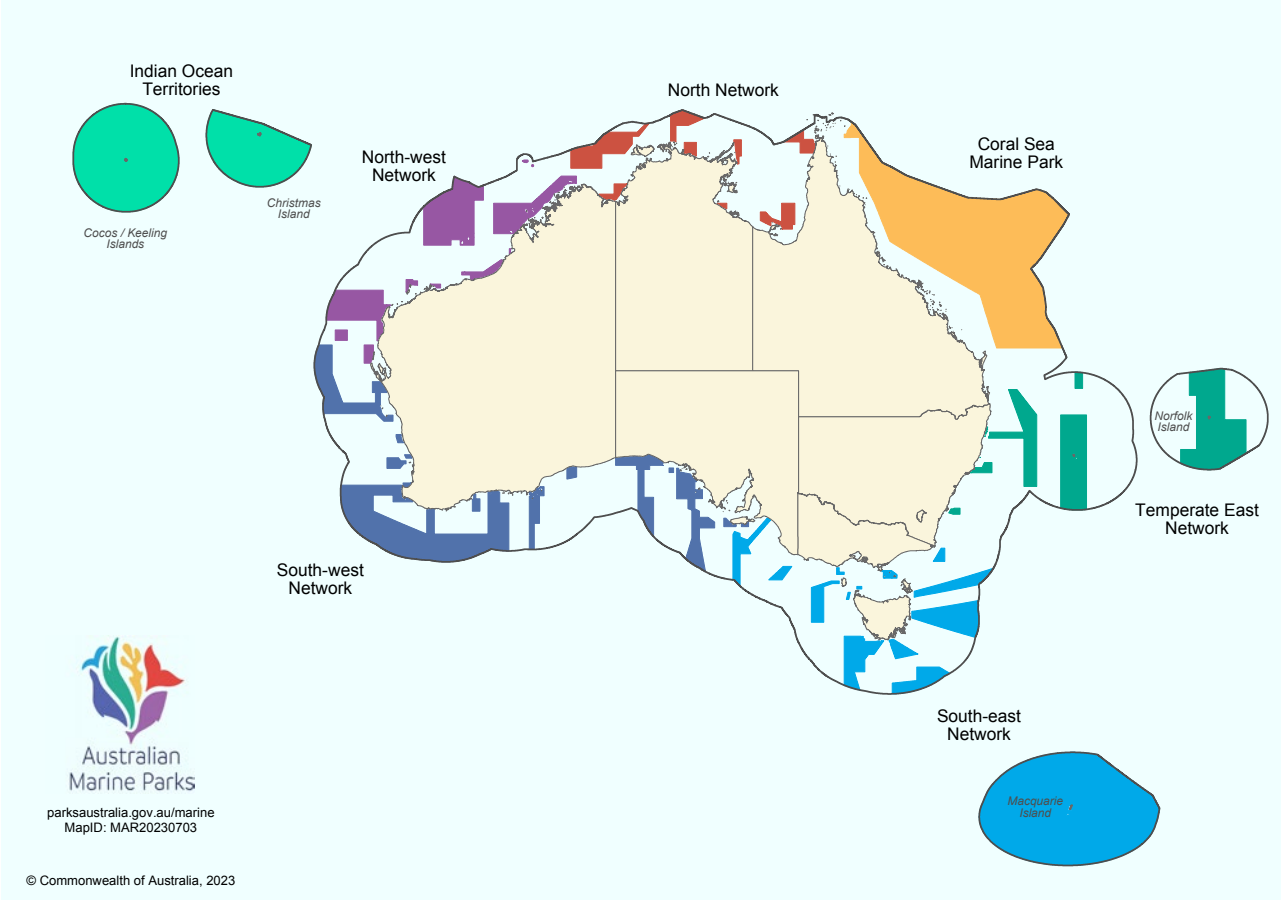


Figure 3 Map of Australian Marine Parks

Heard and McDonald Islands

The remote, uninhabited Heard and McDonald Islands are an external territory of the Commonwealth of Australia and consist of a 71,000 km^2 large reserve, which is mainly marine. The Heard Island and McDonald Islands (HIMI) Marine Reserve hosts unique sub-Antarctic and Antarctic marine life of high conservation value. The entire reserve is a Category 1a Strict Nature Reserve, which means any extractive activities are prohibited and other uses are strictly controlled. As an external territory, the HIMI reserve is not part of the Australian Marine Parks; instead, it is managed by the Australian Antarctic Division (AAD) in collaboration with various stakeholders, including the Australian Border Force, the Australian Fisheries Management Authority and the Australian Marine Safety Authority.

Great Barrier Reef Marine Park

The Great Barrier Reef Marine Park (GBRMP) is one of Australia's oldest marine protected areas. Established in 1975, the GBRMP spans 344,440 km^2 and is Australia's best known marine park. The Great Barrier Reef (GBR) is a world heritage area and the world's largest coral reef ecosystem. The park attracts tourism to Queensland and Australia, has cultural value for Traditional Owners and supports a fishing and aquaculture industry. Through its various direct and indirect ecosystem services, the total economic value of the GBR has been estimated to exceed $56 billion per year (Deloitte Access Economics 2017).

The GBRMP is also a Commonwealth park, but it is managed by the Great Barrier Reef Marine Park Authority (GBRMPA), an Australia Government agency that is part of DCCEEW. GBRMPA works closely with

different stakeholders to manage the park, and currently uses a long-term sustainability plan as a framework to guide its management practices. The GBRMP is divided into different use zones with specific priorities and rules. Monitoring in the GBRMP is conducted by a range of stakeholders following the Reef 2050 Integrated Monitoring and Reporting Program (RIMReP).

State and territory marine parks

All Australian states and territories that border the ocean have marine parks. However, there are large jurisdictional differences in the number of parks, the area covered, zoning arrangements, and management priorities and approaches. State parks generally run from the high tide line up to 5.5 km (3 nautical miles) from shore. An estimated 98 state parks or reserves have been declared, covering more than 95,000 km^2. The size of individual parks ranges from less than 1 km^2 to 18,450 km^2, with a median size of 673 km^2. State and territory parks are coastal, and compared to parks managed by the Australian Government, the ecosystems represented in state parks more commonly include shallow habitats such as beaches, estuaries, mangroves and shallow reefs.

Management and monitoring approaches differ markedly between states and territories. Multiple state and territory governments have departments responsible for managing parks, which typically includes marine parks. In some states, however, marine park management is the responsibility of fisheries or primary industry departments.

State parks are typically smaller than those managed by the Australian Government, and see more recreational use. This is reflected in stronger interactions with stakeholders such as Traditional Owners, recreational fishers and tourism operators. Increasingly, state parks are co-managed by Traditional Owner groups, as is the case with the recently established Bardi Jawi Gaarra and Lalanggaddam marine parks in Western Australia (DBCA 2022a,b). Most state parks also consist of multi-use zones, but there is a stronger emphasis on the use of no-take zones than in Commonwealth marine parks. These no-take zones can be integrated within parks or, in some states, can be entirely separate parks, often called reserves or sanctuary zones.

Stakeholders in marine parks

Australia's marine parks are used by a range of stakeholders (Table 2). Many stakeholders contribute directly or indirectly to monitoring or have strong interests in its outcomes and the resulting management actions. While state and Commonwealth marine parks are managed separately, many stakeholders are common to both.

Stakeholders active in the Australian marine environment include government agencies, industry bodies, Traditional Owner groups and research organisations. The nature and relevance of monitoring and the potential applications of eDNA methods vary greatly between stakeholders. Certain bodies are already using eDNA methods or planning to increase their use (e.g. biosecurity departments; McDonald et al. 2019), while eDNA methods might not be relevant to other agencies for the foreseeable future (e.g. marine heritage groups). The level of integration of monitoring activities between stakeholders and parks management likewise differs considerably. The activities of monitoring bodies can be closely aligned with the marine parks they take place in, but monitoring or research results do not always feed back into management practices.

Table 2 Stakeholders active in Australia's marine parks and their monitoring roles

Agency	Type	Role	Monitoring capability
Parks Australia	Parks management agency	Manage Australia's Commonwealth Marine Parks	Commissions monitoring
Great Barrier Reef Marine Park Authority	Parks management agency	Manage the Great Barrier Reef Marine Park	In-house capability Commissions monitoring
State parks agencies	Parks management agency	Manage state marine parks	In-house capability Commissions monitoring
State fisheries agencies and primary industries agencies	Government agency	Manage state-based fisheries (e.g. Western Australian Department of Primary Industries and Regional Development, South Australian Research and Development Institute, New South Wales Department of Primary Industries)	In-house capability
Australian Fisheries Management Authority	Government agency	Manage Commonwealth fisheries	Commissions monitoring
Australian Maritime Safety Authority	Government agency	Oversee maritime safety, protect the marine environment and operate maritime aviation search and rescue	Commissions monitoring
Biosecurity departments	Government agency	Monitor and respond to biosecurity threats	In-house capability Commissions monitoring
Environmental Protection Agencies	Government agency	Protect, restore and enhance the environment through the regulation of pollution, waste, noise and radiation	Variable
Australian Antarctic Division	Government agency: research organisation	Coordinate Australia's activities in Antarctica, from scientific research through to logistics and transport	In-house capability Commissions monitoring

continues

Table 2 *continued*

Agency	Type	Role	Monitoring capability
Australian Institute of Marine Science	Government agency: research organisation	Australia's tropical marine research agency: conduct research	Supplies capability
CSIRO	Government agency: research organisation	Australia's national science research agency: conduct research	Supplies capability
National Environmental Science Program	Government agency: research funding body	Fund research	Supplies capability
Integrated Marine Observing System	Research organisation	Provide open access marine observation data	Supplies capability
Universities	Research organisation	Conduct research	Supplies capability
Industry research organisations (e.g. Western Australian Marine Science Institution)	Research organisation Industry body	Investigate and inform governments, industry and the wider community about the management of specific industries	In-house capability
Fisheries Research and Development Corporation	Industry body Research funding body	Manage research and development investment by the Australian Government and the Australian fishing and aquaculture sectors	Supplies capability
Australian Petroleum Production & Exploration Association	Industry body	Represent the interests of the oil and gas industry	No monitoring
Clean Energy Council	Industry body	Represent the interests of companies that work in or support the clean energy sector	No monitoring
Commercial shipping bodies (e.g. Shipping Australia Limited, Australian Peak Shippers Association)	Industry body	Represent the interests of shipowners, shippers, and shipping agents	No monitoring

continues

Table 2 *continued*

Agency	Type	Role	Monitoring capability
National Offshore Petroleum Safety and Environmental Management Authority	Industry body	Regulate health and safety, well integrity, and environmental management for all oil and gas operations, offshore renewable infrastructure and greenhouse gas storage activities	No monitoring
Ports Australia	Industry body	Represent the port sector and associated maritime services	Commissions monitoring
Recreational fishing bodies (e.g. OzFish, RecFishWest)	Industry body	Represent recreational fishers' interests	Commissions monitoring
Tourism bodies (e.g. Association of Marine Park Tourism Operators, Tourism Australia)	Industry body	Represent tourism industry interests	No monitoring
Scientific organisations (e.g. Australian Coral Reef Society, Australian Marine Science Association, Australian Society for Fish Biology)	Industry body	Represent research fields active in Australia's marine estate	Supplies capability
Seafood industry associations (e.g. Commonwealth Fisheries Association, Seafood Industry Australia, Seafood Industry Victoria)	Industry body	Represent fishery, aquaculture and seafood industry interests	No monitoring
Traditional Owner groups	Private	Represent the interests of Traditional Owners	Variable
The Minderoo Foundation	Philanthropic research organisation	Conduct research and promote marine conservation activities	In-house capability

continues

Table 2 *continued*

Agency	Type	Role	Monitoring capability
Conservation organisations (e.g. Australian Marine Science Conservation Society, Surfrider Foundation)	NGOs	Promote ocean conservation	Variable
Limited current relevance for eDNA methods			
Geosciences Australia	Government agency Research organisation	Conduct geoscientific research, provide technical geoscience advice and act as custodian of geographic and geological data and knowledge	Supplies capability
Australian Border Force	Government agency	Protect Australia's border and enable legitimate travel and trade	N/A
Australian Defence Force	Government agency	Defend the Commonwealth of Australia and its national interests	N/A
Maritime Heritage organisations	Government agency	Manage and protect marine heritage and their associated artefacts	N/A
DCCEEW Underwater Cultural Heritage	Government agency	Manage and protect underwater heritage and their associated artefacts	N/A

Marine park monitoring priorities

Biodiversity monitoring principles

Successful resource management relies on realistic predictions about future conditions, which in turn requires a clear understanding of past and current states. To achieve such understanding, well-designed monitoring and research programs are essential. Monitoring is defined as the systematic collection of data over time to detect changes in a system (Gerber et al. 2005) and can include information on a range of factors, including environmental (e.g. temperature), ecological (e.g. species abundance), biological (e.g. health of organisms), social (e.g. visitor rates) and economic (e.g. ecosystem value). Monitoring of physical and chemical environmental data, and socioeconomic data are beyond the scope of this roadmap.

Scientific activities in marine parks extend beyond typical monitoring-only surveys and include a range of applied research programs. Applied research directly relevant to the management of marine parks mainly focuses on the characterisation of marine communities (i.e. biodiversity surveys) for purposes such as establishing baselines or approving permits for activities in marine parks. The suggestions in this roadmap are equally applicable to these applied research activities as to the more typical mandated monitoring programs.

For monitoring to be successful in the long term, it needs to be well designed with clear priorities (Hedge et al. 2022). Monitoring priorities are set by management and policy makers, who often commission external stakeholders to undertake the actual monitoring. In other cases, external stakeholders conduct their own question-driven monitoring or applied research, with results fed back to management indirectly.

Many marine parks are moving towards an adaptive management approach, which integrates program design, management and monitoring and allows systematic testing of assumptions. The exact application of this framework varies between parks, and the variables being monitored differ depending on each park's nature, use and current pressures.

Priorities in federally managed marine parks

To manage the Australian Marine Parks (Commonwealth) network, Parks Australia uses an adaptive management approach based on a monitoring and evaluation framework (Figure 4) and informed by targeted monitoring and applied research programs.

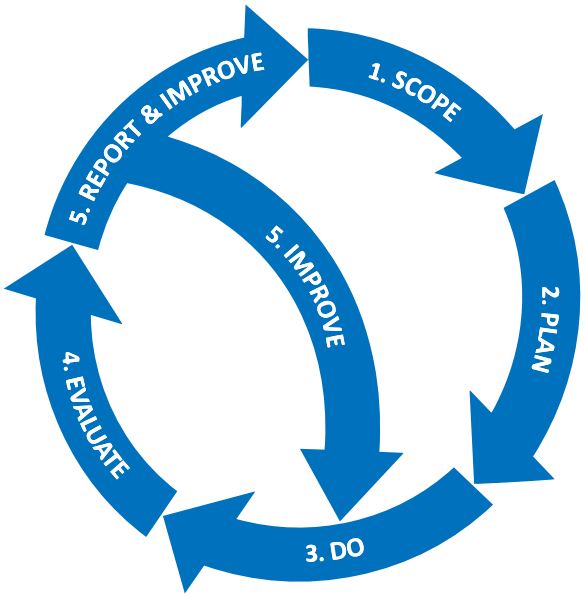


Figure 4 Adaptive management cycles in Australian Marine Parks

This framework aims to address the most pressing management issues and questions and increase the effectiveness of park management (Hayes et al. 2021). Science plays a critical role in each step of the adaptive management cycle. The marine science program also underpins Parks Australia's management effectiveness

approach, which helps measure implementation of management strategies, evaluate effectiveness and identify opportunities for improvement. Within this framework, the monitoring cycle uses a standardised approach (Hayes et al. 2021).

In the Heard and McDonald Islands, monitoring objectives are written into the general management plan and aim to increase baseline knowledge, assess impacts and changes in the reserve's ecosystems, and inform management decisions (DOE 2014). A recently released eDNA biosecurity framework outlines how eDNA methods can be used to monitor biosecurity threats to the Heard and McDonald Islands and other Antarctic regions (Clarke et al. 2023).

Monitoring in the GBRMP is directed and managed by the GBRMPA through RIMReP, which is part of the strategic Reef 2050 Long-Term Sustainability Plan (Commonwealth of Australia 2015, GBRMPA & Queensland Government 2015). RIMPReP aims to measure the condition and trends of key values in the park; inform the evaluation of management effectiveness; and inform stakeholders of progress relative to the Reef 2050 Plan (GBRMPA & Queensland Government 2015). As part of RIMReP, the *Great Barrier Reef Outlook Report* is published every 5 years, most recently in 2019. These reports present:

- the current state of key park environmental values
- threats, management responses, resilience and risk
- the long-term outlook for the GBR Marine Park.

Monitoring priorities within RIMReP include specific habitats such as coral reefs and mangrove forests, as well as population dynamics of taxa such as bony fishes, seagrasses and dugongs (GBRMPA 2019).

Priorities in state and territory marine parks

State and territory management bodies set monitoring priorities for their marine parks. These can be detailed and prescriptive, as is the case for New South Wales, which recently established a new Integrated monitoring and evaluation framework for the Marine Integrated Monitoring Program (MIMP). This framework, similar to RIMReP, was co-designed with key stakeholders and is managed by the New South Wales Department of Primary Industries. The program's aims are to monitor the condition of the environmental assets and the community benefits from these; evaluate the effectiveness of management initiatives and actions; and fill knowledge gaps in the parks. MIMP has specific monitoring indicators that inform a 5-year health check, which will be reported publicly.

The Department of Biodiversity, Conservation and Attractions (DBCA) in Western Australia uses a different approach to address similar monitoring goals. DBCA has individual management plans for each of its marine parks, but a coordinated framework that considers all marine reserves as a part of a network. Monitoring evaluates park-specific KPIs, with a monitoring prioritised based on a framework designed by Simpson et al. 2015. Monitoring activities are further influenced by factors such as the natural variability in ecological values, current conditions and trends, existing pressures on ecological values and the availability of internal expertise.

South Australia's marine park network was proclaimed in 2012 and a 10-year evaluation of the marine program is currently underway. Marine park management plans include a monitoring, evaluation and reporting (MER) component that describes how to assess whether the park is achieving the aims of the *Marine Parks Act 2007.*

The Marine Science Team (within the Department for Environment and Water) implements the MER program, which focuses on 6 questions that evaluate conservation outcomes, ecological processes, management effectiveness, public engagement and cultural heritage. The individual management plans for South Australia's marine parks use the same strategies that are applied to the network, but each focuses implementation on local needs and capacity. Monitoring predominantly occurs in sanctuary zones and comparison sites.

Parks Victoria has adopted an adaptive management framework and conservation planning process to manage natural assets and threats to marine parks. Challenges include limited baseline knowledge and addressing practical management questions. Parks Victoria uses its Signs of Healthy Parks (SHP) program to monitor the health of marine protected areas and inform management (Ierodiaconou et al. 2022). The SHP monitoring program uses environmental indicators of natural values and ecological processes, as well as potential threats within the parks. Indicators for marine ecosystems and key habitats in parks are outlined under the SHP program, with monitoring activities focusing on key ecological attributes and threats within at least one marine park with each bioregion in the state.

Among all Australian states, Tasmania has the lowest proportion of marine parks (1.1% of state waters). Tasmania's marine parks do not have official monitoring programs or published management priorities, and there are no active marine parks management activities in place. Monitoring occurs solely through research initiatives of parks stakeholders (e.g. Reef Life Survey), without a formal government strategy.

Marine parks in Queensland are divided into 2 management units: the GBRMP, managed by the GBRMPA (see above), and state marine parks, which are managed by the Queensland Government Department of Environment and Science. The department is working towards an improved framework based on monitoring, evaluation, reporting and improvement principles, which will complement current zoning plan reviews. Monitoring programs in Queensland's state marine parks are primarily set through a risk-based approach, with monitoring focused on vulnerable habitats (e.g. seagrass, coral), threatened species (e.g. turtles, dugongs, grey nurse sharks, migratory shorebirds), and areas of high and competing uses.

The context for marine parks in the Northern Territory is strongly shaped by Traditional Owner stakeholders. Indigenous peoples own more than 78% of the Territory's coastline, including the waters that overlay them. This is recognised in the *Coastal and marine management strategy 2019–2029*, which outlines a 10-year vision for managing and protecting the territory's marine environment. The strategy aims to enable activities that address knowledge gaps by building on existing monitoring initiatives while maximising participation from stakeholders. Of the 5 high-level objectives in the strategy, objectives 2 ('Safeguard our coasts and seas') and 5 ('Build our knowledge') are most relevant to monitoring programs. These objectives include actions such as developing and implementing an integrated monitoring program; identifying knowledge gaps and research priorities to support decision-making; and improving knowledge about key ecological processes, impacts and threats.

Regulatory monitoring needs

In addition to the priorities set by marine park management bodies, the different regulatory stakeholders active in parks may have specific monitoring needs (Table 2). For example, resource industries conduct environmental baseline surveys prior to starting activities and are regulated by the National Offshore Petroleum Safety and Environmental Management Authority (NOPSEMA); the Australian Fisheries Management Authority

regulates fisheries and has strong interest in the abundance and condition of target species; and biosecurity departments closely monitor incursions of invasive pests (Table 2).

Despite the differences in priorities and management approaches, stakeholders in Australia's state and Commonwealth marine parks share 4 general monitoring goals:

- Provide baseline data, including through applied research programs, to fill knowledge gaps (e.g. characterising marine communities, effective regulation of activities).
- Measure the current condition of natural values such as ecosystems, ecosystem components and species.
- Monitor changes in environmental values over time in response to management actions, human impacts and natural fluctuations.
- Report results to inform management and stakeholders.

The natural values or assets that are monitored differ between parks according to local ecosystem-dependent variation. Frequently, monitoring programs focus on:

- key species that sustain ecosystem functioning (e.g. habitat-forming species, key invertebrate communities)
- threatened species (e.g. handfish, skates)
- charismatic species (e.g. turtles, whales)
- pest species (introduced and native)
- harvested species (commercially and recreationally).

Monitoring of these natural values can measure a range of variables or indicators depending on the type of value, its function, known impacts, and so on. Commonly measured variables are:

- species diversity
- species abundance
- percent cover (sessile organisms)
- organism size or size distribution across populations
- patchiness or fragmentation.

Marine park monitoring strategies

Monitoring programs in marine parks measure a wide range of ecosystem characteristics relevant to management priorities, requiring a broad range of expertise and techniques (DCCEEW 2022, Hedge et al. 2022). Monitoring often includes non-biological metrics such physical (e.g. temperature, wave height), chemical (e.g. pH, salinity), and geological metrics (e.g. depth, benthic structure). Here, however, we focus solely on strategies used to monitor biological and ecological metrics, that is, 'biomonitoring'.

Metrics

Monitoring strategies vary according to site-specific priorities, but most aim to measure trends in the status of species and ecosystems of interest. Commonly used metrics include the abundance of mobile species or percent cover for benthic, habitat-forming species such as corals or macro-algae. Data on biological or physiological characteristics (e.g. size, weight, disease status) are also frequently collected. Budgetary and logistical considerations mean that monitoring efforts usually focus either on recognised keystone species or species that are of importance to managers or stakeholders, such as TEP species and charismatic or easily detected macro-organisms. For these reasons, small, cryptic species in remote or hard-to-access locations are monitored less frequently.

Methods

Marine park managers use a variety of established methods to achieve their monitoring objectives. While managers recognise that innovative methods can offer considerable benefits, budgetary restrictions may leave limited room for experimentation. At present, monitoring strategies across Australia most commonly rely on visual, indirect or collection-based methods (e.g. Bryars et al. 2017, Ierodiaconou et al. 2022). Commonly used methods include direct observational

techniques (e.g. underwater visual census [UVC], photo-quadrats, manta tows), video methods (e.g. baited remote underwater video [BRUV], diver operated video [DOV], ROV), remote sensing, sediment grabs and multibeam sonar (Przeslawski & Foster 2020, Young et al. 2022). The use of well-established methods facilitates data inter-operability. For example, the NESP field manuals allow for improved spatial and temporal comparisons to support management decisions (Przeslawski & Foster 2020).

Capacity and implementation

Which organisation carries out monitoring depends on the location and aim of monitoring programs. None of Australia's marine park agencies rely solely on in-house monitoring capacity. Complementary monitoring data is frequently sourced from external agencies or stakeholders to support management (Table 2). For example, Parks Australia, which manages Australia's largest marine parks network, does not have in-house monitoring capacity and commissions its monitoring activities.

Regions differ in the types and number of science partners and stakeholders, methodological approach and the level of monitoring input provided. There is a wide spectrum of involvement with external organisations. For example, in Tasmania, monitoring is designed and conducted wholly by independent external partners, whereas Western Australia and New South Wales both have centrally coordinated regional monitoring teams.

However, even parks with strong internal monitoring capacity often collaborate with local stakeholders. Compared to the larger Australian Marine Parks, state parks tend to involve local stakeholders (e.g. universities, Traditional Owner groups, citizen scientists) more directly due to their proximity to the coastline (e.g. Scholz et al. 2017, Browne et al. 2018, Northern Territory Government 2019, DBCA 2022a,b). Parks Australia does work more closely with Traditional Owners in the North network, and is set to begin an Indigenous science program for the Australian Marine Parks. Collaboration with Traditional Owners is evolving from solely employing people as park rangers towards co-management (DBCA 2022a,b). While larger networks such as the GBRMP still work closely with small stakeholder groups, large-scale and long-term monitoring activities are often conducted in collaboration with large agencies such as Australian Institute of Marine Science (AIMS) or CSIRO.

How eDNA can be used in marine parks: applications, benefits, challenges and opportunities

Monitoring Australia's marine environment is challenging. Successful adaptive management depends upon well-designed monitoring programs to meet mandated monitoring requirements and fulfil specific, research-driven goals. However, the extent, diversity and remoteness of the Australian marine estate make it difficult to design monitoring frameworks that can effectively assess impacts and support management and policy decisions.

This section answers questions about the practical applications of eDNA, and discusses the benefits and challenges of eDNA technologies. It looks at how eDNA could be used in existing monitoring programs, and at eDNA opportunities beyond monitoring.

How eDNA can be used in monitoring

To be used in monitoring programs, eDNA technology must be reliable. The growing scientific consensus is that eDNA methods are ready for deployment in typical marine monitoring applications, although different management needs might require additional validation processes (Hajibabaei 2022). Decisions with more consequential implications (e.g. legal use in biosecurity) require a stronger level of evidence and method validation (as is also the case with conventional methods). The recently published *Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand* and eDNA test validation protocols provide a framework for designing such validation processes that is accepted by the Australian Government (De Brauwer et al. 2022a,b).

Environmental DNA methods have the potential to answer questions relevant to monitoring goals. In aquatic environments, eDNA has even been shown to be more sensitive for detecting pest species than conventional approaches (Dejean et al. 2012, Zaiko et al. 2016). Presently available eDNA methods can readily be applied to monitor:

- ecosystem biodiversity
- temporal and spatial changes in species assemblages
- the presence of TEP species
- the presence of pest species.

Once calibrated to the local environment and monitoring needs, eDNA methods can also be deployed to model species distributions or measure anthropogenic impacts and ecosystem health.

Monitoring approaches fit within a larger management framework, and are not solely determined by scientific evidence. To be integrated into day-to-day management practice, eDNA methods must adhere to the tenets of successful marine park management (Table 3) (Elliot 2013).

Technological advancements, improved cost-effectiveness and increased public understanding of molecular methods due to COVID-19 pandemic reporting mean that there are now few practical barriers to the integration of eDNA methods into management practices.

Table 3 10 tenets of successful marine management and their relevance to eDNA technology

Tenet	Relevance to eDNA technology
Ecologically sustainable	Can deliver ecological information relevant to management
Technologically feasible	Technology is feasible for species detection
Economically viable	Cost-effective, but may need initial additional funding
Socially desirable or tolerable	Non-invasive and can be safely deployed, likely to be accepted by stakeholders and general public
Legally permissible	Best practice guidelines provide a framework for the design of legal validation processes
Administratively achievable	Only minor adaptations required to existing systems
Politically expedient	Can contribute to Australia's image as leader in cutting-edge science and best practice resource and conservation management
Ethically defensible	More suitable than many conventional methods as it is a non-destructive method
Culturally inclusive	Non-eDNA experts can collect samples so possible to include Traditional Owner groups and the general public
Effectively communicable	Possible if done well, as shown by science communication during COVID-19 wastewater detections and PCR testing

Source: Elliot 2013

Frequently asked questions about the applications of eDNA

- **Can eDNA provide data about species abundance or density?**
  Currently, eDNA methods cannot provide measures of abundance in terms of numbers of individuals, although this is an active area of research. It is possible to estimate the relative abundance of some species using qPCR (single-species assays). This approach requires case-by-case, species-specific assay design, extensive validation of limits of detection and quantification.
  The recent literature suggests that in some cases, abundance estimates obtained from metabarcoding (multi-species) assays are correlated to those from conventional survey methods. However, these correlations do not hold across all studies (see Abundance and Long-term future developments).
- **How long does eDNA last in the environment? Is it possible that eDNA detects species that are no longer present?**
  It depends. In general, eDNA degrades quickly in the marine environment. Studies that measured eDNA persistence in the ocean found that in most cases eDNA can no longer be detected after 48 hours. However, the rate of degradation can vary depending on a range of environmental and biological factors such as temperature and UV radiation (see Sample collection).

- **How far can eDNA travel in the marine environment?**
  The dispersion of eDNA through the water column depends on factors such as currents, waves and thermoclines. While eDNA could theoretically travel long distances, studies in the marine environment repeatedly show highly localised eDNA signatures often less than 500 m from the sampling source. This is likely due to factors such as dilution and degradation. In certain environments, such as rivers, eDNA can sometimes travel further and be detected multiple kilometres from its source (see Sample collection).
- **Can eDNA methods be used to detect TEP species or invasive and pest species?**
  Yes. In cases where detecting a species would be highly significant, it is important to develop and validate eDNA assays to ensure they have appropriate sensitivity and accuracy (see eDNA opportunities in existing monitoring programs and Assay development and calibration).
- **Do different species shed different amounts of DNA?**
  Yes, eDNA shedding rates can vary significantly between species, according to life stage, physiological activity and other factors. Understanding how much or how little DNA different species shed can be important for designing sampling strategies with sufficient power to detect target species (see Sample collection).
- **How many eDNA samples should I take?**
  This can vary depending on the purpose of the study, the type of environment, the characteristics of the target species or communities, and the known abundance of target species. An absolute minimum of 3 replicate samples per site is advised, but higher replication is often desirable for environments with high biodiversity or to accommodate analytical approaches such as occupancy modelling.
  A pilot study may be needed to determine minimum sample size for long-term monitoring projects, particularly in novel habitats or with target taxa for which limited empirical eDNA data exist.
- **Can eDNA methods be used for population genetics studies?**
  While studies have shown eDNA can provide some information about levels of population genetic diversity and geographic differentiation in specific situations, at present it is largely a topic of research rather than a routine monitoring approach (see Long-term future developments).
- **Is there a risk of obtaining false negatives?**
  Yes, like other survey methods, eDNA analyses can produce false negatives. To minimise the risk of false negatives and correctly interpret results, it is important to understand method-specific limitations, use assays that are fit for purpose and suitably validated, and take sufficient samples (see Result interpretation and Long-term future developments).
- **Is there a risk of obtaining false positives?**
  Yes, false positives can occur, but their causes are well understood. The risks can be mitigated by using strict contamination controls and well-validated assays (see Result interpretation and Long-term future developments).
- **How long does it take to process eDNA samples?**
  Sample processing times depend on which method is used (e.g. qPCR or metabarcoding); whether assays already exist and have been validated; and whether processing is done in-house or through commercial service providers.
  In general, single-species approaches can be finished in as few as 2–3 days, or even in the field if suitable equipment is available. In the metabarcoding process, completing laboratory and bioinformatics procedures can take weeks to months.
  Depending on the project aims, novel assays might need to be developed and validated before sampling. This process can take several months to years, depending on species and validation requirements.

Current uses of eDNA methods in Australia's marine environment

Various agencies are already using eDNA methods to monitor Australia's marine environment (Table 4). So far, most eDNA projects in parks have been limited to biosecurity applications and to research-focused or short-term pilot projects. However, there are examples of successful long-term monitoring projects that have integrated eDNA methodology in the workflow, or will do so in the near future.

For example, the recently announced collaboration between Parks Australia and the Minderoo Foundation aims to use eDNA methods to monitor marine parks in the future. These existing programs highlight the recognised potential of eDNA methods and can help guide wider integration into future monitoring programs.

Table 4 Examples of active and planned environmental DNA monitoring projects in the Australian marine estate

Agency	Location	Project	Reference
Western Australian Department of Primary Industries and Regional Development	Western Australian ports	Biosecurity monitoring: State Wide Array Surveillance Program	McDonald et al. 2019
Queensland Department of Agriculture and Fisheries	Queensland ports	Biosecurity monitoring: Q-SEAS project	Biosecurity Queensland
Victorian Department of Primary Industries	Victorian ports	Victorian Ports Marine Surveillance Pilot Program	Agriculture Victoria 2023
Great Barrier Reef Marine Park Authority and the Australian Institute of Marine Science	Great Barrier Reef Marine Park	Crown of thorns seastar monitoring (see Box 2)	Uthicke et al. 2022
Australian Antarctic Division	Antarctica	eDNA biosecurity framework	Clarke et al. 2023
Parks Australia	Norfolk Island	Norfolk Marine Park habitat mapping project	Australian Marine Parks 2021
Minderoo Foundation	Australian Marine Parks	Ocean Discovery and Restoration Program	Australian Marine Parks 2023
UNESCO	Ningaloo, Shark Bay and Lord Howe Island	Environmental DNA Expeditions in UNESCO World Heritage Marine Sites	UNESCO 2023

Benefits of integrating eDNA methods in marine parks

Scaling up

Australia's marine park network is one of the world's largest, and covers a diversity of habitats and ecosystems. Managing marine environments on such a large scale is challenging. Typically, conventional survey methods can only cover a small geographic area. Commonly used methods such as visual surveys, ROVs, AUVs, towed video and even BRUVs can take hours to investigate the biodiversity of even small reefs. Large-scale ocean observation is possible through remote sensing, though it lacks resolution to address many monitoring requirements.

One of the biggest benefits of eDNA methods is that samples can potentially be collected across a large area more quickly and with fewer monitoring personnel (Mori et al. 2023). Staff can be trained more quickly to collect eDNA samples than to conduct visual surveys, handle ROVs, or use other field data collection methods. Advances in automated collections systems and linking eDNA data to remote sensing data will make scaling up monitoring programs over large spatial and temporal time scales even easier (Budd et al. 2023).

Scaling up any monitoring program depends on standardised methods across spatial and temporal scales. Environmental DNA collection methods can be standardised easily, and the development of national best practice guidelines and SOPs mean spatial inter-operability and temporal continuity of eDNA-derived data are realistic near-future prospects (Mori et al. 2023). Introducing formal certifications for eDNA laboratories or developing measures to validate eDNA processes used in marine park monitoring will increase trust in methods and facilitate their broader use.

Accessing the inaccessible – health and safety

Marine monitoring comes with risks associated with going out onto, and often into, the ocean. Scuba diving and snorkelling surveys require extensive training and risk mitigation protocols to manage environmental risks (e.g. hypothermia, currents, depth limits) and dangerous marine life (e.g. jellyfish, sharks, crocodiles) (DiBattista et al. 2019, West et al. 2021, Muff et al. 2023). Conversely, eDNA samples can be collected without entering the water, removing the risks associated with in-water surveys.

These risks can also be avoided by using remote video survey systems such as BRUVs or ROVs. However, systems such as BRUVs are usually heavy and bulky, requiring winches and additional care to avoid manual handling injuries. The risks of handling injuries are smaller when collecting water samples for eDNA analysis. Furthermore, while video methods are efficient in clear waters, they are less effective in waters with low visibility. While turbid waters can also complicate eDNA methods when sediments clog up filters or inhibit PCR reactions, such limitations can be addressed by optimising methods (e.g. Williams et al. 2017, Takasaki et al. 2021).

Cost

The initial uptake of eDNA methods in monitoring programs will require extra investments, but once established, operational costs of eDNA surveys are lower than those of many conventional methods (Gilbey et al. 2021). Studies have shown that compared to other methods, metabarcoding monitoring methods can reduce costs by 55% and lower monitoring time by 72% (Aylagas et al. 2018). Single-species surveys can be up to 67% less expensive, depending on which methods are used (Evans et al. 2017).

The average per sample cost for eDNA surveys is lower than conventional methods, largely

because of boat times are reduced and fewer trained staff are needed in the field. While the costs of lab processing, sample sequencing and bioinformatics are substantial, costs are similar for taxonomic experts to analyse videos or process bulk samples. Using automation, sequencing DNA can process hundreds of samples in a short time frame, further increasing cost-effectiveness.

Ethical considerations

Because eDNA can detect species at low concentrations, it has been used to detect rare, threatened, or endangered species. Environmental DNA sample collection is non-lethal and non-invasive, making it particularly relevant for monitoring threatened species. In Australian Marine Parks, destructive sampling methods (e.g. ichthyocides) are generally prohibited or subject to strict regulation. Methods such as seine netting or long lining also come with the inherent risk of decreased survival of the species studied, and removing the need to catch, handle or euthanise organisms to detect their presence is advantageous when species are rare or threatened (Simpfendorfer et al. 2016, Nester et al. 2023). Recent advances in eDNA technologies also open the future possibility of better understanding population structure of threatened species without the need for invasive sampling (Sigsgaard et al. 2016, Adams et al. 2022).

Stakeholder inclusion

Marine parks aim to educate the public, communicate the state of the marine environment and sometimes even co-manage parks with stakeholders. As COVID-19 pandemic communications have shown, molecular methods can be clearly communicated to the public. Practical international examples of clear eDNA communication include New Zealand's Wai Tuwhera o te Taiao – Open Waters Aotearoa program and the ANEMONE program in Japan (Box 3; EPA 2023, Suzuki-Ohno et al. 2023). For marine parks, focusing on the potential to discover charismatic or endangered species can be a particularly valuable engagement approach.

Furthermore, if well designed, monitoring programs can include stakeholders such as industry and community groups directly in sample collection (see Engagement with the general public). Collecting eDNA water samples to detect single species or monitor a suite of species is relatively simple, and as a result, this approach has been used in various successful citizen science projects both nationally and abroad (Biggs et al. 2015, Griffiths et al. 2022).

Challenges to integration of eDNA methods in marine parks

Fragmented monitoring landscape

One of the biggest challenges to the successful integration of novel eDNA methods in monitoring programs is the heterogeneity of monitoring approaches across Australian Marine Parks. Jurisdictional differences in monitoring frameworks are recognised as a challenge, particularly when monitoring priorities would benefit from a coordinated approach (e.g. large-scale ecological processes) (Addison et al. 2018). These differences reflect the large geographic extent, wide range of stakeholders and differences in monitoring needs. Management in different states and territories have different priorities, funding sources and research capacities, which are different still from those associated with the even larger parks and reserves managed by the Commonwealth. Designing a national approach to establish baselines and marine

monitoring was a key recommendation of the National Marine Science Plan and a lack of harmonisation across disciplines and jurisdictions is seen as a significant challenge to achieving this (NMSC 2015, Hedge et al. 2022).

These challenges make standardised and comparable monitoring on a large scale difficult to achieve (Hedge et al. 2022). While integrating eDNA into individual management zones can have local benefits, without strategic national coordination, the full potential of the methods may not be realised (Kelly et al. 2023).

The fundamental solutions to these challenges are beyond the scope of this roadmap. However, the effectiveness of eDNA methods can be maximised by harmonising their adoption across parks, as occurred with the development of SOPs for marine park monitoring (Przeslawski & Foster 2020, Hedge et al. 2022). Harmonised integration of eDNA methods may create opportunities for inter-operability. Method standardisation is preferable, provided it does not reduce the power to detect a change of interest or answer a specific monitoring question. Failure to align methods will inevitably incur a loss of data comparability on small and large scales. The recent development of national best practice guidelines can act as a focal point for a unified, trusted approach.

Cost

Increasing the cost-effectiveness of marine park monitoring and research is universally seen as desirable. It is therefore challenging to add new methods to existing programs, particularly where this entails extra costs. Environmental DNA methods have repeatedly been shown to be cost-efficient on large scales (Aylagas et al. 2018). However, for the foreseeable future, these methods will be complementary, entailing increased costs, including the cost of establishing protocols and calibrating eDNA methods with existing monitoring methods.

Given the evidence that eDNA analysis can yield cost-effective monitoring data, there may be an argument for strategic investment to establish operational monitoring protocols and programs, as occurred with the eDNA program to detect crown of thorns seastars (CoTS) at the GBRMP (Uthicke et al. 2022, see Box 2). Such investment could happen through local or cross-jurisdictional collaborations that address shared needs, such as IMOS, ARC linkage projects or even philanthropic projects (Forrest 2020).

In the absence of investment, there are avenues to maximise the cost-effectiveness of eDNA-based monitoring. For example, scaling up projects through cross-park or cross-stakeholder collaboration can reduce the cost per sample while increasing data output. The rapid evolution of automated eDNA workflows, from sample collecting to data analyses, means costs will decrease further in the future. Finally, where the costs of immediate analysis are too high, samples could be stored in biobanks (Jarman et al. 2018). Baseline samples collected now could be analysed in future projects, when budgets allow.

Expertise

Although the collection of eDNA samples is straightforward, downstream processing is highly specialised and relatively novel, meaning most monitoring teams currently lack relevant expertise. In regional areas, relevant expertise may be even more scarce. Lack of understanding of the capabilities and limits of eDNA methods may hinder the uptake and effective use of eDNA approaches.

These challenges can be addressed in multiple ways. To enable clearer communication, eDNA researchers should build their management and policy literacy, while MPA science managers should develop their understanding of eDNA. This could be supported by the newly established SeDNAS, which advises stakeholders on the best-practice application

and interpretation of eDNA methods. Developing mutual understanding and shared language will help managers and eDNA experts to collaborate more closely.

Alternatively, molecular experts could be employed into monitoring program teams for some jurisdictions. Existing eDNA research activities within the DBCA, New South Wales Department of Primary Industries and the AAD show how this can be achieved. When it is not feasible to employ molecular experts, critical eDNA workflows can be outsourced to molecular experts. Finally, eDNA experts could be embedded in major initiatives and consortiums such as NESP or the National Marine Science Committee to provide oversight and advice.

Reference sequence libraries

To characterise species assemblages, DNA sequences collected from the environment are assigned to a species by interrogating DNA reference libraries. The quality of eDNA results depends on the completeness of reference libraries; yet global and Australian DNA reference libraries are incomplete (Weigand et al. 2019, Yao et al. 2022, CSIRO 2023). As a result, most sequences obtained through eDNA analysis cannot be assigned to species level, but are limited to higher taxonomic ranks such as genus or, more commonly, family or even order (Weigand et al. 2019).

The construction of DNA reference libraries faces 2 main hindrances: the difficulty of extracting sequences from known species and making the sequences publicly accessible; and the lack of taxonomic expertise to link sequences to the correct species. The lack of complete reference libraries is most strongly felt in poorly studied regions (e.g. deep sea canyons) or taxa (e.g. invertebrates), and areas with high biodiversity (e.g. coral reefs).

This challenge links taxonomy, the most fundamental biological science, with cutting-edge technology, and as such, the solution must involve both fields.

New methods to more rapidly extract sequences from species are being developed and improved (e.g. CSIRO's NBDL). However, these still need taxonomic expertise to ensure accuracy – a concern which is addressed through the NBDL project (CSIRO 2023). Investment is needed in both taxonomy and sequencing programs for the creation of reference sequences. Recognising this, the European Union aims to publish the DNA sequences of 50% of the organisms in its ecosystems by 2030 (Lamy et al. 2020).

An alternative already in use by eDNA researchers is the application of taxonomy-independent analyses. The use of OTUs or ASVs (see Bioinformatics) removes the need for taxonomic identification, but still has the potential to measure changes in the ecosystem through, for example, custom health indices. It is important to note, however, that without translation to taxonomic identities, OTUs have limited ability to inform practical management interventions.

Abundance

Most environmental monitoring programs include a focus on measuring abundance and trends in abundance for species of interest. At present, eDNA methods cannot provide measures of abundance in terms of numbers of individuals. This can be perceived as a major challenge to the integration of eDNA methods into monitoring projects (Jerde 2021, Rourke et al. 2021, Norros et al. 2022). Addressing this challenge is the subject of extensive research. A potential way forward would be to examine methods that provide trends without requiring absolute estimates of abundance.

In relation to abundance measures, however, 2 important observations must be made. Firstly, most conventional survey methods only offer a relative measure of abundance, such as MaxN or estimated percentage cover (McCormick & Choat 1987, Benedetti-Cecchi et al. 1996, Campbell et al. 2015). Whether visual, video or catch methods are used, each

method has limitations that affect how well estimates of abundance reflect true abundance. Secondly, the goal of monitoring abundance is generally to measure how it changes over time or in response to management, so that the effects of impacts or interventions can be determined. Precise knowledge of exact population numbers is rarely needed (with some exceptions, such as highly threatened species) (IUCN 2012).

Environmental DNA methods can address some of the challenges around abundance measures by using the relative abundance of DNA sequences as a proxy for species abundance. When conducting single-species assays, it is possible to estimate relative abundance for some species using qPCR (see Laboratory analysis). This approach, however, requires case-by-case, species-specific assay design, extensive validation of limits of detection and quantification. Multiple environmental and species-specific variables can influence whether quantification is feasible. While estimations of relative abundance are possible for many species, they do not work for all species (Rourke et al. 2021, 2023). However, when assays can be validated, the relative abundance results from qPCR methods can be used to measure changes in the population of interest. If necessary, this approach can then be backed up by conventional methods.

Measuring relative abundance for metabarcoding (multi-species) methods is more difficult than conducting single-species assays. The relative abundance reads in metabarcoding results can be affected by a range of technical issues, which complicate their interpretation. A number of studies and reviews have shown strong correlations between relative eDNA abundance and the abundance estimated by conventional methods such as UVCs, BRUVs, trawls and acoustic surveys (Fediajevaite et al. 2021, Keck et al. 2022). However, other studies have failed to find such correlations.

As an alternative to measuring relative abundance, statistical approaches are being developed to infer abundance from eDNA presence/absence data (see Applications). Methods such as multi-species occupancy models use high levels of spatial sampling to infer overall abundance of species and how they might be affected by environmental variables (McClenaghan et al. 2020).

Organismal biology and health

Life history attributes such as the size, life stage, age, weight and health of monitored species are other metrics used when monitoring ecosystems. For example, monitoring organism health, fecundity and size is standard practice for fisheries species. Similarly, in pest species surveillance it is important to be able to distinguish between established populations and the brief presence of larvae. Currently, eDNA methods cannot provide such biological information.

A potential solution is measuring environmental RNA (eRNA) rather than eDNA, or investigating other biomarkers for, for example, age (Mayne et al. 2021). RNA, which is only shed by living organisms, transfers information within cells to produce specific proteins. Theoretically, measuring eRNA offers the potential to study which proteins are being expressed and how these relate to the biological state of an organism (e.g. disease, fecundity, age). The potential applications of eRNA could be significant, and are increasingly the subject of research. However, it is unlikely that such methods will be operational in the short-to-medium term (5–10 years).

Result interpretation

Marine resource managers are accustomed to interpreting estimates of species numbers, percent cover and other similar abundance metrics. The methodological limitations of survey techniques are understood well enough to help interpret the ecological meaning of such monitoring results.

The results of eDNA technologies, however, pose new challenges, such as interpreting data that only conveys presence or absence; determining if ecological interpretations can be made when taxonomic units are presented at the resolution of genus or family, or are replaced entirely by units such as OTUs or ASVs; and understanding how much confidence can be placed in eDNA-based detections or non-detections if it is not possible to visually confirm the presence of a species.

False negatives can occur in eDNA analyses. False negatives are also commonly encountered when using other survey methods (e.g. UVC and BRUV methods generally ignore cryptobenthic species as they cannot be reliably detected) (Samoilys & Carlos 2000, Watson et al. 2010, De Brauwer et al. 2018). Understanding method-specific limitations is therefore key to correct data interpretation. For eDNA, a large body of research has studied the factors influencing false negatives, such as primer design, laboratory protocols and the ecology of eDNA prior to collection. Limitations are increasingly well understood, and will only become clearer in the future.

While there is always a risk of false positives, their causes are also well understood and can be mitigated by strict contamination controls throughout the eDNA workflow (Burian et al. 2021). To further decrease the risk of contamination, eDNA monitoring should ideally be done by experts. At minimum, staff collecting samples should be adequately trained.

Limited taxonomic resolution is not unique to eDNA: methods such as invertebrate surveys regularly identify morphological groups rather than species or even genus or family (Berman et al. 2013). Improved reference libraries will increasingly improve resolution in eDNA metabarcoding studies, alleviating this challenge (see Reference sequence libraries). However, methods that bypass taxonomy such as taxonomy-free biotic indices can be developed to assess the health of ecosystems or indicate specific anthropogenic impacts, allowing for targeted management action (e.g. Cordier et al. 2018, Wilkinson et al. 2023).

Addressing results interpretation challenges will require continued research in method limitations and the ecology of eDNA; stringent contamination controls; and well-designed training protocols for sample collection. Many of these challenges already being addressed through innovations in the field, and there are national guidelines to help non-experts address contamination concerns (De Brauwer et al. 2023). Particularly when monitoring single species, it can be helpful to collaborate with an expert to design a management decision framework that sets out decision triggers linked to specific results (De Brauwer et al. 2022a).

Spatial and temporal comparability of eDNA datasets

Environmental DNA practices and capabilities can differ considerably between users and laboratories. This variability is present in all scientific disciplines, but may be particularly acute for eDNA, since it is developing so rapidly. The use of different protocols has the potential to limit the inter-operability of independently collected data, including time series. Variations in collection methods, bioinformatic protocols and laboratory and service provider procedures can all reduce comparability between studies.

In response, countries around the world are establishing national guidelines for best practice in the use of eDNA in different circumstances (Loeza-Quintana et al. 2020, De Brauwer et al. 2023, Gagné et al. 2021). Creating SOPs for eDNA applications within and between parks is an important first step. Where SOPs are not yet possible, following best practice guidelines and collecting comprehensive metadata should be mandatory. Complete metadata will improve current

inter-operability of data and allow future comparisons when methods have improved (Fediajevaite et al. 2021). Developing a national system to store standardised (meta) data, like the recently developed platform for fish survey image annotation, would greatly improve inter-operability and comparability (Langlois & Friedman 2018).

A long-term solution could be to design a nationally coordinated system to guarantee eDNA samples from monitoring activities across all parks are processed in a reliable, comparable manner, as has been suggested for other countries (Norros et al. 2022, Kelly et al. 2023). Service providers should be given clear guidelines on sample processing standards. Designing proficiency testing systems for service providers, as has been done for the crested newt eDNA project in the UK, would ensure quality and reliability of results (Biggs et al. 2015). Existing testing programs in Australia, such as those for biosecurity applications run through the National eDNA Reference Centre, could potentially be adapted for this purpose in the future (Trujillo-González et al. 2021).

Existing and new opportunities

eDNA opportunities in existing monitoring programs

Environmental DNA methods are already used for monitoring in Australian Marine Parks, and there is opportunity for wider deployment under suitable circumstances. It is likely that initially, eDNA methods will be used to complement existing methods and to establish comparability for future inter-operability. Below is an overview of how existing eDNA capacity in Australia could be used to address the 4 goals of monitoring programs in marine parks (Table 5).

Ecosystem characterisation

Available eDNA applications can generate biodiversity data to fill knowledge gaps in marine parks, particularly in areas where conventional methods are not feasible due to, for example, poor visibility, remoteness or OH&S risks (Table 4). Ecosystem characterisation to establish baseline data is often a condition for approval of activities inside marine parks. Furthermore, in well-known parks, eDNA methods can provide baseline information about a range of cryptic taxa that cannot be detected using conventional survey methods. This use of eDNA is currently limited by the quality of available reference databases (Table 5) and presently only produces reliable presence/absence data, not abundance data.

Measuring the current condition of natural values

There is capacity in Australia to use eDNA methods to monitor the condition of ecosystems through the presence of specific species (Tables 4, 5). This is particularly relevant for pest or TEP species. The AAD's eDNA biosecurity framework identifies benefits from surveillance of, for example, nearshore environments and biofouling species on boats in the Antarctic region (Clarke et al. 2023). Successful ongoing biosecurity monitoring programs, such as the State Wide Array Surveillance Program in Western Australia (McDonald et al. 2019), have been used as a template for different regions (Table 4). With relatively minor adjustments to current systems, eDNA methods could be applied to detect native pest species such as CoTS (Box 2) or TEP species such as sharks or seahorses, as demonstrated by recent research and successful programs in the GBRMP (Uthicke et al. 2022, van Rooyen et al. 2021, Nester et al. 2023). Integrating genetic methods has the potential to reduce costs and increase the scale of monitoring programs, but will require the development and validation of relevant species-specific assays (Table 4; De Brauwer et al. 2022a).

Monitoring changes in environmental values over time

eDNA methods can be used to detect changes in the environment over time, across geographic scales, or through anthropogenic drivers (Berry et al. 2019, DiBattista et al. 2020, West et al. 2021). Environmental DNA methods can detect a wider range of taxa, especially short-lived indicator taxa (e.g. bacteria or cryptobenthic fishes). Because these indicator taxa are more responsive to changing conditions, eDNA methods can detect environmental changes more quickly than monitoring of highly visible but slower growing species.

Report results to inform management and stakeholders

For reporting, eDNA methods offer both challenges and benefits. The sophisticated laboratory methods are highly technical and can be challenging to fully understand, even for those with scientific training. Classic reporting of detailed procedures with extensive results and caveats are unlikely to be understood by management and the public.

However, the COVID-19 pandemic has increased public understanding of certain molecular methods and demonstrated that these methods and their results can be conveyed clearly with the right communication. Furthermore, the core results of most eDNA methods, that is, the presence or absence of species in a location, are easily explained. Interactive maps in the Wai Tuwhera o te Taiao – Open Waters Aotearoa program are an example of how eDNA monitoring data can be communicated effectively to the general public (EPA 2023, Suzuki-Ohno et al. 2023; see also Box 3).

Box 2 Detecting crown of thorn seastars using eDNA methods

Author: Sven Uthicke, Principal Research Scientist, AIMS

Coral-eating crown of thorn seastars (CoTS) on the GBR and elsewhere in the Indo–Pacific region are natural members of coral reef ecosystems, but are prone to extreme population explosions (Uthicke et al. 2015). During these outbreaks the number of corals they consume outweighs coral growth rates, resulting in reef degradation. Overfishing of predators or increased larval survival triggered by high food (plankton algae) through increased land-runoff ('eutrophication') alone or in combination are the most likely causes of outbreaks (Babcock et al. 2016).

Given other reef stressors such as climate change need global long-term effort to address, management authorities endorse and support culling of CoTS as one way to directly protect corals. There are 6–7 vessels involved in culling, with > 1 million CoTS culled in the last 10 years.

To understand outbreaks and guide culling activities, outbreaks need to be detected early. This is difficult to achieve with standard monitoring techniques because CoTS are often cryptic juveniles only visible by experts, and outside active outbreaks, densities are less than 10 animals per hectare.

To assist with these issues, scientists at AIMS developed CoTS-specific DNA markers initially used to detect and quantify CoTS larvae (Uthicke et al. 2015, Doyle et al. 2017). This larvae work is ongoing and supported by tourism operators and CoTS control boats collecting plankton samples. New eDNA techniques, developed later, use these markers to detect post-settlement CoTS in small water samples. The new methods can detect very low densities, and sample occupancy and concentration show a direct relationship to CoTS densities (Uthicke et al. 2018, Uthicke et al. 2022). Method development and testing was supported by the Australian Government through funding to the AIMS and NESP funding, but also by grants from the GBRMPA and the tourism industry.

AIMS is part of the CoTS control innovation program (CCIP) which seeks to improve monitoring and control of CoTS. The project is tasked with operationalising an eDNA-based CoTS monitoring program. Although eDNA will not be the only monitoring tool, the ongoing close collaboration with managers and reef users allows scientists to identify where methods are most efficient and can most effectively guide management effort. The most likely areas for eDNA methods will be in early detection of outbreaks on individual reefs and in monitoring potential population growth after culling on specific reefs has pushed densities below outbreak levels. Other methods for achieving this would require significantly more time and financial effort, and thus more reefs can be surveyed with eDNA methods in less time. In addition, the ease with which samples can be obtained by citizen scientist or managers/enforcement makes eDNA monitoring for CoTS attractive for citizen scientists.

Table 5 Opportunities to integrate eDNA methods in existing marine monitoring frameworks

Attributes	Monitoring goals			
	Characterise communities (baseline studies)	**Measure current condition**	**Monitor environmental change**	**Report**
Technical feasibility	• Possible for single species and whole communities	• Possible depending on which values or indicators are measured	• Needs method calibration and baseline data	• Feasible
Potential monitoring applications	• Areas unsafe or difficult to access using conventional methods • Biosecurity surveillance • Complementary with existing methods	• Presence of Threatened, Endangered and Protected species • Species assemblages • Biosecurity applications • Native pest species presence	• Temporal studies • Measure effects of management • Rapid data collection after disasters and human impact events	• Novel data visualisation options • Wider range of taxa to report than conventional monitoring
Benefits	• Samples easy and safe to collect • Possible to conduct large-scale surveys • Can monitor wider range of species compared to other methods (including cryptic species and microbial diversity) • Non-lethal data collection • Reduced vessel and fieldwork costs	• Samples easy and safe to collect • Can monitor wider range of target species compared to other methods • Non-lethal data collection • Potential multi-species health indices • Reduced vessel and fieldwork costs	• Samples easy and safe to collect • Can monitor wider range of species compared to other methods • Sensitive to detect rapid changes in cryptic organisms • Potential multi-species health indices • Reduced vessel and fieldwork costs	• Presence/absence data is easy to understand • Can easily be visualised in map form • Can be done rapidly

continues

Table 5 *continued*

Attributes	Monitoring goals			
	Characterise communities (baseline studies)	**Measure current condition**	**Monitor environmental change**	**Report**
Limitations	• Incomplete reference databases mean not all taxa identifiable to species • Currently no abundance data	• Incomplete reference databases mean not all taxa identifiable to species • Species-specific assays need to be developed and validated • Limited information on abundance and population structure	• Incomplete reference databases mean not all taxa identifiable to species • Limited information on abundance and population structure • Indices need to be developed and ground truthed • No information on size/biomass/ health of species	• Molecular underpinnings hard to explain to non-experts • Workflows for specific visualisation applications need to be developed

Environmental DNA opportunities beyond monitoring

Co-management with Traditional Owners

First Nations peoples' long history of caring for country is increasingly recognised in the management and monitoring of marine parks. Parks Australia and many state and territory marine parks are beginning to incorporate the knowledge of First Nations peoples in management decisions and the co-design of projects and programs on Sea Country. Some jurisdictions are also moving towards co-managed parks with local Traditional Owners, such as the Bardi Jawi Gaarra and Maiyalam parks in WA, or the ongoing partnership approaches of AIMS (Evans-Illidge et al. 2020, DBCA 2022a,b). For First Nations groups in remote regions, eDNA methods offer a way of collecting data on the entire ecosystem without the need for expensive, hard-to-deploy equipment or expert training. Indigenous ranger groups are already using eDNA methods in the Torres Strait Islands and the Kimberley to detect invasive and threatened species (Villacorta-Rath et al. 2021, Villacorta-Rath & Burrows 2021). Increased uptake of eDNA methods could further improve the quantity and quality of data provided by and to First Nations peoples.

While new methods provide new opportunities for co-management, they also present new challenges. To improve sample collection and minimise contamination, training is needed. The remoteness of some regions co-managed by Traditional Owner groups increases the risk of degradation before samples reach processing facilities. To maximise uptake and efficiency, collection methods should be adapted to local conditions and needs, for example, with adapted protocols or mobile facilities (Villacorta-Rath & Burrows 2021, Forrest 2020).

Engagement with the general public

Most if not all marine parks aim to engage with the general public (e.g. GBRMPA & Queensland Government 2015, Director of National Parks 2018, Aither 2021). This engagement can be limited to simply communicating park rules, but is often more involved. Many marine parks aim to educate the public about local marine life and how parks can help conserve it. Other parks have gone further, engaging with the public through citizen science – the results of which are sometimes used to inform park management (e.g. Browne et al. 2018). Engagement with the public also helps marine parks to maintain productive relationships with certain stakeholders, such as tourism or recreational fishing bodies. Redmap Australia is one example of successful engagement with recreational fishers to inform both climate change researchers and the public (Pecl et al. 2019).

The ease of collecting eDNA samples has led to the development of numerous eDNA citizen science projects in Australia and across the world. In one of the earliest established projects, eDNA methods have been used by citizen scientists since 2015 to detect the great crested newt (*Triturus cristatus*) in the UK. Government has sanctioned use of the resulting data for planning applications (Biggs et al. 2015). A more recent Japanese project worked with 168 volunteers to map coastal fish assemblages across the country (Suzuki-Ohno et al. 2023). The Wai Tuwhera o te Taiao – Open Waters Aotearoa program in New Zealand exemplifies how eDNA methods can be effectively used by and communicated with the public (Box 3). On an even larger scale, the new eBioAtlas is an international partnership between the IUCN and a UK-based service provider that has the ambitious goal of filling critical conservation knowledge gaps by mapping global riverine biodiversity with local stakeholders and citizen scientists (IUCN & NatureMetrics 2023).

In Australia, notable citizen science projects that use eDNA include the Great Australian Platypus Search, which collected samples from 1,649 sites to improve knowledge of platypus distribution across Victoria (Griffiths et al. 2022). Recreational fishing bodies have also run eDNA projects to help recreational fishers detect threatened freshwater species in New South Wales, Queensland and Victoria (OzFish Unlimited 2022).

Box 3 Wai Tuwhera o te Taiao – Open Waters Aotearoa

Author: Vanessa Crowe, Principal Community Engagement Lead, Environmental Protection Authority, New Zealand

Wai Tuwhera o te Taiao – Open Waters Aotearoa is a nationwide community science program designed by the New Zealand Environmental Protection Authority (EPA). The project supports and empowers collaborative environmental protection at the flax roots of Aotearoa. The program has supported over 300 community groups, schools and Māori-led groups to undertake eDNA testing.

Participants can use eDNA findings to advocate for their local environment and make connections with science, technology and mātauranga (Māori knowledge) to inform environmental management decisions. This can include collecting baseline data, comparing sites, monitoring the distribution of species within a catchment or area, and tracking changes over time. The test results can provide useful evidence that can be used in submissions, advocacy and environmental decision-making, such as the extension to trapping zones and reserve areas and improvements to fish passage. The EPA encourages match-funding and collaboration with other support organisations such as councils to increase resources and strengthen place-based relationships.

We prioritise Māori participation and acknowledge the inherent rights and interests that Māori have in relation to the collection, ownership and application of Māori data, including data relating to te taiao (the environment). Some steps we have taken so far towards recognising Māori rights to data and involvement in how that data is managed include:

- ensuring collected samples stay within Aotearoa
- encouraging participants to talk with mana whenua (the indigenous people who have historic and territorial rights over the land) before taking samples
- making sure data is password-protected, unless people wish to make it public
- ensuring full ownership and rights to the sample data sit with the participant
- taking time to speak transparently about how data is stored, used, and managed.

Learn more about Wai Tuwhera o te Taiao.

Using eDNA technologies to engage with the public poses risks that should be considered before programs begin. Clear communication of methods and results is vital to optimise and sustain engagement with non-scientists. Messaging should be in plain language; focus on discovery or species of interest; and ideally include engaging images and video. Higher potential for sample contamination should be considered in downstream analyses for results to also be of use to management. Although some contamination is unavoidable when samples are taken by non-experts, steps can be taken to reduce the initial contamination risk, filter out doubtful samples, and follow up results of interest (Biggs et al. 2015, Griffiths et al. 2022).

Action plan for managers: practical steps to integrate eDNA in individual projects

Resource managers can use the following framework to inform decision-making and determine how eDNA methods could fit within their monitoring programs. The open access *Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand* provide detailed information about designing and executing eDNA projects in the Australian context (De Brauwer et al. 2023).

The framework steps begin with identifying priorities and opportunities, and then move from pilot study to implementation of monitoring in a cyclical approach (Figure 5).

Marine park objectives

First, assess existing monitoring objectives and budget (Hayes et al. 2021). This will ensure the adoption of a cost-effective, targeted approach with the highest chance of successful integration. Detailed monitoring objectives should be accompanied with clear metrics and expectations of which (if any) changes are expected to be detected.

eDNA opportunities

Next, cross-reference park-specific monitoring objectives with existing eDNA methods and capability. Simple decision frameworks can be used to assess the potential implementation of eDNA methods (Figure 6, Darling 2020). Consulting with eDNA experts is strongly recommended at this stage to ensure crucial technical nuances and region- or taxon-specific limitations are taken into account (Gleeson 2021, Berry et al. 2023).

Use this information to identify the monitoring objectives to which eDNA methods are most applicable. The feasibility of different eDNA monitoring opportunities can then be assessed in reference to the existing monitoring framework. This assessment should consider logistical requirements, budgets, available in-house expertise, added value and processing timelines. To reduce contamination risk and ensure sample quality, personnel must be trained in sample collection.

The assessment at this stage may identify multiple opportunities, which can then be prioritised in line with integration objectives or to maximise added value. Alternatively, the assessment may show that eDNA methods are not yet feasible for integration into the existing monitoring framework.

Source: redrawn from Darling 2020, reused with permission.

Figure 5 Action plan: flowchart of the practical steps to integrate eDNA methods in monitoring programs

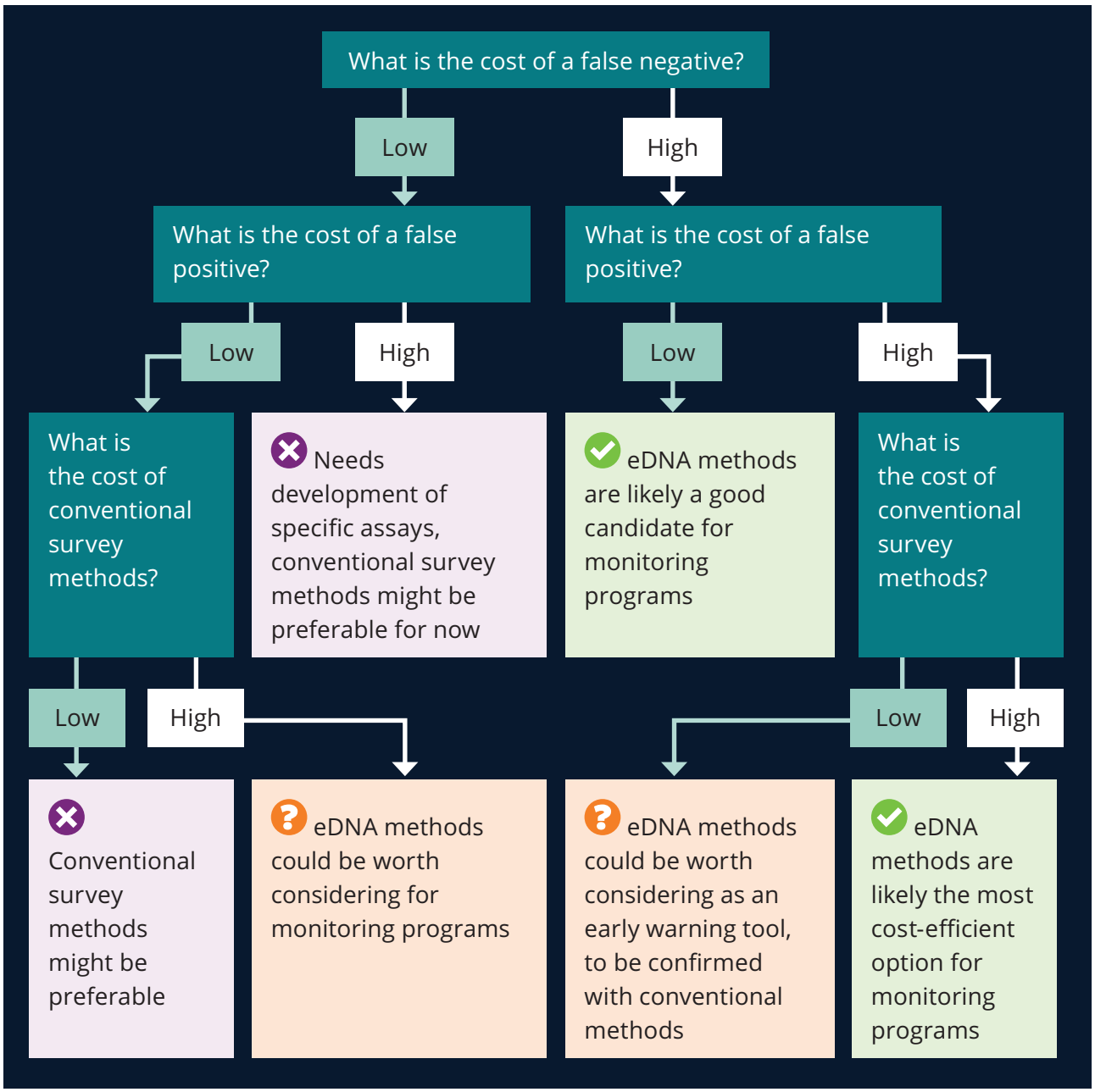


Figure 6 Example of a decision tree used when considering eDNA methods in species-specific monitoring programs (e.g. for endangered or pest species)

Pilot study

Strongly consider collaborating with eDNA experts to conduct a pilot study or eDNA method calibration test. This pilot study should be tailored to match the specific objective, including exact method, habitat and target species. Pilot studies might not be necessary where existing assays have been used and validated for target taxa in the intended ecosystem (Thalinger et al. 2021).

Monitoring

Monitoring programs need a well-designed workflow able to meet project goals. During project design, plan each step in the eDNA workflow, ensuring it is fit for purpose and can deliver the data required. Developing an appropriate experimental design suited for the monitoring objective, which considers strengths and limitations of the planned eDNA method, is essential at this stage. This should consider factors such as representative sampling, replication needs, spatial and temporal relevance, field logistics, data analysis needs, and data storage (De Brauwer et al. 2022a – Table 1; Guri et al. 2023). If ongoing monitoring is planned, specific SOPs should be developed for the monitoring program. Consulting with molecular experts at this stage is advised to ensure planned methods are suitable and realistic.

Plan ahead for how eDNA results will inform management, before monitoring activities and data analyses. Decision frameworks can aid result interpretation, identify potential follow-up surveys, or set trigger values for specific management actions (see **Result interpretation**, Sepulveda et al. 2020, De Brauwer et al. 2022a). The decision framework can incorporate potential sources of error, management objectives or regional factors affecting monitoring actions.

Also at this stage, define what will be considered successful outcomes from eDNA monitoring activities to facilitate method and result evaluation.

So that the effectiveness of eDNA surveys can be evaluated regularly, consider integrating eDNA technologies in adaptive management frameworks, such as the management effectiveness system. This approach ensures that improvements to eDNA monitoring programs can be implemented early.

Budget considerations

The costs of implementing molecular monitoring methods may be considerable. Recent research provides guidance on estimating eDNA survey costs and comparing them with other methods (Andres et al. 2022). Depending on project specifications, there may be eDNA-specific costs such as assay design and specialised sampling. Therefore, we strongly suggest consulting with molecular experts to assess budgetary needs.

Ideally, monitoring budgets would be designed to accommodate the integration of eDNA methods, but this might not be immediately feasible. Cost-effectiveness can be improved by designing cross-stakeholder or cross-park monitoring programs to decrease the per-unit processing cost of individual samples. Coordinating programs across jurisdictions could be used as leverage to decrease costs with eDNA service providers. If done correctly, samples can also be extracted and frozen to be analysed when budget is available or when there are enough samples to reduce the per-unit cost. However, if samples are not stored in appropriate facilities, there is a risk of degraded sample quality or even sample loss.

Research priorities and future developments: supporting integration into monitoring programs

Ongoing research and development are needed if eDNA and other molecular methods are to reach their full potential as part of marine monitoring programs. To align method improvements with monitoring priorities, the needs of monitoring agencies must be clearly communicated to eDNA researchers.

This section considers eDNA research priorities, based on monitoring needs in Australian Marine Parks.

Research priorities

Based on the monitoring needs and priorities of different marine park jurisdictions and stakeholders, we consider research priorities that will support the uptake of eDNA technologies (Table 6). Although we expect this research to be conducted by molecular experts rather than marine park departments, collaboration between these two groups will maximise the benefits of advances in eDNA technology.

Infrastructure and logistics

Quality control

Optimising essential infrastructure and workflow logistics should be an important focus for development. For the foreseeable future, most laboratory processing of eDNA samples will likely be conducted by external service providers, not in-house. Designing systems for intercalibration and proficiency testing would help guarantee reliable results across different providers, increasing managers' trust in sample quality (Trujillo-González et al. 2021). A National eDNA Reference Centre has recently been established to provide these services for eDNA used in biosecurity monitoring, and could potentially be adapted as a model for marine monitoring programs (Trujillo-González et al. 2021). Countries such as the UK have developed laboratory certifications for specific eDNA applications. This approach could be of interest for certain monitoring activities in Australian Marine Parks, such as impact assessments. More simply, designing national standardised positive controls for metabarcoding studies could improve quality assessment of survey results.

Reference libraries

Recognised globally as a research priority, complete reference libraries are needed to optimise assays and accurately identify eDNA samples to species level (Beng & Corlett 2020, Norros et al. 2022, Takahashi et al. 2023). The NBDL is a collaborative initiative that will provide a comprehensive and authoritative data resource for all Australian species, based on expertly identified voucher specimens held in institutional collections. Complete reference sequences for all Australian marine vertebrates are expected by 2025 (CSIRO 2023). Efforts to create reference sequences for other marine species have been initiated but will require sustained investment and collaboration with stakeholders.

Table 6 Research priorities for environmental DNA methods to assist in monitoring

Focus area	Technology	Applications	Timeline*
Infrastructure and logistics	Quality control methods	Increased trust in results from service providers	2030
	Passive sample collection	All eDNA projects, particularly remote, long-term, large-scale	2030
	Automatised sample collection	All eDNA projects, particularly remote, long-term, large-scale	2030
	Point-of-need technologies for sample collection	Projects needing rapid turnaround times	2030
Assay development and calibration	National standards and SOPs	Spatial and temporal data comparability between park monitoring programs	2025
	International reporting and analyses standards and SOPs	Increased international comparability	2030
	Assay validation	Increased trust in results	2025–2030
	Technical improvements in assay validation	Improved understanding of limits and results interpretation	2025–2030
	Calibration with conventional methods	Comparability across spatial and temporal scales	2025–2030
	Ecology of eDNA (e.g. persistence, transport)	All eDNA projects: improved experimental design and interpretation of results	2025–2030
Applied research	Microbial ecosystem health indicators	Monitor base food chain and early indication impacts	2025
	Custom parks ecosystem health indicators	Monitor changes in ecosystem health	2030
	Early warning systems to detect health risks	Monitor presence of hazardous jellyfish, toxic algae blooms and other public health risks	2030
	Early warning systems to detect pests and nuisance species	Monitor presence of species such as crown of thorns seastars or invasive pests	2025

continues

Table 6 *continued*

Focus area	Technology	Applications	Timeline*
Analyses	Data inter-operability for bioinformatics	Spatial and temporal data comparability between park monitoring programs studies	2030
	Machine learning bioinformatics	Metabarcoding surveys, large-scale monitoring	2030
	Novel modelling methods for analyses	Improved understanding of marine ecosystems	2030
	Application-based packages for analyses	Improved efficiency for targeted surveys	2025
Reporting	Data visualisation and communication	All eDNA projects that need to be communicated with the public	2030

* Timeline is based on expected technological readiness for widespread integration in management.

Sample collection

Monitoring programs will benefit from continued efforts to simplify and improve sample collection methods (Formel et al. 2021, Truelove et al. 2022, Hendricks et al. 2023). Increased automatisation and methods such as passive sampling are particularly relevant when surveying remote or large areas, and when collection is done by non-experts. Portable point-of-need tools capable of providing near-instant species identifications could be used to assess significant biosecurity risks or responses to catastrophic environmental impacts (Gleeson et al. 2022). These management needs could also be addressed by ships with mobile laboratory and sequencing facilities (Forrest 2020). Incorporating eDNA monitoring into the existing IMOS framework of national ocean observations is feasible and would benefit long-term observations. To further accommodate long-term comparisons of eDNA data, custom data platforms could be designed to allow Australian eDNA monitoring data to be stored and accessed for re-analysis.

Assay development and calibration

Standard operating procedures

Researchers are investigating how to improve the performance of eDNA assays that serve specific purposes, such as detection surveys of specific taxonomic groups. Establishing and regularly updating SOPs based on the latest research should be a priority. Implementing national SOPs for specific eDNA survey methods and metadata will improve the quality and inter-operability of eDNA monitoring programs (Samuel et al. 2021, Fediajevaite et al. 2021). NESP initiatives have developed standardised fieldwork protocols for a number of marine monitoring methods (Przeslawski & Foster 2020). These initiatives illustrate how such protocols can be developed, but do not yet include eDNA methods. The *Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand,* which will be reviewed frequently, could be used to design SOPs to expert-approved quality standards (De Brauwer et al. 2023).

Assay validation

So that results can be interpreted correctly and used to inform management, assays used to detect single species of interest need to be adequately validated and their limitations well understood (Klymus et al. 2020, De Brauwer et al. 2022b). Assays used to inform management should be validated to the level where limits of detection and assay sensitivity are well understood (i.e. levels 4–5, following Thalinger et al. 2021). It will also be important to continue calibrating single-species assays with conventional methods to inform quantitative measures and ensure temporal continuity of methods (Rourke et al. 2021).

Calibration with conventional methods

Calibration with existing methods is equally important for metabarcoding (multi-species) assays. A next step in calibrating metabarcoding methods is to investigate if, how and when conventional and eDNA methods can be combined for different monitoring purposes (Andres et al. 2022). To optimise the benefits of different survey methods, monitoring frameworks should include robust guidelines on the best use of available methods based on goals, budget, staff and other logistical considerations (Andres et al. 2022). An important area of research relevant to marine park management is understanding how estimates of abundance can be derived from eDNA data (see Long-term future developments).

Ecology of eDNA

Refining the understanding of the ecology of eDNA, that is, 'the origin, state, transport, and fate of eDNA within the environment' (Barnes & Turner 2016) is of relevance to all eDNA applications. While such studies can be highly technical with seemingly limited immediate benefits to management, they are essential to improve survey design, increase sampling efficiency and reduce sources of error from data analysis (Barnes & Turner 2016, Scriver et al. 2023). This is particularly relevant considering the size and diversity of Australia's marine parks and the need to understand if and how the behaviour of eDNA molecules differs across the vast range of conditions in parks.

Applied research

A wide range of new practical eDNA applications are being developed globally. These range from highly specific applications designed to detect single species in a particular context (e.g. Uthicke et al. 2022, Griffiths et al. 2022) to projects designed to detect multiple species relevant to nationwide monitoring (e.g. McDonald et al. 2019). Here, we consider promising developments relevant to large-scale marine monitoring programs.

Ecosystem health indices

Nearly all monitoring programs aim to detect changes in ecosystem health over time to help assess the effectiveness of policy and management interventions (Lindenmayer & Likens 2010). The complexity associated with managing large, highly biodiverse ecosystems can make it harder to determine which signals are important for detecting meaningful long-term trends or impacts. Ecosystem health indices based on a set of indicator taxa are commonly used to monitor freshwater habitats (Borja et al. 2015). Such indices can provide a reliable indication of the condition of an ecosystem, but morphological species identification is time-consuming and dependent on dwindling taxonomic expertise (Cordier et al. 2019, Wilkinson et al. 2023).

Multi-species molecular health indices could provide invaluable information for marine park managers to rapidly assess environmental changes (Hering et al. 2018, Cordier et al. 2019). By using metabarcoding methods, such indices could incorporate hundreds or even thousands of additional taxa with different sensitivities to a range of environmental pressures. A benefit of using molecular versus morphological indices is that data from macro-organisms

(e.g. fish or habitat-forming species), smaller cryptic invertebrates (e.g. meiofauna, plankton) and even bacteria can be collected simultaneously and included in the same index, providing a much more complete and sensitive measure of health than is currently possible (Chariton et al. 2015, Aylagas et al. 2017). Importantly from a management perspective, biodiversity metrics alone are of limited use without the ability to link changes to pressures or management interventions (Hillebrand et al. 2018).

Current research in molecular biological indices has primarily focused on freshwater species assemblages or marine microbial communities (Aylagas et al. 2017, Borja 2018, Apothéloz-Perret-Gentil et al. 2020, Beale et al. 2022a). While metabarcoding studies of marine eukaryote species assemblages can accurately reflect anthropogenic impact gradients (e.g. Chariton et al. 2015, DiBattista et al. 2020), they have yet to be tested against existing conventional biological indices or put into practice in monitoring programs. The development of custom molecular assays and workflows for marine parks could be done relatively rapidly; however, this will require a standardised environmental health status rating for marine park sites as a reference.

Early warning systems

The ability to detect micro-organisms or cryptic species has other potential applications beyond health indices. Monitoring trends in cryptic species communities could serve as an early warning system for shifting ecosystem dynamics, potentially allowing for more rapid, successful interventions (Beale et al. 2022b). A better understanding of the dynamics of these species could allow researchers to diagnose how major impacts might have knock-on effects through the food chain, or to closely monitor recovery after major disturbances (Lanzén et al. 2021, Wolfe et al. 2023).

The same approach could be applied as a warning system for public health threats from species that are currently hard to monitor. Standardised or automated systems could be designed to detect harmful algal blooms, the presence of nuisance species such Irukandji jellyfish, or even dangerous sharks (Bolte et al. 2021, Rolton et al. 2022).

Applications could be developed to monitor compliance and the impacts of different industries active within parks. These could include improvements to existing methods for the early detection of invasive pest species in ballast water, as biofouling, or settling in new regions (Zaiko et al. 2016, Shaw et al. 2019). Assays can also be developed to detect the presence of bacteria or other species indicative of oil spills or sediment run-off (Lanzén et al. 2021).

Analytical improvements

Environmental DNA methods have the potential to generate vast quantities of data. The datasets typical for eDNA surveys are challenging to analyse with standard ecological methods. Challenges include the large quantities of data generated, the complexity of assigning sequences to taxonomical species, and inferring ecological meaning from eDNA datasets. Novel analytical methods are already being developed to address some of these challenges.

Bioinformatics pipeline efficiency

The first, essential, step when analysing eDNA metabarcoding results is to process raw sequencing data using bioinformatics pipelines. However, there is a lack of consensus on best practice or standardised methods and a subsequent lack of consistency between pipelines (Pauvert et al. 2019). Bioinformatics pipelines are often designed for specific study goals or specific labs, which results in limited inter-operability between datasets. Data analysed using different bioinformatics protocols are rarely directly comparable, even if samples were processed with the same laboratory workflow (Pauvert et al. 2019, Mathon et al. 2021).

Project-specific needs mean that strict (inter) nationally standardised bioinformatics pipelines are often not desirable or feasible. However, designing guidelines on minimal metadata reporting would be a first step towards increasing data inter-operability (Samuel et al. 2021). More research is needed to clarify how specific steps in bioinformatics protocols affect data variability. Until these factors are better understood, raw data and scripts should be made available to allow for spatiotemporal comparisons, especially when samples were collected for monitoring purposes. Improved reference libraries are also essential (see Infrastructure and logistics).

Machine learning methods

Processing large quantities of data in metabarcoding workflows is computationally intensive (Mathon et al. 2021, Flück et al. 2022). One way of improving the speed and accuracy of taxonomic assignation is applying machine learning methods, such as supervised and semi-supervised models or convolutional neural networks (Crisci et al. 2012, Cordier et al. 2019, Flück et al. 2022). Different machine learning methods are increasingly used to process eDNA data and have been used to develop biological indices (Cordier et al. 2018, Wilkinson et al. 2023). The main benefits of machine learning methods are the much faster speed (minutes instead of hours), increased accuracy, and potential large-scale applications (Bohan et al. 2017, Cordier et al. 2019, Flück et al. 2022). While these methods show promise, they require strong expertise in computational modelling and are not yet ready for use in routine monitoring programs. Future work should investigate how precision can be further optimised, and how methods can be used in user-friendly applications.

Occupancy modelling

Species occupancy models aim to make inferences about alpha (local scale), beta (landscape scale), and gamma (macroscale) diversity and can be based on Bayesian or frequentist statistics (Bailey et al. 2014, Ferguson et al. 2015), with the former seen as more flexible (Burian et al. 2021). Although such models have predominantly been used to predict single-species dynamics to date, multispecies applications are increasingly being trialled (e.g. Fukaya et al. 2022).

Occupancy modelling applied to eDNA surveys aims to predict the probability of:

- species occurring at a site (occupancy)
- capturing the eDNA at a site (capture)
- detecting a species at a site (detection) (McClenaghan et al. 2020, Burian et al. 2021).

Advantages of this approach include the ease of incorporating covariates related to probability of detection (e.g. PCR amplification biases), false negative measurements errors, temporal dynamics of species-level occurrence, and species interactions (McClenaghan et al. 2020).

While these approaches show promise, further ground truthing across diverse systems is needed. The benefits of incorporating error measurement and capture probabilities can only be maximised if the data informing models is accurate (Willoughby et al. 2016). To achieve this accuracy, factors such as the ecology of eDNA (e.g. transport, degradation) and the effects of variations in lab protocols need to be better understood (Willoughby et al. 2016, McClenaghan et al. 2020).

Network analysis

Ecological network analyses are another class of models that have been applied to eDNA data (e.g. Evans et al. 2016, DiBattista et al. 2020, Seymour et al. 2020, Djurhuus et al. 2020). This approach assesses network properties that arise from analysing which taxa co-occur. Properties such as network connectivity, modularity and nestedness can convey information about the stability of ecological communities and how they respond to different impacts or management (Tulloch et al. 2018, DiBattista et al. 2020, Djurhuus et al. 2020).

The benefit of network analyses, particularly species co-occurrence networks, is that they do not necessarily require abundance data or taxonomic identification to describe communities and detect impacts or changes (Djurhuus et al. 2020, Seymour et al. 2020, Codello et al. 2022). However, to maximise these benefits, research is needed to determine which network properties (or combinations thereof) best predict community shifts or stability, how they correlate with conventional metrics, and how they can be used in real-world predictive modelling (Codello et al. 2022).

Application-based software

Finally, there is increasing demand for user-friendly, application-based packages to speed up analyses for different monitoring purposes. Software packages or apps that directly address management needs without requiring specialist coding skills, specialist software or supercomputing resources would free up time and resources, which could instead be used to respond to the results.

One such application is Pest Alert, a new online app that automatically screens eDNA datasets for invasive species in New Zealand, even if the primary purpose of the dataset is not invasive species detection (Zaiko et al. 2023). Applications that make it easier to re-use existing data for different purposes could be helpful to resource managers. For example, raw metabarcoding data initially collected to detect invasive taxa could be reanalysed for biodiversity studies, as a baseline to assess impacts, or to conduct long-term temporal studies. Such reanalysis would be supported by the development of custom data storage platforms that allow datasets to be archived, explored and extracted.

Reporting

Environmental DNA methods offer the potential to collect ever-larger amounts of monitoring data; however, this data only has value when used appropriately. Reporting is essential to ensure eDNA-based monitoring programs have real-world outcomes.

Depending on the audience, monitoring results are communicated in various formats. Reports written for management agencies are common, and publications in peer reviewed journals can be used to inform both the broader scientific community and marine park managers. Results can be shared with other stakeholders such as policy makers, industry and the public via white papers, public forums, websites and even social media.

Communicating detailed molecular results to non-experts can be challenging. Reporting must be clear and avoid jargon where possible, while still addressing the questions asked by the target audience. Examples of effective reporting to broad audiences include:

- the assessment summaries used in the GBR Outlook Reports (GBRMPA 2019)
- interactive survey maps, such as the Nonindigenous Aquatic Species map by the United States Geological Survey and the Reef Dashboard Monitoring Map by AIMS (Ferrante et al. 2022, AIMS 2023)
- biotic indices that clearly show ecosystem health (Wilkinson et al. 2023).

Developing clear communications often means engaging with professional science communicators to produce reports that are suited for the target audience.

Long-term future developments

The key to ensuring the integration of eDNA technologies into monitoring, is to ask what outcomes resource managers and policy makers (and by extension society) want to see from eDNA surveys. In other words, what does a finished eDNA product look like? In the long term, technological improvements must respond to societal needs and be developed as operational systems and tools.

Abundance: single species

Resource managers are interested in the ability to estimate the relative abundance of different species. Improvements in abundance estimates would increase the usefulness and uptake of eDNA methods in many marine parks.

The current literature suggests that relative measures of abundance can already be estimated from some, but not all, species-specific (qPCR) assays (Rourke et al. 2021, Skelton et al. 2022, Rourke et al. 2023). Such assays need to be thoroughly validated and ideally compared to abundance estimates from conventional methods before operational use (Thalinger et al. 2021). Multiple environmental and species-specific biological factors can influence the appropriateness and effectiveness of abundance estimates for single-species assays. The routine use of eDNA methods to monitor single-species abundance is unlikely to become widespread without significant methodological developments. Better understanding of species-specific factors influencing DNA shedding rates (e.g. season, life stage, body size) and the ecology of eDNA (e.g. eDNA decay and transport) is needed to inform correlations through, for example, allometric scaling (linking organism biomass to eDNA shedding rates) (Yates et al. 2021)

Abundance: species communities

There has been comparatively less research into the abundance measures associated with metabarcoding (multi-species) assays (Rourke et al. 2021). The recent literature suggests that in some cases, abundance estimates obtained from metabarcoding assays are correlated to those from conventional survey methods (e.g. Yates et al. 2019, Fraija-Fernández et al. 2020). However, these correlations do not hold across all studies (e.g. Lim et al. 2016) and scientific publishing bias means that failures to detect correlations could remain unpublished (Yates et al. 2019).

Therefore, the use of eDNA metabarcoding to reliably monitor multi-species abundance will require sustained research effort and method improvements over several years. Parallel surveys with conventional methods across temporal scales will aid calibration of these methods and might allow for operational use in the next decade. These should, however, be combined with controlled experiments and a better understanding of the mechanisms underlying the PCR process (e.g. PCR bias, amplification efficiencies) that might affect PCR read abundance (Kelly et al. 2019, Rourke et al. 2021, Shelton et al. 2023).

Physiological condition

Being able to understand the physiological states of species of interest would assist marine resource managers in some circumstances, particularly when managing fisheries or endangered species. Early research is exploring how data such as the biomass, body size, age, fecundity or health of individuals can be derived from eDNA surveys. Some progress has been made using data based on eRNA to indicate gene expression state (i.e. animal condition; Yates et al. 2019). Also under development are methods for isolating and analysing individual cells from the environment ('emCells') to make inferences about abundance and other characteristics of target species (Miller et al. 2023).

Population genetics

Another long-term research frontier for eDNA monitoring is characterising the genetic population structure of species (Adams et al. 2019). Studying population genetics can answer questions related to genetic diversity, the occurrence of hybridisation, effective population size and extent of dispersal, which can inform marine spatial planning decisions and restoration programs (Sigsgaard et al. 2016, Bani et al. 2020). Environmental DNA methods have been used to survey genetic variation in populations of species such as

abalone, porpoises, fishes and whale sharks (Parsons et al. 2018, Tsuji et al. 2020, Dugal et al. 2022, Adams et al. 2022). Extensive field and laboratory research is required to resolve questions such as: how can sequences be assigned to individual organisms? What DNA markers are most appropriate for these questions? How does allelic abundance reflect real abundance? And can current statistical assumptions (e.g. the ability to differentiate between individuals) be applied to eDNA data (Parsons et al. 2018, Adams et al. 2019)? Many of the advancements in other fields of eDNA research discussed in this section will also feed into eDNA applications in population genetics.

Cross-technological integration

The integration of large-scale eDNA monitoring programs with other survey methods, such as remote sensing, may offer opportunities for improved spatial planning and management (Bani et al. 2020). Automated eDNA monitoring systems could feed into spatial datasets to create nationwide, near-real-time monitoring networks (Bohan et al. 2017). Designing workflows that feed into online platforms or spatial planning frameworks would be of high relevance to dynamic ocean planning, incident responses and permit applications (Lewison et al. 2015, Dunn et al. 2016). New data management infrastructure will need to be designed to handle increasingly large volumes of data, but could be supported by novel data architecture developments such as data fabrics or meshes (Machado et al. 2022, Hechler et al. 2023).

Global perspectives and the inter-operability of eDNA data

The increased integration of molecular methods in monitoring and natural resource management is not restricted to Australia. Government and resource managers globally are adopting eDNA surveys as part of their monitoring toolbox, with levels of uptake varying from initial trials to standardised long-term programs (Table 7).

Many developed countries have integrated or are planning to formally integrate eDNA and other omics methods into government monitoring programs in the next decade (Goodwin et al. 2020, Norros et al. 2022). The successful EU-funded DNAqua-Net program consisted of nearly 600 research and policy members from 36 European countries and aimed to improve eDNA methods and their uptake (Leese et al. 2016). Finland recently published a roadmap that aims to implement molecular monitoring methods (including eDNA) in national monitoring programs by 2030 (Norros et al. 2022). In the USA, NOAA recently released a strategic 5-year plan to facilitate the uptake of various omics technologies in its monitoring programs (Goodwin et al. 2020).

Current applications centre around biosecurity (e.g. McDonald et al. 2019, Jerde 2021), single-species monitoring (Biggs et al. 2015) and freshwater biodiversity (e.g. Leese et al. 2016, Hering et al. 2018). Standardisation of methods and results reporting is seen as central to the reliability and interoperability of this global movement towards omics-based monitoring (Berry et al. 2020, Samuel et al. 2021, Zaiko et al. 2022). Developing international initiatives that support best practices and help increase interoperability of the data generated by eDNA surveys would maximise the applications for management at both regional and international scales (Shea et al. 2023).

The need for such initiatives is recognised in the United Nations Ocean Decade framework (e.g.in relation to the challenge of expanding the global ocean observing system). International programs such as the Ocean Biomolecular Observing Network (OBON) are addressing the Ocean Decade challenges by hosting a suite of projects aimed at improving monitoring methods (Table 7). OBON projects of particular relevance to eDNA technologies include the NBDL, Better Biomolecular Ocean Practices and the Minderoo Foundation's eDNA program, OceanOmics.

Standards and best practice guidelines are also being developed on national and regional levels (Table 6). The diversity in methods and applications used to detect eDNA means that standardisation attempts often focus on either broad principles or very specific workflows (e.g. metabarcoding in marine environment, diatom detection in freshwater systems). National initiatives tend to focus more on general best practice guidelines, with currently only a few initiatives aiming to develop formally accredited standards such as International Organization for Standardization standards (Table 7).

The establishment of national and international scientific societies focused on environmental omics methods reflects the increased use of eDNA methods and the demand for standardisation. These societies are well-connected internationally and are likely to drive formalised standardisation and collaboration initiatives. SeDNAS aims to ensure that Australia and New Zealand guidelines are in harmony with international developments (De Brauwer et al. 2023).

Table 7 Recent global initiatives for eDNA standardisation and interoperability

Type	Region	Title	Goal	Organisation
Standard	Canada	Environmental DNA (eDNA) reporting requirements and terminology (Gagne et al. 2021)	Provide minimum reporting requirements for the planning, execution, analysis, interpretation, and reporting of eDNA projects	Canadian Standards Association
Standard	Europe	CEN/TC230/WG28	Create ISO-approved standard for eDNA water sampling methods	DNAqua-Net and the European Standardisation Committees
Guidance document	Global	Minimum Information for an Omic Protocol (Samuel et al. 2021)	Orient protocols for the discovery of suitable protocol suites on the Ocean Best Practices System	Ocean Best Practices System Omics/eDNA Protocol Management Task team
Guidance document	Australia and New Zealand	Best Practice Guidelines for environmental DNA biomonitoring in Australia and New Zealand (De Brauwer et al. 2023)	Set minimum standards to support a consistent and best practice approach to eDNA testing	Southern eDNA Society
Guidance document	Canada	Environmental DNA Standardization Needs for Fish and Wildlife Population Assessments and Monitoring (Helbing & Hobbs 2019)	Guide, solicit support, and encourage development of rigorous standards by providing a comprehensive overview of current understanding and anticipated challenges	Canadian Standards Association
Guidance document	Canada	Guidance on the use of targeted environmental DNA (eDNA) analysis for the management of aquatic invasive species and species at risk (Abbott et al. 2021)	Provide a model for consistent and transparent communication and reporting of eDNA results aimed at fisheries and oceans managers	Department of Fisheries and Oceans Canada

continues

Table 7 *continued*

Type	Region	Title	Goal	Organisation
Guidance document	Europe	A practical guide to DNA-based methods for biodiversity assessment (Bruce et al. 2021)	Summarise the scientific consensus relating to every step of the eDNA field and laboratory workflows involved in the most common types of samples and analyses	DNAqua-Net
Guidance document	Europe	A validation scale to determine the readiness of environmental DNA assays for routine species monitoring (Thalinger et al. 2021)	Describe the measures and tests necessary for successful validation of targeted eDNA assays to form the basis of guidelines	DNAqua-Net
Guidance document	Japan	Environmental DNA sampling and experiment manual	Promote and standardise eDNA analysis methods	The eDNA Society
Guidance document	Switzerland	Environmental DNA applications in biomonitoring and bioassessment of aquatic ecosystems (Pawlowski et al. 2020)	Provide detailed protocols and best practices for processing eDNA samples	Federal Office for the Environment
Platform	Global	Ocean Best Practices System	Enhance management of methods and support the development of ocean best practices through a global, sustained system comprising technological solutions and community approaches	Ocean Best Practices System
Platform	Global	Omic Biodiversity Observation Network	Promote intercalibration of biomolecular observing technologies, the development of globally harmonised practices, standards and protocols to help establish global coordinated biomolecular observations	Biodiversity Observation Network

continues

Table 7 *continued*

Type	Region	Title	Goal	Organisation
Platform	Global	Biomolecular Ocean Observing Network	Develop a global system that will allow science and society to understand ocean using biomolecular methods	UN Ocean Decade action
Platform	Canada	Pathway to Increase Standards and Competency of eDNA Surveys	Explore and inform public policy, industry strategies and future research on eDNA	University of Guelph
Platform	Europe	Bioscan Europe	Create a shared European perspective and framework for effective DNA-based biodiversity monitoring, connecting and enhancing national DNA barcoding infrastructures and initiatives	Bioscan Europe


LEGEND
DIVE RITE

Acronyms, initialisms and glossary

Term	Definition
AAD	Australian Antarctic Division
AIMS	Australian Institute of Marine Science
Assay	The laboratory workflow from DNA extraction to sequence outputs. Often refers more specifically to the target gene and taxonomic group (e.g. 16S_Fish, 18S universal, COI).
AUV	Autonomous underwater vehicle
ASV	Amplicon sequence variant
BRUV	Baited remote underwater video
CoTs	Crown of thorns seastar
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DBCA	Department of Biodiversity, Conservation and Attractions
DCCEEW	Department of Climate Change, Energy, the Environment and Water
ddPCR	Digital Droplet PCR, a quantitative PCR method based on water-oil emulsion droplet technology.
DOV	Diver operated video
EEZ	Exclusive economic zone
Environmental DNA or RNA (eDNA or eRNA)	DNA or RNA present in environmental samples (soil, sediment, water, etc.), often without observation of the original organism. DNA carries genetic instructions for the development, functioning, growth and reproduction of organisms, while RNA transfers information within cells to produce specific proteins and is believed to be only shed by physiologically active (living) organisms.
EPA	Environmental Protection Authority
FAIR (data)	Findable, accessible, inter-operable, and reusable (data)
GBR	Great Barrier Reef
GBRMP	Great Barrier Reef Marine Park
GBRMPA	Great Barrier Reef Marine Park Authority

Term	Definition
HIMI	Heard Island and McDonald Islands
HTS	High-throughput sequencing
IMOS	Integrated Marine Observing System
IUCN	International Union for Conservation of Nature
Library (also Reference library, Reference database)	Database with DNA sequences of specific species
MER	Monitoring, evaluation and reporting
Metabarcoding	Simultaneous taxonomic identification of multiple species (or OTUs ASVs in eDNA samples with millions of sequences, generated by PCR amplification using high-throughput sequencing techniques
MIMP	Marine Integrated Monitoring Program
MinION	A type of portable DNA and RNA sequencing device manufactured by Oxford Nanopore Technologies
mOTU	Molecular operational taxonomic units
NBDL	National Biodiversity DNA Library
NESP	National Environmental Science Program
NOAA	National Oceanic and Atmospheric Administration
NOPSEMA	National Offshore Petroleum Safety and Environmental Management Authority
OBON	UN Ocean Biomolecular Observing Network
Operational taxonomic unit (OTU)	Uniquely recurring DNA sequences used as a proxy for diversity. These taxonomically similar units are grouped according to their close DNA sequence similarity. Used a unit of diversity when conventional biological classification is not possible or desirable.
Polymerase chain reaction (PCR)	A molecular technique that allows the exponential amplification of a target fragment or region of DNA from a mixture of DNA fragments. The desired fragment is selected from the other fragments in the mixture by specific primers (small single-strand oligonucleotides) complementary to the desired sequence.

Term	Definition
Primer	Short DNA fragments used in PCR amplification that bind adjacent to the target region or gene. They enable the amplification and sequencing of specific parts of a genome and from specific groups of organisms.
Quantitative PCR (qPCR; also real-time PCR)	A variant of PCR. The main difference is that qPCR quantifies the amount of DNA in the original sample.
RIMReP	Reef 2050 Integrated Monitoring and Reporting Program
ROV	Remotely operated underwater vehicle
SeDNAS	Southern eDNA Society
Sequencing	Determining the order of nucleotides in DNA or RNA; this can be done with a variety of methods
SHP	Signs of Healthy Parks
SOP	Standard operating procedure
TEP	Threatened, Endangered and Protected (species)
UNESCO	United Nations Educational, Scientific and Cultural Organization
UVC	Underwater visual census
zOTU	Zero-radius Operational Taxonomic Unit (see OTU)

References

Abbott C, Coulson M, Gagné N, Lacoursière-Roussel A, Parent GJ, Bajno R, Dietrich C & May-McNally S (2021). *Guidance on the use of targeted environmental DNA (eDNA) analysis for the management of aquatic invasive species and species at risk*, Fisheries and Oceans Canada. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/40960791.pdf.

Adams CIM, Hepburn C, Jeunen GJ, Cross H, Taylor HR, Gemmell NJ, Bunce M & Knapp M (2022). Environmental DNA reflects common haplotypic variation. *Environmental DNA* 00:1–14. https://doi.org/10.1002/edn3.352.

Adams CIM, Knapp M, Gemmell NJ, Jeunen GJ, Bunce M, Lamare MD & Taylor HR (2019). Beyond biodiversity: can environmental DNA (eDNA) cut it as a population genetics tool? *Genes* 10(3):192. https://doi.org/10.3390/genes10030192.

Addison PFE, Collins DJ, Trebilco R, Howe S, Bax N, Hedge P, Jones G, Miloslavich P, Roelfsema C, Same M, Stuart-Smith RD, Scanes P, von Baumgarten P & McQuatters-Gollop A (2018). A new wave of marine evidence-based management: emerging challenges and solutions to transform monitoring, evaluating, and reporting. *ICES Journal of Marine Science* 75(3):941–52. https://doi.org/10.1093/icesjms/fsx216.

Agriculture Victoria (2023). *Testing the waters – Victorian Ports Marine Surveillance Pilot Program*, Agriculture Victoria: Biosecurity. https://agriculture.vic.gov.au/biosecurity/protecting-victoria/strengthening-victorias-biosecurity-system-program/biosecurity-stories/testing-the-waters-victorian-ports-marine-surveillance-pilot-program.

AIMS (Australian Institute of Marine Science) (2023). *The AIMS Index of Marine Industry*. https://www.aims.gov.au/sites/default/files/2023-05/AIMS_IndexOfMarineIndustry_24May2023FINAL.pdf.

Aither (2021). *Integrated monitoring and evaluation framework for the marine integrated monitoring program*, report to the NSW Department of Primary Industries and Environment. https://www.marine.nsw.gov.au/marine-estate-programs/marine-integrated-monitoring-program.

Andres KJ, Lambert TD, Lodge DM, Andrés J & Jackson JR (2022). Combining sampling gear to optimally inventory species highlights the efficiency of eDNA metabarcoding. *Environmental DNA* 5(1):146–57. https://doi.org/10.1002/edn3.366.

Apothéloz-Perret-Gentil L, Bouchez A, Cordier T, Cordonier A, Guéguen J, Rimet F, Vasselon V & Pawlowski J (2020). Monitoring the ecological status of rivers with diatom eDNA metabarcoding: a comparison of taxonomic markers and analytical approaches for the inference of a molecular diatom index. Molecular Ecology 30(13):2959–68. https://doi.org/10.1111/mec.15646.

Apothéloz-Perret-Gentil L, Cordonier A, Straub F, Iseli J, Esling P & Pawlowski J (2017). Taxonomy-free molecular diatom index for high-throughput eDNA biomonitoring. *Molecular Ecology Resources* 17(6):1231–42. https://doi.org/10.1111/1755-0998.12668.

Australian Marine Parks (2021). Norfolk Marine Park habitat mapping projects. *Australian Marine Parks: news*. https://parksaustralia.gov.au/marine/news/norfolk-marine-park-habitat-mapping-projects/.

Australian Marine Parks (2023). $11m partnership a game-changer in monitoring ocean biodiversity. *Australian Marine Parks: news*. https://parksaustralia.gov.au/marine/news/11m-partnership-a-game-changer-in-monitoring-ocean-biodiversity/.

Aylagas E, Borja A, Tangherlini M, Dell'Anno A, Corinaldesi C, Michell CT, Irigoien X, Danovaro R & Rodríguez-Ezpeleta N (2017). A bacterial community-based index to assess the ecological status of estuarine and coastal environments. Marine Pollution Bulletin 114(2):679–88. https://doi.org/10.1016/j.marpolbul.2016.10.050.

Aylagas E, Borja A, Muxika I & Rodríguez-Ezpeleta N (2018). Adapting metabarcoding-based benthic biomonitoring into routine marine ecological status assessment networks. *Ecological Indicators* 95:194–202. https://doi.org/10.1016/j.ecolind.2018.07.044.

Babcock RC, Dambacher JM, Morello EB, Plagányi EE, Hayes KR, Sweatman HPA & Pratchett MS (2016). Assessing different causes of crown-of-thorns starfish outbreaks and appropriate responses for management on the Great Barrier Reef. *PloS ONE* 11(12):e0169048. https://doi.org/10.1371/journal.pone.0169048.

Bailey LL, MacKenzie DI & Nichols JD (2014). Advances and applications of occupancy models. *Methods in Ecology and Evolution* 5(12):1269–1279. https://doi.org/10.1111/2041-210X.12100.

Bani A, De Brauwer M, Creer S, Dumbrell AJ, Limmon G, Jompa J, von der Heyden S & Beger M (2020). Informing marine spatial planning decisions with environmental DNA. In: Dumbrell AJ, Turner EC & Fayle TM (eds), *Advances in ecological research*, Tropical Ecosystems in the 21st Century. Academic Press, 62:375–407. https://doi.org/10.1016/bs.aecr.2020.01.011.

Barnes MA & Turner CR (2016). The ecology of Environmental DNA and implications for conservation genetics. *Conservation Genetics* 17(1):1–17. https://doi.org/10.1007/s10592-015-0775-4.

Beale DJ, Crosswell J, Shah RM, Hillyer KE, Stephenson S, Karpe AV, Palombo EA, Jones OAH, Gorman D, Cook S, Bodrossy L, van de Kamp J, Bissett A, Whiteley AS & Steven ADL (2022a). Establishing a regional microbial blueprint of metabolic function in sediment collected from pristine tropical estuarine systems. In: Beale DJ, Hillyer K, Warden A & Jones OAH (eds), *Applied environmental metabolomics*, Academic Press, 337–357. https://doi.org/10.1016/B978-0-12-816460-0.00023-X.

Beale DJ, Jones OAH, Bose U, Broadbent JA, Walsh TK, Van De Kamp j & Bissett A (2022b). Omics-based ecosurveillance for the assessment of ecosystem function, health, and resilience. *Emerging Topics in Life Sciences* 6(2):185–199. https://doi.org/10.1042/etls20210261.

Beentjes KK, Speksnijder AGCL, Schilthuizen M, Schaub BEM & van der Hoorn BB (2018). The influence of macroinvertebrate abundance on the assessment of freshwater quality in the Netherlands. *Metabarcoding and Metagenomics* 2:e26744. https://doi.org/10.3897/mbmg.2.26744.

Benedetti-Cecchi L, Airoldi L, Abbiati M & Cinelli F (1996). Estimating the abundance of benthic invertebrates: a comparison of procedures and variability between observers. *Marine Ecology Progress Series* 138:93–101. https://doi.org/10.3354/meps138093.

Beng KC & Corlett RT (2020). Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodiversity and Conservation* 29(7):2089–2121. https://doi.org/10.1007/s10531-020-01980-0.

Berman J, Burton M, Gibbs R, Lock K, Newman P, Jones J & Bell J (2013). Testing the suitability of a morphological monitoring approach for identifying temporal variability in a temperate sponge assemblage. *Journal for Nature Conservation* 21(3):173–82. https://doi.org/10.1016/j.jnc.2012.12.003.

Berry O, Bulman C, Bunce M, Coghlan M, Murray DC & Ward RD (2015). Comparison of morphological and DNA metabarcoding analyses of diets in exploited marine fishes. *Marine Ecology Progress Series* 540:167–81. https://doi.org/10.3354/meps11524.

Berry O, Holleley CE & Jarman SN (2023). *Applied Environmental Genomics*, CSIRO Publishing, Melbourne.

Berry O, Jarman S, Bissett A, Hope M, Paeper C, Bessey C, Schwartz MK, Hale J & Bunce M (2020). Making environmental DNA (eDNA) biodiversity records globally accessible. *Environmental DNA* 3(4):699–705. https://doi.org/10.1002/edn3.173.

Berry TE, Saunders BJ, Coghlan ML, Stat M, Jarman S, Richardson AJ, Davies CH, Berry O, Harvey ES & Bunce M (2019). Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. *PloS Genetics* 15(2):e1007943. https://doi.org/10.1371/journal.pgen.1007943.

Bessey C, Gao Y, Truong YB, Miller H, Jarman SN & Berry O (2022). Comparison of materials for rapid passive collection of environmental DNA. *Molecular Ecology Resources* 22(7):2559–72. https://doi.org/10.1111/1755-0998.13640.

Bessey C, Jarman SN, Berry O, Olsen YS, Bunce M, Simpson T, Power M, McLaughlin J, Edgar GJ & Keesing J (2020). Maximizing fish detection with eDNA metabarcoding. *Environmental DNA* 2(4):493–504. https://doi.org/10.1002/edn3.74.

Bessey C, Jarman SN, Simpson T, Miller H, Stewart T, Keesing JK & Berry O (2021). Passive eDNA collection enhances aquatic biodiversity analysis. *Communications Biology* 4(1):1–12. https://doi.org/10.1038/s42003-021-01760-8.

Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Foster J, Wilkinson JW, Arnell A, Brotherton P, Williams P & Dunn F (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation* 183:19–28. https://doi.org/10.1016/j.biocon.2014.11.029.

Biosecurity Queensland (2021). *Queensland Seaports eDNA Surveillance (Q-SEAS) Marine Pest Pilot Program 2019–2020: Port of Gladstone*, Queensland Department of Agriculture and Fisheries, Brisbane.

Bissett A, Fitzgerald A, Meintjes T, Mele PM, Reith F, Dennis PG, Breed MF, Brown B, Brown MV, Brugger J, Byrne M, Caddy-Retalic S, Carmody B, Coates DJ, Correa C, Ferrari BC, Gupta VVSR, Hamonts K, Haslem A, Hugenholtz P, Karan M, Koval J, Lowe AJ, Macdonald S, McGrath L, Martin D, Morgan M, North KI, Paungfoo-Lonhienne C, Pendall E, Phillips L, Pirzl R, Powell JR, Ragan MA, Schmidt S, Seymour N, Snape I, Stephen JR, Stevens M, Tinning M, Williams K, Yeoh YK, Zammit CM & Young A (2016). Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database. *GigaScience* 5(1):21. https://doi.org/10.1186/s13742-016-0126-5.

Blackman RC, Walser J-C, Rüber L, Brantschen J, Villalba S, Brodersen J, Seehausen O & Altermatt F (2023). General principles for assignments of communities from eDNA: open versus closed taxonomic databases. *Environmental DNA* 5(2):326–42. https://doi.org/10.1002/edn3.382.

Bohan DA, Vacher C, Tamaddoni-Nezhad A, Raybould A, Dumbrell AJ & Woodward G (2017). Next-generation global biomonitoring: large-scale, automated reconstruction of ecological networks. *Trends in Ecology & Evolution* 32(7):477–87. https://doi.org/10.1016/j.tree.2017.03.001.

Bolte B, Goldsbury J, Huerlimann R, Jerry D & Kingsford M (2021). Validation of eDNA as a viable method of detection for dangerous cubozoan jellyfish. *Environmental DNA* 3(4):769–79. https://doi.org/10.1002/edn3.181.

Borja Á, Franco J & Pérez V (2000). A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin 40(12): 100–1114. https://doi.org/10.1016/S0025-326X(00)00061-8.

Borja Á (2018). Testing the efficiency of a bacterial community-based index (microgAMBI) to assess distinct impact sources in six locations around the world. *Ecological Indicators* 85:594–602. https://doi.org/10.1016/j.ecolind.2017.11.018.

Borja Á, Marín SL, Muxika I, Pino L & Rodríguez JG (2015). Is there a possibility of ranking benthic quality assessment indices to select the most responsive to different human pressures? *Marine Pollution Bulletin* 97(1):85–94. https://doi.org/10.1016/j.marpolbul.2015.06.030.

Brandt MI, Trouche B, Quintric L, Günther B, Wincker P, Poulain J & Arnaud-Haond S (2021). Bioinformatic pipelines combining denoising and clustering tools allow for more comprehensive prokaryotic and eukaryotic metabarcoding. *Molecular Ecology Resources* 21(6):1904–21. https://doi.org/10.1111/1755-0998.13398.

Brown MV & Bodrossy L (2021). *SoE 2021 marine expert assessments: state and trend assessment – microbial communities and processes*, Australian Ocean Data Network, Hobart. https://soe.dcceew.gov.au/views/reference/48930.

Brown MV, van de Kamp J, Ostrowski M, Seymour JR, Ingleton T, Messer LF, Jeffries T, Siboni N, Laverock B, Bibiloni-Isaksson J, Nelson TM, Coman F, Davies CH, Frampton D, Rayner M, Goossen K, Robert S, Holmes B, Abell GCJ, Craw P, Kahlke T, Sow SLS, McAllister K, Windsor J, Skuza M, Crossing R, Patten N, Malthouse P, van Ruth PD, Paulsen J, Fuhrman JA, Richardson A, Koval J, Bissett A, Fitzgerald A, Moltmann T & Bodrossy L (2018). Systematic, continental scale temporal monitoring of marine pelagic microbiota by the Australian Marine Microbial Biodiversity Initiative. *Scientific Data* 5(1):180130. https://doi.org/10.1038/sdata.2018.130.

Browne M, Pocklington J, Franklin A, Howe S, Rodrigue M, Stevenson J & Krak I (2018). *Sea Search Manual*, Parks Victoria, Melbourne.

Bruce K, Blackman R, Bourlat SJ, Hellström AM, Bakker J, Bista I, Bohmann K, Bouchez A, Brys R, Clark K, Elbrecht V, Fazi S, Fonseca V, Hänfling B, Leese F, Mächler E, Mahon AR, Meissner K, Panksep K, Pawlowski J, Yáñez PS, Seymour M, Thalinger B, Valentini A, Woodcock P, Traugott M, Vasselon V & Deiner K (2021). *A practical guide to DNA-based methods for biodiversity assessment*. Advanced Books. https://doi.org/10.3897/ab.e68634.

Bryars S, Page B, Waycott M, Brock D & Wright A (2017). *South Australian marine parks monitoring, evaluation and reporting plan: technical report*, South Australian Department of Environment, Water and Natural Resources, Adelaide.

Budd AM, Schils T, Cooper MK, Lyons MB, Mills MS, Deinhart ME, Le Port A, Huerlimann R & Strugnell JM (2023). Monitoring threatened species with environmental DNA and open ecological data: local distribution and habitat preferences of scalloped hammerhead sharks (*Sphyrna lewini*). *Biological Conservation* 278:109881. https://doi.org/10.1016/j.biocon.2022.109881.

Burian A, Mauvisseau Q, Bulling M, Domisch S, Qian S & Sweet M (2021). Improving the reliability of eDNA data interpretation. *Molecular Ecology Resources* 21(5):1422–33. https://doi.org/10.1111/1755-0998.13367.

Campbell MD, Pollack AG, Gledhill CT, Switzer TS & DeVries DA (2015). Comparison of relative abundance indices calculated from two methods of generating video count data. *Fisheries Research* 170:25–33. https://doi.org/10.1016/j.fishres.2015.05.011.

Capo E, Giguet-Covex C, Rouillard A, Nota K, Heintzman PD, Vuillemin A, Ariztegui D, et al. (2021). Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: overview and recommendations. *Quaternary* 4(1):6. https://doi.org/10.3390/quat4010006.

Casas L & Saborido-Rey F (2022). *State of the art review of bioinformatics analysis of environmental DNA*, Institute of Marine Research, Spain. FishGenome-D1.3b. eDNA_Bioinformatics_SoA_Review.pdf (csic.es).

Chariton AA, Stephenson S, Morgan MJ, Steven ADL, Colloff MJ, Court LN & Hardy CM (2015). Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. *Environmental Pollution* 203:165–74. https://doi.org/10.1016/j.envpol.2015.03.047.

Chrismas N, Allen R, Allen MJ, Bird K & Cunliffe M (2023). A 17-year time-series of fungal environmental DNA from a coastal marine ecosystem reveals long-term seasonal-scale and inter-annual diversity patterns. *Proceedings of the Royal Society B: Biological Sciences* 290(1992):20222129. https://doi.org/10.1098/rspb.2022.2129.

Clark DR, Ferguson RMW, Harris DN, Nicholass KJM, Prentice HJ, Randall KC, Randell L, Warren SL & Dumbrell AJ (2018). Streams of data from drops of water: 21st century molecular microbial ecology. *Wiley Interdisciplinary Reviews: Water* 5(4):e1280. https://doi.org/10.1002/wat2.1280.

Clarke LJ, Shaw JD, Suter L, Atalah J, Bergstrom DM, Biersma E, Convey P, Greve M, Holland O, Houghton MJ, Hughes KA, Johnston EL, King CK, McCarthy AH, McGaughran A, Pertierra LR, Robinson SA, Sherman CDH, Stark JS, Stevens MI, Strugnell JM, von Ammon Ulla, Wilson NG, Zaiko A & Macdonald AJ (2023). An expert-driven framework for applying eDNA tools to improve biosecurity in the Antarctic. *Management of Biological Invasions* 14(3):379–402. https://doi.org/10.3391/mbi.2023.14.3.01.

Codello A, Hose GC & Chariton A (2022). Microbial co-occurrence networks as a biomonitoring tool for aquatic environments: a review. *Marine and Freshwater Research* 74:409–422. https://doi.org/10.1071/MF22045.

Collins RA, Baillie C, Halliday NC, Rainbird S, Sims DW, Mariani S & Genner MJ (2022). Reproduction influences seasonal eDNA variation in a temperate marine fish community. *Limnology and Oceanography Letters* 7(5):443–49. https://doi.org/10.1002/lol2.10271.

Commonwealth of Australia (2015). *Reef 2050 Long-Term Sustainability Plan*, Commonwealth of Australia. dcceew.gov.au.

Cordier T, Esling P, Lejzerowicz F, Visco J, Ouadahi A, Martins C, Cedhagen T & Pawlowski J (2017). Predicting the ecological quality status of marine environments from eDNA metabarcoding data using supervised machine learning. *Environmental Science & Technology* 51(16):9118–26. https://doi.org/10.1021/acs.est.7b01518.

Cordier T, Forster D, Dufresne Y, Martins CIM, Stoeck T & Pawlowski J (2018). Supervised machine learning outperforms taxonomy-based environmental DNA metabarcoding applied to biomonitoring. *Molecular Ecology Resources* 18(6):1381–91. https://doi.org/10.1111/1755-0998.12926.

Cordier T, Lanzén A, Apothéloz-Perret-Gentil L, Stoeck T & Pawlowski J (2019). Embracing environmental genomics and machine learning for routine biomonitoring. *Trends in Microbiology* 27(5):387–97. https://doi.org/10.1016/j.tim.2018.10.012.

Crisci C, Ghattas B & Perera G (2012). A review of supervised machine learning algorithms and their applications to ecological data. *Ecological Modelling* 240:113–22. https://doi.org/10.1016/j.ecolmodel.2012.03.001.

CSIRO (2023). *About the National Biodiversity DNA Library.* National Biodiversity DNA Library. https://research.csiro.au/dnalibrary/.

Darling JA (2020). How to learn to stop worrying and love environmental DNA monitoring. *Aquatic Ecosystem Health & Management* 22(4):440–51. https://doi.org/10.1080/14634988.2019.1682912.

DBCA (Department of Biodiversity, Conservation and Attractions) (2022a). *Lalang-Gaddam Marine Park Joint Management Plan 98*, Western Australian Department of Biodiversity, Conservation and Attractions, Perth.

DBCA (Department of Biodiversity, Conservation and Attractions) (2022b). *Bardi Jawi Gaarra Marine Park Joint Management Plan 99*, Western Australian Department of Biodiversity, Conservation and Attractions, Perth.

DCCEEW (Department of Climate Change, Energy, the Environment and Water) (2022). *Nature Positive Plan: better for the environment, better for business*, Australian Government Department of Climate Change, Energy, the Environment and Water, Canberra.

De Brauwer M, Chariton A, Clarke L, Cooper M, Dibattista J, Furlan E, Giblot-Ducray D, Gleeson DM, Harford AJ, Herbert S, MacDonald AJ, Miller A, Montgomery K, Mooney T, Noble L, Rourke ML, Sherman C, Stat M, Suter L, West K, White NE, Villacorta-Rath C, Zaiko A & Trujillo-González A (2022a). *Environmental DNA protocol development guide for biomonitoring*, National eDNA Reference Centre, Canberra. https://doi.org/10.13140/RG.2.2.12118.11849.

De Brauwer M, Chariton A, Clarke L, Cooper M, Dibattista J, Furlan E, Giblot-Ducray D, Miller A, Montgomery K, Mooney T, Noble L, Rourke ML, Sherman C, Stat M, Suter L, West K, White NE, Villacorta-Rath C, Zaiko A & Trujillo-González A (2022b). *Environmental DNA test validation guidelines*, National eDNA Reference Centre, Canberra. https://doi.org/10.13140/RG.2.2.33928.49927.

De Brauwer M, Clarke LJ, Chariton A, Cooper MK, de Bruyn M, Furlan E, MacDonald AJ, Rourke ML, Sherman CDH, Suter L, Villacorta-Rath C, Zaiko A & Trujillo-González A (2023). Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand. *Environmental DNA* 5(3):417–23. https://doi.org/10.1002/edn3.395.

De Brauwer M, Hobbs JPA, Ambo-Rappe R, Jompa J, Harvey ES & McIlwain JL (2018). Biofluorescence as a survey tool for cryptic marine species. *Conservation Biology* 32(3):706–15. https://doi.org/10.1111/cobi.13033.

Deagle BE, Kirkwood R & Jarman SN (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology* 18(9):2022–38. https://doi.org/10.1111/j.1365-294X.2009.04158.x.

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME & Bernatchez L (2017). Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology* 26(21):5872–95. https://doi.org/10.1111/mec.14350.

Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E & Miaud C (2012). Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49(4):953–59. https://doi.org/10.1111/j.1365-2664.2012.02171.x.

Deloitte Access Economics (2017). *At what price? The economic, social and icon value of the Great Barrier Reef*, report to the Great Barrier Reef Foundation. https://apo.org.au/node/96031.

DiBattista JD, Berumen ML, Priest MA, De Brauwer M, Coker DJ, Sinclair-Taylor TH, Hay A, Bruss G, Mansour S, Bunce M, Goatley CHR, Power M & Marshell A (2022). Environmental DNA reveals a multi-taxa biogeographic break across the Arabian Sea and Sea of Oman. *Environmental DNA* 4(1):206–221. https://doi.org/10.1002/edn3.252.

DiBattista JD, Reimer JD, Stat M, Masucci GD, Biondi P, De Brauwer M & Bunce M (2019). Digging for DNA at depth: rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef biodiversity across a depth gradient. *PeerJ* 7 : e6379. https://doi.org/10.7717/peerj.6379.

DiBattista JD, Reimer JD, Stat M, Masucci GD, Biondi P, De Brauwer M, Wilkinson SP, Chariton AA & Bunce M (2020). Environmental DNA can act as a biodiversity barometer of anthropogenic pressures in coastal ecosystems. *Scientific Reports* 10(1):1–15. https://doi.org/10.1038/s41598-020-64858-9.

Director of National Parks (2018). *Temperate East Marine Parks Network Management Plan 2018*, Director of National Parks, Canberra.

Djurhuus A, Closek CJ, Kelly RP, Pitz KJ, Michisaki RP, Starks HA, Walz KR, Andruskieicz EA, Oleson E, Hubbard K, Montes E, Otis D, Muller-Karger FE, Chavez FP, Boehm AB & Breitbart M (2020). Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nature Communications* 11:254. https://doi.org/10.1038/s41467-019-14105-1.

DOC (Department of Conservation) and Toitū Te Whenua Land Information New Zealand (2023). *Long-term insights briefing: how can we help biodiversity thrive through the innovative use of information and emerging technologies?*, Conservation House, Wellington. https://www.doc.govt.nz/globalassets/documents/about-doc/long-term-insights-briefings/2023/ltib2023-doc-linz.pdf.

DOE (Department of the Environment) (2014). *Heard Island and McDonald Islands Marine Reserve Management Plan 2014–2024*, Australian Government Department of the Environment, Canberra. http://heardisland.antarctica.gov.au/.

Doyle J & Uthicke S (2021). Sensitive environmental DNA detection via lateral flow assay (dipstick): a case study on corallivorous crown-of-thorns sea star (*Acanthaster cf. solaris*) detection. *Environmental DNA* 3(2):323–42. https://doi.org/10.1002/edn3.123.

Doyle JR, McKinnon AD & Uthicke S (2017). Quantifying larvae of the coralivorous seastar *Acanthaster* cf. *solaris* on the Great Barrier Reef using qPCR. *Marine Biology* 164(8):176. https://doi.org/10.1007/s00227-017-3206-x.

Dugal L, Thomas L, Jensen MR, Sigsgaard EE, Simpson T, Jarman S, Thomsen PF & Meekan M (2022). Individual haplotyping of whale sharks from seawater environmental DNA. *Molecular Ecology Resources* 22(1):56–65. https://doi.org/10.1111/1755-0998.13451.

Dunn DC, Maxwell SM, Boustany AM & Halpin PN (2016). Dynamic ocean management increases the efficiency and efficacy of fisheries management. *Proceedings of the National Academy of Sciences* 113(3):668–73. https://doi.org/10.1073/pnas.1513626113.

Egeter B, Veríssimo J, Lopes-Lima M, Chaves C, Pinto J, Riccardi N, Beja P & Fonseca NA (2022). Speeding up the detection of invasive bivalve species using environmental DNA: a nanopore and illumina sequencing comparison. *Molecular Ecology Resources* 22(6):2232–47. https://doi.org/10.1111/1755-0998.13610.

Elliott M (2013). The 10-tenets for integrated, successful and sustainable marine management. *Marine Pollution Bulletin* 74(1):1–5. https://doi.org/10.1016/j.marpolbul.2013.08.001.

EPA (Environmental Protection Authority) (2023). *Wai Tuwhera o Te Taiao – about the programme*. Environmental Protection Authority, New Zealand. https://www.epa.govt.nz/community-involvement/open-waters-aotearoa/the-programme/.

Evans DM, Kitson JJN, Lunt DH, Straw NA & Pocock MJO (2016). Merging DNA metabarcoding and ecological network analysis to understand and build resilient terrestrial ecosystems. *Functional Ecology* 30(12):1904–16. https://doi.org/10.1111/1365-2435.12659.

Evans NT, Shirey PD, Wieringa JG, Mahon AR & Lamberti GA (2017). Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. *Fisheries* 42(2):90–99. https://doi.org/10.1080/03632415.2017.1276329.

Evans-Illidge E, Forester T, Depczynski M, Duggan E & Souter D (2020). *AIMS Indigenous Partnerships Plan: from engagement to partnerships*. Australian Institute of Marine Science, Townsville.

Fediajevaite J, Priestley V, Arnold R & Savolainen V (2021). Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. *Ecology and Evolution* 11(9):4803–15. https://doi.org/10.1002/ece3.7382.

Ferguson PFB, Conroy MJ & Hepinstall-Cymerman J (2015). Occupancy models for data with false positive and false negative errors and heterogeneity across sites and surveys. *Methods in Ecology and Evolution* 6(12):1395–1406. https://doi.org/10.1111/2041-210X.12442.

Ferrante J, Daniel W, Freedman J, Klymus K, Neilson M, Passamaneck Y, Rees C, Sepulveda A & Hunter M (2022). Gaining decision-maker confidence through community consensus: developing environmental DNA standards for data display on the USGS Nonindigenous Aquatic Species Database. *Management of Biological Invasions* 13(4):809–32. https://doi.org/10.3391/mbi.2022.13.4.15.

Ficetola GF, Miaud C, Pompanon F & Taberlet P (2008). Species detection using environmental DNA from water samples. *Biology Letters* 4(4):423–25. https://doi.org/10.1098/rsbl.2008.0118.

Ficetola GF, Pansu J, Bonin A, Coissac E, Giguet-Covex C, De Barba M, Gielly L, Lopes CM, Boyer F, Pompanon F, Rayé G & Taberlet P (2015). replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources* 15(3):543–56. https://doi.org/10.1111/1755-0998.12338.

Flanigan D, Bowman J, Connors E, Flanigan M & Trenaman N (2021). The development and deployment of a programmable water sampling system using an autonomous surface vehicle. *OCEANS 2021: San Diego – Porto*, 1–5. https://doi.org/10.23919/OCEANS44145.2021.9705708.

Flück B, Mathon L, Manel S, Valentini A, Dejean T, Albouy C, Mouillot D, Thuiller W, Murienne J, Brosse S & Peillissier L (2022). Applying convolutional neural networks to speed up environmental DNA annotation in a highly diverse ecosystem. *Scientific Reports* 12(1):10247. https://doi.org/10.1038/s41598-022-13412-w.

Formel N, Enochs IC, Sinigalliano C, Anderson SR & Thompson LR (2021). Subsurface automated samplers for eDNA (SASe) for biological monitoring and research. *HardwareX* 10:e00239. https://doi.org/10.1016/j.ohx.2021.e00239.

Forrest A (2020). The Panthalassa Project: the future of ocean research for conservation. *Conservation Letters* 13(6):e12743. https://doi.org/10.1111/conl.12743.

Fraija-Fernández N, Bouquieaux MC, Rey A, Mendibil I, Cotano U, Irigoien X, Santos M & Rodríguez-Ezpeleta N (2020). Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. *Ecology and Evolution* 10(14):7560–84. https://doi.org/10.1002/ece3.6482.

Fukaya K, Kondo NI, Matsuzaki SS & Kadoya T (2022). Multispecies site occupancy modelling and study design for spatially replicated environmental DNA metabarcoding. *Methods in Ecology and Evolution* 13(1):183–93. https://doi.org/10.1111/2041-210X.13732.

Furlan EM, Gleeson D, Hardy CM & Duncan RP (2016). A framework for estimating the sensitivity of eDNA surveys. *Molecular Ecology Resources* 16(3):641–54. https://doi.org/10.1111/1755-0998.12483.

Gagné N, Bernatchez L, Bright D, Côté G, Coulson M, Gurney K, Hanner R, Helbing C, Hobbs J, Hocking M, Khan I, Naumann C, Parent G, Richter C, Silverio C, Skinner M, Weir A, Wilcox T, Wilson C & Clogg-Wright K (2021). *Environmental DNA (eDNA) reporting requirements and terminology*, Canadian Standards Association, Toronto, Canada.

GBRMPA (Great Barrier Reef Marine Park Authority) (2019). *Great Barrier Reef Outlook Report 2019*, Great Barrier Reef Marine Park Authority, Townsville. http://hdl.handle.net/11017/3474.

GBRMPA (Great Barrier Reef Marine Park Authority) and Queensland Government (2015). *Reef 2050 Integrated Monitoring and Reporting Program Strategy*. Great Barrier Reef Marine Park Authority, Townsville.

Gerber LR, Beger M, McCarthy MA & Possingham HP (2005). A theory for optimal monitoring of marine reserves. *Ecology Letters* 8(8):829–37. https://doi.org/10.1111/j.1461-0248.2005.00784.x.

Gilbey J, Carvalho G, Castilho R, Coscia I, Coulson MW, Dahle G, Derycke S, Francisco SM, Helyar SJ, Johansen T, Junge C, Layton KKS, Martinsohn J, Matejusova I, Robalo JI, Rodríguez-Ezpeleta N, Silva G, Strammer I, Vasemägi A & Volckaert FAM (2021). Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring. *Marine Policy* 124:104331. https://doi.org/10.1016/j.marpol.2020.104331.

Gleeson D (2021). Zoological applications for environmental DNA: detection, diversity, and health. *New Zealand Journal of Zoology* 48(3–4):185–87. https://doi.org/10.1080/03014223.2021.1961562.

Gleeson D, Trujillo-González A, Rojahn J, Duncan R & Furlan E (2022). *Real Time eDNA tools to improve early detection and response approaches for high-risk pest animals: final report for Project P01-I-004*. Centre for Invasive Species Solutions, Canberra. https://pestsmart.org.au/wp-content/uploads/sites/3/2023/1004-Final-release-uploaded.pdf.

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-McCance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS, Strickler KM, Waits LP, Fremier AK, Takahara T, Herder JE & Taberlet P (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution* 7(11):1299–1307. https://doi.org/10.1111/2041-210X.12595.

Goodwin K, Certner R, Strom M, Arzayus F, Bohan M, Busch S, Canonico G, Cross S, Davis J, Egan K, Grieg T, Kearns E, Koss J, Larsen K, Layton D, Nichols K, O'Neil J, Parks D, Poussard L & Werner C (2020). *NOAA 'Omics white paper: informing the NOAA 'Omics Strategy and Implementation Plan*, National Oceanic and Atmospheric Administration, United States. https://doi.org/10.25923/BD7Z-ZB37.

Green M, Craw P, Hardesty B, Deagle BE & Wilcox C (2021). *Using eDNA to reconstruct logbook information and improve estimates of by-catch: information paper*, https://iotc.org/documents/using-edna-reconstruct-logbook-information-and-improve-estimates-catch.

Griffiths J, Impey R, Marwood S, van Leeuwen H & Weeks A (2022). *Platypus results report: the Great Australian Platypus Search Victoria 2021*, EnviroDNA, Brunswick. https://static1.squarespace.com/static/60c19413cc35f9526d23997a/t/63101710cdafe532fbf5f39c/1661998872495/GAPS_RESULTS_REPORT_20220901.pdf.

Guri G, Westgaard JI, Yoccoz N, Wangensteen OS, Præbel K, Ray JL, Kelly RP, Shelton AO, Hanebrekke T & Johansen T (2023). Maximizing sampling efficiency to detect differences in fish community composition using environmental DNA metabarcoding in subarctic fjords. *Environmental DNA* 00:1–15. https://doi.org/10.1002/edn3.409.

Hajibabaei M (2022). Demystifying eDNA validation. *Trends in Ecology & Evolution* 37(10):826–28. https://doi.org/10.1016/j.tree.2022.06.015.

Harrison JB, Sunday JM & Rogers SM (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B: Biological Sciences* 286(1915):20191409. https://doi.org/10.1098/rspb.2019.1409.

Hayes KR, Dunstan P, Woolley S, Barret N, Howe S, Samson C, Bowling R, Ryan MP, Foster S, Monk J, Peel D, Hosack GR & Francis SO (2021). *Designing a targeted monitoring program to support evidence-based management of Australian Marine Parks: a pilot on the South-East Marine Parks network*, Parks Australia, University of Tasmania and CSIRO. https://www.nespmarine.edu.au/document/designing-targeted-monitoring-program-support-evidence-based-management-australian-marine.

Hechler E, Weihrauch M & Wu Y (2023). Evolution of data architecture. In: Hechler E, Weihrauch M and Wu Y (eds) *Data fabric and data mesh approaches with AI: a guide to AI-based data cataloging, governance, integration, orchestration, and consumption*, Apress, Berkeley, 3–15. https://doi.org/10.1007/978-1-4842-9253-2_1.

Hedge P, Souter D, Treblico R, Ward T, van Ruth P, Cowlishaw M, Lara-Lopez A, Barrett NS, Thornborough K, Nichol SL, Przeslawski R, Parr A, Kendrick A, Holmes T, Pattiaratchi C, Ferns L & Jordan A (2022). *Establishing and supporting a national marine baselines and monitoring program: working group report: technical report*. https://doi.org/10.13140/RG.2.2.16944.23043.

Helbing CC & Hobbs J (2019). *Environmental DNA standardization needs for fish and wildlife population assessments and monitoring,* CSA Group, Canada. https://www.csagroup.org/wp-content/uploads/CSA-Group-Research-Environmental-DNA.pdf.

Hendricks A, Mackie CM, Luy E, Sonnichsen C, Smith J, Grundke I, Tavasoli M, Furlong A, Beiko RG, LaRoche J & Sieben V (2023). Compact and automated eDNA sampler for in situ monitoring of marine environments. *Scientific Reports* 13(1):5210. https://doi.org/10.1038/s41598-023-32310-3.

Hering D, Borja A, Jones JI, Pont D, Boets P, Bouchez A, Bruce K, Drakare S, Hänfling B, Kahlert M, Leese F, Meissner K, Mergen P, Reyjol Y, Segurado P, Vogler A & Kelly M (2018). Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water Research* 138:192–205. https://doi.org/10.1016/j.watres.2018.03.003.

Hillebrand H, Blasius B, Borer ET, Chase JM, Downing JA, Eriksson BK, Filstrup CT, Harpole WS, Hodapp D, Larsen S, Lewandowska AM, Seabloom EW, van de Waal DB & Ryabov AB (2018). Biodiversity change is uncoupled from species richness trends: consequences for conservation and monitoring. *Journal of Applied Ecology* 55(1):169–84. https://doi.org/10.1111/1365-2664.12959.

Holmes V, Aman J, York G & Kinnison MT (2022). Environmental DNA detects spawning habitat of an ephemeral migrant fish (anadromous rainbow smelt: *Osmerus mordax*). *BMC Ecology and Evolution* 22(1):121. https://doi.org/10.1186/s12862-022-02073-y.

Ierodiaconou D, Young M, Wines S, Carnell P, Tinkler P, Blake A, Whitmarsh S, Howe S & Pocklington J (2022). *An integrated monitoring program for Port Phillip Heads Marine National Park*, Parks Victoria, Melbourne.

IUCN (2012). *IUCN Red List Categories and Criteria: version 3.1*, 2nd edn, IUCN, Gland and Cambridge. https://portals.iucn.org/library/node/10315.

IUCN and NatureMetrics (2023). *About eBioAtlas*, eBio Atlas. https://ebioatlas.org/about/.

Jarman SN, Berry O & Bunce M (2018). The value of environmental DNA biobanking for long-term biomonitoring. *Nature Ecology & Evolution* 2(8):1192–93. https://doi.org/10.1038/s41559-018-0614-3.

Jerde CL (2021). Can we manage fisheries with the inherent uncertainty from eDNA? *Journal of Fish Biology* 98(2):341–53. https://doi.org/10.1111/jfb.14218.

Kawakami T, Yamazaki A, Asami M, Goto Y, Yamanaka H, Hyodo S, Ueno H & Kasai A (2023). Evaluating the sampling effort for the metabarcoding-based detection of fish environmental DNA in the open ocean. *Ecology and Evolution* 13(3):e9921. https://doi.org/10.1002/ece3.9921.

Keck F, Blackman RC, Bossart R, Brantschen J, Couton M, Hürlemann S, Kirschner D, Locher N, Zhang H & Altermatt F (2022). Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. *Molecular Ecology* 31(6):1820–35. https://doi.org/10.1111/mec.16364.

Kelly RP, Shelton AO & Gallego R (2019). Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. *Scientific Reports* 9(1):12133. https://doi.org/10.1038/s41598-019-48546-x.

Kelly RP, Lodge DM, Lee KN, Theroux S, Sepulveda AJ, Scholin CA, Craine JM, Allan EA, Nichols KM, Parson KM, Goodwin KD, Gold Z, Chavez FP, Noble RT, Abbott CL, Baerwald MR, Naaum AM, Thielen PM, Levi Simons A, Jerde AC, Duda JJ, Hunter ME, Hagan JA, Meyer RS, Steele JA, Stoeckle MY, Bik HM, Meyer CP, Stein E, James KE, Thomas AC, Demir-Hilton E, Timmers MA, Griffith JF, Weise MJ & Weisberg SB (2023). Toward a national eDNA strategy for the United States. *Environmental DNA*. https://doi.org/10.1002/edn3.432.

Kjær KH, Pedersen MW, De Sanctis B, De Cahsan B, Korneliussen TS, Michelsen CS, Sand KK, Jelavić S, Ruter AH, Schmidt AMA, Kjeldsen KK, Tesakov AS, Snowball I, Gosse JC, Alsos IG, Wang Y, Dockter C, Rasmussen M, Jørgensen ME, Skadhauge B, Prohaska A, Kristensen JÅ, Bjerager M, Allentoft ME, Coissac E, PhyloNorway Consortium, Rouillard A, Simakova A, Fernandez-Guerra A, Bowler C, Macias-Fauria M, Vinner L, Welch JJ, Hidy AJ, Sikora M, Collins MJ, Durbin R, Larsen NK & Willersley E (2022). A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. *Nature* 612(7939):283–91. https://doi.org/10.1038/s41586-022-05453-y.

Klymus KE, Merkes CM, Allison MJ, Goldberg CS, Helbing CC, Hunter ME, Jackson CA, Lance RF, Mangan AM, Monroa EM, Piaggio AJ, Stokdyk JP, Wilson CC & Richter CA (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA* 2(3):271–82. https://doi.org/10.1002/edn3.29.

Koziol A, Stat M, Simpson T, Jarman S, DiBattista JD, Harvey ES, Marnane M, McDonald J & Bunce M (2019). Environmental DNA metabarcoding studies are critically affected by substrate selection. *Molecular Ecology Resources* 19(2):366–376.

Lamy P, Citores A, Deidun A, Evans L, Galgani F, Heffernan P, Karageorgis A, Kauppi L, Manakovski D, Meissner G, Moldoveanu V, Ramm K, Pedicchio MC, Pitta e Cunha T, Slat B & Pons G (2020). *Mission Starfish 2030: restore our ocean and waters*, Directorate-General for Research and Innovation, Publications Office, European Commission. https://data.europa.eu/doi/10.2777/70828.

Langlois TJ & Friedman A (2018). *GlobalArchive: an online repository of ecological data and science communication.* https://globalarchive.org.

Lanzén A, Dahlgren TG, Bagi A & Hestetun JT (2021). Benthic eDNA metabarcoding provides accurate assessments of impact from oil extraction, and ecological insights. *Ecological Indicators* 130:108064. https://doi.org/10.1016/j.ecolind.2021.108064.

Leese F, Altermatt F, Bouchez A, Ekrem T, Hering D, Meissner K, Mergen P, et al. (2016). DNAqua-Net: developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe. *Research Ideas and Outcomes* 2:e11321. https://doi.org/10.3897/rio.2.e11321.

Lewison RL, Hobday AJ, Maxwell S, Hazen EL, Hartog JR, Dunn DC, Briscoe D, Fossette S, O'Keefe CE, Barnes M, Abecassis M, Bograd S, Bethoney ND, Bailey H, Wiley D, Andrews S, Hazen L & Crowder LB (2015). Dynamic ocean management: identifying the critical ingredients of dynamic approaches to ocean resource management. *Bioscience* 65(5):486–98. https://doi.org/10.1093/biosci/biv018.

Lim NKM, Tay YC, Srivathsan A, Tan JWT, Kwik JTB, Baloğlu B, Meier R & Yeo DCJ (2016). Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. *Royal Society Open Science* 3(11):160635. https://doi.org/10.1098/rsos.160635.

Lindenmayer DB & Likens GE (2010). The science and application of ecological monitoring. *Biological Conservation* 143(6):1317–28. https://doi.org/10.1016/j.biocon.2010.02.013.

Lodge DM (2022). Policy action needed to unlock eDNA potential. *Frontiers in Ecology and the Environment* 20(8):448–49. https://doi.org/10.1002/fee.2563.

Loeza-Quintana T, Abbott CL, Heath DD, Bernatchez L & Hanner RH (2020). Pathway to increase standards and competency of eDNA surveys (PISCeS): advancing collaboration and standardization efforts in the field of eDNA. *Environmental DNA* 2(3):255–60. https://doi.org/10.1002/edn3.112.

Machado IA, Costa C & Santos MY (2022). Data mesh: concepts and principles of a paradigm shift in data architectures. *Procedia Computer Science*, International Conference on ENTERprise Information Systems / ProjMAN - International Conference on Project MANagement / HCist - International Conference on Health and Social Care Information Systems and Technologies 2021, 196:263–71. https://doi.org/10.1016/j.procs.2021.12.013.

Maiello G, Talarico L, Carpentieri P, De Angelis F, Franceschini S, Harper LR, Neave EF, Rickards O, Sbrana A, Shum P, Veltre V, Mariana S & Russo T (2022). Little samplers, big fleet: eDNA metabarcoding from commercial trawlers enhances ocean monitoring. *Fisheries Research* 249:106259. https://doi.org/10.1016/j.fishres.2022.106259.

Mathieu C, Hermans SM, Lear G, Buckley TR, Lee KC & Buckley HL (2020). A systematic review of sources of variability and uncertainty in eDNA data for environmental monitoring. *Frontiers in Ecology and Evolution* 8. https://www.frontiersin.org/articles/10.3389/fevo.2020.00135.

Mathon L, Valentini A, Guérin PE, Normandeau E, Noel C, Lionnet C, Boulanger E, Thuiller W, Bernatchez L, Mouillot D, Dejean T & Manel S (2021). Benchmarking bioinformatic tools for fast and accurate eDNA metabarcoding species identification. *Molecular Ecology Resources* 21(7):2565–79. https://doi.org/10.1111/1755-0998.13430.

Mayne B, Espinoza T, Roberts D, Butler GL, Brooks S, Korbie D & Jarman S (2021). Nonlethal Age Estimation of three threatened fish species using DNA methylation: Australian lungfish, Murray cod and Mary River cod. *Molecular Ecology Resources* 21(7):2324–32. https://doi.org/10.1111/1755-0998.13440.

McClenaghan B, Compson ZG & Hajibabaei M (2020). Validating metabarcoding-based biodiversity assessments with multi-species occupancy models: a case study using coastal marine eDNA. *PloS ONE* 15, no. 3 (March 2020): e0224119. https://doi.org/10.1371/journal.pone.0224119.

McCormick MI & Choat JH (1987). Estimating total abundance of a large temperate-reef fish using visual strip-transects. *Marine Biology* 96(4):469–78. https://doi.org/10.1007/BF00397964.

McDonald JI, Wellington CM, Coupland GT, Pedersen D, Kitchen B, Bridgwood SD, Hewitt M, Duggan R & Abdo DA (2019). A united front against marine invaders: developing a cost-effective marine biosecurity surveillance partnership between government and industry. *Journal of Applied Ecology* 57(1):77–84. https://doi.org/10.1111/1365-2664.13557.

Miller H, Takahashi M, Hui H, Fuller K, Jarman SN & Berry O (2023). Single cell sequencing of environmental cells (emCells). In: *Proceedings of the 1st Australian and New Zealand Environmental DNA (eDNA) Conference: Innovation and Application*, Hobart, 14–17 February 2023.

Minamoto T, Miya M, Sado T, Seino S, Doi H, Kondoh M, Nakamura K, Takahara T, Yamamoto S, Yamanaka H, Araki H, Iwasaki W, Kasai A, Masuda R & Uchii K (2021). An illustrated manual for environmental DNA research: water sampling guidelines and experimental protocols. *Environmental DNA* 3(1):8–13. https://doi.org/10.1002/edn3.121.

Mori AS, Suzuki KF, Hori M, Kadoya T, Okano K, Uraguchi A, Muraoka H, Sato T, Shibata H, Suzuki-Ohno Y, Koba K, Toda M, Nakano S, Kondoh M, Kitajima K & Nakamura M (2023). Perspective: sustainability challenges, opportunities and solutions for long-term ecosystem observations. *Philosophical Transactions of the Royal Society B: Biological Sciences* 378(1881):20220192. https://doi.org/10.1098/rstb.2022.0192.

Muff M, Jaquier M, Marques V, Ballesta L, Deter J, Bockel T, Hocdé R, Juhel J-B, Boulanger E, Guellati N, Fernández AP, Valentini A, Dejean T, Manel S, Albouy C, Durville P, Mouillot D, Holon F & Pellissier L (2023). Environmental DNA highlights fish biodiversity in mesophotic ecosystems. *Environmental DNA* 5(1):56–72. https://doi.org/10.1002/edn3.358.

Nagarajan RP, Bedwell M, Holmes AE, Sanches T, Acuña S, Baerwald M, Barnes MA, Blankenship S, Connon RE, Deiner K, Gille D, Goldberg CS, Hunter ME, Jerde CL, Luikart G, Meyer RS, Watts A & Schreier A (2022). Environmental DNA methods for ecological monitoring and biodiversity assessment in estuaries. *Estuaries and Coasts* 45(7):2254–73. https://doi.org/10.1007/s12237-022-01080-y.

Nester GM, De Brauwer M, Koziol A, West KM, DiBattista JD, White NE, Power M, Heydenrych MJ, Harvey E & Bunce M (2020). Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (Family: *Syngnathidae*). *Environmental DNA* 2(4):614–26. https://doi.org/10.1002/edn3.93.

Nester GM, Heydenrych MJ, Berry TE, Richards Z, Wasserman J, White NE, De Brauwer M, Bunce M, Takahashi M & Claassens L (2023). Characterizing the distribution of the critically endangered estuarine pipefish (*Syngnathus watermeyeri*) across its range using environmental DNA. *Environmental DNA* 5(1):132–45. https://doi.org/10.1002/edn3.365.

NMSC (National Marine Science Committee) (2015). *National Marine Science Plan 2015–2025: driving the development of Australia's blue economy*, National Marine Science Committee, Canberra. https://www.marinescience.net.au/wp-content/uploads/2021/08/NMSP-2015-2025-reportREDUCED.pdf.

NMSC (National Marine Science Committee) (2021). *National Marine Science Plan 2015–2025: the midway point*, National Marine Science Committee, Canberra. https://www.marinescience.net.au/wp-content/uploads/2021/08/NMSC_Midway_Point_Report_Card_FINAL_WEB_July27_2021.pdf.

Norros V, Laamanen T, Meissner K, Iso-Touru T, Kahilainen A, Lehtinen S, Lohtander-Buckbee K, Nygård H, Pennanen T, Ruohonen-Lehto M, Sirkiä P, Suikkanen S, Tolkkinen M, Vainio E, Velmala S, Vuorio K & Vihervaara P (2022). *Roadmap for implementing environmental DNA (eDNA) and other molecular monitoring methods in Finland: vision and action plan for 2022–2025*, Finnish Environment Institute. https://jukuri.luke.fi/handle/10024/552344.

Northern Territory Government (2019). *Coastal and Marine Management Strategy: Northern Territory – 2019–2029*, Northern Territory Government, Darwin. https://depws.nt.gov.au/__data/assets/pdf_file/0004/729472/coastal-marine-management-strategy-2019-2029.pdf.

OzFish Unlimited (2022). EDNA sampling uncovering threatened fish species at scale. *OzFish Unlimited*. https://ozfish.org.au/2022/05/edna-sampling-uncovering-threatened-fish-species-at-scale/.

Parsons KM, Everett M, Dahlheim M & Park L (2018). Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. *Royal Society Open Science* 5(8):180537. https://doi.org/10.1098/rsos.180537.

Pauvert C, Buée M, Laval V, Edel-Hermann V, Fauchery L, Gautier A, Lesur I, Vallance J & Vacher C (2019). Bioinformatics matters: the accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* 41:23–33. https://doi.org/10.1016/j.funeco.2019.03.005.

Pawlowski J, Apothéloz-Perret-Gentil L, Mächler E & Altermatt F (2020) *Environmental eDNA applications in biomonitoring and bioassessment of aquatic ecosystems: guidelines*, Federal Office for the Environment, Swiss Confederation.

Pawlowski J, Bruce K, Panksep K, Aguirre FI, Amalfitano S, Apothéloz-Perret-Gentil L, Baussant T, Bouchez A, Carugati L, Cermakova K, Cordier T, Corinaldesi C, Costa FO, Danovaro R, Dell'Anno A, Duarte S, Eisendle A, Ferrari BJD, Frontalini F, Frühe L, Haegerbaeumer A, Kisand V, Krolicka A, Lanzén A, Leese F, Lejzerowicz F, Lyautey E, Maček I, Sagova-Marečková M, Pearman JK, Pochon X, Stoeck T, Vivien R, Weigand A & Fazi S (2022). Environmental DNA metabarcoding for benthic monitoring: a review of sediment sampling and DNA extraction methods. *Science of The Total Environment* 818:151783. https://doi.org/10.1016/j.scitotenv.2021.151783.

Pawlowski J, Kelly-Quinn M, Altermatt F, Apothéloz-Perret-Gentil L, Beja P, Boggero A, Borja A, Bouchez A, Cordier T, Domaizon I, Feio MJ, Filipe AF, Fornaroli R, Graf W, Herder J, van der Hoorn B, Jones II, Sagova-Mareckova M, Moritz C, Barquín J & Kahlert M (2018). The future of biotic indices in the ecogenomic era: integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. *Science of The Total Environment* 637–638:1295–1310. https://doi.org/10.1016/j.scitotenv.2018.05.002.

Pecl GT, Stuart-Smith J, Walsh P, Bray DJ, Kusetic M, Burgess M, Frusher SD, Gledhill DC, George O, Jackson G, Keana J, Martin VY, Nursey-Bray M, Pender A, Robinson LM, Rowling K, Sheaves M & Moltschaniwskyj N (2019). Redmap Australia: challenges and successes with a large-scale citizen science-based approach to ecological monitoring and community engagement on climate change. *Frontiers in Marine Science* 6:349. https://doi.org/10.3389/fmars.2019.00349.

Pochon X, Wood SA, Atalah J, Laroche O, Zaiko A & Keeley N (2019). A validated protocol for benthic monitoring of New Zealand's salmon farms using environmental DNA. Report to Seafood Innovation Ltd, New Zealand King Salmon Company Ltd, Ministry for Primary Industries and Marlborough District Council. Cawthron Institute, New Zealand.

Pochon X, Wood SA, Keeley NB, Lejzerowicz F, Esling P, Drew J & Pawlowski J (2015). Accurate assessment of the impact of salmon farming on benthic sediment enrichment using foraminiferal metabarcoding. *Marine Pollution Bulletin* 100(1):370–82. https://doi.org/10.1016/j.marpolbul.2015.08.022.

Przeslawski R & Foster S (2020). *Field manuals for marine sampling to monitor Australian waters*, Deakin University. https://doi.org/10.11636/9781925297669.

Richards ZT, Stat M, Heydenrych M & DiBattista JD (2022). Environmental DNA for biodiversity monitoring of coral reefs. In: van Oppen MJH and Lastra MA (eds) *Coral Reef Conservation and Restoration in the Omics Age*, Springer, Cham, 203–224. https://doi.org/10.1007/978-3-031-07055-6_13.

Rolton A, Rhodes L, Hutson KS, Biessy L, Bui T, MacKenzie L, Symonds JE & Smith KF (2022). Effects of harmful algal blooms on fish and shellfish species: a case study of New Zealand in a changing environment. *Toxins* 14(5):341. https://doi.org/10.3390/toxins14050341.

Rourke ML, Fowler AM, Hughes JM, Broadhurst MK, DiBattista JD, Fielder S, Walburn JW & Furlan EM (2021). Environmental DNA (eDNA) as a tool for assessing fish biomass: a review of approaches and future considerations for resource surveys. *Environmental DNA* 4(1):9–33. https://doi.org/10.1002/edn3.185.

Rourke ML, Walburn JW, Broadhurst MK, Fowler AM, Hughes JM, Fielder DS, DiBattista JD & Furlan EM (2023). Poor utility of environmental DNA for estimating the biomass of a threatened freshwater teleost; but clear direction for future candidate assessments. *Fisheries Research* 258:106545. https://doi.org/10.1016/j.fishres.2022.106545.

Samoilys MA & Carlos G (2000). Determining methods of underwater visual census for estimating the abundance of coral reef fishes. *Environmental Biology of Fishes* 57(3):289–304. https://doi.org/10.1023/A:1007679109359.

Samuel RM, Meyer R, Buttigieg PL, Davies N, Jeffery NW, Meyer C, Pavloudi C, Pitz KJ, Sweetlove M, Theroux S, van de Kamp J & Watts A (2021). Toward a global public repository of community protocols to encourage best practices in biomolecular ocean observing and research. *Frontiers in Marine Science* 8:758694. https://doi.org/10.3389/fmars.2021.758694.

Scholz G, von Baumgarten P, Wilson H, Wright A & Bryars S (2017). *Monitoring, Evaluation and Reporting Framework for Marine Parks Program: DEWNR Technical note 2017/06*, South Australian Department of Environment, Water and Natural Resources, Adelaide. https://data.environment.sa.gov.au/Content/Publications/MER_Framework_MarineParks.pfd.

Scriver M, Zaiko A, Pochon X & von Ammon U (2023). Harnessing decay rates for coastal marine biosecurity applications: a review of environmental DNA and RNA fate. *Environmental DNA* 00:1–13. https://doi.org/10.1002/edn3.405.

Sepulveda AJ, Nelson NM, Jerde CL & Luikart G (2020). Are environmental DNA methods ready for aquatic invasive species management? *Trends in Ecology & Evolution* 35(8):668-678. https://doi.org/10.1016/j.tree.2020.03.011.

Seymour M, Edwards FK, Cosby BJ, Kelly MG, de Bruyn M, Carvalho GR & Creer S (2020). Executing multi-taxa eDNA ecological assessment via traditional metrics and interactive networks. *Science of The Total Environment* 729:138801. https://doi.org/10.1016/j.scitotenv.2020.138801.

Shaw JLA, Weyrich LS, Hallegraeff G & Cooper A (2019). Retrospective eDNA assessment of potentially harmful algae in historical ship ballast tank and marine port sediments. *Molecular Ecology* 28(10):2476–85. https://doi.org/10.1111/mec.15055.

Shea MM, Kuppermann J, Rogers MP, Smith DS, Edwards P & Boehm AB (2023). Systematic review of marine environmental DNA metabarcoding studies: toward best practices for data usability and accessibility. *PeerJ* 11:e14993. https://doi.org/10.7717/peerj.14993.

Shelton AO, Gold ZJ, Jensen AJ, D' Agnese E, Andruszkiewicz Allan E, Van Cise A, Gallego R, Ramón-Laca A, Garber-Yonts M, Parsons K & Kelly RP (2023). Toward quantitative metabarcoding. *Ecology* 104(2):e3906. https://doi.org/10.1002/ecy.3906.

Siano R, Lassudrie M, Cuzin P, Briant N, Loizeau V, Schmidt S, Ehrhold A, Mertens KN, Lambert C, Quintric L, Noël C, Latimier M, Quéré J, Durand P & Penaud A (2021). Sediment archives reveal irreversible shifts in plankton communities after World War II and agricultural pollution. *Current Biology* 31(12):2682–2689. https://doi.org/10.1016/j.cub.2021.03.079.

Sigsgaard EE, Nielsen IB, Bach SS, Lorenzen ED, Robinson DP, Knudsen SW, Pedersen MW, Jaidah MA, Orlando L, Willerslev E, Møller PR & Thomsen PT (2016). Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature Ecology & Evolution* 1(1):1–5. https://doi.org/10.1038/s41559-016-0004.

Simpfendorfer CA, Kyne PM, Noble TH, Goldsbury J, Basiita RK, Lindsay R, Shields A, Perry C & Jerry DR (2016). Environmental DNA detects critically endangered largetooth sawfish in the wild. *Endangered Species Research* 30: 09–16. https://doi.org/10.3354/esr00731.

Simpson C, Beger M, Colman JG, Friedman K, Hill AK, Kendrick AK, Waples K, Whiting S & Wilson S (2015). Prioritisation of conservation research and monitoring for Western Australian protected areas and threatened species. *Conservation Science Western Australia* 9:227–37.

Skelton J, Cauvin A & Hunter ME (2022). Environmental DNA metabarcoding read numbers and their variability predict species abundance, but weakly in non-dominant species. *Environmental DNA*. https://doi.org/10.1002/edn3.355.

Stat M, Huggett MJ, Bernasconi R, DiBattista JD, Berry TE, Newman SJ, Harvey ES & Bunce M (2017). Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports* 7(1):12240. https://doi.org/10.1038/s41598-017-12501-5.

Stepien CA, Theroux S & Weisberg SB (2022). The second national workshop on marine eDNA: a workshop to accelerate the incorporation of eDNA science into environmental management applications. *Environmental DNA*. https://doi.org/10.1002/edn3.379.

Suzuki-Ohno Y, Tanabe AS, Kasai A, Masuda R, Seino S, Dazai A, Suzuki S, Abe T & Kondoh M (2023). Evaluation of community science monitoring with environmental DNA for marine fish species: fish survey project using environmental DNA. *Environmental DNA* 5(3):613–23. https://doi.org/10.1002/edn3.425.

Taberlet P, Coissac E, Pompanon F, Brochmann C & Willerslev E (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* 21(8):2045–50. https://doi.org/10.1111/j.1365-294X.2012.05470.x.

Takahashi M, DiBattista JD, Jarman S, Newman SJ, Wakefield CB, Harvey ES & Bunce M (2020). Partitioning of diet between species and life history stages of sympatric and cryptic snappers (*Lutjanidae*) based on DNA metabarcoding. *Scientific Reports* 10(1):4319. https://doi.org/10.1038/s41598-020-60779-9.

Takahashi M, Saccò M, Kestel JH, Nester G, Campbell MA, van der Heyde M, Heydenrych MJ, Juszkiewicz DJ, Nevill P, Dawkins KL, Bessey C, Fernandes K, Miller H, Power M, Mousavi-Derazmahalleh M, Newton JP, White NE, Richards ZT & Allentoft ME (2023). Aquatic environmental DNA: a review of the macro-organismal biomonitoring revolution. *Science of The Total Environment* 873:162322. https://doi.org/10.1016/j.scitotenv.2023.162322.

Takasaki K, Aihara H, Imanaka T, Matsudaira T, Tsukahara K, Usui A, Osaki S & Doi H (2021). Water pre-filtration methods to improve environmental DNA detection by real-time PCR and metabarcoding. *PLoS ONE* 16(5:e0250162. https://doi.org/10.1371/journal.pone.0250162.

Thalinger B, Deiner K, Harper LR, Rees HC, Blackman RC, Sint D, Traugott M, Goldberg CS & Bruce K (2021). A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. *Environmental DNA* 3(4):823–36. https://doi.org/10.1002/edn3.189.

Thomas AC, Nguyen PL, Howard J & Goldberg CS (2019). A self-preserving, partially biodegradable eDNA filter. *Methods in Ecology and Evolution* 10:1136–1141. https://doi.org/10.1111/2041-210X.13212.

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M & Willerslev E (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PloS ONE* 7(8):e41732. https://doi.org/10.1371/journal.pone.0041732.

Truelove NK, Patin NV, Min M, Pitz KJ, Preston CM, Yamahara KM, Zhang Y, Raanan BY, Kieft B, Hobson B, Thompson LR, Goodwin KD & Chavez FP (2022). Expanding the temporal and spatial scales of environmental DNA research with autonomous sampling. *Environmental DNA* 4(4):972–84. https://doi.org/10.1002/edn3.299.

Trujillo-González A, Villacorta-Rath C, White NE, Furlan EM, Sykes M, Grossel G, Divi UK & Gleeson D (2021). Considerations for future environmental DNA accreditation and proficiency testing schemes. *Environmental DNA* 3(6):1049–58. https://doi.org/10.1002/edn3.243.

Tsuji S, Shibata N, Sawada H & Ushio M (2020). Quantitative evaluation of intraspecific genetic diversity in a natural fish population using environmental DNA analysis. *Molecular Ecology Resources* 20(5):1323–32. https://doi.org/10.1111/1755-0998.13200.

Tulloch AIT, Chadès I & Lindenmayer DB (2018). Species co-occurrence analysis predicts management outcomes for multiple threats. *Nature Ecology & Evolution* 2(3):465–474. https://doi.org/10.1038/s41559-017-0457-3.

Turrell WR (2018). Improving the implementation of marine monitoring in the Northeast Atlantic. *Marine Pollution Bulletin* 128:27–38. https://doi.org/10.1016/j.marpolbul.2018.01.067.

UNESCO (2023). Environmental DNA expeditions in UNESCO World Heritage marine sites, *UNESCO eDNA Expeditions,* UNESCO. https://www.unesco.org/en/edna-expeditions.

Urban P, Bekkevold D, Hansen BK, Jacobsen MW, Nielsen A & Nielsen EE (2022). Using eDNA to estimate biomass of bycatch in pelagic fisheries. *Environmental DNA* 00:1–16. https://doi.org/10.1002/edn3.377.

Uthicke S, Doyle J, Duggan S, Yasuda N & McKinnon AD (2015). Outbreak of coral-eating crown-of-thorns creates continuous cloud of larvae over 320 km of the Great Barrier Reef. *Scientific Reports* 5(1):16885. https://doi.org/10.1038/srep16885.

Uthicke S, Lamare M & Doyle JR (2018). eDNA detection of corallivorous seastar (*Acanthaster* cf. *solaris*) outbreaks on the Great Barrier Reef using digital droplet PCR. *Coral Reefs* 37(4):1229–39. https://doi.org/10.1007/s00338-018-1734-6.

Uthicke S, Robson B, Doyle JR, Logan M, Pratchett MS & Lamare M (2022). Developing an effective marine eDNA monitoring: eDNA detection at pre-outbreak densities of corallivorous seastar (*Acanthaster* cf. *solaris*). *Science of The Total Environment* 851:58143. https://doi.org/10.1016/j.scitotenv.2022.158143.

van de Kamp J, Borja A & Bissett A (2023). DNA-based microbial bioindication of environmental state. In: Berry OF, Hollely CE & Jarman SN (eds), *Applied Environmental Genomics*, CSIRO Publishing, Melbourne, 368.

van Rooyen A, Miller AD, Clark Z, Sherman CDH, Butcher PA, Rizzari JR & Weeks AR (2021). Development of an environmental DNA assay for detecting multiple shark species involved in human–shark conflicts in Australia. *Environmental DNA* 3(5): 40–49. https://doi.org/10.1002/edn3.202.

Villacorta-Rath C & Burrows D (2021). *Standard operating procedure for environmental DNA field sample collection.* Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville.

Villacorta-Rath C, Hoskin CJ, Strugnell JM & Burrows D (2021). Long distance (>20km) downstream detection of endangered stream frogs suggests an important role for eDNA in surveying for remnant amphibian populations. *PeerJ* 9:e12013. https://doi.org/10.7717/peerj.12013.

Watson DL, Harvey ES, Fitzpatrick BM, Langlois TJ & Shedrawi G (2010). Assessing reef fish assemblage structure: how do different stereo-video techniques compare? *Marine Biology* 157(6):1237–50. https://doi.org/10.1007/s00227-010-1404-x.

Weigand H, Beermann AJ, Čiampor F, Costa FO, Csabai Z, Duarte S, Geiger MF, Grabowski M, Rimet F, Rulik B, Strand M, Szucsich N, Weigand AM, Willassen E, Wyler SA, Bouchez A, Borja A, Čiamporová-Zaťovičová Z, Ferreira S, Dijkstra KDB & Ekrem T (2019). DNA barcode reference libraries for the monitoring of aquatic biota in Europe: gap-analysis and recommendations for future work. *Science of the Total Environment* 678:499–524. https://doi.org/10.1016/j.scitotenv.2019.04.247.

West KM, Stat M, Harvey ES, Skepper CL, DiBattista JD, Richards ZT, Travers MJ, Newman SJ & Bunce M (2020). eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical island ecosystem. *Molecular Ecology* 29(6):1069–86. https://doi.org/10.1111/mec.15382.

West KM, Travers MJ, Stat M, Harvey ES, Richards ZT, DiBattista JD, Newman SJ, Harry A, Skepper CL, Heydenrych M & Bunce M (2021). Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical North-Western Australia. *Diversity and Distributions* 27(10):1942–57. https://doi.org/10.1111/ddi.13228.

Wilkinson SP, Gault AA, Welsh S, Smith J, David B, Hicks A, Fake D, Suren A, Bunce M, Lust B & Shaffer MR (2023). A robust Taxon-Independent Community Index (TICI) for freshwater ecological health assessment. Accessed May 31, 2023. https://www.wilderlab.co.nz/tici.

Williams KE, Huyvaert KP & Piaggio AJ (2017). Clearing muddied waters: capture of environmental DNA from turbid waters. *PloS ONE* 12(7):e0179282. https://doi.org/10.1371/journal.pone.0179282.

Williams JW, Spanbauer TL, Heintzman PD, Blois J, Capo E, Goring SJ, Monchamp M-E, Parducci L & Eggers JM (2023). Strengthening global-change science by integrating eDNA with paleoecoinformatics. *Trends in Ecology & Evolution* 38(10):946–960. https://doi.org/10.1016/j.tree.2023.04.016.

Willoughby JR, Wijayawardena BK, Sundaram M, Swihart RK & DeWoody JA (2016). The importance of including imperfect detection models in eDNA experimental design. *Molecular Ecology Resources* 16(4):837–44. https://doi.org/10.1111/1755-0998.12531.

Wolfe K, Desbiens AA & Mumby PJ (2023). Emigration patterns of motile cryptofauna and their implications for trophic functioning in coral reefs. *Ecology and Evolution* 13(3):e9960. https://doi.org/10.1002/ece3.9960.

Yao M, Zhang S, Lu Q, Chen X, Zhang S-Y, Kong Y & Zhao J (2022). Fishing for fish environmental DNA: ecological applications, methodological considerations, surveying designs, and ways forward. *Molecular Ecology* 31(20):5132–64. https://doi.org/10.1111/mec.16659.

Yates MC, Derry AM & Cristescu ME (2021). Environmental RNA: a revolution in ecological resolution? *Trends in Ecology & Evolution* 36(7):601–9. https://doi.org/10.1016/j.tree.2021.03.001.

Yates MC, Fraser DJ & Derry AM (2019). Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. *Environmental DNA* 1(1):5–13. https://doi.org/10.1002/edn3.7.

Young MA, Porskamp P, Critchell K, Treml E, Ierodiaconou D, Pocklington J & Sams M (2022). *Statewide assessment of Victorian Marine Protected Areas using existing data,* Parks Victoria Technical Series: 118, Parks Victoria, Melbourne.

Zaiko A, Greenfield P, Abbott C, von Ammon U, Bilewitch J, Bunce M, Cristescu ME, Chariton A, Dowle E, Geller J, Gutierrez AA, Hajibabaei M, Haggard E, Inglis GJ, Lavery SD, Samuiloviene A, Simpson T, Stat M, Stephenson S, Sutherland J, Thakur V, Westfall K, Wood SA, Wright M, Zhang G & Pochon X (2022). Towards reproducible metabarcoding data: lessons from an international cross-laboratory experiment. *Molecular Ecology Resources* 22(2):519–38. https://doi.org/10.1111/1755-0998.13485.

Zaiko A, Scheel M, Schattschneider J, von Ammon U, Scriver M, Pochon X & Pearman JK (2023). Pest alert tool: a web-based application for flagging species of concern in metabarcoding datasets. *Nucleic Acids Research* 51(W1):W438–W442. https://doi.org/10.1093/nar/gkad364.

Zaiko A, Schimanski K, Pochon X, Hopkins GA, Goldstien S, Floerl O & Wood SA (2016). Metabarcoding improves detection of eukaryotes from early biofouling communities: implications for pest monitoring and pathway management. *Biofouling* 32(6):671–84. https://doi.org/10.1080/08927014.2016.1186165.

As Australia's national science agency, CSIRO is solving the greatest challenges through innovative science and technology.

CSIRO. Creating a better future for everyone.

Contact us
1300 363 400
+61 3 9545 2176
csiro.au/contact
csiro.au

For further information
Environomics Future Science Platform
Dr Maarten De Brauwer
maarten.debrauwer@csiro.au
csiro.au/eDNA-roadmap

B&M | 23-00541