Marine Genomics 4 Users

MG4U Workshop Report

Friday 28 June 2013

Venue: Radisson Blu Hotel, Dublin

"THE POTENTIAL OF GENOMICS FOR MARINE MONITORING AND BIODIVERSITY MAPPING: FROM CONCEPT TO REALISATION"

COOPERATION

ucc
Coláiste na hOllscoile Corcaigh, Éire
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AQUATT
Science. Communication. Knowledge. Innovation.

Aim of the Workshop

This workshop brought together relevant stakeholders to debate and discuss genomic tools and techniques and their potential applications in marine monitoring. Industry representatives, leading genomic researchers, actors with a governmental mandate to carry out marine monitoring, and marine decision makers took part in the workshop. As the MG4U project was scheduled to finish at the end of June 2013, the workshop also included representatives from similar projects who were likely to disseminate the information and knowledge arising from the workshop to key end-users as well as building on the work started by MG4U.

Background

The Dublin workshop was designed to build on the outputs of the previous MG4U workshop held in Oxford, UK, in September 2012, which focused on the potential of marine genomics in relation to the implementation of the Marine Strategy Framework Directive (MSFD)[1] . The Oxford workshop brought together representatives from policy, genomics research and the marine monitoring community. It was held in conjunction with an international meeting of the Genomics Standards Consortium (GSC) and a number of experts attending that conference observed and participated in the workshop.

The overall conclusion arising from the Oxford workshop was that genetic and genomic methods have a high potential to address marine environmental monitoring gaps in a standardised way. As there are no genetic or genomic standards in the current monitoring programmes, it was inferred by the participants that it would be relatively straightforward to introduce the standards developed by the GSC into marine monitoring.

The six methods considered by the participants to have promise were as follows: DNA bar-coding, microarrays, quantitative polymerase chain reaction (qPCR) and single-nucleotide polymorphism (SNP) based methods, metagenomics and transcriptomics. These were identified as being relevant to six of the MSFD descriptors: D1 (Biological diversity), D2 (Non-indigenous species), D3 (Population of fish/shellfish), D4 (Elements of marine food webs), and D5 (Eutrophication), and D6 (Sea floor integrity). Further details of the methods are given in the MG4U white paper entitled Genomics in marine monitoring: new opportunities for assessing marine health status, which was published in the Marine pollution bulletin in May 2013 and can be found online through the MG4U website.

WORKSHOP
Introduction

The workshop methodology combined informative presentations and interactive participatory sessions to facilitate the participants' exchange of ideas and input into discussions.

The Chair of the workshop, Prof John Benzie, University College Cork and partner in the MG4U project, welcomed all participants and speakers and opened the day's proceedings. Prof Benzie provided an introduction to the MG4U project, the background of the workshop, and summarised the main elements for discussion during the day. The terms of reference for the workshop were explained and participants were asked to introduce themselves and their areas of interest. Please see Annex 1 for the full list of workshop participants.

Session 1 Setting the Scene

The morning session consisted of two rounds of presentations, with the first speakers setting the scene for the workshop. Naiara Rodriguez-Ezpeleta from the DEVOTES[2] (Development of innovative tools for the understanding of marine biodiversity and assessing Good Environmental Status) project presented the marine legislative requirements of the MSFD which could be informed by genomic techniques.

Following this, Johanna Wesnigk, a partner in the MG4U project from the Environmental & Marine Project Management Agency, Germany, explored the use of

[1]The MSFD is a legal framework that requires EU Member States to assess the overall state of their marine environments and to achieve Good Environmental Status (GES) of marine waters by 2020. The EU has established 11 qualitative descriptors that, together with associated criteria and indicators, will be used to determine the status of marine ecosystems and how GES can be achieved and maintained in the future. MSFD is described in greater detail in the report of the Oxford workshop which can be found on the MG4U website.

[2]http://www.devotes-project.eu/

ecosystem biology as an important element in understanding the marine environment and addressed possible infrastructure and expertise requirements that may need to be integrated into marine monitoring systems.

Geoffrey O'Sullivan, chief advisor to the CEO of the Marine Institute in Ireland then provided an overview of the funding landscape, including current possibilities and potential future international funding collaborations. This session closed with a brief video from the STAGES[3] (Science and Technology Advancing Governance on Good Environmental Status) project, which summarised the MSFD legislative requirements and implementation timeline.

Session 2 Practical experiences of genomic applications

The second round of presentations featured international examples of practical experiences of using genomic techniques for marine research on a large scale. Iratze Zarraonaindia from the Argonne National Laboratory, USA, began with an introduction to the Earth Microbiome[4] project. This case study showed how genomic approaches are being used for a systematic characterisation of global microbial diversity. The final presentation of the day was given by Dan Distel from Ocean Genome Legacy[5], USA, a not-for-profit marine research institute and genome bank. He explained Ocean Genome Legacy's philosophy on the significant storage of marine genomic material collection for future analysis.

INTERACTIVE SESSIONS

Session 3: What is the Current Status of Marine Genomic Tools?

In this first interactive session of the workshop, the participants were divided into four groups. There was an expert in genomic techniques in each group to provide explanations and clarification where needed. Each participant was given a draft policy brief developed for the workshop and based on a white paper written by the MG4U partnership and other genomic experts, some of whom were present at the workshop.

The draft policy brief summarised the findings of the MG4U paper, specifically laying out the comparison of six genomic tools which were deemed to have the highest potential for application in marine monitoring, with a focus on the MSFD. Each group was assigned two tools to review and analyse:

- Each group was given either metagenomics or transcriptomics
- Each group was also given one of the following: DNA bar-coding, microarrays, qPCR or SNP based methods

Participants had time to review the factsheet for each tool within the policy brief before discussing it as a group. The groups were specifically asked to consider the following:

- The validity of the details within the factsheet, e.g. advantages, disadvantages, cost and application of the tool
- The potential applications of the tool
- The most promising application (only one choice allowed)

Results:

At the end of the session each group presented their feedback to the room, resulting in discussion and interaction. The results of the session can be viewed in Annex 2.
The general topics discussed at this point included the difficulties that can arise in the understanding and promotion of genomic techniques as a result of a lack of standard definitions for the different tools and techniques. The general opinion during the discussions was that marine genomics has great potential to fill the gaps in marine monitoring and biodiversity mapping which are not currently being addressed by conventional methods. Questions on the timelines for integration as well as infrastructure needs were put forward and debated. Many of the participants were of the opinion that some genomic tools and techniques could be used immediately, while others were uncertain about the methods and means required for a novel methodology or technique to be used in a new setting. For example, with marine genomics there needs to be standardisation of protocols, inter-laboratory validation and buy-in, and infrastructure investment - all pre-empted by an evidence-based cost benefit analysis.

[3]http://www.stagesproject.eu/
[4]http://www.earthmicrobiome.org/
[5]http://www.oglf.org/

After each group had nominated the most promising application of the tools they reviewed, they voted on the top two tools they would like to investigate in more detail in Session 4. The two chosen tools and applications were:

Tool	Application
Metagenomics	Biodiversity
DNA Barcoding	Common Fisheries policy: Assessment of Fish stocks

Session 4: From Theory to Application?

In the second interactive session, the four previous working groups were combined to create two groups containing an equal mix of genomic experts, policy makers and entrepreneurs. Using a highly facilitated and interactive step by step process, the session explored the value chain which would lead to the realisation of the potential of the two examples voted on in the previous session, namely DNA bar-coding and metagenomics.

In this case, a value chain was defined as the order of events, developments, transformations and stages through which the tool would need to travel in order to reach its ultimate impact. Interestingly, at the start of the session, the general consensus of the group was that many of the genomic tools being considered were either ready or almost ready for market with regards to use in relation to marine monitoring. The methodology for this session involved three steps and required participation and dialogue between all group members. The template used for this exercise is included in Annex 3.

Step one involved the identification of the ultimate function the tool may have within marine monitoring when considering the most promising application identified in Session 3. This required consideration of the specific activities which would then be carried out, actors who would be responsible, and the ultimate outcome. An example from the DNA bar-coding tool would be the final function of DNA bar-coding would be the assessment of the relative abundance of fish gametes as part of the Stock assessment of fish species under the CFP. This would be the responsibility of government monitoring agencies and would provide a faster, cheaper and more in depth result than current methods.

In step two, the participants were asked to focus on the value chain for the specific tool. Facilitators aided the flow of the discussion by asking probing questions and directing participants to consider the relevant systems surrounding the tool. The participants were again asked to consider the specific activities, responsible actors, and the outcome for each stage in the value chain. This process was repeated until the ultimate outcome was reached.

Step three involved determining a timeline and conducting a cost exercise. Each group worked back through the value chain that they had just mapped and discussed and agreed upon approximations for the timeline and cost that would be required for each stage. Finally, these estimates were tallied to give the overall timeline and costs required to bring the tool from its current status to its ultimate potential.

Results:

Many of the participants were surprised by the final results of this exercise. The cost and timeline results for the DNA bar-coding techniques in particular were far greater than what had been assumed at the end of Session 3. After reflection on, and examination of, the steps involved, it was determined that it could take a minimum of 10 years for this tool to become available for marine policy makers to incorporate it into their monitoring programmes.

The timeline for metagenomics to influence biodiversity monitoring was less surprising as both areas are still in the early stages of formulation.

The discussion of the developmental pathways of these two applications showed that several years of work was likely to be necessary before robust validated tools, of known cost, could be delivered. A discussion on how to best speed up development of promising applications highlighted a need for the development of interdisciplinary teams in future research to ensure that projects focus on practical applications as

well as better defined cost structures and strategies for integration into existing monitoring systems. The discussion also highlighted the critical role legislation plays in creating new markets and driving technology development.
Please see Annex 4 for full results.

Session 5: What are the next steps for realisation?

In the final session of the workshop, the two groups created for Session 4 added a final step to their respective value chain mapping exercises, before coming back together for a plenary discussion.

After reviewing the value chain for their particular tools, each group was asked to carry out a two step exercise. The first step was to identify three major bottlenecks which could affect the realisation of the ultimate impact of the tool. The second step was to consider what actions could overcome each bottleneck identified, providing an indication of the type of action where possible, e.g. RTD, Policy, Knowledge Transfer (KT), etc.

Results:

Each group identified three bottlenecks for their tool (full results can be seen in Annex 5). There were two bottlenecks that were determined to affect the progression of both tools. The first was the validation of the method as a robust tool capable of performing its role as well as, or better than, current methods. The second concerned the motivation and potential uninterest at a policy level with regard to the benefits and advantages of investing in and incorporating the new methods.

Conclusions

- The genomic methods identified by the Oxford meeting and analysed during this workshop clearly have high potential, and provide capability not provided in current approaches.
- Proof of concept of genomic approaches has been demonstrated clearly in several types of generic application.
- However, several steps (likely over several years) are required to develop robust, validated tools of known cost.
- There is a need for focused research on practical applications to speed up the development of specific applications.
- Practical steps to speed up the development of these technologies are needed to gain the advantage of their added capability in surveys, and should include: 1) the use interdisciplinary teams and 2) possible industry led technology development projects. This would help focus on practical applications, better defined cost structures and strategies for integration.
- There is a need for increased focus on the development of bioinformatics and supporting systems needed to handle the magnitude of data which could be produced.

Annex 1: List of Participants:

Name	Organisation	Country
John Benzie	University College Cork	Ireland
Tim Cummins	IIBD Associates	Ireland
Daniel Distel	Ocean Genome Legacy	USA
Raquel Diez	Cetmar	Spain
Thomas Furey	Marine Institute	Ireland
Francesca Mapelli	University of Milan	Italy
Pauhla McGrane	SMART - GMIT	Ireland
Bruno Meola	MedPan	France
David Murphy	AquaTT	Ireland
Cliona Ní Cheallachain	AquaTT	Ireland
Glenn Nolan	Marine Institute	Ireland
Brian O'Farrell	IIBD Associates	Ireland
Maria O'Mahoney	University College Cork	Ireland
Geoffrey O'Sullivan	Marine Institute	Ireland
Andreas Palialexis	Joint Research Centre (JRC)	Italy
Margaret Rae	Martin Ryan Institute	Ireland
Naiara Rodríguez-Ezpeleta	AZTI-Tecnalia	Spain
Malin Strand	Swedish Species Information Centre	Sweden
Tim Sullivan	DCU	Ireland
Hilde van Pelt-Heerschap	IMARES Wageningen UR	The Netherlands
Johanna Wesnigk	EMPA Environmental & Marine Project Management Agency	Germany
Iratxe Zarraonaindia	Argonne National Laboratory, Chicago	USA

Annex 2: Results of Session 3: What is the current status of marine genomic tools?

qPCR	
Comments and Observations on Tool	• Much simpler technology (than transcriptomics) • More portable (than transcriptomics) • Standardisation is equally important for qPCR (although possibly easier to achieve) as compared with transcriptomics
Potential Applications for Tool	• Pathogens • Invasive species detection • Quantification of biomass (with caveats) • Small samples (larvae etc.) • Samples in poor condition (degraded DNA) • Impact assessment (perturbation of bio-indicator species)
Most Promising Application	• Invasive species or any application where you want to sensitively detect a well defined target DNA

Microarrays	
Comments and Observations on Tool	• Established technology • You need to know what you are looking for and its DNA sequence information • Portability is a question • Established cost basics • May estimate abundance
Potential Applications for Tool	• Detecting specific species in a sample → Algal blooms → Pathogens → Invasive species
Most Promising Application	• High priority testing

DNA Bar-coding	
Comments and Observations on Tool	• Increasing barcode library: More barcodes = Quantification of biomass
Potential Applications for Tool	• Fish stock assessment, Common Fisheries Policy (CFP) • Mapping stomach, ecosystem functioning contents of fish • Harmful Algal Bloom (HAB) identification
Most Promising Application	• Fish stock assessment

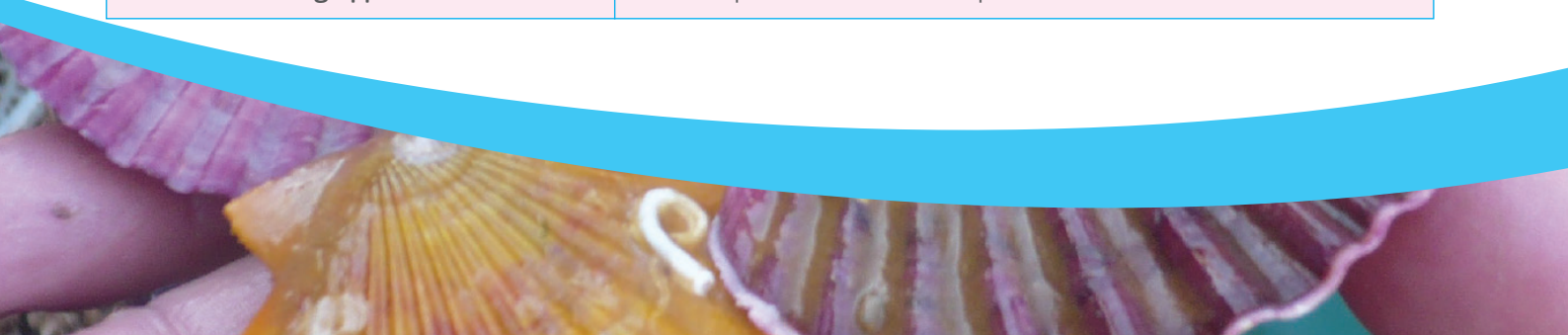
SNP based method	
Comments and Observations on Tool	• Requires references for the targeted species (reference library) • High cost-benefit ratio initially to create reference library but later more cost effective • Seasonality • SNP widely available in human genomics • Doesn't capture directly a descriptor in MSFD but perhaps best used in commercial fisheries management and traceability
Potential Applications for Tool	• Aquaculture escapees: Traceability • Sustainability of fisheries (geospatial distribution of species life stages) • Commercial fisheries management • Regulatory • Branding
Most Promising Application	• Population genetic structure in MSFD (1.3.2)

Metagenomics (1)	
Comments and Observations on Tool	• Rare species suffer from this approach • Qualitative, at best semi-quantitative • Point of samples should always be conserved for future purposes. Once conserved it allows future testing when you know what to look for
Potential Applications for Tool	• Biodiversity rather than seafloor integrity → Species distribution → Population size → Stress related genes → Relative abundance → Filtration → Species shift
Most Promising Application	• Biodiversity

Metagenomics (2)	
Comments and Observations on Tool	• Biodiversity assessment: method provides lots of information that is not necessary, meta bar-coding is the alternative • Need to develop standard procedures • Without knowing anything about the sample beforehand, we cannot estimate costs
Potential Applications for Tool	• Provides information about the potential metabolic activity of the community
Most Promising Application	• Low priority- generally answers more research questions

Transcriptomics (1)	
Comments and Observations on Tool	• Microarray limited for ecosystem monitoring • Problems with RNA gene activity for monitoring of samples handled badly/inappropriately • Transcriptomics good to identify new features of organism, functions and applications e.g. for biodiscovery • Advantages include: → Signs for predicting effect at physiological level and population level → Mode of Action (adaptation to environmental change)
Potential Applications for Tool	• Applied research on newly identified functional genes (biodiscovery) e.g. Pharmaceuticals • Environmental monitoring to determine pollutants and stresses • Determine increase in acidification • Determine chemical pollution - Eutropication • Environmental change and responses to environmental change
Most Promising Application	• MSFD: Pollution (8.2.1- levels of pollution)

Transcriptomics (2)	
Comments and Observations on Tool	• Not portable: Strong laboratory component • More information rich results: available for later mining and reinterpretation • Biases due to turnover rates or different transcripts • Requires serious effort for sampling standardisation • Interpretation of results is complicated
Potential Applications for Tool	• Exploring biological activity in sample • Addresses what is actually happening in the environment • Response to environment change • Exploring biodiversity in environment
Most Promising Application	• Response to environment perturbations



Annex 3: Example of template for Session 4

B	Stage	Activity	Actor(s)	Outcome	C	Timeline	Cost
A	Application						

Annex 4: Session 4: From Theory to Application results

1: Metagenomics	Biodiversity Monitoring

Step A: What is the ultimate application of the tool?

Application	Using biodiversity as a means to comply with MSFD requirements
Activity	Biodiversity monitoring in MSFD
Actors	Government mandated authorities
Outcome	Meeting MSFD requirements for marine monitoring

Step B: What are the steps between the current status of the tool and its ultimate application?

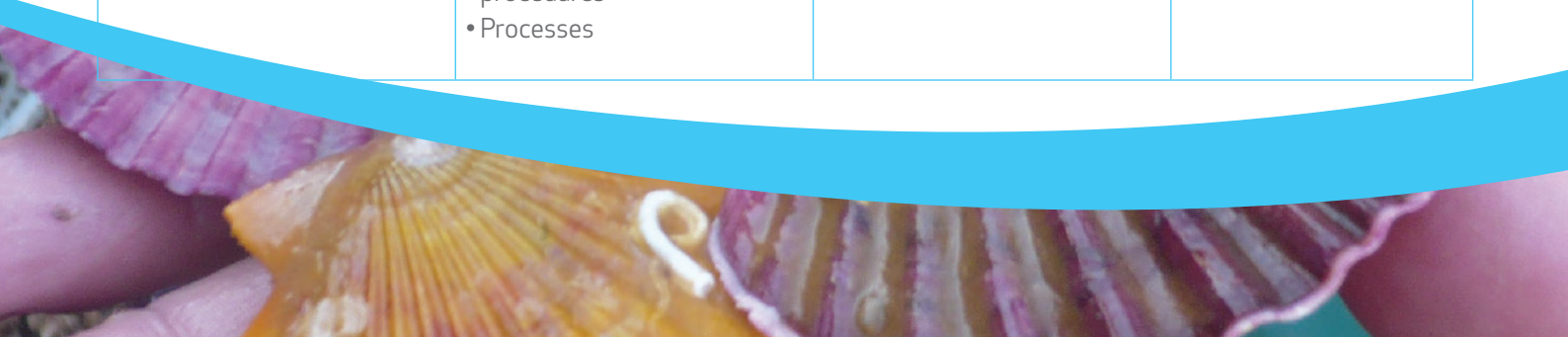
STAGE	ACTIVITY	ACTORS	OUTCOME
RTD	International: Definition of biodiversity National: Pilot studies/ demonstration	Researchers/funding agencies	Understanding the benefits and potential applications of biodiversity monitoring
RTD	Infrastructure: • Labs/equipment • H.R (automation) • Processes • Data management	Research institutes Universities Funding agencies	Increased capacity to carry out biodiversity monitoring
RTD	Standardisation: • Sites of frequency • Standard operating procedures • Processes	Research institutes Universities Funding agencies	A robust test officially recognised by technical community
Demonstration	Case studies in Member State and at an EU level showing: • Applications • Costs • What to monitor	Researchers and monitoring bodies and Industry	Proof of Principle
Policy	Transnational validation: Uptake metagenomic approach	Metagenomic experts DG Env	Uptake by Member State governments

Step B: What are the steps between the current status of the tool and its ultimate application?

STAGE	ACTIVITY	ACTORS	OUTCOME
Policy	Issue tender for monitoring Define frequency	DG Env	Assign contract Carry out monitoring and analysis of results
Policy	Scope: Currently: Limited locations and indicators of species and abundances Future: Increased species monitored	Competent authorities	Increased usage of the method
Policy	Monitoring • Planning • Collection • Sample handling/ splitting/ transport/labelling • Analysis • Storage • Interpretation • "DB" • Reporting	Competent authorities	Ultimate impact: Use of methodologies to indicate what GES is and also provide results to remain compliant with MSFD requirements

STEP C: Timelines and Cost

STAGE	OUTCOME	TIMELINE	COST
RTD	International: Definition of biodiversity National: Pilot studies/ demonstration		
RTD	Infrastructure: • Labs/equipment • H.R (automation) • Processes • Data management		
RTD	Standardisation: • Sites of frequency • Standard operating procedures • Processes		



STEP C: Timelines and Cost

STAGE	OUTCOME	TIMELINE	COST
Demonstration	Case studies in Member State and at an EU level showing: • Applications • Costs • What to monitor	<1 year - ongoing	~ €250 million
Policy	Transnational validation: Uptake Metagenomic approach	2-3 years	€10,000
Policy	Issue tender for monitoring Define frequency		
Policy	Scope: Currently : Limited locations and indicators of species and abundances Future: Increased species monitored		
Policy	Monitoring • Planning • Collection • Sample handling/ splitting/ transport/labelling • Analysis • Storage • Interpretation • "DB" • Reporting		

Annex 4: Session 4: From Theory to Application

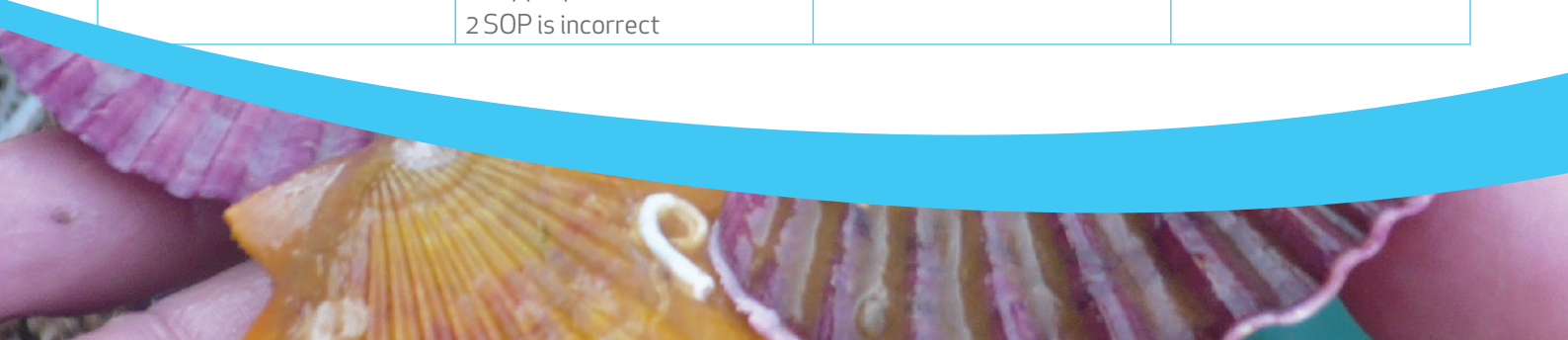
2: DNA Barcoding	Common Fisheries Policy: Assessment of fish stocks

Step A: What is the ultimate application of the tool?

Application	Assessment of the relative abundance of fish gametes as part of the Stock assessment of fish species under the CFP.
Activity	Set formal method for assessing planktonic fish species (designated in legislation) which provides information on relative abundance.
Actors	National Fisheries Institutes/Governmentally mandated organisations
Outcome	Faster, Cheaper, More accurate method requiring less technical expertise in gametes (fish).

Step B: What are the steps between the current status of the tool and its ultimate application?

STAGE	ACTIVITY	ACTORS	OUTCOME
RTD	Testing a known set of genes a) Protocol development for the biomass abundance gene b) Development of bioinformatics pipeline	Researchers: There is an identified need for this research	Identify the genes that show biomass
RTD	Validation: 1. Compare conventional tests (naked eye/microscopy) with the same sample 2. Test for accuracy using synthetic samples Feedback loop: If results are unfavourable then step one needs to be re-visited.	Researchers	• Reliable test for biomass and identification • Standard Operating Procedure (SOP)
Other	Cost benefit analysis	Researchers/social economists in research institute/university/external consultants	Official declaration of benefits compared to traditional methods
RTD	Inter-laboratory Validation: Similar to second step but using external laboratories to test the method. Feedback loop: If results are variable then two possibilities: 1 Individual laboratories procedures are incorrect or inappropriate 2 SOP is incorrect	Researchers/technical groups	A robust test officially recognised by technical community



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2: DNA Barcoding	Common Fisheries Policy: Assessment of fish stocks

Step B: What are the steps between the current status of the tool and its ultimate application?

STAGE	ACTIVITY	ACTORS	OUTCOME
Policy	Official policy acceptance of method: • Need a clear report of inter-laboratory validation plus expert advice • Informing stakeholders could also play a large role in uptake	Researchers, JRC, other related policy actors, Common Fisheries Forum.	Accepted into official monitoring programmes on a Member State level
Education	Training on a Member State level	Government authorities on an EU and Member State level. Currently EU Member States take it in turns to provide training on monitoring techniques	Ultimate application: Official use as the designated method for the assessment of the relative abundance of fish gametes as part of the stock assessment of fish species under the CFP.

STEP C: Timelines and Cost

STAGE	OUTCOME	TIMELINE	COST
RTD	Identify the genes that show biomass	2-4 years	Ran out of time
RTD	• Reliable test for biomass and identification • SOP	4-6 years	Ran out of time
Other	Official declaration of benefits compared to traditional methods	>1 year	Ran out of time
RTD	A robust test officially recognised by technical community	1 year	Ran out of time
Policy	Accepted into official monitoring programmes on a Member State level	1-5 years +	Ran out of time
Education	Ultimate application: Official use as the designated method for the assessment of the relative abundance of fish gametes as part of the Stock assessment of fish species under the CFP.	1-2 years	Ran out of time

Result: It could take at least 10 years and up to 19+ years for DNA bar-coding to replace current methods.

Annex 5: Session 5: What are the next steps for realisation?

1: Metagenomics	Biodiversity Monitoring

BOTTLENECK	ACTION TO OVERCOME
Validation of Method and application to MSFD	RTD /KT: Cost Sell multi- benefits
Convince Member State governments	Policy: Lobby – small add-ons
Sufficient capacity	Centralise? • Training (bioinformatics) • Infrastructure • Investment

2: DNA Barcoding	Common Fisheries Policy: Assessment of Fish stocks

BOTTLENECK	ACTION TO OVERCOME
Identification of the biomass gene: If unsuccessful then there this application is invalid	KT: Share research results and methodologies to learn more and decrease duplication
Effective validation: You might find a gene but it might not work in the SOP	KT: Share research results and methodologies to learn more and decrease duplication
Legislative approval: Actually making the change	Other: Confidence in tool and in benefits – no maybes or possibly. Lobbying and marshalling evidence in a clear manner