Translational Molecular Ecology in practice: linking DNA-based methods to actionable marine environmental management

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The adoption of DNA-based methods by marine environmental agencies remains limited. Defining scope and possibilities ensures realistic DNA-based management applications. Sustained dialogue among stakeholders is often critical to success. Collaborative working will foster the uptake of DNA methods in marine policies. Several case studies confirm the value of Translational Molecular Ecology to achieve policy impact.

Abstract

Molecular-based approaches can provide timely biodiversity assessments, showing an immense potential to facilitate decision-making in marine environmental management. However, the uptake of molecular data into environmental policy remains minimal. Here, we showcase a selection of local to global scale studies applying molecular-based methodologies for environmental management at various stages of implementation. Drawing upon lessons learned from these case-studies, we provide a roadmap to facilitate applications of DNA-based methods to marine policies and to overcome the existing challenges. The main impediment identified is the need for standardized protocols to guarantee data comparison across spatial and temporal scales. Adoption of Translational Molecular Ecology – the sustained collaboration between molecular ecologists and stakeholders, will enhance consensus with regards to the objectives, methods, and outcomes of environmental management projects. Establishing a sustained dialogue among stakeholders is the key to accelerating the adoption of molecular-based approaches for marine monitoring and assessment.

Keywords:
Molecular methods, marine policy, translational molecular ecology, metabarcoding, marine bioassessments

1. The need to adopt new molecular tools for routine bioassessment
The rapid loss of marine biodiversity due to various anthropogenic pressures (Rands et al., 2010) calls for the urgent protection and conservation of marine communities and habitats (IPBES, 2019). Conservation efforts require the assessment of these communities to establish a baseline for future comparisons. Traditional methods to assess community and species composition based on morphology have well-known drawbacks that constrain biodiversity surveys. They often require multiple specialized taxonomists, have long processing times, and are time-intensive, limiting the extent of measurements in space and time (Maurer, 2000; Lejzerowicz et al., 2015). Recent advances in molecular-based approaches offer unprecedented opportunities for time- and cost-effective monitoring of the marine environment (Ribeiro et al., 2017; Goodwin et al., 2019; Ruppert et al., 2019).

DNA-based approaches provide information on species and communities via genetic material obtained from the environment (e.g., water, sediment, air, bulk samples of multiple organisms). These include various techniques such as metabarcoding to describe whole communities (Taberlet et al., 2012; Taberlet et al., 2018) and quantitative PCR (qPCR) or droplet digital PCR (ddPCR) to enable real-time detection of target species (Wood et al., 2019). Although these approaches rely on previous DNA information of the studied organisms deposited in DNA-reference libraries (Ortega et al., 2020), in contrast to traditional methods, molecular-based approaches (i) are observer-independent, (ii) allow simultaneous identification of a broad range of taxa from multiple samples (Wangensteen and Turon, 2016), (iii) can provide near real-time identification of target species (Bourlat et al., 2013), (iv) allow minimal and non-intrusive sampling (Zaiko et al., 2018), and (v) can reduce the costs associated with environmental assessments (Stein et al., 2014; Smart et al., 2016; Aylagas et al., 2018). These benefits have opened the door for an “ecogenomic” era (Pawlowski et al., 2018), where molecular techniques are increasingly being applied to study responses of organisms to natural and human-induced environmental changes (Cordier et al., 2020).

Recent studies show how molecular tools can be applied to comprehensively characterize biodiversity, relative to traditional methods, by improving the efficacy of routine marine monitoring programs,
facilitate environmental management practices, and inform marine spatial planning decisions (e.g., Lanzen et al., 2016; Laroche et al., 2016; Wood et al., 2018; Carvalho et al., 2019; Cordier et al., 2019; Stat et al., 2019; Bani et al., 2020; West et al., 2020). However, the transition from promising scientific studies to the adoption of new molecular tools for routine bioassessment still must overcome multiple challenges (Table 1). From a scientific perspective, a detailed understanding of the scope of the monitoring goal (Bani et al., 2020), and the integration of existing conventional monitoring databases with new molecular data (Hering et al., 2018), is essential. Also, the new molecular-based methods require a standardization process across different laboratories and timeframes to produce comparable and robust data (Zinger et al., 2019). Equally important, active participation of practitioners in framing the overall policy challenges and setting specific goals is often essential to success (Evans and Cvitanovic, 2018). Yet, a transdisciplinary dialogue during the policy process (i.e., discussions about science policy interfaces and policy impact) is probably the most apparent challenge in our ability to convert basic molecular research into actionable marine management (Leese et al., 2018).

The existing gap between research and policy requires “constant two-way communication between scientists and stakeholders” to “connect environmental science providers (i.e., field and laboratory scientists) with end-users (i.e., environmental managers) for the adoption of research results into action”; this definition is termed “Translational Ecology” (Schlesinger, 2010). We, therefore, suggest that this term be extended to “Translational Molecular Ecology (TME)”, whereby molecular ecologists collaborate with industry and policy-makers on the use of molecular approaches to ensure that the data meet policy-maker needs and expectations. Ideally, TME would involve the active engagement of (i) policy-makers, (ii) a technical counterpart (molecular ecologists), and (iii) an end-user providing a practical application perspective, such as industry partners, municipal governments, non-government organizations (NGOs), citizen science groups or private companies. In essence, the collaboration and communication will be the starting point to allow researchers to tailor DNA methodologies to the extent of delivering accurate and
meaningful data to stakeholders and regulators. The outcome will be the adoption of methods that support resource conservation and management of the marine environment.

Here, we selected several case studies focusing on the marine environment where TME is being implemented at multiple levels of jurisdiction. Case studies include projects from the local level in New Zealand and the US, the regional level in the European Union, and global challenges regulated by international-level conventions. In each of case study, we provide examples of how engagement of scientists and other stakeholders can lead to improved products, facilitating their ultimate adoption via the “translational” component. Based on these case studies, we provide a roadmap to guide scientists, managers, industry, and policymakers through the implementation of DNA-based methods into policy frameworks that regulate marine environmental monitoring and assessment.

2. Case studies

2.1. Validation and uptake of a DNA-based tool for monitoring benthic impacts of salmon farming in New Zealand.

When fish farming was first introduced in New Zealand in 1985, the government implemented a routine monitoring program. This monitoring program, however, became insufficient due to the large application for new aquaculture farming space, and a robust environmental management plan was needed. The need of a robust plan led to the formulation of the Enrichment Stage (ES) index, that combined conventional benthic indicators including biological (benthic macroinvertebrates) and geochemical (organic matter content, total free sulfides or redox potential) parameters to produce a single quantitative index value in a semi-automated manner (Keeley et al., 2012a; Keeley et al., 2012b; Keeley et al., 2013). The new approach was heavily scrutinized in 2013 through the Board of Enquiry of the Environmental Protection Authority (EPA), and the outcome of this review led to a modification of the ES with a new version proposed. This new index subsequently became a valuable metric against which DNA-based information could be interpreted and validated.
The increased interest by the government to develop and implement a standard benthic monitoring program for salmon farms in New Zealand led to the formation of a benthic standards working group (BSWG) to provide guidance for the monitoring program. The standardization process involved open discussions and obtaining consensus from all interested parties of the BSWG, including a single nominated representative from the resource user (industry), relevant government agencies (Ministry for Primary Industry and Regional Council), science providers (Cawthron Institute and National Institute of Water and Atmospheric Research), and community stakeholders. The debates around this process resulted in the formation of the Marlborough Sounds Best Practice Management (BMP) guidelines (MPI, 2015), the formal acceptance of the ES index, and a mechanism for reviewing the process in the future to ensure that protocols and standards remain optimal.

Back in 2012, when the potential of metabarcoding was first realized, the New Zealand government funded a project to begin a parallel benthic monitoring approach, combining traditional and DNA-based monitoring. After three consecutive years of sampling at target farms situated in different bioregions and water-flow environments, the DNA-based data proved to be consistent with the ES index (Keeley et al., 2018a). The results of the parallel monitoring revealed that microbial communities characterized using DNA metabarcoding could be used as a proxy for ES (Dowle et al., 2015; Pochon et al., 2015a; Pochon et al., 2015b). Later, a multi-trophic Metabarcoding Biotic Index (mt-MBI), combining microbial and eukaryotic bioindicators, demonstrated an improved accuracy for this purpose (Keeley et al., 2018b).

It soon became apparent that the multi-year, multi-site validation, and inter-calibration of methods was key to validate the inclusion of the new method. The opportunity for review within the BMP guidelines (MPI, 2018) allowed the new method to be presented to the working group, which resulted in additional funding for final refinements and validation. Fortunately, parallel sampling (DNA and conventional-based benthic variables) had been continued in the interim and also expanded to more farm locations.
contributing to a seven-year, multi-farm, multi-region dataset, which provided a solid basis for the final validation step. The final outcome of this project was the elaboration of a fully standardized protocol, which will be applied unchanged during a ‘phase-in’ period of up to five years as part of regular compliance monitoring (Pochon et al., 2020). We anticipate that after this ‘phase-in’ period, DNA-based assessments will be sufficiently grounded (i.e., robust, consistent, and interpretable) for routine use and supported by environmental legislation in New Zealand.

A critical component of this study was the open and transparent involvement and support by representatives from the local regional council, national ministry for Primary Industries and the end-user (fish farming company). The direct involvement of the end-user and relevant government agencies in the project from its outset was critical in helping scientists develop a protocol that, after several trials, resulted in a product that satisfied all parties. Moreover, the framework of balanced cross-representation under the BSWG was integral in obtaining a relatively rapid evaluation of the results. Furthermore, a mechanism for reviewing the protocols every year has been established to ensure that standards remain optimal.

2.2. Assessment of ichthyoplankton metabarcoding for routine monitoring of the Southern California Bight

Like many urban coasts, the Southern California Bight is subject to extensive monitoring associated with regulatory and management requirements (Schiff et al., 2016). However, existing marine monitoring programs are biased to the evaluation of demersal (bottom-dwelling) and rocky reef/kelp-associated fish communities, while the pelagic (water column) fish communities are often overlooked. Although these pelagic communities are of ecological and commercial importance (e.g., foodweb regulation and profitable fish catch), their diversity is difficult to assess due to large spatial scales and extreme variability in species composition. Quantification of the ichthyoplankton (i.e., early life history stages, such as larvae and eggs) is a useful way to understand the composition of the pelagic community, as well as to provide
insights into their levels of reproduction and recruitment, and the impacts and efficacy of management actions (Auth and Brodeur, 2013). However, traditional microscopy-based biomonitoring of ichthyoplankton is laborious, costly, and requires highly specialized technical expertise.

Over the past decade, DNA metabarcoding has become an increasingly popular method to identify ichthyoplankton, given its ability to process several samples in parallel and to distinguish morphologically similar species. In Southern California, the National Oceanic and Atmospheric Administration (NOAA) Fisheries manages the California Cooperative Oceanic Fisheries Investigations (CalCOFI) long-term monitoring program. This program has experienced delays in sample processing due to the reliance on morphology-based taxonomic identification and a paucity of available taxonomists. Thus, NOAA fisheries requested the development of a molecular approach to relieve their taxonomic capacity limitation. Likewise, regional wastewater dischargers have long sought tools to help assess the potential effects of their discharges on nearshore fisheries production, and molecular tools presented this opportunity. In partnership with these agencies, the Southern California Coastal Water Research Project (SCCWRP) led an effort to develop a framework for cost-effective and informative ichthyoplankton monitoring within the coastal waters of Santa Monica Bay and beyond (Kacev et al., 2018). The main objectives of this study were to compare traditional microscopy-based approaches to DNA metabarcoding to determine whether metabarcoding is a viable alternative to traditional methods and could be used for status and trends monitoring programs in coastal California.

A total of 74 ichthyoplankton samples were collected from 37 sampling stations throughout the Southern California Bight in May and June of 2016 using pairovet nets. This sampling effort was done in collaboration with multiple regional monitoring programs including the Los Angeles’ Environmental Monitoring Division, Los Angeles County Sanitation Districts, and the University of California at Santa Barbara Marine Biodiversity Observation Network. Samples from each station were sorted, enumerated, and identified to the lowest possible taxonomic level by expert taxonomists at the NOAA Southwest
Fisheries Science Center in La Jolla, California. After morphological identification, samples were homogenized and used for DNA extraction. The 12S barcoding region was amplified using universal fish primers (Miya et al., 2015) and selected for the first phase of the study. Concordance between metabarcoding and traditional approaches was evaluated by comparing the number of taxa with matching identification to the total number identified in each sample, and then averaged across all the samples in each group. Individual samples were compared to determine if the metabarcoding approach had similar resolving power to identify ichthyoplankton composition within a sample at three different taxonomic levels: family, genus and species level.

Across all samples, there was substantial agreement between the metabarcoding and traditional taxonomy results, with over 96% concordance at the family level, although fewer shared taxa at the genus level (81%) and species level (78%). The microscopy-based taxonomy yielded many unidentified specimens, and processing times were substantially longer due to the fact that only one laboratory in the region had the capability to process and identify ichthyoplankton samples. Overall, the metabarcoding approach was able to resolve all taxa to the family level, but not consistently to genus or species level. The study concluded that, the metabarcoding approach demonstrated clear potential as a rapid, cost-effective, and accurate alternative taxonomic approach.

A key component of this project was to build capacity and coordination among regional monitoring agencies to sample ichthyoplankton and assess the relative health of pelagic ecosystems. To this end, the collaboration between research scientists and monitoring program staff from the early stages of project design was critical for subsequent successful outcomes. Staff from the three monitoring entities were trained to collect samples using pairrovet net tow sampling protocols, including the assembling, deploying, and recovering of sampling equipment, processing samples onboard the ship, and preserving the samples in ethanol for future lab work. Collective training and intercalibration to ensure repeatability among sampling teams resulted in consistency of the sampling approach and acceptance of the new methods.
Training and initial implementation also allowed the field crews to help design protocols in ways that were compatible with existing sample methods and introduced minimal additional effort, thereby increasing willingness to adopt the methods. In addition to collaborating on sample collection and processing, the stakeholder group took part in discussions on data analysis and interpretation, such as how to define operational taxonomic units (OTUs) and the desired attributes of future biotic indices. These discussions helped to reach consensus regarding data analysis and interpretation, which further helped solidify acceptance and appreciation of the resulting data. This study helps illustrating both the power of applying a DNA metabarcoding approach for ichthyoplankton assessment, as well as the power of designing molecular-based biomonitoring approaches alongside monitoring program personnel to help guarantee future method adoption and success.

2.3. Development of DNA-based biotic indices within Aquatic Directives in Europe

Investigations on the use of molecular methods for the assessment of the ecological status of European coastal and estuarine-like water bodies began under the umbrella of the European Framework Programme 7 (FP7) DEVOTES project (2012-2016; www.devotes-project.eu). The two directives under which the status of aquatic systems is monitored in Europe – the Water Framework Directive (WFD; European Commission, 2000) and the Marine Strategy Framework Directive (MSFD; European Commission, 2008) - drove the need for new efficient and low-cost bioindicators (Borja and Elliott, 2013). In response, research focused on the adaptation and development of new indicators based on DNA methods (Wernersson et al., 2015; Hering et al., 2018; Pont et al., 2019; Tapolczai et al., 2019).

As part of the project, the adaptation of AZTI’s Marine Biotic Index (AMBI; Borja et al., 2000) into a DNA-based AMBI was considered. AMBI was developed for the assessment of ecological status in routine marine benthic surveys and is officially implemented in several European countries. AMBI determines the ratio between sensitive and opportunistic macroinvertebrate species (identified based on morphology) in estuarine and coastal waters under the WFD. The need for greater time- and cost-
efficiencies for these assessments led to an index calculated from taxonomic data generated via DNA metabarcoding, the genetics-based AMBI, or gAMBI (Aylagas et al., 2014). Likewise, a bacteria-based biotic index, microgAMBI, was developed following the same principle of gAMBI (Aylagas et al., 2017). The validation of gAMBI was carried out in partnership with the Basque Water Agency monitoring network (Spain), where a parallel sampling program (DNA and morphology-based indices) demonstrated that (i) gAMBI delivered similar assessment conclusions to those obtained via traditional monitoring, and (ii) the DNA-based index was 55% less costly and 72% less time consuming. Thus, gAMBI represents a valuable metric tool that improves decision-making by providing early and cost-efficient responses to ecological changes, allowing for more intensive monitoring programs where finer temporal and spatial scales can be assessed.

A step forward in the application of gAMBI is its official implementation into environmental policy frameworks. The legislative requirement is an important aspect of the TME process as policies were sometimes developed before the applicability of molecular tools in bioassessments was fully tested, as in the case of the WFD and MSFD. For example, the WFD prescribes the use of abundance data in the generation of biotic indices. Secondly, in official WFD assessments, AMBI is used in combination with species richness and diversity to deliver the multivariate AMBI (M-AMBI, Muxika et al., 2007). Currently, a collaborative approach involving many different stakeholders (i.e., researchers, commercial laboratories, policy-makers and end-users) is focused on the incorporation of the molecular data into legislation, and making modifications in the directive, if required. This effort is undertaken within the European COST Action DNAqua-Net project (Leese et al., 2016), where a sustained dialogue is taking place during frequent meetings split into specific organized working groups. Yet, in recognition of the time-consuming process to amend the legislation for accommodating new tools, researchers are refining existing methodologies in order to be considered a valid tool for implementation in current directives. For example, while a presence/absence-based gAMBI was recommended (Aylagas et al., 2014), further research demonstrated that read count data derived from metabarcoding could be assimilated into the calculation of AMBI using either abundance or biomass (Aylagas et al., 2018). Also, a multivariate DNA-
based index (M-gAMBI) is under development, and it includes (i) the result of gAMBI, (ii) richness and (iii) diversity, both based on metabarcoding. A 3-year parallel dataset of gAMBI and AMBI generated within the Basque Water Agency monitoring program has been established for this purpose. It is expected that this assessment will allow the calibration of reference conditions and critical thresholds between molecular and traditional methods.

A critical component in this case study is the sustained, long-term networking between stakeholders. At a local level, years of collaboration between researchers from a non-profit research institution (AZTI) and the governmental Basque Water Agency has built trust and mutual interest. This relationship has enabled a sustained communication during frequent meetings (at least twice a year) at both the government building and the research institution. This dialogue has dramatically boosted the development of ideas to open the window for policy change. The government body has trusted researchers and funded projects for the development of molecular-based methodologies for a time- and cost-efficient assessment of ecological status in routine marine benthic surveys. The example of the TME at a local level is extended in this case study to regional level, involving a large number of stakeholders in the policy process (e.g., at a European level through the DNAqua-Net action). Within a regional jurisdiction, the implementation of M-gAMBI requires intercalibration with the methods proposed by other European Member States, to demonstrate consistency in the designation of ecological status (Borja et al., 2007; European Commission, 2008; Birk et al., 2013). To fulfill this purpose, a consortium of researchers is devising and proposing consistent indices (Buchner et al., 2019; Pont et al., 2019), as part of the of intercalibration required to deliver consistent data. These intercalibration exercises are part of European funded projects (see DNAqua-Net; Leese et al., 2016) that in collaboration with International Partner Countries and several stakeholders (scientists, managers, governmental institutions, manufacturers, and emerging service providers) are identifying major challenges in DNA-based bioassessments and providing standardized best-practice solutions to those. This collaboration is facilitating future amendments in the legislation to account for the benefits and particularities of the novel molecular approaches. This case study provides a realistic
example of how TME is playing a crucial role to increase the potential of DNA metabarcoding methods to be implemented in the EU marine regulatory framework.

2.4. Marine biosecurity monitoring

The introduction of non-indigenous species (NIS) is a global phenomenon and a major human-induced pressure on marine ecosystems (Ojaveer et al., 2018) that has led to the implementation of a broad range of biosecurity policies at local, national, regional and international jurisdictions (e.g., International Convention for the Control and Management of Ships’ Ballast Water, European Strategy on Invasive Alien Species, New Zealand Craft Risk Management Standard, and the United States Ballast Water Regulations).

Robust NIS-related biological information (e.g., on NIS diversity, rate of incursions and environmental impacts) is critical for adequate biosecurity management (Olenin et al., 2011) and, in theory, can be effectively delivered by inventory-based molecular approaches (i.e., metabarcoding; Zaiko et al., 2018). However, the incompleteness of reference sequence databases (Briski et al., 2016; Zaiko et al., 2016; Weigand et al., 2019) and absence of truly universal marker genes (Pochon et al., 2013; Fletcher et al., 2017) place target-specific approaches (e.g., real-time PCR assays) into a more advantageous position for rapid uptake. The downside of the targeted approaches is their applicability for detecting from one to a few species only, with a dedicated effort required to establish an assay for every new species of interest. Hence, these methods are more easily implemented for regional biosecurity management rather than international policies.

For example, targeted approaches are currently applied in New Zealand, and are close to uptake for regional marine biosecurity monitoring. In response to stakeholders’ needs for a faster, more sensitive, and more precise targeted surveillance, individual surveys began in 2017, aiming to (i) test their performance as a complementary surveillance tool, (ii) validate the results of traditional surveillance
(visual detection by divers), (iii) optimize surveillance designs for detection of new arrivals, and (iv) verify eradication success over a constrained area (bay or marina). The continuous dialogue between scientists and other stakeholders (policy makers and biosecurity practitioners) and the ability of New Zealand regional councils to make decisions about additional or complementary methods for biosecurity surveillance in their jurisdictions allowed for the implementation of molecular assays for detecting two high-priority NIS: the Mediterranean fanworm Sabella spallanzanii and clubbed tunicate Styela clava. The wider implementation of DNA-based biosecurity surveillance has been facilitated through the open and continuous dialogue between scientists and stakeholders. The specificities of this communication are highlighted in Table 2 and are presented as “good practices” from both scientists and other stakeholders to achieve policy impact. The ongoing research effort is focusing on improving and optimizing molecular surveillance technologies, specifically fit-for-purpose sampling devices, designs, and strategies, for better harnessing the power of molecular tools in biosecurity.

To broaden the applicability of inventory-based (metabarcoding) techniques for NIS detection, the existing caveats around unified quality-assured methodological protocols, bioinformatics pipelines and, particularly, reliable and exhaustive reference databases, should be resolved. Coordinated international efforts to standardize, calibrate and validate the tool remain a priority. Currently several initiatives have started developing national sequence databases. For example, the State of California (USA), a global hotspot for marine invasions (Ruiz et al., 2011), is developing an extensive NIS sequence database to address current gaps for marine invertebrate NIS already present in the state. Paired morphological and genetic vouchers are collected during traditional surveys and bioblitz campaigns (i.e., dedicated biodiversity surveys to collect and identify as many species as possible within an area). The performance of metabarcoding to detect NIS (and native species) is then tested by comparing results with traditional morphological analyses using replicate samples across multiple locations and years.
While current surveillance efforts in California include traditional survey methods, and this may continue at selected sentinel sites, one goal is to transition to metabarcoding across many additional sites, and ultimately to build a new set of tools to improve spatial coverage, temporal frequency, and associated costs of biosecurity surveillance. This approach to state-wide monitoring and detection has emerged from a collaboration program between a research team and a resource management agency team. The collaboration began after informal discussions over several years at regional conferences and workshops across a wide range of topics about environmental science and management in coastal ecosystems, establishing a baseline of shared interests in marine invasion dynamics and identifying key challenges from the policy and management perspective. This led to more intentional and direct discussions, which aimed to exchange ideas about the potential for genetic methods to meet surveillance challenges, including candid assessment about both strengths and weaknesses of available approaches. We agreed to design and launch a pilot project together in one bay, to compare results from traditional and metabarcoding survey methods, providing initial data for evaluation, continued discussion, and design of a broader program. Based on our pilot project results and experience, we designed an expanded program for coastal waters across the state through iterative group discussions about goals, expectations, performance, interpretation, and experience. The program has advanced over seven years, following a roadmap similar to that below, and includes frequent communication and “collaborative working” on the key elements.

3. Roadmap

Based on the lessons learned from the case studies presented above, we propose a roadmap (Fig. 1) for a meaningful collaboration between researchers and policy-makers towards the implementation of molecular methods in marine policies, integrating the key principles of TME (Lawson et al., 2017).

The case studies described above illustrate how the successful integration of DNA-based assessments into management and monitoring schemes depends upon continuous transdisciplinary dialogue and
collaboration among researchers and stakeholders during the TME implementation process. Building this collaboration, however, requires substantial commitment from both sides. Researchers and policy makers usually differ in their short-term and long-term goals and career paths, have differing attitudes toward scientific novelty and operate at different time scales and spheres (Choi et al., 2005). For these reasons, there has often been a lack of engagement between researchers and policy-makers, and establishing new interaction can require a substantial and intentional investment of both time and effort. Successful integration of new molecular methods into routine use begins with constructive dialogue and bilateral knowledge sharing between policy-makers and researchers, the purpose of which is to understand whether the new method has the potential to address specific policy needs. This represents Phase 1 of the TME process: interaction and engagement.

If potential benefits of the new method are identified in Phase 1, the next step is to carry out a collaborative pilot project consisting of field trials in which the method is implemented in a realistic monitoring context, ideally alongside conventional monitoring so that outputs can be compared. This phase requires considerable commitment from both researchers and policy-makers, and will be an iterative process of calibration, validation and standardization to fully assess whether the potential benefits can be achieved. This represents Phase 2 of TME: communication and commitment.

Where a clear benefit has been demonstrated in Phase 2, and the new method has been validated and calibrated with relevant indices and data from conventional methods, the final aspect of TME consists of operationalization of the new method for use in ecological decision-making. This phase represents Phase 3 of TME: decision framing.

3.1. Interaction and engagement

Collaboration between researchers and stakeholders is essential to evaluate existing needs for effective management policies. It is important to highlight that to achieve this, some existing barriers must be broken. It is commonly believed that researchers are motivated solely by publications and career
development (Pang, 2003). However, the potential of research to be applied and produce a positive impact in society and solutions for societal goals is fast becoming a central driving factor for scientific research. This creates a meaningful space for interaction and engagement between researchers and policy-makers. Yet, the engagement must begin in the early stages of the project, allowing research to be shaped to address real policy needs, while policy-makers stand to gain a deeper understanding of the costs and benefits of the new research through direct involvement in the project from its outset. We have identified two main potential risks when engagement is only sought once the research has been completed. First, there is a likelihood that important key requirements or goals from a policy perspective are left out. And, second, communication becomes increasingly difficult since research scientists are usually expected to disseminate their research in a form of peer-reviewed scientific journal articles, which often lack a clear message for policy-makers. Policy-makers, meanwhile, often lack the time and subject-matter expertise to remain up to date with the scientific literature and extract the information that is relevant to them. Thus, to build this collaboration towards finding common goals and framing the decision process we identify four categories of engagement. These categories are defined as ‘no communication’, ‘opportunistic interaction’, ‘sustained dialogue’ and ‘collaborative working’ (Fig. 2).

**Opportunistic interaction** is important for forging new links between different groups of stakeholders and is most easily facilitated via events such as conferences and workshops. To establish opportunistic interaction researchers can intentionally seek out relevant policy and management-orientated conferences, in addition to purely scientific meetings. Conversely, policy-makers can be drawn to scientific meetings through mechanisms such as special sessions focusing on policy and implementation, or specific workshops that create a forum for multi-stakeholder discussions. Such interactions lay the foundations for deeper relationships to be developed, leading to sustained dialogue and collaborative working.

**Sustained dialogue** refers to an ongoing relationship between stakeholders where opportunities for discussion are routinely created, providing opportunities for research ideas and project proposals to be considered.
Collaborative working represents the highest level of commitment from all parties and is usually established around a particular research project with potential policy benefits. Collaborative working can be promoted with frequent meetings of the project team with each party (i.e., researchers and policy-makers) having a defined role and responsibilities in delivering the project. This is the engagement level that is required for effective TME.

The first stage of the TME process is bilateral knowledge exchange. It is crucially important for researchers to understand the legal and practical context in which monitoring is applied and the specific challenges or limitations faced. It is equally important for policy-makers to understand the capabilities of the new molecular tools. Here we present key aspects that researchers and policy-makers should seek to understand from one another in order to assess the suitability of the method for implementation in marine policies.

Researchers should seek to understand from policymakers:

- How monitoring is conducted at present, including the time and cost involved in sample acquisition and analysis;
- The main drivers for interest in a new method, which may be related to factors such as time and cost savings, statistical power or resolution of data, or availability of relevant expertise;
- The legal context for monitoring and reporting, and whether there are specific indices or pieces of information that have to be reported at national or international levels;
- Practical constraints in terms of access to equipment or personnel for monitoring, or restrictions around the use or transportation of dangerous goods such as ethanol.

Policy-makers should seek to understand from researchers:

- The extent to which the method has already been trialed and validated, as well as potential weaknesses;
The type of data generated by the new method and how this differs from conventional methods in factors such as taxonomic coverage, data resolution, and the ability to make quantitative inferences;

Whether there is the potential to replicate existing indices using the new method, or if new indices will need to be created and calibrated with existing ones;

Approximate times, costs and forms of expertise involved in sample acquisition and analysis.

Addressing these questions at the outset of the project is critical to understanding whether the new methods have the potential to address real policy needs. For instance, a method that offers to significantly improve taxonomic resolution of biomonitoring data will only be attractive to policy-makers if (a) taxonomic resolution of conventional methods limits their ability to make robust decisions, or (b) the new method can also address another need (e.g., cost reduction). Thus, this process of knowledge exchange helps to align priorities and goals to evaluate whether novel assessment methods can help to improve management outcomes given realistic practical, financial and legal constraints. Once a decision about the potential suitability of the new methods is made (implementation, non-suitable, or suitable but requires further validation), Phase 2 may commence.

### 3.2. Communication and commitment

Phase 2 of TME involves calibrating and validating the new method, which is then standardized prior to operational implementation. This usually centers around a collaborative field validation study to trial the new molecular methods alongside conventional ones, facilitating a detailed cost-benefit analysis. The process requires substantial commitment from both parties and should be expected to be an iterative process of adapting and optimizing research methods, to account for the realistic constraints and requirements of the end-use. Yet, building this communication relies as much on the soft skills as on the robustness of the scientific methods (Cvitanovic et al., 2016). The case studies presented here highlight some personal attributes shown by both scientists and policy-makers that are key for the alignment of the
monitoring goals, including honesty, transparency, flexibility and openness. Thus, a strong and transparent relationship between the parties facilitates collaborative work by creating an environment in which open, honest and constructive feedback can be communicated so that methods can be fully optimized. Researchers must be open and responsive to such feedback, while policy-makers must recognize the scope for adaptation and optimization, understanding that initial results might fail so that further work might be needed to address the issues encountered. Thus, projects should allocate sufficient time and funding for multiple rounds of testing during this validation process. In the case studies summarized here, both parties understood that several trials might be necessary for full optimization and standardization of the workflow. Collaborative working was maintained throughout the entire validation process, allowing preemptive identification of potential issues and better optimized solutions before agreeing the final protocol.

Once validated, the method needs to be standardized prior to operational implementation. Standardization can take place informally or through formal bodies such as the European Committee for Standardization (CEN) or the International Standards Organization (ISO), both of which already include working groups for standardizing biological survey methods. Indeed, there is even a CEN working group specifically for standardization of molecular methods for biological monitoring (Technical committee 230: working group 28). Agreed protocols and best practice guidance should be generated for each step of the workflow: (i) sample collection, preservation and processing; (ii) DNA extraction and processing (i.e. PCR amplification incorporating minimum quality control procedures, and sequencing; (iii) data analyses (where applicable) and; (iv) communication of the results, ideally via a publicly available data archive (see Ebentier et al., 2013; Deiner et al., 2017; Zinger et al., 2019). The validation and standardization process can span a wide range of timescales, which will depend greatly on the starting point of the project (i.e., whether previous validation studies exist in the region or not), on any previous work to standardize certain parts of the process and the spatial scale required for the standardization (e.g., national monitoring programs or cross-national projects). The optimized protocols should be reviewed and accepted by as
many labs and scientists as possible through established consortia, whilst maintaining frequent communication with end-users. Protocols should then be shared and made public to enhance international collaboration towards unified standards (e.g., Eco-AlpsWater Project, https://www.alpinespace.eu/projects/eco-alpswater/en/home). An excellent example of this is the Molecular Methods Workgroup run by the California Water Quality Monitoring Council, which brings together multiple stakeholders to build consensus and standardize molecular protocols for key aquatic management applications.

Frequent changes in the protocol will diminish stakeholder confidence in molecular methods, especially where this leads to variation in the resolution of data output, which needs to be comparable over long timeframes. Thus, the methods should be standardized for consistency in routine monitoring applications whilst recognizing that this fast-moving research field will continue to generate methodological improvements. We suggest that TME should be undertaken with the goal of developing a protocol that will be consistent during a specific timeframe (including sample processing, database, and analytical pipeline), but that includes expected revision of the method. We further propose that particular emphasis should be placed on development and standardization of the initial stages of the workflow (specifically, sample collection, preservation and DNA extraction), since this provides the foundation for a non-biased DNA archive that can be used for evaluation and intercalibration of new methods. This can facilitate methodological changes that increase efficiency, without having to restart the whole process. Once a clear benefit of the method has been demonstrated and agreed by both parties the final aspect of TME consists of operationalization of the new method for use in ecological decision-making.

3.3. Decision framing

The adoption of the new tools into policy arrives when Phases 1 and 2 are completed to satisfaction, and will depend upon how well the DNA-based methods addressed the management and policy priorities identified in Phase 1, how robust the data is, and whether it is able to suitably support making decisions
on marine policies. The discussions around these issues must take place during Phase 1 and 2, and Phase 3 represents only the final decision of its implementation into policy, which by this point is mostly out of the hands of researchers.

The roadmap presented here shows 3 distinct phases that are not always independent of each other or static. Thus, because policymaking in practice might not follow a strict sequence (Cairney et al., 2013), the order of the phases presented here might be adapted or combined depending on the specific nature of each project. However, it is still helpful to identify the different “phases” within the process to ensure that the crucial aspects of the TME identified here are addressed to deliver robust tools and facilitate the ultimate adoption in management practices. Whenever DNA methods are suitable, they can be implemented as a standalone tool or ideally as a complementary tool to traditional or historical methods for a specific period of time. TME also plays a relevant role in the delivery of the results, as they must be practical enough to be interpreted and visualized by both researchers and end-users.

4. Conclusions

With the increased inclusion of DNA-based technologies in marine research, environmental monitoring and assessment may undergo a rapid transition from phenotypic- to genotypic-based frameworks. Translational Molecular Ecology (TME) is a key process in linking DNA-based research to actionable marine environmental management. The promotion of TME could begin through funded projects from local or national agencies in collaboration with end-users who may benefit from DNA-based methods. Fortunately, the transition from scientific proof-of-concept studies to tangible management purposes has already begun with funded projects in several countries (e.g., EU: GeDNA, Eco-Alps Water project, COST Action Ca 15219; US: California Molecular Methods Agency Workgoup; California Department of Fish & Wildlife, Marine Invasive Species Program; Canada: Canadian Ecogenomics Consortium; New Zealand: Biosecurity Toolbox and Aqua Health Endeavour programmes). These projects might serve as examples of how TME can be brought into practice in regions where this interaction is still lacking.
Additionally, the fall in sequencing costs might allow for substantial savings in monitoring budgets (when the same number of samples are maintained) or promote the analysis of more samples across spatial and/or temporal scales (at similar costs). Also, as new technologies to collect environmental samples are rapidly evolving (e.g., autonomous underwater vehicles), they can be easily coupled with molecular analytical methods and contribute to advancing future marine monitoring programmes.

DNA metabarcoding and targeted methods, such as qPCR, have revealed their suitability to address policy-relevant and society-based questions. However, their implementation into routine programs as single tools in the future may require several years of validation. Therefore, it is recommended that traditional and innovative DNA-based approaches are jointly applied over a sufficient amount of time to evaluate and assure robust performance. During this process, the pros and cons of each method should be carefully evaluated; for example, quantitative information cannot be derived from metabarcoding data or reference databases are incomplete. In some scenarios, TME might provide the opportunity for adjustments in policy if necessary (e.g., remove the requirement for quantitative biodiversity information or revising/updating indices to be more compatible with molecular data). The context of the program and the question addressed are crucial to determine whether molecular methods should be adopted. If so, comprehensive analysis within molecular methods should be performed to decide which method best suits emerging questions (i.e., targeted methods may present more advantages than metabarcoding for particular issues such as detection of NIS or rare species because they provide quantitative information).

Although we have focused on DNA-based methods in the marine environment, our framework is potentially applicable to other aquatic environments and other novel assessment methods, which may improve our ability to monitor and conserve natural resources. We are confident that increasing engagement participation in TME will boost the transferability of DNA-methods from the research to the management domain.
Acknowledgments

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AUTHORS’ CONTRIBUTIONS

All authors conceived the main ideas and contributed to the collective writing process led by E.A. and S.C. All authors revised the manuscript and gave final approval for publication.

Figure captions

Figure 1. Roadmap for effective implementation of molecular methods in legislation and regulations for ecosystem assessment

Figure 2. ‘Science-stakeholders’ interfaces that may occur among different parties while implementing new environmental policies. Opportunities to remove the gap between scientific proof of
concept and applied research begin when two or more parties (researchers and one or multiple end-users) put Translational Molecular Ecology into practice.
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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Table 1: Existing challenges for effective implementation of molecular methods in policy and regulations for ecosystem assessment. The challenges highlighted here have been compiled from the literature in order to synchronize the language among groups and sectors. Facilitating steps to overcome the challenges are proposed. TME: Translational Molecular Ecology; WFD: Water Framework Directive; MSFD: Marine Strategy Framework Directive.

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Current situation</th>
<th>Facilitating steps</th>
<th>References</th>
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<tr>
<td>Transdisciplinary collaboration</td>
<td>Finding the motivation to increase collaboration between researchers and policy-makers is often limited due to conflicting interests of two sectors. This often results in one-way communication, limiting the application of molecular tools for bioassessment by regulatory agencies. In general, the</td>
<td>Substantial progress has been made with funding agencies supporting existing applied research. As a result, researchers benefit from sampling programs with large spatial and temporal scope, whilst applying their</td>
<td>Friberg et al., 2011b; Leese et al., 2016; Leese et al., 2018</td>
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</table>
The incorporation of scientific studies into regulation has been ineffective and as a result, the management tools developed are sometimes inappropriate and poorly designed. Managers benefit from improved scientific surveys, which can translate into better management practices. Maintaining this collaboration is essential for an effective implementation of molecular tools in marine environmental management.

<table>
<thead>
<tr>
<th>Scope and possibilities</th>
<th>The definition of scope and possibilities should include the analysis of the cost and time-frame required to deliver results, not only the resolution.</th>
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<tr>
<td>Marine environmental management requires comprehensive data on the status of marine biodiversity. Before establishing a pilot or routine study to conduct temporal and spatial surveys using molecular methods, the scope and possibilities should include the analysis of the cost and time-frame required to deliver results, not only the resolution.</td>
<td></td>
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Bani et al., 2020
resolution at which the method can inform on the issue addressed needs to be discussed and validated by previous studies.

<p>| Robustness       | Robustness requires the calibration, validation and standardization of the proposed method across different temporal and spatial scales. Similarly, the new methods need comparability with existing data, generally collected using traditional methodologies (i.e. morphological inventories). | International initiatives to calibrate and standardize data across laboratories and countries is encouraged (see Ocean Sampling Day consortium). Ideally, technical considerations should be precisely reported in any study (pilot or routine) to assess the relevance and quality of the data that drives the monitoring conclusions. | Kopf et al., 2015; Deiner et al., 2017; Hering et al., 2018; Blackman et al., 2019; Zinger et al., 2019 |</p>
<table>
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<tr>
<th>Feasibility</th>
<th>Policy makers are starting to visualize molecular tools as a promising alternative to traditional methods due to the relatively simplicity in sampling. However, many constraints are found when the method is to be applied in routine monitoring contexts. For example, sometimes the equipment required for sample collection or preservation is not immediately accessible or affordable.</th>
<th>Methods employed in scientific research should be transferable to routine monitoring contexts. Several commercial companies have developed protocols towards the simplicity to collect and preserve environmental samples. This aspect should be more often considered in academia when the aim is to facilitate biodiversity surveys using molecular tools.</th>
<th>Pilliod et al., 2014; Biggs et al., 2015; Goldberg et al., 2015; Deiner et al., 2017</th>
</tr>
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<tr>
<td>Policy impact</td>
<td>An important component of TME is to demonstrate that the impacts arising from proof of concept studies are tangible and applicable to management</td>
<td>Empowering science communication positions in both academia and governmental institutions</td>
<td>Evans and Cvitanovic, 2018; Catalano et</td>
</tr>
</tbody>
</table>
networks. Sometimes scientists lack of skills to translate an approach that is otherwise obscure for regulators as it involves the inclusion of traditionally overlooked biological elements (microbial communities) or fashionable analysis such as Supervised Machine Learning.

**Legislative requirements**

Most of the current policies have been developed before the applicability of molecular tools in bioassessments was tested. For example, the WFD and MSFD specifically require quantitative biodiversity information, which can sometimes be unachievable using DNA-methods. Once a government body adopts a policy, it takes time and a close dialogue between scientists and managers is required to show the benefits of the novel approach for marine assessments and transfer the perception of the new concepts brought by molecular methods. The dialogue will allow...

| Networks | will likely help the interactions between science and managers and make the biggest impact with respect to making TME real. | Friberg et al., 2011a; Leese et al., 2018 | Seymour, 2019 |
| demands much new evidence before changes are accepted. | evaluation of tradeoffs, opportunities, and possible modifications (applications) to the existing legislative acts. These revisions need to accommodate the possibility of new and powerful tools becoming available, despite current limitations. |
Table 2: Implementation of target-specific approaches for pest detection in several New Zealand regions: local (sub-national) implementation

Prerequisites (Alignment of demands and opportunities between researchers and end-users):
- High demand for a rapid, sensitive, precise and efficient tool for targeted surveillance, as the end-users unanimously agree that current surveillance approach based on visual surveys by divers presents several downsides (e.g. time and resource demanding, poses a hazard for diving teams working around ports and busy waterways, lacks sensitivity especially for detecting early incursions or populations of small and taxonomically obscure species);
- Opportunities for local implementation: New Zealand regional councils are in a position to make decisions about additional/complementary methods for marine pest surveillance in their jurisdictions;
- Targeted detection of few known high-priority pests allows a straightforward validation of the assays and demonstrate their robustness.

Current status - close to uptake, individual surveys are performed to:
- Test the performance of molecular methods as a complimentary marine biosecurity surveillance tool;
- Validate the results of traditional surveillance over various spatial scales;
- Optimize surveillance designs for improved probability of detection of new arrivals (low-density populations);
- Verify eradication success over a constrained area (bay or marina).

The “Art of small steps” for successful implementation - the researchers’ perspective:
- Focus on assays of the species of primary importance/concern to end-users;
- Inform end-users about the tools available, showcase their application and results of the proof-of-concept studies;
- Perform 1-2 trial studies in local jurisdictions - the good examples of real-life applications help things rolling, facilitate the outreach and demand for more case studies;
- Maintain close and fair communication with end-users, being open about what the method can do and what it cannot; inform
- Use each case study or independently-funded research opportunity to improve/refine your methods and approaches;
- Inform the stakeholders on the directions of the further developments and take into account their feedback (very valuable moment, as at this point you can actually focus on resolving their concerns and addressing main needs, to make sure your tool is fit-for-purpose).

Main concerns communicated by end-users and further steps to address those:
- False positive detections – one of the biggest fears of decision-makers as might potentially infer unnecessary management efforts and elicit inadequate legislative response.
  - Provide stakeholders with information on possible sources of false-positive eDNA-based detections (see e.g. Darling and Mahon 2011);
  - Probabilistic estimation of false detections in the context of particular study design and developing fit-for-purpose strategy of mitigating those risks;
  - Ensure rigorous SOP for field and lab workflow with appropriate contamination control established.
- Target-specific approaches are only applicable from one-to-few species.
  - Complement the molecular surveillance toolkits with validated assays for a range of priority species;
- Elaborate regional reference libraries and combine targeted assays with the community-level molecular screening (e.g. using High Throughput Sequencing metabarcoding).

- Inability to locate the actual source population based on eDNA signal from water, i.e. inability to implement focused response (mitigation or eradication).
  - Coupling eDNA studies with oceanographic and particle transport models and developing predictive probability maps of source populations (see e.g. Sansom & Sassoubre 2017).

- Need for robust and reproducible sampling and analytical pipelines.
  - Work collaboratively with other national and international teams towards establishing best practice guidelines and standardization of protocols for specific biosecurity applications;
  - Co-develop with end-users and adhere to quality criteria and reporting standards.
Graphical abstract

**Highlights:**

- The adoption of DNA-based methods by marine environmental agencies remains limited
- Defining scope and possibilities ensures realistic DNA-based management applications
- Sustained dialogue among stakeholders is often critical to success
- Collaborative working will foster the uptake of DNA methods in marine policies
- Several case studies confirm the value of Translational Molecular Ecology to achieve policy impact
Interaction and Engagement
Phase 1

Private sector
Citizens
Government

Academia
Research Institutions
Commercial Laboratories

Is the DNA-based method suitable for policy-relevant and society-based marine research?

Yes, but requires validation

Use non DNA-based methods

Communication and Commitment
Phase 2

Tests for standardization, calibration and validation

DNA Archive

Data Archive

Sample collection & processing → DNA extraction & processing → Data analysis → Communication of results

Has the method been validated?

Further validation and tests

Decision Framing
Phase 3

Use of non DNA-based methods

Implementation of DNA-based methods

Figure 1
<table>
<thead>
<tr>
<th>Communication</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td>No Communication</td>
<td>Research not specifically aligned with goals of policy-makers</td>
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<tr>
<td>Opportunistic Interaction</td>
<td>Foundation for deeper relationships towards the development of policy-relevant research</td>
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<tr>
<td>Sustained dialogue</td>
<td>Comprehensive engagement and interaction to explore projects with potential to improve policies</td>
</tr>
<tr>
<td>Collaborative working</td>
<td>TME in practice to develop a particular research project. High probability of successful implementation in marine environmental policies</td>
</tr>
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