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Interim Report of the Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM)

11–13 May 2016

Belfast, UK

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM) convened at the School of Biological Sciences, Queens University, Belfast UK, 11–13 May 2015. Thirteen delegates from 7 nations, discussed four Terms of Reference (ToRs), and associated matters. In addition there were ongoing discussions about mechanisms for enhancing the role of WGAGFM within the ICES community, and beyond, including potential contributions to the ICES Open Science session, and the ICES Science Fund, as well as specific responses to designated questions from the Working Group on Integrated Morphological and Molecular Taxonomy (WGMIT) and the Stock Identification Methods Working Group (SIMWG). The latter two Expert Groups had considered topics complimentary to those within WGAGFM, including the evidence base for genetic structuring in commercially exploited species, and the burgeoning role of DNA meta-barcoding in marine ecosystems. During 2015, and in conjunction with the ICES secretariat, there has been a review of WGAGFM membership, resulting in several new members joining the WGAGFM: Harri Vehvilainen, Finland; Johan Östergren, Sweden; Malte Damerau, Germany.

Four multi-annual ToRs were considered, including a new 2-year ToR focusing on a novel approach for estimating abundance of deep sea fishes (ToR d). Members continued deliberations on the range of molecular techniques to evaluate infectious disease and parasite spread from seafood into wild populations (ToR a). A tool for assessing infection risk across host-pathogen scenarios was proposed, categorised according to a scale based on infection and transmission potential. Such benchmarking yields a tractable method for identifying high risk situations. Key challenges for screening pathogens in seafood were discussed in relation to recent developments, most notably the ability for standardised high throughput taxonomic identification, and distinction between pathogen presence and pathogen viability. A realistic workflow was identified for robust molecular screening of pathogens in seafood based on DNA barcoding and DNA metabarcoding. The mechanisms for integrating WGAGFM advice into fisheries assessment and management (ToR b) were extended by identifying case studies of impact, including the role of genetics in promoting detection of species and stocks within an ecosystem-wide perspective, aspects of risk assessment and adoption of tools to support development of economically important traits and enforcement of international regulations. New approaches likely to promote integration of WGAGFM outputs within the ICES community include environmental DNA (eDNA) as tools for detecting biodiversity and function at the community and ecosystem levels, the role of microbiomes in fish health, performance and aquaculture, and identification of key genes and metabolic pathways underpinning response to environmental change. The challenges of elucidating the genetic basis of adaptive shifts in exploited species was considered further in relation to new opportunities arising from quantitative genetic analyses (ToR c). Additional recent advances were considered in relation to key challenges such as climate change, fisheries induced evolution, biological invasions, and aquaculture impacts. Such impacts on marine resources generate different types of population responses. A key objective of ToR c in 2016 was to consider how contemporary and emergent tools, such as genome-wide association studies and trait-gene associations can be employed to assess and monitor impact. A new ToR (d), focused on the use of a recently developed approach for estimating population abundance in blue-

fin tuna, and the potential for transfer to deep sea fishes. The utility of the approach was discussed in the context of the high vulnerability of many deep sea fishes to exploitation and environmental change, as well as the intractability of obtaining robust and representative data from sampling programmes. In particular, a range of molecular based markers and an associated statistical genetic framework were evaluated for: 1) their utility in detecting close-kin genetic relationships (genetic tagging) within wild stocks and 2) using this information within a mark-recapture framework to estimate stock abundance in the context of yielding scientific advice implemented under the remit of the Common Fisheries Policy (CFP). Initial simulations indicated some ambiguity in underlying assumptions affecting robustness of estimates. Further tests and simulations under a range of scenarios will be tested further in Year 2.

Other activities, reported elsewhere, included further consideration of opportunities for promoting training in fisheries and conservation genetics, engagement of the WGAGFM in the 2016 ICES ASC, value of related activities supported by the ICES Science Fund, and preparations for the 2017 WGAGFM, to be hosted by the Centre of Marine Sciences (CCMAR) at the University of Algarve, Portugal.

1 Administrative details

Working Group name

Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM)

Year of Appointment

2015

Reporting year within current cycle (1, 2 or 3)

2

Chair(s)

Gary Carvalho, UK

Meeting venue

Queens University, Belfast, UK

Meeting dates

11–13 May 2016

2 Terms of Reference a) – z)

ToR a) A review of existing and potential molecular techniques to evaluate infectious disease and parasite spread from transferred seafood into wild populations

Contributors: Claudia Junge, Pierre Boudry, Daria Zelenina, Martin Llewellyn, Naiara Rodriguez-Ezpeleta & Filip AM Volckaert

Large volumes of live or frozen seafood products are transferred between continents and regions, hence crossing biological barriers. These products may contain communities of harmful micro-organisms (viruses, bacteria and eukaryotic unicellular parasites) and multicellular parasites, which upon establishment in the new environment, can entail multiple (often underestimated) consequences such as i) economic losses for fisheries and aquaculture due to infections, ii) substantial impact on local biodiversity, and iii) biosecurity issues, such as appearance of zoonoses. Yet, despite the scale of the seafood business, the inventory and monitoring of these biological hitchhikers is at best incomplete, and therefore merits close scrutiny. Current (meta) genomic and genetic methods represent potentially cost-effective and accurate approaches for routine screening of harmful organisms in seafood, but few of them have been implemented. Hence, a review of existing and potentially applicable genetic tools for disease and parasite spread in seafood is needed, which might be further corroborated by WGPDMO and WGAGFM.

ToR b) Review and map decision channels for integrating WGAGFM advice into fisheries assessment and management

Contributors: Geir Dahle, Gary Carvalho, Jann Martinsohn, Dorte Bekkevold, Tom Cross, Phil McGinnity, Torild Johansen, Martin Taylor (3-year)

It is a scientific aim to integrate genetic monitoring and assessment methods into advice and management. There is, in principle, particular potential to implement advances in salient concepts and technologies into fisheries resource management, governance and policy formulation. The overall aim is to enhance the integration of genetic monitoring and assessment methods into advice and management. The nature and effectiveness of implementation processes, as well as a consideration of strategies to promote such integration within the context of the ICES structure and community and beyond will be considered. As such, the ToR provides an opportunity to review past and current impact of outputs generated via the annual WGAGFM meetings and associated activities.

ToR c) Review application of quantitative genetic techniques into non-mariculture marine species

Contributors: Sarah Helyar, Dorte Bekkevold, Ian Bradbury, John Gilbey, Phil McGinnity, Paulo Prodohl, Malte Damerau (3-year)

Quantitative genetics has been utilised by the aquaculture industry for many years to improve a range of traits relevant for the industry; including morphometric traits and increased resistance to parasites. Advances in molecular technology and statistical analyses are now making the application of quantitative genetics a realistic possibility for wild-capture fisheries. Some of the key challenges that remain in the conservation and management of wild fishes are understanding and predicting adaptive responses, in particular, in response to human activities including fishing, human-modified ecosystems, conservation efforts and the effects of climate change. There is growing recognition that these influences are important in shaping the evolution of fish populations, but there is still little knowledge of the quantitative responses of populations. This ToR will summarise the research to date, and explore the major role that quantitative genetics can play in the key issues of conservation and management of fish populations: the evolutionary effects of fishing and adaptation to climate change.

ToR d) Close-kin mark recapture approaches to estimate abundance and population parameters of deep-sea marine fish species in support of enhance management under the Common Fisheries Policy

Contributors: Jann Martinsohn, Ernesto Jardim & Naiara Rodriguez-Ezpeleta, Jens Carlsson, Paulo Prodohl, Ilaria Coscia

According to the European Commission, particular attention is needed to secure the sustainable exploitation of deep-sea stocks in view of their vulnerable nature. For many stocks, knowledge and data remain insufficient for scientific analysis (COM (2007) 30 final), which is also reflected in recent TAC and Quota setting. Moreover, according to the European Commission, the poor state of key deep-sea stocks and the lack of scientific data clearly demonstrates the need for an improved management framework for deep-sea fisheries, as proposed by the Commission in 2012 (see IP/12/813). Based on recent research by CSIRO Australia, using close-kin analysis, a method that has particular potential for generating abundance for the management of Southern Bluefin Tuna, utility

for transfer to deep-sea species will be assessed. In particular a range of genetic techniques and their utility for close-kin mark-recapture applications will be evaluated with respect to feasibility and utility in the context of yielding scientific advice implemented under the remit of the CFP.

3 Summary of Work plan

ToR a) Year 1: Review of the literature on molecular detection of infectious agents to identify the widely used ones and assess their advantages and disadvantages; Review available high-throughput sequencing and genotyping techniques potentially applicable for infectious agent identification/detection; identify advantages and disadvantages. **Year 2:** Identify the challenges for screening seafood and produce genetic/-omic tools roadmap. Share produced review with WGPDMO to get insights into new avenues for the application of molecular methods to improve early detection of infectious agents in transferred seafood and share their applicability with policy makers and managers. **Year 3:** Continue knowledge exchange with WGPDMO as well as external experts. Evaluate various screening methods and give recommendations. Produce a final report and publish a position paper.

ToR b) Year 1: Document the history and goal of the WGAGFM within the ICES structure and science mission; to collate detailed information on past WGAGFM ToRs in relation to potential and actual impacts within the ICES community and beyond; to identify the framework of potential range of synergies and overlap among ICES EGs and WGAGFM remit. **Year 2**: Send questionnaire to other Expert Groups concerning awareness of the WGAFM; identify two or three relevant Benchmark meetings for potential engagement; continue evolving the network description of the WGAGFM and salient EGs within ICES; to examine the nature of impacts of WGAGFM ToRs within the ICES community, science mission and beyond; to identify potential obstacles influencing impact within ICES and beyond. **Year 3**: Contact selected bodies (externally) to investigate response to previous year's recommendations; survey the recommendation relating to the less successful – "database" ToRs; classify the range of recommendations relevance; synthesis and identification of key constraints and opportunities for realising impact of WGAGFM actions within and outside the ICES community in relation to management advice and policy formulation.

ToR c) Year 1: Detailed justification of importance for ICES and initial literature review; Review of literature relevant to the application of quantitative genetic methods to wild capture fisheries. **Year 2**: Continuation of literature review with addition of papers to shared online library. Review WGEVO ToRs from recent years to assess complementarity (and contact if appropriate); Contact Dr Kerry Naish (School of Fishery and Aquatic Sciences, University of Washington, Seattle) with a view to collaboration on review paper; Production of conceptual figure illustrating how quantitative genetic approaches as applied to fisheries issues. **Year 3:** Finalising synthesis and applications, with any new case studies; Production of review paper.

In 2015/16 a JRC Technical report was produced, offering a reflection and review of the close-kin approach suggested by CSIRO in Australia in the context of commercially exploited deep-sea fish species. This report served as a starting point for further evaluation and first simulations documented in this interim report. In June 2016 a web-conference

meeting will be convened to establish a strategy that allows to move closer to a practical project type of approach. Also ICES WGDEEP will be contacted to learn more about their work on deep sea species and knowledge gaps and needs that could be covered by a genetic close-kin approach. Outcomes of these activities will be documented together with recommendations in 2017.

4 List of Outcomes and Achievements of the WG in this delivery period

Ongoing.

5 Progress report on ToRs and workplan

5.1 ToR a) Review of existing and potential molecular techniques to evaluate infectious disease and parasite spread from transferred seafood into wild populations

Rationale

Contact between transferred seafood and wild populations may introduce non-local pathogens into previously naïve populations. These newly introduced pathogens (viral, microbial or multicellular) therefore exhibit similar characteristics to invasive species, leading to some similar problems, like an uncontrolled spread due to the lack of predators and/or superiority over other species, on top of the eminent danger of transmitting diseases into the wild.

Trade in live and processed seafood products has augmented worldwide translating into increased opportunities for infectious diseases to cross natural biological barriers, potentially causing important economic losses, impacts on local diversity, and biosecurity issues. It is therefore important to have biosecurity tools that not only reliably detect pathogens' presence in seafood, but furthermore assess their viability and potential epidemiological risk. Those tools need to be sufficiently fast, accurate, reliable, robust and cost-effective to be implemented into routine screening programs for seafood. International frameworks for promoting effective disease management such as the World Organisation for Animal Health (OIE – https://oie.int) strongly endorse such developments.

Such needs can be achieved through molecular techniques (DNA or RNA based), particularly, with the increased feasibility of high-throughput sequencing and genotyping. Although some examples of successful application of DNA-based methods for pathogen detection and identification exist, there is much more potential for further development, notably for multicellular parasites. In this group, immunological methods remain the state of the art, whereas bacterial and viral diseases are increasingly screened with molecular techniques. This is especially crucial for non-cultivable parasites, bacteria and viruses where the use of molecular methods is either the only or the only cost-effective solution.

Progress report

A number of host-pathogen situations leading to different infection risks were characterized and their infection risks (on a scale from 0 to 10) were calculated by multiplying their infectious potential with their transmission potential (Figure 1). We consider this a useful tool to highlight the highest priority situations.

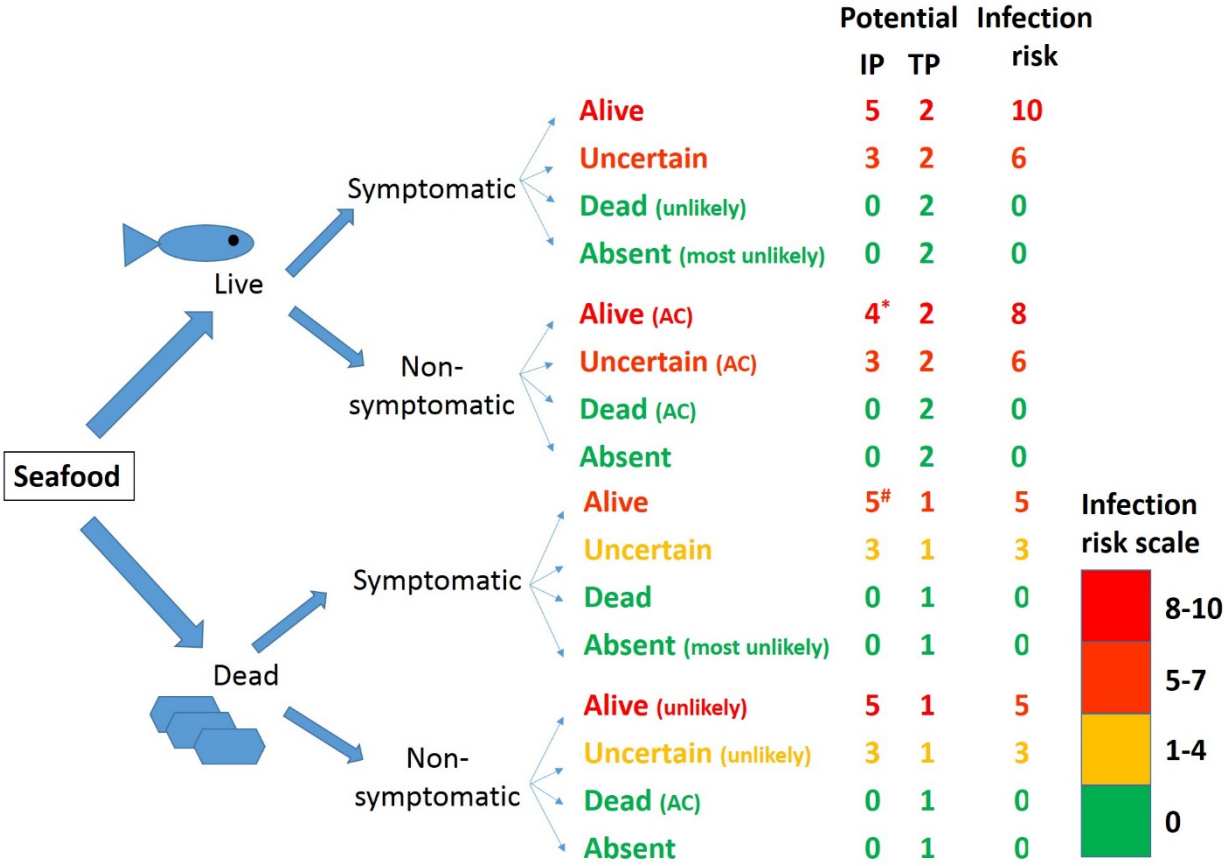

Figure 1. Host-pathogen infection situations with respective infection risks. AC= asymptomatic carrier, IP= infectious potential, TP= transmission potential; *condition dependent: threshold, reservoir function, state of immune system, # condition dependent: biology of the pathogen (i.e. requirements from host), type of processing.

Six challenges with respect to screening pathogens in seafood were identified by the team (Box 1), and further explained and potential solutions suggested (part I). In part II, available molecular methods were evaluated based on the purpose and sampling material for screening and a work flow chart was created (Figure 2).

Challenges for screening of pathogens in seafood
1. Screening units
2. Uncultivable pathogens
3. Detection vs. Disease
4. Sampling material
5. Viability of pathogens
6. Infection threshold

Part I: Screening challenges for pathogens in seafood

The taxonomic units of identification

Identification of pathogens must be performed at the most relevant taxonomic level, i.e. genus, species, or strains/genotypes. For some pathogens however, like bacteria and viruses the species boundaries are not very clear-cut.

Sometimes identification of the genus can be useful if the distinction between species is not readily possible. Most PCR based methods allow for this level of detection.

In many cases it will be necessary to accurately identify the individual species of pathogen. There are two possibilities for identification based on PCR assessing 1) presence/absence using species-specific primers, 2) sequencing of a PCR product using universal primers, e.g. barcoding at COI, and comparison against a genetic database (Genbank: www.ncbi.nlm.nih.gov, BOLD: www.barcodeoflife.org)

For haplosporidian oyster parasites *Bonamia exitiosa* / *ostreae*, for example, the two species can be distinguished with a single locus approach is commonly used (Hill *et al.* 2014). However, species-level identification is not epidemiologically informative for some pathogens where strain-specific features underlie differential levels of pathogenicity. *Vibrio aesturianus,* for example is a commonly reported marine bacterium (Tison *et al.,* 1983) - not all strains are pathogenic. Distinguishing strains that do represent a threat is challenging and it is therefore necessary to make distinctions based on strain genotypes, as well as the presence / absence of plasmids and other mobile genetic elements, especially if they encode virulence factors. One novel approach, which exploits the functional disconnect between taxonomy and pathogenicity is the PathoChip (10.1038/ismej.2013.88.) a hyrbidisation array enriched for virulence factors derived from over 2000 different strains.

As well as detecting functionally significant variants on the context of disease, high-resolution genotyping can also facilitate phylogeographic and demographic modeling to provide source attribution for pathogen transfer. Infectious Salmon Anemia Virus outbreaks in Chile in the early 2000s were fairly unequivocally traced back to Northern Europe by this means (10.1186/1471-2148-11-3490).

Non-cultivable pathogens

Non-cultivable pathogens dominate in the marine realm. Therefor a widely used alternative to cultivation is amplicon sequencing of environmental samples (eDNA) or samples

of pathogens infecting a host. Here there is no need for any incubation time on specific media; sample concentration and DNA extraction suffice. Identification of the OTUs relies on genomic data banks. For example, Lohan *et al.* (2016) pyrosequenced ship's ballast water to detect putative pathogens.

Detection vs. Disease

The detection of a pathogen is not confirming the associated disease. There are several factors that play a role. Firstly, if the detection is based on DNA techniques, the presence of pathogen DNA does not imply that the pathogen is active and alive, therefore it is uncertain if it has infectious potential (see Figure 1). Secondly, even if the pathogen is alive, its ability to provoke an infection is uncertain, and its host could be an asymptomatic carrier merely functioning as a pathogen reservoir.

Molecular methods targeted to the activity (e.g. gene expression) rather than detecting presence are the methods of choice to detect an ongoing infection by the pathogen in question. The combination with e.g. biological validations through culturing and incubation is recommended, but not possible for uncultivable pathogens. For those pathogens, molecular techniques are therefore the methods of choice.

Sampling

Important for sampling is knowledge on the full lifecycle such that pathogens can be sampled in the environment, and the intermediate and final hosts. Lack of such knowledge requires broad–scale sampling. Important is to consider material transferred alongside seafood, such as seaweeds and epifauna.

Table 1. Sampling context of pathogens, including example, technique and comments.

Sample context	Example	Technique	Comments
Environmental water	Virus (herpes virus in *C. gigas*) Amoebic gill disease (*P. perurans*)	eDNA Metabarcoding	Virus/pathogen can be present in the absence of disease
Environmental sediment	Microsporidian (*Bonamia*??)	eDNA Metabarcoding	Ditto
Biological organism (primary host or secondary / reservoir host)	Reservoir host e.g *C. gigas* & *Bonamia*, widespread screening.	Multiple approaches	Can *C. gigas* function as a vector for *Bonamia* spread when transplanting across areas?
Unprocessed seafood (fillets,organs, roe, …)	Molecular epidemiology - ancestry reconstruction - e.g. ISAV - identify origin	Multiple approaches	
Processed seafood	Listeria in smoked salmon	RNA or DNA based; high-throughput NGS amplicon sequencing	Processing of the seafood increases the challenge to detect the primary pathogen

Viability of pathogens in dead hosts

In the wild, different pathogens have variable survival times within their natural habitat, which constitutes of either their hosts and/or their environment, i.e. seawater, sediment, air. While in their hosts, in this case seafood, they are dependent on the condition of their host, to various extents. When their hosts die, they face a number of different challenges associated with e.g. lack of nutrients and circulation, decaying matter, as well as different seafood processing procedures. Those include e.g. freezing, boiling, smoking, frying, drying, and fermenting. Many processing methods are in fact meant to ensure seafood safety for human consumption through killing all harmful pathogens. Therefore properly distinguishing between presence and viability is crucial in processed seafood, e.g. parasite presence in dried seafood vs. e.g. presence of viable cysts/eggs. During the processing, there might be several opportunities to transfer pathogens to wildlife through e.g. water, an accurate record keeping of the chain of processing is therefore vital in order to track movements of seafood, pathogens and associated diseases. Based on their applications, certain types of processed seafood, like fishmeal, might be more dangerous for spreading pathogens.

Detection of pathogen DNA does not necessarily mean that there are viable, infective propagules in any material that tests positive. As well as experimental infections / inoculations of susceptible hosts using such material, several genetic tests may also prove informative (10.2217/17460913.4.1.45). Among these, RT-qPCR targeting actively transcribed mRNA is among the most promising approaches.

Threshold decision/detection

In case of pathogen detection in seafood, its quantification might be necessary in order to evaluate the infection risk. This is particular the case where a given pathogen is naturally present in small quantities in the environment but where a concentration above a certain threshold is a sign of infection enabling effective disease transmission, therefore posing an infection risk to the environment. In any case, when it comes to seafood's infectious nature and seafood safety, a cautionary principle is usually applied with low respective thresholds. The infection thresholds are highly pathogen specific and if a pathogen has the capacity to infect multiple host species, they might be even host-parasite system specific, and need to be determined carefully by pathologists. For this reason, quantitative methods of detection are necessary. Molecular techniques that are appropriate for this application are qPCR and hybridisation arrays.

Part II: Molecular tools for screening pathogens in seafood

We developed a hands-on workflow to aid decision-making when analysing pathogen samples from seafood (Figure 2). Decisions are based on i) a priori knowledge of the pathogen Y/N, and ii) application, i.e. identification, detection, quantification, or viability.

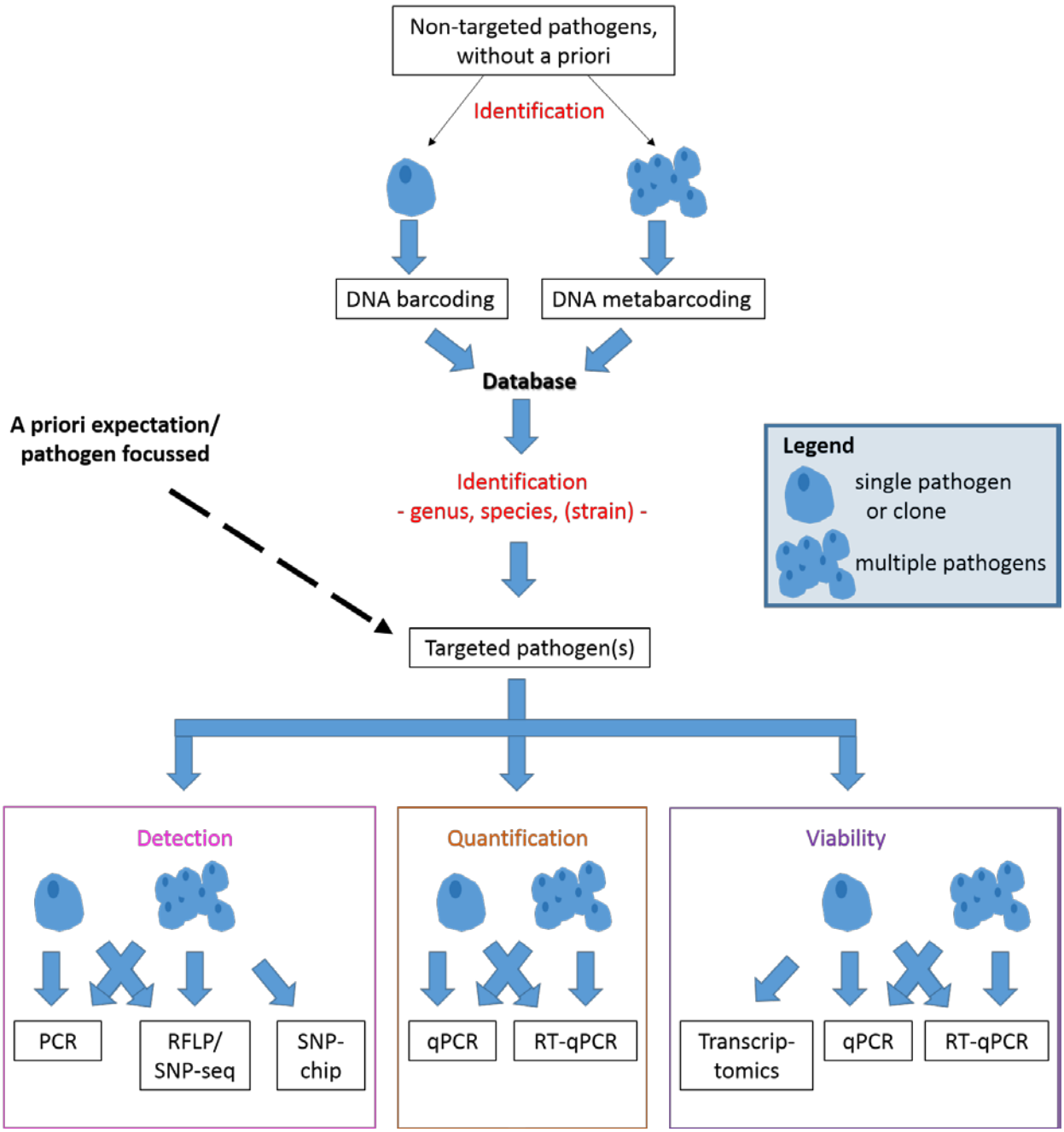

Figure 2. Workflow diagram to illustrate pathogen analysis pathways. Methods are described elsewhere.

DNA–based method

DNA-based methods rely on the positive identification of a unique (set of) marker(s) linked to the identity of the species. For multicellular eukaryotes this is linked to a mitochondrial marker and organized in the context of the Barcoding of Life project (http://www.barcodeoflife.org). Protists are documented in the protists ribosomal reference database (Guillou *et al.* 2016). Bacteria are barcoded based on a combination of sequences.

Most methods are PCR-based such as the confirmation of the presence/absence of a specific pathogen or a combination of pathogens (DNA-chip). An alternative is the detection of a specific pathogen based on PCR-RFLP method, which is widely used in case of a suspected pathogen. Positive identification of a pathogen is possible with DNA barcoding. A sample is amplified with universal primers for a diagnostic marker (COI, ribosomal RNA) and sequenced.

"PCR-free" methods are gaining importance because of the high-throughput. Next Generation methods targeting specific amplicons (genetic markers) allow for the detection of a wide range of pathogens in a large number of samples. Metabarcoding screens for "all" potential pathogens.

Metabarcoding, i.e., the taxonomic identification of individuals present in an environmental sample based on a small fraction of the genome (barcode) is a promising approach. This method allows sequencing the same homologous fragment of the genome in the organisms present in a complex sample using so-called universal primers (they are able to amplify a wide range of taxonomic groups); once sequenced, the fragments can be compared against a database in order to identify the species present.

Microfluidic high-throughput genotyping based on probes of known infection agents has also proven useful for example in the identification of parasites of several salmon species. This method consists of amplifying by RT-PCR simultaneously hundreds of samples using hundreds of different primer pairs. In a single assay the presence of multiple potential infectious agents, as well as their abundance, can be determined.

RNA–based method

RT-PCR – reverse transcriptase polymerase chain reaction is a highly sensitive technique for the detection and quantitation of mRNA (messenger RNA). The technique consists of two steps: 1) the synthesis of cDNA (complementary DNA) from RNA by reverse transcription (RT) and 2) the amplification of a specific cDNA by the polymerase chain reaction (PCR). RT-PCR may be used for detection of expression of pathogen-specific genes for DNA viruses, bacteria and multicellular parasites as well as for measuring viral load with RNA viruses. The fact of expression means that the pathogen is alive and active.

Real-Time qRT-PCR – real time quantitative reverse transcription PCR allows not only to detect the presence of pathogen-specific mRNA but to evaluate the level of gene expression as well.

Transcriptomics is one of fast developing approaches in next-generation sequencing era focusing on analysis of the transcriptome i.e. the complete set of RNA-transcripts is a tissue or individual cell. Comparison of transcriptome sequencing results with genetic

databases allows for the simultaneous detection of multiple pathogen-specific genes expression.

Conclusion

There is a need for coordinated approaches between seafood pathologists and genomics experts as chances for zoonoses are steadily increasing. This requires the combination of fundamental, applied and operational research.

5.2 ToR b) Review and map decision channels for integrating WGAGFM advice into assessment and management of aquatic resources

Aim: To enhance the integration of genetic monitoring and assessment methods into advice and management. ToRb will identify implementation processes and advise on how the impact of potential obstacles can be reduced.

5.2.1 Introduction

Key features of the WGAGFM in the context of ICES management priorities

The role of WGAGFM was discussed further and can be summarised as:

1) to promote the inclusion of genetics and evolutionary concepts and methods as important elements in the management of fisheries, mariculture and aquaculture.

2) to establish a representative, sustained and engaged scientific forum across ICES countries to discuss technological and statistical developments and new ideas in genetics/genomics, salient opportunities for research consortia, and exchange at the science-policy interface.

We believe that the ever-increasing importance of genetics in fisheries and aquaculture development, illustrated recently by the FAO decision to establish the State of the World's Aquatic Genetic Resources, 2016 (http://www.fao.org/fishery/AquaticGeneticResources/en), endorses the integration of genetics and genomics into management practice at the international level. Previously it was perceived that demographic and evolutionary changes in response to natural (climate induced) or anthropogenic phenomena were slow and consequently of little relevance to fisheries managers. It is now known, however, that many marine species shift their biological characteristics and distribution over short, ecological time-scales (Hauser & Carvalho, 2008). Increased dialogue is urgently required by managers to detect, assess and respond to such genetic changes by appropriate management actions. The role of WGAGFM in relation to aquaculture is equally important as in the case of capture fisheries. For example, genetics underpins breeding programmes, selection for commercially relevant traits and is extremely important for economic development, global food safety and security. Many major breeding companies for fish and invertebrates recognise the central role of genetics for their development, whereas the role of genetics in relation to capture fisheries appears to be less established, though there are notable exceptions as described below.

Another emerging issue of common concern is the interaction between captively bred individuals deliberately (stocking/ranching/enhancement) or accidentally (farm escaped) released into the wild and their direct (by interbreeding with native conspecifics) or indirect (by disease or competition) impacts on wild populations. Furthermore there has been a paradigm shift in the field of genetics, with a progressive move from studies that examine a few genes of often unknown function, to genomics, where the focus is on entire or large parts of genomes, as initiated by the sequencing of the human genome. Such genome-wide approaches are particularly important in fisheries and aquaculture, especially with the availability of entire genome sequences now for many species such as salmon, trout, cod, tilapia, oysters, and sea bream. From these efforts, an ever-increasing number of functional genes have been identified that influence commercially important traits such as growth rate, disease resistance, and domesticity (aggression, stress response). Such knowledge represents an invaluable natural resource for exploitation within the sphere of fisheries and aquaculture.

5.2.2 Why are the activities of WGAGFM relevant to the conservation and management of aquatic resources?

With accelerating advances in methodological and conceptual approaches, there are a corresponding bourgeoning of applications pertinent to fisheries and aquaculture, some of which we highlight below. Successful selected applications of genetics in the context of marine resource management include:

1) Modern molecular methods allow the accurate identification of species, communities and ecosystem processes (Alyagas *et al.*, 2016; Bucklin *et al.*, 2016) and population structure of marine fish and invertebrate species (Nielsen *et al.*, 2013), which has potential to redefine taxonomic and stock boundaries that more closely match biological reality, therefore promoting sustainability.

2) Genetic methods have identified the impact of sub-specific population structuring on productivity (Heath *et al.*, 2015) and vulnerability to local extinctions (Hutchinson *et al.*, 2003).

3) The existence of long-term archived scales and otoliths in most fisheries institutions has yielded new insights into the population dynamics of exploited species in the face of environmental change and harvesting pressures (Hauser *et al.*, 2002; Bonanomi *et al.*, 2015).

4) High throughput and cost-effective genetic analysis has enabled the deployment of real time methodologies such as mixed stock analysis of Pacific salmon (the so-called GSI programme; Larson *et al.*, 2014) and for genetic monitoring of Atlantic salmon, with potential extension to commercially important marine species.

5) Modern genetic markers combined with common garden field experiments have enabled quantification of the negative effects, in terms of production and genetic integrity, of farm-escaped salmon interbreeding with wild individuals (McGinnity *et al.*, 2003; Glover, 2010). The approach has also demonstrated the extent of local adaptation in wild salmon populations, elucidating the dangers associated with enhancement activities.

6) The outputs from the EU-funded FishPopTrace project (https://fishpoptrace.jrc.ec.europa.eu/) that focused on populations of marine fish species (cod, herring, sole, hake), directly influenced the incorporation of genetic approaches to traceability in the Common Fisheries Policy.

7) Genetic approaches have revealed unexpected features of some marine fish populations; most notably the marked disparity between census and effective (number of breeding individuals) population size, with the latter being up to several orders of magnitude smaller (Hauser *et al.*, 2002). Such information is of major importance in predicting the vulnerability of certain commercial species to environmental change and over harvesting.

8) Genetic approaches have revolutionised our ability to confirm authenticity and traceability of fish and other seafood products throughout the food supply chain ("fish to fork"; Martinsohn *et al.*, 2011; Helyar *et al.*, 2014). Such approaches have been extended to tackling illegal fishing (Nielsen *et al.*, 2013) and enforcement of fishing and aquaculture regulations.

9) Integrated molecular and common garden experiments have demonstrated for the first time a genetic basis to fisheries-induced shifts in body size and maturation (Van de Wijk *et al.*, 2013). Such disclosure highlights the need to reconsider the capacity of harvested populations to adapt to, and recover from, harvesting and predation.

In addition, recent technological and conceptual advances have opened new frontiers in the integration of genetic approaches to management and conservation of aquatic resources.

Foremost among these are the following:

- **Metabarcoding**: it is now possible using high throughput techniques to characterise community biodiversity across multiple trophic levels simultaneously (e.g. predator-prey, host-parasite, and producers and consumers), thereby promoting Ecosystem-based approaches.
- **Environmental DNA (e-DNA):** rapid advances in the retrieval of free-floating DNA taken directly from water samples allow the detection of multiple species without the need to sample organisms directly (Bohmann *et al.*, 2014). The integration of metabarcoding and eDNA analyses promotes further the opportunities to examine community and ecosystem-wide dynamics in structure and function, as well as providing robust tools for the early detection of invasive species.
- **Disease diagnosis**: Molecular techniques involving DNA and RNA detection are increasing in sensitivity, to one or a few molecules, so enabling earlier diagnosis of potential disease-causing organisms. Such developments are the subject of a current ToR for the 2016 WGAGFM meeting (ToR a).
- **Analysis of microbiomes** (the microorganisms colonising a particular environment of the host, such as skin, gill, gut). The study of bacterial species occupying the vertebrate gut or skin is of increasing importance in human and veterinary medicine, and is applied to many wild and culture fishes (Llewellyn *et al.*, 2014). Increasing data now confirm the key role that microbiomes play in

vertebrates and beyond, including impacts on disease, nutrition, immunity, development, and life histories.

- **Quantitative genetics of wild populations** [current WGAGFM ToR c)]: molecular markers can be used to construct pedigrees allowing for estimation of aspects such as heritability of commercially important traits and other analyses (e.g. Genome Wide Association Study, Quantitative Trait Loci; Tsai *et al.*, 2015)
- **Targeting specific functional genes** underpinning key physiology processes including immune competence (MHC) and growth maturity axes (VGLL3) (Hemmer-Hansen *et al.*, 2014).
- **Novel genetic methods to estimate population abundance**, such as close kin recapture methods (see ToR d)

5.2.3 Applications of Genetics in the Real World of Aquatic Resource Management: From principles to practise

In the context or aquatic resource management, it should be emphasised that genetics is only one approach in the fisheries managers' repertoire. The WGAGFM endorses strongly the integration of genetic tools with other existing and emerging methods. These include: coupling of oceanographic modelling with population genetics to estimate dispersal and gene flow (e.g. Young *et al.*, 2015), stock assessment, harvesting pressures and population genetics to assess vulnerability of marine fish to overharvesting (Heath *et al.*, 2013), disease biomonitoring and population genetics to explore population variability in disease prevalence (Tysklind *et al.*, 2013); analysis of trophic interactions and feeding relationships of aquatic taxa through combined gut analyses with and metabarcoding (Leray & Knowlton, 2015).

It is further recognised that the effective implementation of any method is dependent on socio-economic and political constraints, including available resources and shifting priorities. The application of genetics in fisheries until recently was perceived often as an expensive luxury. Technological advances have, however, greatly reduced cost per sample to equivalent or below that of other techniques. The single most important remaining constraint to fuller incorporation of genetics into management of aquatic resources is the lack of standardised protocols for collection of samples for genetics alongside traditional biological sampling. The logistic (national research vessel programmes) and data resource (Data Collection Framework) requirements are in place, though there is currently no national or international requirement for such extended routine and inclusive data collection.

5.2.4 Historical narrative and role of the WGAGFM

5.2.4.1 The origins of the WGAGFM within the ICES Expert Group structure

In order to appreciate the evolving role and contributions of the WGAGFM in the context of management and policy formulation for fisheries and aquaculture, it is informative to consider briefly the origins and key milestones influencing its role within and beyond the ICES community. At the 81st Statutory meeting in Dublin, September 1993, the former Working Group on Genetics (WGG) was renamed the Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM), and Dr Jarle Mork was asked to chair the new group. The first meeting was located at ICES Headquarters in Copenhagen in

March 1994. In its justification for suggesting the new Working Group, the Mariculture Committee noted, "...the broad range of expertise required will mean that the Working Group will utilize a sub-group format". In cooperation with the Chairman of the Mariculture Committee, the WGAGFM Chairman established a "core" structure of the Working Group during the autumn of 1993, and towards the end of the year two sub-groups were established; subgroup 1in qualitative genetics and sub-group 2 in quantitative genetics within the WGAGFM.

The new sub-group format of the WGAGFM reflected the broadening of its function as recommended by the Mariculture Committee at the Council at the 81st Statutory Meeting. The primary driver was the rapid growth of mariculture in marine food production that already had taken place, and its anticipated further increase. It was recognised that that a resilient and sustainable mariculture industry be founded on sound genetic management, including breeding programs to increase, for example, production efficiency and disease resistance. Broader considerations of capture fisheries were included, and it was subsequently decided to integrate the qualitative and quantitative sub-group components.

The annual meeting is a forum for WGAGFM members and Chair-invited Guests to discuss salient timely population genetic topics in an informal setting within the context of management and policy implications. For members from small institutions especially, the format is a valuable possibility to enlighten questions or solve problems in a milieu with a broad population genetic and, more recently, genomics competence. The broadened genetic scope of the WGAGFM has been a benefit in this respect.

Typically, participants select most Terms of Reference for annual meetings, in line with advances in the field and perceived policy needs. A valuable by-product of the WGAGFM meeting format is to generate opportunities for publication of ToR topics in peer-reviewed journals. Initially, WGAGFM reported to the Mariculture Committee, though from 2008 internal reorganisation of ICES resulted in the renaming of the former Consultative Committee into the Science Committee (SCICOM), an equal partner to the already existing Advisory Committee (ACOM). The SCICOM directs the scientific programme of ICES on behalf of the Council. All former science committees (including the Mariculture committee) ceased to exist in 2008, and the concept of Expert Groups where introduced for the Working Groups. Within SCICOM there were five steering groups of which the WGAGFM reported to the Steering Group for Human Interactions on Ecosystems (SSGHIE). In 2008 the first Science plan (2009-2013) was implemented. Today the WGAGFM reports to the Steering Group on Ecosystem Pressure and Impacts (SSGEPI).

Although WGAGFM proposes Terms of Reference for the next meeting, ratification is required formally from ICES. In addition, ToRs proposed by SCICOM and other Expert groups are considered, as well as stakeholders (clients) outside ICES, such as OSPAR, NASCO and HELCOM, seeking advice from ICES. The final list of ToRs is ratified in a Council Resolution at the Annual Science Conference (previously Statutory Meeting) in September before the WGAGFM annual meeting. In January, the Chair of WGAGFM starts communicating with the members initiating the preparation process for the annual meeting. From 2015, in line with ICES directives, a new 3-year term with multi-annual ToRs was introduced in WGAGFM tasks.

5.2.4.2 Implementation processes

Normally the end result from the ToRs at the annual WGAGFM has been a synthesis document, together with a summary and list of recommendations intended for stakeholders and the wider ICES community. The Expert group report, including the specific recommendations, is presented to SCICOM (via SSGEPI).

5.2.4.3 Changes through two decades

In common with all ICES Expert Groups, it is important to ensure that primary activities and WGAGFM Terms of Reference (ToRs) adapt to shifts in stakeholder and end-user priorities, as well as exploiting advances in the field. A core feature of fisheries and conservation genetics is the constantly changing repertoire of available molecular tools for characterising individuals, populations and species in the wild. There has been a corresponding effort to exploit technological advances in line with recent ecosystem-based approaches to marine resource management. Thus, new developments encompass not only the application of tools to detect biological integrity from individual to species levels, but also the inclusion of novel DNA sequencing methodologies to investigate interactions across trophic levels and taxa that characterise community and ecosystem dynamics. Correspondingly, new opportunities have emerged to estimate empirically the impact of past and projected perturbations on natural systems in relation to ecosystem services and function. A contribution of the WGAGFM is thus not only to consider critically the range of alternative tools and their application across biological levels, but importantly to identify and monitor those elements of ecosystem structure, diversity and dynamics, most likely to impact sustainable development of fisheries and aquaculture.

5.2.4.4 Enhancing the impact of ToR recommendations

It is acknowledged broadly that genetic information and tools can contribute to fisheries and mariculture management (Dichmont *et al.* 2012; Duncan*et al.* 2013). Nevertheless, its coherent and routine integration into scientific advice for management purposes, similar to fisheries data collected under the Data Collection Framework (Council Regulation (EC) No 199/2008) remains limited, with the notable exception of Pacific salmon (Canada and Canada 2011; Hess *et al.*, 2014). Since the establishment of the WGAGFM, it was structured such that relevant research topics could be discussed in the context of latest advances, as well as exercising a clear focus on implications for marine conservation and management issues. Such recommendations were addressed at a variety of the ICES structural units, such as ACOM, SCICOM and various Working Groups. However, based on the feedback to WGAGFM, the perception is that the impact of our recommendations is somewhat limited: the aim is to implement and monitor strategies to enhance impact. To this end, a number of activities will be pursued, described briefly in the following subsections.

Clearly, there is a need to change from a tendency to remain inward looking towards an outreaching attitude: This includes enhancing interactions with other relevant Working Groups and Benchmarking meetings. WGAGFM will pursue an ICES Expert Group (EG) mapping exercise that will identify and cluster EGs according to their scope and activities. The inventory will greatly facilitate the building of a timely interaction and exchange with the EGs in the contexts of specific ToRs. In line with the inventory, we aim to establish an appropriate network between WGAGFM, other EGs and stakeholders outside

ICES, including policy makers and fishery/aquaculture managers, to better integrate genetic information into management and policy options. We consider developing a questionnaire as to inquire about awareness of other EGs of the WGAGFM and to investigate the perception of fisheries and aquaculture genetics, similar to that used by Ovenden *et al.* (2013). Over the three-year duration, we will additionally review the outcome of a representative range of previous recommendations. An example is given below with a series of ToRs pursued from 2006 to 2012 on the need to centrally compile genetic data on marine species and to render that data publicly accessible. The activity will allow the WGAGFM to identify pitfalls and impediments to impact creation, as well as disclosing examples with impact.

Finally, and to some extent dependent upon ICES support, dissemination of examples of WGAGFM initiatives with measurable impact will be undertaken, within and external to the ICES community. Importantly while pursuing the outlined activities, the WGAGFM will monitor progress and review success to adapt and improve strategy as required.

5.2.5 Impact of previous WGAGFM recommendations

5.2.5.1 Classification of WGAGFM ToRs

We divide the impact from WGAGFM into two main categories, based on the nature of the ToR:

1) Recommendations resulting from ToR targeting specific questions or topics, coming from clients and stakeholders, and bodies within ICES (other EGs, Study Groups etc.)

2) Recommendations resulting from ToRs developed WGAGFM.

Although the impact for category 2 recommendations might not be as easily detectable, their influence is discernible from a wider consideration of genetic contributions to our understanding of the marine environment via scientific papers, research project applications and more generally in the scientific community. The potential impacts are not the specific results of any recommendation, but the result of the "internal" distribution of knowledge within the group and colleagues working in genetics. The WGAGFM must explore ways to enhance accessibility of these recommendations to the wider scientific community.

5.2.5.2 Case Study illustrating low impact

Establishing a Central Public Marine Genetic database – from 2006, the WGAGFM identified the need to establish an international database, hosting genetic data in support of fisheries management (ICES WGAGFM Report 2006). The recommendation was pursued in 2007 (ICES WGAGFM Report 2007) and further specified as "To identify the structural and institutional requirements for developing meta-data bases for genetics of fish species covered under the ICES remit". There was a strong need to collate and standardise where possible the plethora of data generated from numerous studies, mostly funded by the European Union, as well as national governments and research councils, examining the nature and extent of genetic diversity in wild and captive stocks of finfish and shellfish. There was a notable lack of coherence and accessibility of the dispersed data. The technical specifications and system architecture were outlined along with data format re-

quirements, functionalities and measures to ensure public accessibility. Such a database would necessarily require resources and commitment at an institutional or consortium level. Specific recommendations were posited that ICES and the European Commission collaborate closely on such an initiative.

Indeed, the need to establish a coherent database was in effect a multi-annual ToR in nature since the topic was considered sequentially each year until 2012. In addition to the WGAGFM endeavours, complementary external drivers endorsed such needs. Foremost among these were the Data Collection Framework (Council Regulation (EC) No 199/2008), for EU-wide collection of biological and economic fisheries data (but not genetic data), and regional Data Base FishFrame (http://www.ices.dk/marine-data/data-portals/Pages/RDB-FishFrame.aspx), and the set of DCF databases (https://datacollection.jrc.ec.europa.eu), hosted by the European Commission Joint Research Centre, FP7 project FishPopTrace (https://fishpoptrace.jrc.ec.europa.eu). In 2011, in the context of the reform of the DCF, a ToR was dedicated to the possibility of integration of genetic data under the remit of such an EU-wide data fishery and aquaculture data collection scheme.

In retrospect, it has to be acknowledged that despite the commitment of WGAGFM to drive such an endeavour, the impact of the recurrent ToR 'database' was negligible: there is currently no such integrated collective framework encompassing fisheries and aquaculture genetic data at a species or geographic level. Our example is counter to the general acceptance of the value of such endeavours for the provision of scientific advice to marine and maritime governance, including the DCF and as other initiatives such as EMODNET (http://www.emodnet.eu). We do not know to what extent, if at all, the topic was considered within the ICES structure: feedback from SCICOM, ACOM or any other Working Group was not forthcoming. Specific obstacles undoubtedly relate to the need for dedicated resources, though scenarios can be envisaged that incorporate genetic/genomics data on an ongoing basis within existing data collection initiatives. The resource issue was recognised by WGAGFM, and as early as 2008 the WGAGFM embarked in a discussion on possible venues with the ICES data centre, and evaluated the possibility of developing a marine fish genetic database under the remit of EMODNET (http://www.emodnet.eu). The discussion however was inconclusive, mostly because potential resources remained unidentified. When reviewing the ToRs on databases, there was clearly a lack of interaction with other potentially relevant ICES Working Groups, an issue for priority consideration in future strategies.

Besides a lack of dedicated resources, another factor underlies the lack of impact evident from the database ToRs: Under the remit of the DCF, national institutions have staff that are dedicated to collect fisheries and aquaculture data outside the academic realm. The goal is to create information to support scientific advice provision under the Common Fisheries Policy, rather than publishing peer reviewed scientific articles (which might nevertheless result from this activity). Such focus differs fundamentally from fisheries genetics, where all activities emerge from academic institutions with the aim of contributing to the primary literature. Despite the resulting accessibility of such data through publication and portals, information is typically highly dispersed and independent, of uncertain quality control, and frequently not comparable in scope and detail. Without a clear commitment of stakeholders and nations to establish the capacity for a coherent and

persistent compilation of marine species genetic data, similar to fisheries data collection, progress will be impeded.

5.2.6 Potential interactions between the Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM) and other Expert Groups (EGs)

The variety of EGs with potential overlap with WGAGFM is detailed in Figure 1. EGs are structured in relation to respective SCICOM steering groups. Brief details of the potential interaction with each WG are detailed below.

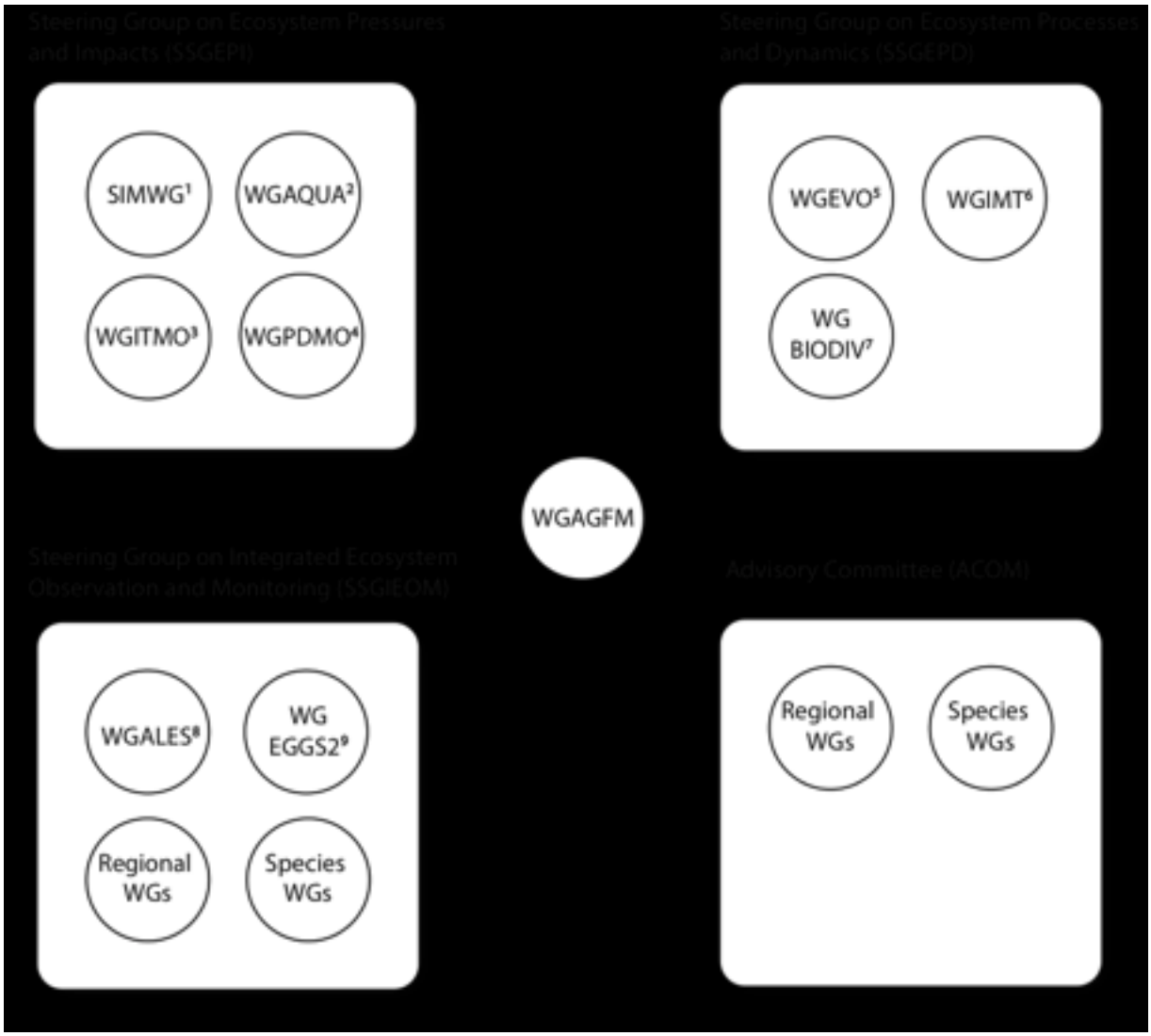

Figure 1. Expert Groups with potential overlap in interest with WGAGFM or past/future interactions with WGAGFM (see text for brief details).

1) **SIMWG: Stock Identification Methods Working Group.** Genetics is one of the key methods used in stock identification and thus there is considerable

overlap with this EG. In particular, developments in sequencing and genotyping technology are disclosing increasingly fine-scaled population structuring.

2) **WGAQUA: Working Group on Aquaculture**. There are a number of genetic methods that are of interest to the WGAQUA EG. Relevant areas shared with WGAQUA include: Genomic selection; pedigree / parentage analysis; traceability; genetic diversity maintenance / inbreeding avoidance; disease / pathogen identification.

3) **WGITMO: Working Group on Introductions and Transfers of Marine Organisms.** Overlap with this group focuses on the detection and monitoring of alien / invasive species using molecular methods including eDNA.

4) **WGPDMO: Working Group on Pathology and Diseases of Marine Organisms.** There are several genetic methods that are used for the identification of diseases in wild and aquaculture species. Thus, there is overlap with this EG, and in particular with the current WGAGFM ToR to 'Review and assess the utility of molecular techniques to evaluate disease and parasite spread from transferred seafood into wild populations'.

5) **WGEVO: Working Group on Fisheries-Induced Evolution.** The basis of phenotypic changes of fish stocks associated with fishing pressure remains an ongoing priority issue. Increasing evidence supports the notion that genetic changes play a key role in the reduction in body size and size at maturity found across many fish stocks and species. Thus, information on population genetic structure and demographics, as well as a need to better assess the quantitative genetic basis of such shifts, represent two complementary fields for interaction.

6) **WGIMT: Working Group on Integrated Morphological and Molecular Taxonomy.** There are clear potential interactions with WGIMT in terms of molecular phylogenetics and species identification.

7) **WGBIODIV: Working Group on Biodiversity Science**. There are many areas where WGAGFM can interact with WGBIODIV. These include elucidation and monitoring of genetic diversity; species identification using DNA barcoding and metabarcoding; eDNA methods for biodiversity assessment; population structure and stock dynamics.

8) **WGALES: Working Group on Atlantic Fish Larvae and Eggs Surveys**. Genetic methods can identify cryptic species in ichthyoplankton surveys, such as the identification of visually indistinguishable gadoid eggs (cod, haddock and whiting). Further, genetic methods have been applied to eggs and larvae to ascertain stock structure at different life history stages and geographic origin.

9) **WGEGGS2: Working Group 2 on North Sea Cod and Plaice Egg Surveys in the North Sea.** Similar scope and complementarity as with the WGALES above are evident.

Progress in Year 2: 2016 (Belfast, Queen's University, Belfast)

In addition to the activities focusing on exemplar case studies (see sections 1–3 above) that demonstrate the value of genetics and genomics in fisheries, the ToR was expanded to include explicitly aquaculture activities, within the broader sphere of aquatic resource management. Moreover, additional consideration, detailed above, was given to the key features of the WGAGFM EG in relation to established successful application of genetics and depiction of new opportunities driven by emergent technologies. Below we summarise additional activities during Year 2 in relation to the role of the WGAGFM in the wider context of the ICES EG structure.

As a first initiative, a simple questionnaire was distributed to 14 Expert Groups to map awareness of the WGAGFM. The following Expert Groups were approached; SIMWG, WGAQUA, WGITMO, WGPDMO, WGEVO, WGIMT, WGBIODIV, WGALES, WGEGGS2, HAWG, PGDATA, WGHANSA, WGMEGS, WGNAS. To date, we have received responses from 3 groups: – WGBIODIV, WGNAS, HAWG. In addition, this simple questionnaire will be sent to three other relevant groups: WGECO, WGFMAC, and WGMASC, not yet included in our network description. The questionnaire was designed based on 4 questions:

1) Are you familiar with the work done by the WGAGFM or the expertise within the group?

2) Are you aware of the recommendations presented by the WGAGFM?

3) Have your EG or anyone in your EG, used / quoted the WGAGFM recommendations - either in work/studies performed by the EG or scientist within the EG?

4) In your opinion - what would be the optimal way to communicate the Recommendations from the ToR in order to target the scientific community as well as stakeholders.

It should be emphasised at this stage that the approach is exploratory only. Due to the unrepresentative number of responses received, we do not provide further details here. A decision on whether to include details of responses in the final report depends upon the number of respondents. Additional mechanisms to promote engagement across EGs are detailed further below.

Various Benchmark meetings have been identified as having potential for synergy with the WGAGFM: WKCOSTBEN 2016, WKIrish2, WKPout 2016, and WKSAND 2106. The latter two are benchmark workshops on Norway pout and sand eel, and might not be of interest as a first opportunity to enhance engagement. We therefore have concentrated on the two first benchmark meetings:

WKCOSTBEN 2016 – Workshop on cost benefit analysis of data collection in support of stock assessment and fishery management. This workshop will meet in ICES HQ 28.06 to 1.07 2016, and the discussions will focus on different aspects that are of interest to WGAGFM. A long-standing quest for WGAGFM has been to promote involvement in the planning of data collection – see text in bold, taken from the description of planned activities.

- Propose options and analytical methods for an objective framework to evaluate the benefits vs. costs of datasets used to support stock assessment and fishery management advice, where the benefits are in terms of accuracy (bias and precision) of assessment results and derived management variables, and risks to stocks associated with management under uncertainty. **This framework should be able to evaluate existing datasets, new data requests from end-users, and options for focusing elements of funding, survey design, spatial and temporal coverage, and sampling effort towards components of data collection that have greatest influence on quality of assessments and management decisions for particular stocks or groups of stocks.**
- Identify a range of stocks for detailed case studies, including those with full analytical age-based assessments and data-limited assessments, and contrasting stock status and biology. Describe the data used in the assessments, the design of fishery-dependent and fishery-independent sampling surveys providing the data, including hierarchical cluster sampling designs and analytical methods for quantifying precision reliably. Evaluate sampling rates and allocation for given survey designs that are required to derive estimates with adequate precision. Specify how simulations of the sampling schemes could be used to relate precision to sampling intensity and costs.
- Develop a proposal for a longer-term (3-year) project to develop a general methodological framework and open-source software to carry out cost–benefit analysis and provide proof of concept using the case study stocks. Identify potential sources of funding.
- Identify the need for follow-up workshops in 2017 onwards in the event of no funding for a dedicated project.
- WKIrish2 (26–29.10.16) - Benchmark Workshop on sharing information on the Irish Sea Ecosystem, stock assessments, and fisheries issues, and scoping needs for assessment and management advice. This WKIrish1 (2015) meeting concluded among several priorities for action that they would like to ascertain how long the truncated age structure has persisted, as well **as improving the understanding of the level of migration of mature fish north and south out of the Irish Sea.** Again the WGAGFM should be able to contribute to this Benchmark workshop.

The work plan for WKIrish is a two-year process focusing on improving single-species stock assessments (principally cod, haddock, whiting, plaice, herring), incorporating a mixed fisheries model, and developing the integration of ecosystem aspects and working towards an integrated assessment and advice.

In relation to remaining activities for potential engagement, discussions are underway with PGDATA, an Expert Group for further potential relevance to WGAGFM within the ICES. PGDATA (ICES Planning Group on Data Needs for Assessments and Advice (PGDATA) is the parent steering group for Expert Groups dealing with surveys (e.g. IBTSWG), fishing technology, fishery data (WGCATCH and WGRFS) and biological data (e.g. WGBIOP). A difference between PGDATA and many of the other EGs is its particular focus on the end use of data, and for this role it requires strong links and communication with EGs dealing with design, implementation and analysis of surveys and other data collection schemes.

PGDATA discussed its role in relation to InterCatch, the Regional Databases (RDB) and the ICES Data Group. The PGDATA recognized the potential of the RDB as a tool for end-users to scrutinize the coverage and quality of fishery sampling data, including the evaluation and documentation of data quality for benchmark and update assessments at ICES. PGDATA recommends that funding be made available for further development of the RDB including routines to provide estimates needed for stock assessments or other end uses together with diagnostics of the quality of data and estimates.

The Working Group on Commercial Catches (WGCATCH), is responsible for documenting national fishery sampling schemes, establishing best practice and guidelines on sampling and estimation procedures, and providing advice on other uses of fishery data. The Expert Group also endorses the workshop WKCOSTBEN.

Currently we are targeting recommendations to ACOM, SCICOM and specific expert groups, but more opportunities are required to promote dissemination of WGAGFM activities, relevance and recommendations. Additional mechanisms will be explored, including:

- Contributions to ICES "News and Events"– short and to the point - highlight of the work in the group;
- Generation of a succinct 4-page PostNote style flyer focusing on the role and relevance of the WGAGFM;
- Enhanced usage of ICES social media;
- To establish an appropriate alert system within the ICES dissemination framework to identify those benchmark meetings of relevance for WGAGFM engagement.

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5.3 ToR c) Review application of quantitative genetic techniques into nonmariculture marine species

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Change to the original title of the ToR to **Review the application of quantitative genetic techniques into exploited marine species**.

Within natural populations, there is an immense diversity of phenotypic variation for traits such as morphology, physiology, behaviour, and disease resistance. Typically, underlying this diversity are multiple genetic loci that interact with each other and the environment. Recognising the scope of genetic and phenotypic diversity, and the genetic basis of adaptation is fundamental to our understanding of how exploited marine populations will respond to changing environmental pressures (e.g. climate change, overfishing, biological invasions, aquaculture) and the effective ecosystem-based management of these important natural resources.

From an evolutionary perspective, the presence and maintenance of genetic variation allows for a selective response to both direct and indirect effects of changing environments. Adaptive genetic diversity influences functional variation and the way populations and ultimately species adapts to a new or changing environment. Recent analytical developments in quantitative genetic approaches now allow the study of the genetic variation underlying the expression of specific phenotypic and life-history traits within and among populations and species. Pedigrees and broader studies of genetic relationships can be exploited to estimate quantitative genetic parameters related to trait evolution, including heritabilities and genetic correlations for which there are very few estimates in wild populations. Information on the extent to which variation in fitness-related traits such as size at age and maturation schedule is transmitted from parents to offspring is also crucial to predicting the genetic and demographic consequences of fisheries, climate variation, hybridisation and introgression with captive bred conspecifics, invasive species and habitat change.

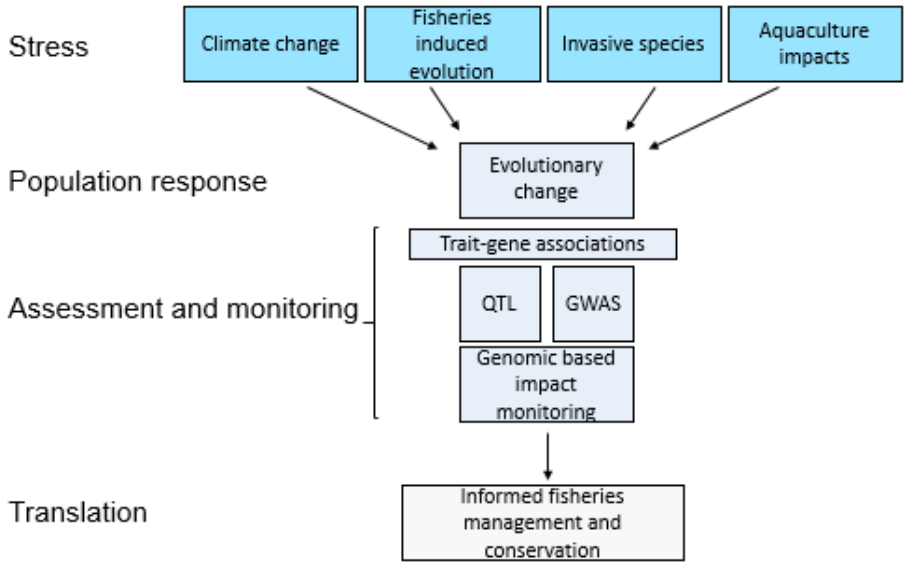
Most recent literature in evolutionary population genetics has focused on models of selection at one, or a small number of loci. This is in contrast to classical models of natural and artificial selection in quantitative genetics, where it is assumed that most traits of interest are highly polygenic, and are influenced to a small degree by standing variation at many loci. Over the last year a number of papers have been published which try to identify the 'lost' proportion of heritable variation that is missed by the strict statistical tests used in GWAS studies, and resolve the differences between the theory and current results using novel methods (Hassl & Payseur 2016, Jensen *et al.* 2016). A recent review by Wellenreuther & Hansson (2016) reviewed analytical advances, including improved mixed models (such as mvLMM and BSLMM), which allow for the incorporation of confounding factors (environmental noise) and allow all markers to be modelled simultaneously. Pathway analysis (e.g. Random Forest algorithms; a tree based machine learning tool, which can account for interactions among loci) have also been used in a couple of studies over the last year. While this type of analysis is frequently used in medicine and

agriculture, it has yet to be fully explored in studies of wild populations. Recent studies, however, in Sitka spruce (Holliday 2012), Chinook salmon (Brieuc *et al.* 2015) and Eels (Parvey 2015; Laport 2016) clearly illustrate the potential of this analytical approach.

In addition to the work updating the literature review from last year (ICES WGAGFM 2015), the most relevant and current papers have been added on am online library which can be accessed by all members of the ToR group, to facilitate continuing work on the literature review over the coming months.

Following discussions within the group, the ToR has been restructured from last year, to give a greater emphasis on environmental stressors (climate change, fisheries induced evolution, biological invasions, aquaculture impacts – see diagram below) and how quantitative genetics techniques can be used to address key management related questions. Current key papers were identified in these areas, including how species will adapt to the effects of climate change (Latimer *et al.* 2015; Mäkinen *et al.* 2015; Stillman & Armstrong 2015; Waples & Audzijonyte 2016), fisheries induced evolution (Heino *et al.* 2015; Uusi-Heikkilä *et al.* 2015), invasive species (Chown *et al.* 2015) and the impacts of aquaculture (Besnier *et al.* 2015). A number of additional relevant reviews elaborating on the roles of plasticity, the basis of adaptation and the use of transcriptomics, were also identified as covering aspects pertinent to this report (Alvarez *et al.* 2015; Pardo-Diaz *et al.* 2015; Hendry 2016). An additional section on applications within a management context has also been included in the working draft report.

A conceptual figure illustrating how quantitative genetic approaches as applied to fisheries management has also been developed, and will be further developed for inclusion in the final report and associated manuscript (see below).



WGEVO ToRs from recent years were assessed for complementarity and at this point they were not contacted as their current ToRs are focused in a different direction.

Prof Kerry Naish (School of Fishery and Aquatic Sciences, University of Washington, Seattle) was contacted concerning collaboration on review paper. She has responded and is keen to collaborate.

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5.4 ToR d) Close-kin mark recapture approaches to estimate abundance and population parameters of deep-sea marine fish species in support of enhanced management under the Common Fisheries Policy.

Rationale

In view of their vulnerable nature, the European Commission has indicated that particular attention is needed to secure the sustainable exploitation of deep-sea fish stocks. For many of these stocks, knowledge and data remain insufficient for adequate scientific analysis and management advice (COM(2007) 30 final). This scarcity of information is reflected in recent Total Allowable Catches (TACs) and Quota setting. Basically, the poor state of key deep-sea stocks and the lack of scientific data clearly demonstrates the need for an improved management framework for deep-sea fisheries, as proposed by the European Commission in 2012 (see IP/12/813). For logistic reasons, one of the main difficulties managing deep-see stocks is linked to the difficult to obtain reliable estimates of stock assessment. A recent research report, published by CSIRO Australia, has indicated that genetic close-kin analysis can be potentially used for assessing abundance of Southern Bluefin Tuna. This would greatly facilitate gathering of relevant information for fisheries management. In here, the possibility of transferring this method to estimate abundance of deep-sea stocks will be assessed. In particular, a range of molecular based markers and associated statistical genetic framework will be evaluated for: 1) their usefulness in detecting close-kin genetic relationships (genetic tagging) within wild stocks and 2) using this information within a mark-recapture framework to estimate stock abundance in the context of yielding scientific advice implemented under the remit of the Common Fisheries Policy (CFP). The interim reporting of this ToR is largely based on a recent Technical Report on the subject, published by the Joint Research Centre.

Introduction

Deep-sea fish species live in waters deeper than 400 metres, characterised by extremes in terms of pressure, food availability and lack of sun light but are, at the same time, very stable environments, which are very susceptible to disturbances. Fish in this area tend to live long, grow slowly and mature late (i.e. k selected species). Taking also in consideration their generally low fecundity render them particularly vulnerable to over-fishing. Deep-sea fisheries management is currently challenged by a lack of reliable scientific data to ensure sustainable exploitation. Furthermore, as deep-sea fisheries operate at considerable distances from the coast, and, hence, in the context of complex governance frameworks, their management is both difficult and complex. To add to this complexity, current knowledge about the biology of deep-sea fish is very limited. Currently most deep-water stocks are considered to be below safe biological limits for exploitation. In the

European Union (EU) deep-sea fisheries are covered by Council regulation No. 2347/2002 on “establishing specific access requirements and associated conditions applicable to fishing for deep sea stocks", which lists 24 species. For the European Union, the setting of total allowable catches (TACs) for deep-sea fish is based on the scientific advice developed by the International Council for the Exploration of the Sea (ICES) and the Scientific, Technical and Economic Committee for Fisheries (STECF). According to both advisory bodies, the majority of deep-sea stocks are subject to unsustainable exploitation. Therefore, the management trend is to reduce fishing opportunities. The assessment of abundance and state of deep-sea stocks is difficult, due to the lack of data mentioned above, which greatly impedes quantitative population modelling of exploited stocks. It is clear that alternative approaches are urgently required to assess the status and/or the risk exposure of populations that cannot be assessed by more traditional fisheries science approaches. This ToR assesses the feasibility of transferring genetic close-kin abundance estimation, a method that has been recently applied to translate biomass estimates of Southern Bluefin Tuna, into fisheries management advice for marine deep-sea fish species. In this interim report, which is also based on a recently published Technical Report of the Joint Research Centre, the principle of genetic close-kin analysis will be considered and the feasibility of generating abundance estimates for deep sea species will be assessed.

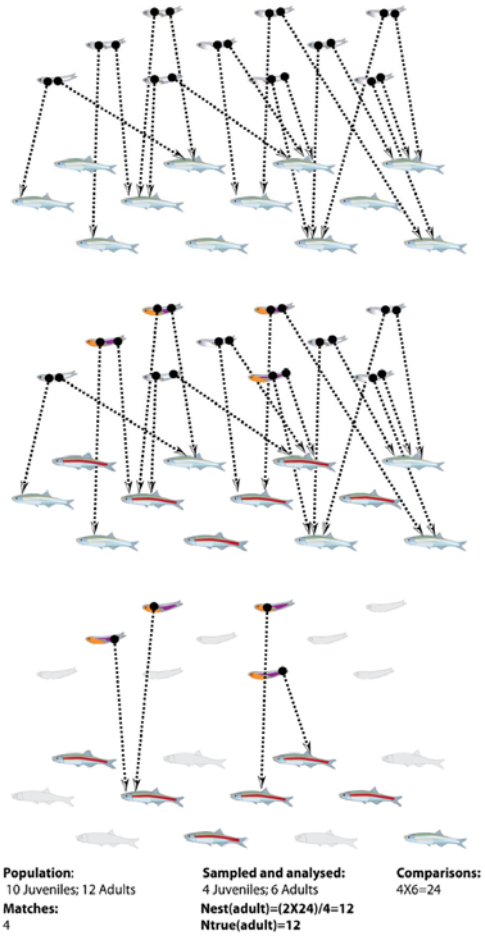

Figure 1. Close-kin abundance estimate: The principle (see text for explanation).

The close-kin method is a genetic based approach aimed at detecting parent-offspring pairs in the random sample of a population using molecular markers (genetic tagging).

The approach uses the same principle of a 'paternity' test: an offspring always has two parents, from whom it will inherit half of its DNA from. By comparing the genetic make-up of fish representing different generations (i.e. offspring against a pool of candidate parents), the likelihood of an adult being the parent of a give offspring can then be estimated.

In a relatively recent report published by CSIRO Australia, Bravington *et al.,* (2014) suggested that this cost-effective method could be applied in fisheries to estimate Spawning Stock Biomass. The approach involves the random sampling of adult spawners and their associated juvenile and the genotyping of a sufficiently high number of genetic markers in order to conduct parentage analysis (27 microsatellites, in this specific case).

The genetic DNA profile (i.e. multilocus genotype) of the offspring (in our case juvenile fish) are compared to that of the available adults (putative parents). Following parentage analysis, the most likely adult individuals (based on matching probabilities) are identified as the biological parents of a given offspring. The rationale is that when analysing a

sufficiently high number of genetic markers (i.e. loci), the probability that two individuals sharing the same alleles at all loci by chance only will be extremely low. The number of juveniles that have at least one parent in the sampled adult pool (or Parent-Offspring-Pairs, POPs) will be inversely proportional to the absolute spawning stock (see below for details of calculations involved).

The method is schematically illustrated in Figure 1. In this example, two generations of a fish population comprised of 12 adults and 10 juveniles are been considered. From these, six adults and four juveniles are sampled and genotyped. If four POPs are found, the estimated population census size is 12 (see figure), which is the true N.

Calculations are as follows:

- True Nc=12 (adults stock)
- 4 juveniles and 6 adults sampled
- 4 POPs found
- number of adult-juvenile comparisons > 6 x 4=24
- Nc estimated=(2 x 24)/4=12 (See Bravington *et al.*, 2014 for details)

Close-kin abundance estimate for deep sea species: A practical approach

As discussed in Martinsohn *et al.* (2015) for the deep sea species, listed in Annex I of Council Regulation No. 2347/2002, a considerable lack of information on biology and stock status prevails. In particular, while frequently the simultaneous catch of spawners and juveniles is possible, knowledge about spawning areas is very limited. Moreover juvenile samples are often rare to the point that the collection of enough samples to sufficiently support a close-kin abundance estimate study on deep-sea species would require a major effort and significant resources.

To overcome this logistic complication, Martinsohn *et al.*, (2015) suggested the use of a proxy species to assess the usefulness of the method for stock assessment. The authors argued that white anglerfish (*Lophius piscatorius*) might be a good test species. While it is not a deep-sea species, it shares some common features and also supports a highly commercial fishery that is endowed with valuable information. Its assessment in ICES divisions VIIIc and IX (Cantabrian Sea, Atlantic Iberian waters), provides a reference point supporting a feasibility assessment of close-kin analysis. The ICES technical advice on white anglerfish states that the adult stock size is unknown, and that fishing pressure is too high to ensure an optimal use in the long term. Hence, the stock, exploited above MSY in 2014 (International Council for the Exploration of the Sea, 2015c), would benefit from an independent estimation approach such as the close-kin analysis. Obtaining a sufficient number of samples seems feasible. In summary, it appears that knowledge about *Lophius piscatorius* and its commercial exploitation as well as management combines with features which render this species a good candidate for a close-kin abundance estimate study which might ultimately also serve as a paradigm for deep-sea species.

In VIIIc and IXa Spawning Stock Biomass in 2016 was estimated at about 7500t (http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2015/2015/anp-8c9a.pdf). Assuming an average weight of 5kg for mature white anglerfish (Ofstad *et al.* 2013) this translates into a population size N of $1.5X10^6$ individuals. Applying this estimated N in the equations developed by Bravington *et al.* (2014), we tested a number of simulated

scenarios to assess the sampling effort required that would be required to obtain a reliable estimation of stock abundance based on close-kin analysis.

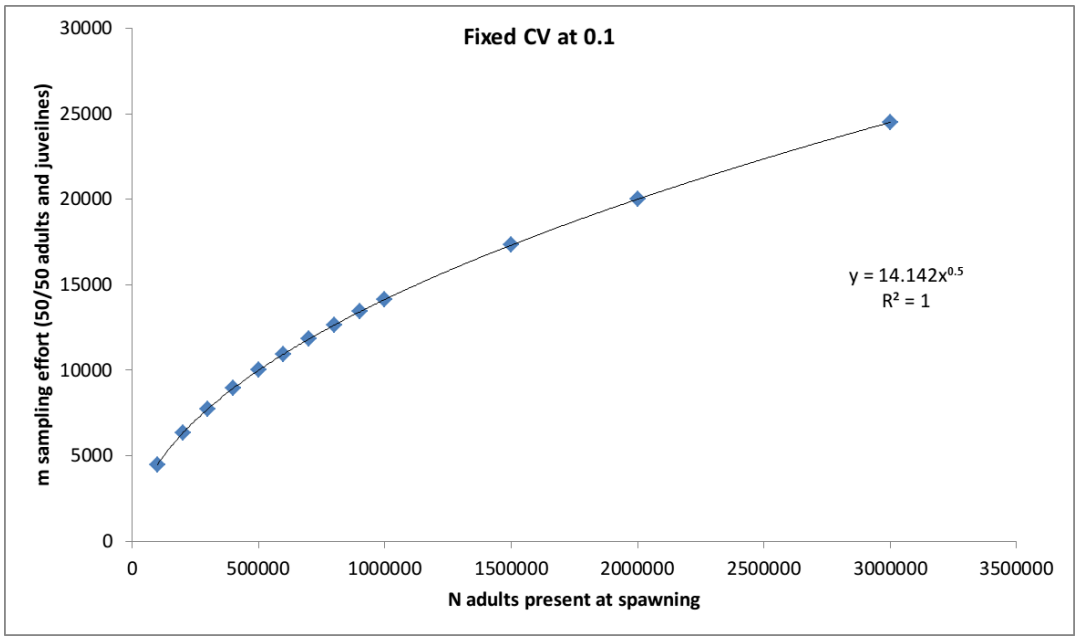

Figure 2. Relationship between the assumed population size (as number of adults at the breeding area) and the sampling effort needed (m) to achieve accurate estimates of numbers of adults (N) with a coefficient of variance at 10%.

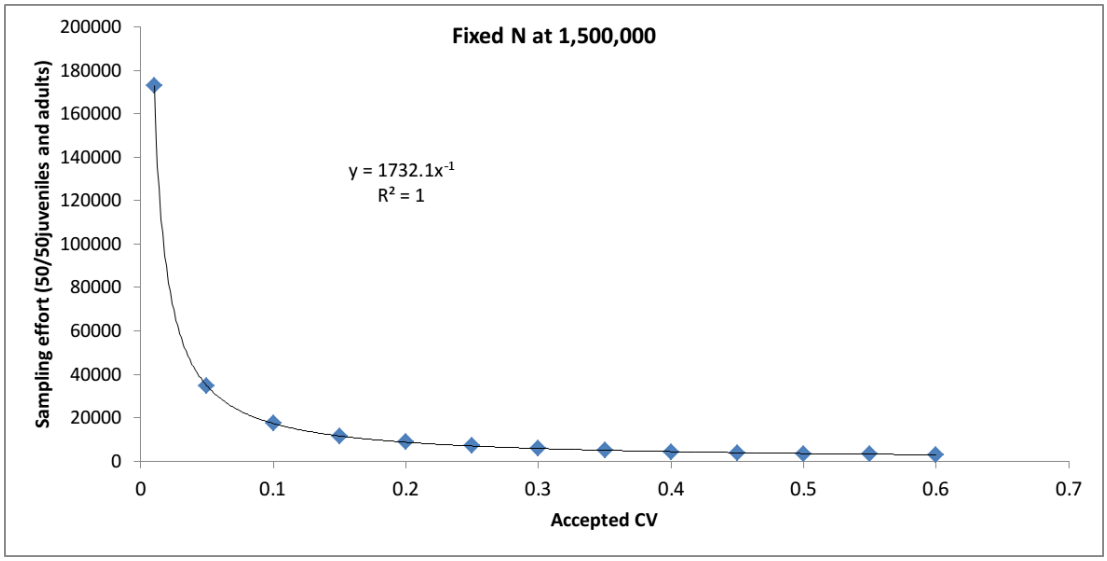

Figure 3. Effect of coefficient of variance on the sampling effort needed to achieve accurate estimates of numbers of adults (N) with a total number of adults breeding at 1 500 000.

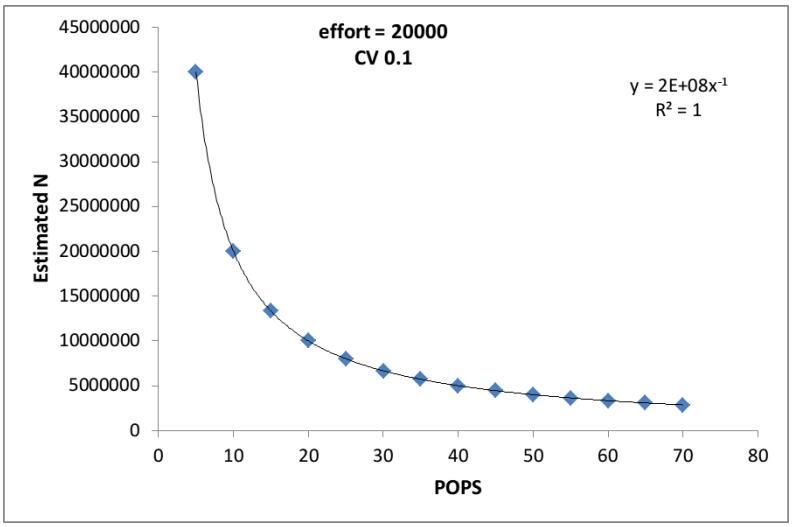

Figure 4. Relationship between the number of POPs (parent offspring pairs) and the estimation of adults at the breeding are with a sampling effort of 20 000 and variance of 10%.

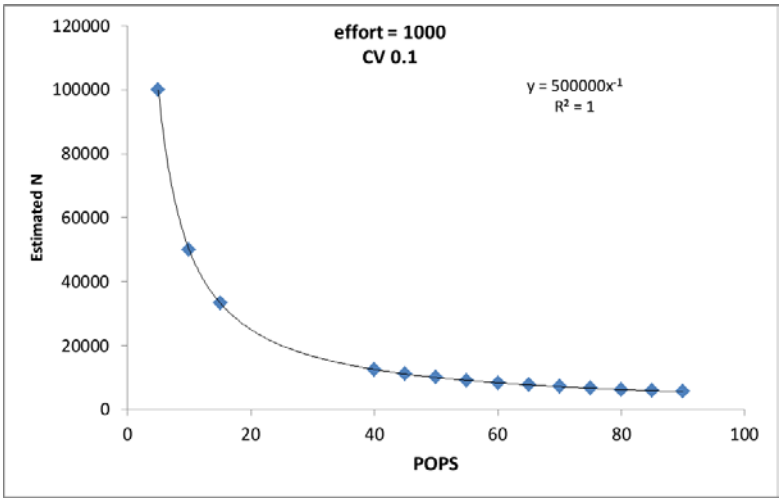

Figure 5. Relationship between the number of POPs (parent offspring pairs) and the estimation of adults at the breeding are with a sampling effort of 1000 and variance of 10%.

Table 1. Biological data used for estimating sampling effort needed for assessing the number of adults at breeding sites for White Angler fish (*Lopius piscatorius*) in ICES areas VIIIc and IXa.

White Angler fish (*Lopius piscatorius*)	
Biomass	7500000kg
Mean weight at sexual maturation	5kg
Expected number of mature individuals	1500000
Accepted CV	10%
Estimated sampling effort needed	17320
Juveniles	8660
Adults	8660
Expected number of POPs	100

To assess the usefulness of the Close-Kin method to recover reliable estimated of stock abundance on White Angler fish, different scenarios considering the effect of different variables and sampling efforts were performed. The number of samples needed to be genetically analysed to assess adult population sizes ranging from 10 000 to 3 000 000 are shown in Figure 2. Not surprisingly, the sampling effort needed to obtain reliable estimates of abundance will increase with increasing adult population sizes. Further, efforts to reduce the coefficient of variance are very demanding on the number of samples needed and there is little return for effort at CVs below 10% (Figure 3). Figure 4 and 5 shows the relationship between the number of observed POPs and the change in adult population estimates. Increased sampling efforts will allow for more accurate estimations of large adult population sizes while smaller efforts only allow for precise estimations of smaller adult population sizes.

Conclusions and outlook

In this ToR d) interim report we aimed to further explore the feasibility of applying genetic close-kin analysis, as suggested by Bravington *et al.* (2014), to estimated abundance of commercially exploited deep-sea fish. Using the white anglerfish data (ICES divisions VIIIc and IX) as a model species, simulations indicate that a sample size of about ~17.000 individuals, of which 8.500 adults and 8.500 juveniles, would be required to obtain reliable estimates of abundance based on the close-kin method. Preliminary discussions indicate that such a sample size is achievable, but this has to be further explored.

In general, the feeling is that the genetic close-kin abundance estimate approach has not been sufficiently tested under fully controlled conditions. Hence, a more conservative approach would be to carry out additional pilot studies in an experimental framework using a well described and assessed species. This would allow to ground truth the approach under coherent and stringent conditions.

During the final year of this ToR, we envision to apply further simulation-based approaches to test the rigour of the close-kin analysis for marine fish stocks. Moreover this analysis will be extended to estimate resources needed to carry out the genetic analysis. Currently, due to its increasingly widespread use and advantages discussed elsewhere (Helyar *et al.* 2011), we assume that the genetic marker of choice will be Single Nucleotide Polymorphisms (SNPs), but in any case genetic markers with sufficient statistical power

for parentage analysis will be employed. . We will also engage with WGDEEP as to better understand current needs and challenges inherent to deep-sea fisheries management.

Recommendations

To be formulated in 2017. They might include:

- Simulations testing a variety of scenarios (generation time, mortality, sex rations etc.)
- Metanalysis (tapping into existing data; data mining of other studies)
- Controlled experiments (*Danio rerio*; Salmon; stickleback – might not be needed if simulations plus metaanalysis - conclusive)

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International Council for the Exploration of the Sea, 2015c. ICES Advice on fishing opportunities, catch, and effort Bay of Biscay and the Iberian Coast Ecoregion: 7.3.39 - White anglerfish (Lophius piscatorius) in Divisions VIIIc and IXa (Cantabrian Sea, Atlantic Iberian waters). ICES Advice on fishing opportunities, catch, and effort Bay of Biscay and the Iberian Coast Ecoregion. *Published 30 June 2015 ICES Advice 2015, Book 7.*

6 Revisions to the work plan and justification

ToR a) After careful evaluation of the work needed to complete a comprehensive review of existing and potential molecular techniques to evaluate infectious disease and parasite spread from transferred seafood into wild populations and, particularly, after considering the interaction with WGPDMO, critical we recommend extending this 2-year ToR into a 3-year ToR. Furthermore, the team varied substantially across years, so an extra year to synthesise different approaches and materials is needed. Completion of ToR a) will therefore be delayed until May 2017, though reported fully alongside remaining ToRs.

ToR b) Additional effort was devoted to summarizing the relevance of key conceptual and technological advances in genetics and genomics in the context of key challenges in managing marine resources. While the questionnaire designed to explore awareness and role of the WGAGFM across other Expert Groups was distributed, the response rate was too low (x3) to allow a meaningful synthesis of responses. During year 3 it will be decided whether to incorporate or exclude questionnaire outputs depending upon number of respondents.

7 Next meetings

The 2017 meeting of the WGAGFM will take place at the Centre of Marine Sciences (CCMAR) at the University of Algarve, Portugal, 2–5 May 2017. To accommodate the additional time required for the Final Report for the period 2015–2017, we aim to include an additional afternoon in the workshop, commencing on Tuesday, 2 May at 15.00.

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