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

2–5 May 2017

Olhão, Portugal

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Executive summary

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met in Olhão, Portugal, 2–5 May 2017. Nineteen participants from 11 countries discussed the four Terms of Reference (ToR) and associated matters. Specifically, there was a focused discussion concerning the proposal by the ICES Science Committee to establish Aquaculture Steering Group (ASG). It was agreed unanimously that the WGAGFM, would wish to be affiliated to the new steering group, while retaining collective interest in capture fisheries. It was further agreed that the WGAGFM would submit an application to coordinate an ICES training course broadly on the *Application of Genetics and Genomics to Fisheries and Aquaculture Science*.

Four multiannual ToRs were considered and finalised. **ToR (a)** finalised consideration of infectious disease and parasite spread from seafood into wild populations. A schematic overview was designed to provide a tool for assessing infection risk based on infectious and transmission potential across specified scenarios of host-pathogen infection. The highest infection risk (value 10) corresponds to symptomatic live seafood carrying live pathogens. Moreover, a workflow to aid decision-making when analysing pathogen samples from seafood was constructed, depicting the most appropriate methods to employ, while enhancing detection, robust quantification, and assessment of viability. The mechanisms for integrating WGAGFM advice into fisheries assessment and management, **ToR (b),** focused on additional mechanisms for enhancing awareness of WGAGFM activities, expertise, and contributions to ICES and beyond. Such mechanisms were discussed in relation to practical applications of advances in genetics and genomics, most notably considering such issues as meta-barcoding, environmental DNA (eDNA), disease diagnosis, analysis of microbiomes, quantitative genetics of wild populations, targeting specific functional genes, and novel genetic methods to estimate population abundance. In relation to the promotion of WGAGFM activities, while interactions among potential complimentary ICES working groups is not extensive, several information requests between 2015–2017 (e.g. from the Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT), Benchmark Workshop on Northern Haddock Stocks (WKHAD) and the Stock Identification Methods Working Group (SIMWG), indicates active complementarity. To promote awareness and impact of WGAGFM, two new methods of dissemination, in addition to existing ICES channels were identified: First, creation of a "Project" in "Researchgate.com" to reach the scientific community in a more targeted way (https://www.researchgate.net/project/ICES-WGAGFM-Working-Group-on-Application-of-Genetics-in-Fisheries-and-Mariculture), and design of a 2-page leaflet for targeting industry, management, national governments, EU, FAO, research councils etc. (attached herein). The challenges of elucidating the genetic basis of adaptive shifts in exploited species was considered further in relation to advances in methods and application of quantitative genetic analysis **(ToR (c)).** Particular emphasis was based on the range of phenotypic traits relevant to exploitation, captive propagation and environmental change, and the potential for rapid genetically-based shifts in such traits across both natural and farmed environments. An outline was produced of the scope of quantitative genetic based methods, of pedigree- and pedigree-free genomic mapping approaches, and how they can be applied in planning for promotion of evolutionary resilience, sustainable stock exploitation at MSY and in predictions for stock recovery. In particular, it

was emphasised that the routine collection of appropriate tissue samples for DNA coupled with phenotypic measures on the same individuals, and associated environmental data, would enable improved monitoring of quantitative genetic change and predictions for response for shifts in harvesting practices, breeding scenarios, and ongoing influences of climate change, invasive species, and habitat deterioration. Finally, **ToR (d)** focused on recently developed approaches for estimating population abundance in the context of deep sea fisheries. The feasibility of the close-kin approach was assessed by consideration of a model system with sufficient background information, and representative of several deep sea species: the white anglerfish (*Lophius piscatorius*). Basic simulations using white anglerfish fishing data covering ICES divisions VIIIc and IX (Iberian region), indicate that, assuming a coefficient of variation (CV) of 10%, a sample size of about 17 000 individuals, 8500 adults and 8500 juveniles, would be required to obtain reliable estimates of abundance (i.e. breeding population that in this case, based on existing stock assessments, is believed to be ~1.5 million individuals) based on the close-kin method**.** Using the same estimate of abundance (i.e. 1.5 million individuals), further simulations were subsequently carried out to investigate sampling requirements under distinct CV levels and, more specifically, the minimum number of parent-offspring pairs (POPs) to obtain reliable estimates of abundance (i.e. close to "real" value) based on the close-kin approach. The most important parameter was the number of POPs that need to be identified to obtain reliable estimates of abundance. A simple Excel based guide was developed to assist users in choosing the optimal sampling design, together with resource requirements. We propose that the genetic marker of choice will be Single Nucleotide Polymorphisms (SNPs) or microsatellites, ensuring that markers deployed exhibit sufficient statistical power for parentage analysis.

1 Administrative details

Working Group name

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

Year of Appointment

2015

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

3

Chair(s)

Gary R Carvalho, UK

Meeting venue

Olhão, Portugal

Meeting dates

2–5 May 2017

2 Terms of Reference a) – z)

(ToR leaders in bold)

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, Pierre Boudry, Ian Bradbury, Malte Damerau, John Gilbey, Phil McGinnity, Kerry Naish, Paulo Prodohl, Tom Reed, Jochen Trautner, Harri Vehviainen, Daria Zelenina (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, Naiara Rodriguez-Ezpeleta, Jens Carlsson, Ernesto Jardim, Rita Castilho, Paulo Prodohl, Gary Carvalho, Francis Neat & 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: Historic narrative role and impact of the WGAGFM: To verify the position of Consideration of the implementation process, and mapping of potential interactions between the WGAGFM and other Expert groups. **Year 2 – Questionnaire and benchmark meetings.** Consideration of key features of the WGAGFM EG in relation to established successful application of genetics and depiction of new opportunities driven by emergent technologies. Distribution of a simple questionnaire to 14 Expert Groups to map awareness of the WGAGFM, including SIMWG, WGAQUA, WGITMO, WGPDMO, WGEVO, WGIMT, WGBIODIV, WGALES, WGEGGS2, HAWG, PGDATA, WGHANSA, WGMEGS, WGNAS. In addition, this simple questionnaire was sent to three other relevant groups: WGECO, WGFMAC, and WGMASC, not yet included in our network description. **Year 3 - The way forward:** To identify methods for promoting dissemination of the recommendations or expert advice to the scientific community, stakeholders; industry, management, "decision makers". First, creation of a "Project" in "Researchgate.com" to reach the scientific community in a more targeted way (https://www.researchgate.net/project/ICES-WGAGFM-Working-Group-on-Application-of-Genetics-in-Fisheries-and-Mariculture). Second, design of a 2-page leaflet for targeting industry, management, national governments, EU, FAO, research councils etc. The leaflet includes a schematic presentation of areas of interest and key expertise available.

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.

ToR d) Year 1: 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. **Year 2:** Outcomes of these activities will be documented together with recommendations in 2017.

4 Summary of Achievements of the WG during 3-year term

- **Coordination of Theme Session at the ICES Annual Science Conference (21-25 September, 2015, Copenhagen):** "A holistic ecosystem approach for marine management and conservation: Opportunities through the application of genetic and genomic approaches". The theme session was well attended and included 14 oral presentations and 6 flash talks, and 10 posters. The aim was to raise awareness across researchers and stakeholders and to facilitate the integration of genetics and genomics into a holistic approach to an ecosystem based marine resource management. The session considered the current status of the application of genetic and genomic approaches to marine management, their benefits as well as obstacles to their routine application. We included a broad spectrum of natural renewable resource management targets and aspects, including aquaculture. Studies spanned the integration of genetic analyses with approaches such as habitat mapping and fisheries modelling, as well as linking genetic diversity with ecosystem function and resilience of harvested species, and also cost-benefit estimates.
- **Tool to assess the infection risk of a range of scenarios of host-pathogen infection (ToR a, Figure 1).** A scheme was developed in which several host-pathogen scenarios leading to different infection risks were characterized. We consider this a simple but useful tool to highlight the highest priority situations. It is based on successive parameters characterizing the state of seafood and its eventual pathogenic agents, such as live versus dead, fresh versus frozen, cooked or processed and symptomatic versus asymptomatic. Each case was associated with an infection potential arbitrary ranging from a value of 0 to 5 and a transmission potential ranging from 1 to 2, leading to an infection risk on a scale from 0 to 10, calculated by multiplying their infectious potential with their transmission potential. The highest infection risk (value 10) corresponds to symptomatic live seafood carrying live pathogens. Special attention must be paid to the international trade of live seafood which is common for numerous species that can be consumed alive (e.g., oysters), or alive before cooking (e.g., lobsters and most fishes in Asia). In that case, non-local seafood is often maintained in recirculating-systems for which seawater must be sterilized (using U.V., chlorine, ozone…) prior to release into the wild in order to prevent the transfer of pathogens.
- **Workflow design to aid decision-making when analysing pathogen samples from seafood using molecular tools (ToR a, Figure 2).** The workflow is de-

signed to assist in the choice of the most effective molecular approaches to employ in analysing pathogen samples from seafood, highlighting detection, quantification and viability assessment. It is important to recognise the link between the two types of approach; whereby novel targets identifying pathogen species, strains, virulence factors are first identified via genome-wide approaches and later developed in to rapid screening tools. Furthermore, both cost and efficiency of deployment are major considerations. As such, 'classic' molecular tools (e.g. PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) may be preferable over more technologically advanced approaches in many circumstances. Furthermore, novel methodologies like loop-mediated isothermal amplification (LAMP), PCR-dipstick tests as well as several commercially available portable qPCR machines means that screening can be carried out and acted upon on-site. In Figure 2 we supply a diagram to assist in the appropriate deployments of different tests.

- **Establishment of a WGAGFM Project in "Researchgate.com" (ToR b)** (https://www.researchgate.net/project/ICES-WGAGFM-Working-Group-on-Application-of-Genetics-in-Fisheries-and-Mariculture). In addition to existing methods for ICES dissemination of expertise and activities of the WGAGFM, a project using social media based on the Researchgate.com site was established with the aim of extending our reach. The new site will allow linking to the ICES webpage (WGAGFM), where in addition to the background for the group, the annual reports and list of group members would be available. Within less than 1 month of set-up, we have over 30 followers and over 130 reads, which indicates a wider interest and the fact that we are now reaching a broader audience of researchers.
- **Design of a 2-page flyer summarising the objectives, interests, activities and expertise of the WGAGFM (ToR b).** WGAGFM has created a 2-page leaflet for targeting industry, management, national governments, EU, FAO, research councils etc. (see design draft). The leaflet includes a schematic presentation of the scope of expertise and services the EG offers, designed around a key question: "what can genetics do for fisheries and aquaculture?". New frontiers of application in fisheries and aquaculture are highlighted, including environmental DNA (eDNA), microbiomes, transcriptomics, adaptive diversity, population sizes, meta-barcoding, and epigenetics. The key objectives of the WGAGFM in the context of ICES, is highlighted. To profile the target users, a database will be constructed, for electronic dissemination, and potential production of hard copies for distribution.
- **Simulations using white anglerfish (*Lophius piscatorius*) to identify appropriate sampling design for estimating reliable estimates of abundance using close-kin mark recapture approaches (ToR d)**. Basic simulations using white anglerfish fishing data covering ICES divisions VIIIc and IX (Iberian region), indicate that, assuming a coefficient of variation (CV) of 10%, a sample size of about 17 000 individuals, 8500 adults and 8500 juveniles, would be required to obtain reliable estimates of abundance (i.e. breeding population that in this case, based on existing stock assessments, is believed to be ~1.5 million individuals) based on the close-kin method. Using the same estimate of abundance (i.e. 1.5 million individuals), further simulations were subsequently carried out

to investigate sampling requirements under distinct CV levels and, more specifically, the minimum number of parent-offspring pairs (POPs) that would have to be identified to obtain reliable estimates of abundance (i.e. close to "real" value) based on the close-kin approach. Variation of CV levels had a considerable impact on sample size requirement. For instance, a CV of 12% reduced sampling requirements to about 14 500 individuals (7250 adults and 7250 juveniles). The most important parameter, however, was found to be the number of POPs that need to be identified to obtain reliable estimates of abundance. Assuming a breeding population of ~1.5 million individuals, and a CV of 10%, 70 POPs need to be identified among samples to truly reflect "real" abundance. Under this criteria, a lower number of identified POPs will result in greatly inflated measures of abundance while larger numbers will result in major overestimations.

- **A quantitative assessment of the optimal sampling design and resource requirements for implementation of the close-kin mark recapture method for extension to deep sea fishes (ToR d)**. It is recognised that a crucial component of the utility and implementation of molecular tools, is the transfer of quantifiable information on optimal design and resource requirements. A detailed outline considering sampling, DNA extraction and marker identification, and the statistical evaluation to identify parent-offspring pairs allowed to deduce an abundance estimate, is presented. In addition to precise estimates of the number of samples, capture locations, time, personnel and infrastructure requirements, specific suggestions are provided on the choice of DNA extraction methods and statistical analyses. Overall, analysis of approximately 17 000 individuals, would require an estimated 24 person months for execution.
- **Agreement to submit a proposal to coordinate an ICES training course for submission, September 2017:** Following correspondence with the ICES Secretariat, it was agreed to submit a proposal in advance of September 2017, to coordinate a training course, broadly on the Applications of Genetics/Genomics to Management of Fisheries and Aquaculture. While the detail is yet to be can finalised, several guiding principles were agreed: 1) to focus on clear practical questions of importance to the ICES mission and beyond; 2) to consider critically, with a full cost-benefit analysis the optimal design and choice of tools to tackle key questions; 3) to consider underpinning concepts relating to environmental change and harvesting, and consequences for stock resilience, recovery and sustainability; 4) to focus on opportunities for integration of molecular tools with traditional estimates of stock status and abundance, including population modelling, oceanography, biomonitoring; and non-genetic methods of stock ID; 5) to illustrate the above principles and applications with reference to salient case studies demonstrating utility, impact and constraints; 6) The science : policy interface - mechanisms to promote impact and uptake of genetic/genomic tools.

5 Final report on ToRs, workplan and Science Implementation Plan

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

Claudia Junge, Pierre Boudry, Martin Llewellyn, Naiara Rodriguez-Ezpeleta, Daria Zelenina, Johann Hofherr, Filip A.M. Volckaert

5.1.1 Summary

Worldwide trade in seafood involves shipping of associated microbes and parasites. The many opportunities for introducing non-indigenous species, and especially the risk for infection, are considerable. Once infection risk has been determined and the screening challenges overcome, molecular tools for the screening of pathogenic microbes and parasites are the prime tool for research and monitoring purposes. Cases of successful application show that operational opportunities should be considered and used to understand the risks involved with the mostly involuntary transfer of organisms.

5.1.2 Background

Human society consumes 146.3 million tonnes of seafood annually (FAO, 2016). The European Union produces an estimated 6.149 million tonnes of seafood with consumers spending 54 billion euro in 2015 (EUMOFA, 2016). These large amounts of seafood, representing a wide range of exploited biodiversity, host also a wide diversity of viruses, bacteria, archaea, unicellular and multicellular eukaryotic organisms. While taxon diversity is considered to be at least of the same order of magnitude as their hosts (Price, 1980; Lafferty and Hoffman., 2016), most remain to be described and their biology elucidated. They are highly important as they play key functional roles in nature, regulate population dynamics, affect community structure and influence ecosystem resilience (Marcogliese, 2004; Tompkins *et al.* 2011). However, from a human perspective, intense trading and shipping of seafood products with these associated organisms may have harmful impacts on the natural ecosystem, aquaculture operations and represent a human biohazard (Krkošek, 2017). In that case, they are considered pathogenic and should be monitored and managed.

Globalization has enhanced the transfer rates of organisms between geographical regions. For example, in the USA, 19% of food consumed is imported including 97% of fish and shellfish (Gould *et al.,* 2017). This involves the transfer of organisms throughout the natural distribution range (interpopulation) as well as the introduction of non-indigenous species (NIS) into non-native regions. The establishment of NIS may lead to major ecosystem disturbances and has led to significant economic losses on land and at sea (Carrasco *et al.,* 2010). Most NIS have few chances to survive in the new environment or on the consumer's plate, but they may also contain communities of harmful microorganisms (viruses, bacteria and eukaryotic unicellular parasites) and multicellular parasites (Mattei *et al.* 2014; Weyl *et al.* 2014; Tripathi 2014; Pearce *et al.* 2014). Also frozen and fresh seafood may harbour microbes and metazoans of concern. The introduction of NIS has raised special concerns and is considered one of the five major threats associated with global change (Millennium Ecosystem Assessment, 2005). In particular, trade in live,

fresh and frozen seafood products has provoked increased opportunities for infectious diseases to cross natural biological barriers. This may lead to huge economic losses in fisheries and aquaculture, impacts on local diversity, and biosecurity issues (Lafferty *et al.*, 2015).

The transfer of seafood between producers, from producer to consumer, often with intermediate trading and processing posts, has increased the opportunities for the crossing of natural biological barriers. Economic losses have been huge (Lafferty *et al.*, 2015), but societal awareness limited, responses mixed and most often delayed (Lafferty and Hoffman, 2016). Experts have drawn attention before and pleaded for diagnostic tools to identify pathogens (Carnegie *et al.*, 2016). International and national regulations point increasingly to a precautionary approach. Some regulations have been in vigour, mostly to protect the consumer and aquatic animals for some specific diseases (European Parliament and the Council 2002, Council 2006, European Commission 2008) and in some cases also related to faunal integrity.

With the growing world population, the concomitant threat of zoonoses, i.e. infectious diseases of animals that can naturally be transmitted to humans, has increasingly raised concerns (Cutler *et al.*, 2010; Ivanovic, 2017). Despite the scale of the seafood business and the threat to humans, the inventory and monitoring of these biological hitchhikers is at best incomplete, and therefore merits close scrutiny. Numerous introductions of non-indigenous pathogens (NIP) into natural ecosystems remain undocumented. Moreover, the continuously expanding worldwide seafood trade enforces infection risks throughout the supply chain. This raises the need for detection and monitoring of seafood with diagnostic tools that need to be fast, accurate, reliable, robust, sound and cost-effective. Fast because of the potentially acute nature the response has to be within a short time frame. Accurate because assessments do not tolerate mistakes. Reliable because results have to be sufficiently robust to stand in court. Robust because methods have to be operational under a broad range of conditions. Sound because they have to meet legal and ethical standards. Cost-effective in order to have a broad penetration through society. International frameworks for promoting effective disease management such as the World Organisation for Animal Health (OIE – https://oie.int) strongly endorse these developments.

DNA and RNA based molecular techniques can meet these needs, particularly, with the enhanced accessibility of high-throughput sequencing and genotyping (Goodwin *et al.* 2016; Rius *et al.* 2015). Although examples of successful (commercial) applications of DNA-based methods for pathogen detection and identification exist, there is much more potential for further development, notably for emerging technologies and for multicellular parasites. In parasites, 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.

In this report, we review the molecular monitoring of infectious disease and parasite spread from transferred/shipped seafood into wild populations. We do so in four sections and propose first a procedure to assess infection risk based on molecular and biological information. Then we assess screening challenges for pathogens in seafood. In a third section, we present an inventory of molecular tools already available and under development for monitoring. We complement the review with an introduction to representative cases where molecular monitoring has been or could be implemented. Hence,

this review of existing and potentially applicable genetic and genomic methods for infectious disease and parasite spread in seafood may be equally useful for policy decisions on animal health as for managers in assessing the risks from trade for indigenous populations (Conn 2014).

5.1.3 Assessing infection risk

Most of the research regarding fish and shellfish disease is dedicated either to the sustainability of aquaculture production through disease control and prophylaxis (Austin, 2012), monitoring of fisheries (Maynard *et al.,* 2016) or, to a lesser extent, to the transfer, through escapees or seawater, of pathogens from aquaculture productions to wild populations (Stephen *et al.,* 2008 ; Sepúlveda *et al.,* 2013). Comparatively, infection risk resulting from non-indigenous seafood trade on wild populations remains poorly explored. We developed a scheme in which a number of host-pathogen situations leading to different infection risks were characterized. We consider this a simple but useful tool to highlight the highest priority situations. It is based on successive parameters characterizing the state of seafood and its eventual pathogenic agents, such as live versus dead, fresh versus frozen, cooked or processed and symptomatic versus asymptomatic. Each case was associated with an infection potential arbitrary ranging from a value of 0 to 5 and a transmission potential ranging from 1 to 2, leading to an infection risk on a scale from 0 to 10 calculated by multiplying their infectious potential with their transmission potential (Figure 1). The highest infection risk (value 10) corresponds to symptomatic live seafood carrying live pathogens. Special attention must be paid to the international trade of live seafood which is common for numerous species that can be consumed alive (e.g., oysters), or alive before cooking (e.g., lobsters and most fishes in Asia). In that case, non-local seafood is often maintained in recirculating-systems for which seawater must be sterilized (using U.V., chlorine, ozone…) prior to release into the wild in order to prevent the transfer of pathogens. The efficiency of such treatments can be easily assessed for some cultivable pathogenic agents such as some bacteria (Sharrer *et al.,* 2005) but remains poorly known for others, especially those presenting spores or other resting forms. Leftover of fresh seafood (shells, bones, unconsumed specimens) must also be considered as a potential risk of disease transmission if in contact with wild populations. Live symptomatic seafood carrying dead pathogens or showing a lack of pathogens were considered as unlikely and associated with no risk of further infection. This might correspond to situations where seafood would have been efficiently treated for a pathogen but remained symptomatic. This was considered unlikely because most treatments, when existing for a given pathogen, cannot guarantee to be 100 % efficient (e.g., most antibiotics against bacteria) and because for many aquatic pathogens, no treatment exists (e.g., bonamiosis affecting flat oysters, Engelsma *et al.,* 2014). Additionally, disease live symptomatic seafood can be marketed and consumed without any problem because concerned pathogenic agents are innocuous to consumers and do not affect seafood marketability until the latest stages of the disease. Conversely, seafood can be healthy carrier of human pathogens, leading to an increasing number of disease outbreaks (Huss *et al.,* 2000, Gould *et al.,* 2017).

Dead seafood can be traded in different states according to its shelf life and consumers' habit: refrigerated, frozen, dried, steamed, caned, fried, mixed with other ingredients and so on. Each type of processing method is likely to affect pathogen's viability and there-

fore influence infection risk. Assessing survival potential of pathogens following different processing methods requires appropriate methods based on cultivation (when possible) or molecular approaches (see below). Most studies concern human pathogenic bacteria that are able to grow at refrigeration or near-refrigeration temperatures (<10°C) (Chintagari *et al.*, 2016) and can be of indirect interest regarding infection risk of wild populations but little is known about fish and shellfish parasites (Spanggaard and Huss, 1996, Kokan *et al.*, 2014) or other types of pathogens.

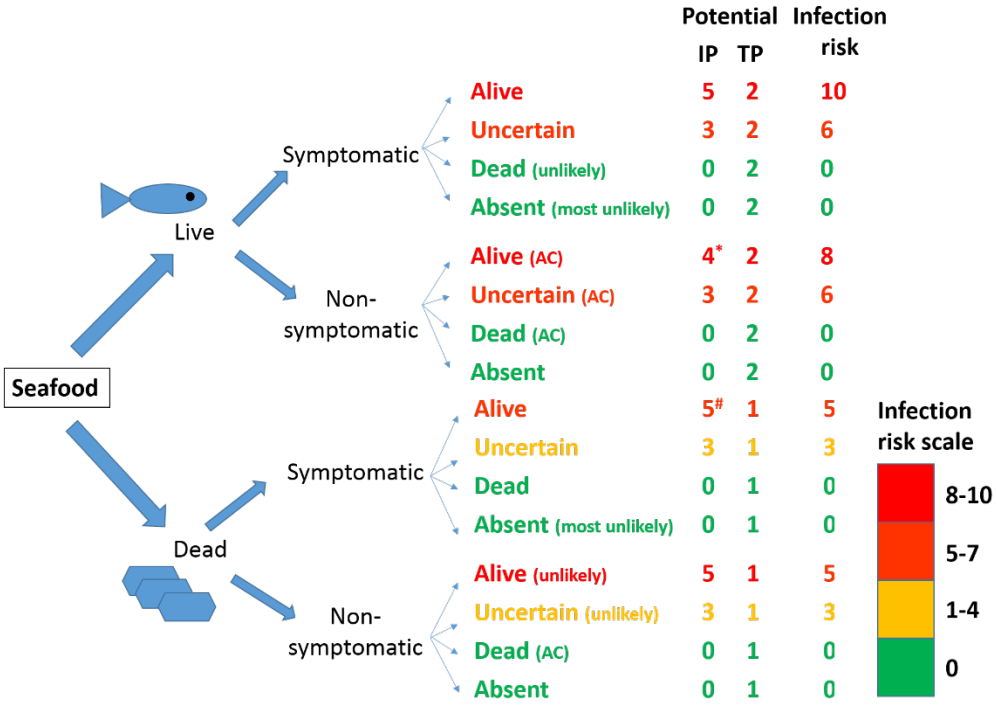

Figure 1 Scenarios of host-pathogen infection with associated respective infection risks. AC= asymptomatic carrier, IP= infectious potential, TP= transmission potential; *condition dependent: threshold, reservoir function, state of immune system, # condition dependent: biology of the pathogen (i.e. requirements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

5.1.4 Challenges for screening pathogens in seafood

The challenges associated with screening pathogens in seafood are numerous and include biological, logistical and methodological. This section highlights some key challenges.

5.1.4.1 The taxonomic units of identification

Identification of pathogens must be performed at the most relevant taxonomic level, i.e. genus, species, or strain/genotype. For some pathogens however, like bacteria and viruses, the species boundaries are not clear-cut. Sometimes identification at the genus level is useful if the pathogeneicity is determined by this taxonomic level. Yet, in some cases, it will be necessary to accurately identify the individual species or even strains. There are two possibilities for identification based on PCR 1) assessing presence/absence using species-specific primers, 2) sequencing a PCR product using universal primers, e.g. bar-

coding at COI, and comparison against a genetic database (Genbank: www.ncbi.nlm.nih.gov, BOLD: www.barcodeoflife.org, EMBL-EBI: http://www.ebi.ac.uk/ena).

For example, for the haplosporidian oyster parasites *Bonamia*, the two species (*B. exitosa* and *B. ostrea*) can be distinguished with a single locus approach (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 and Seidler, 1983) of which not all strains are pathogenic. Distinguishing between strains and identifying those that do represent a threat is challenging. 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 (Lee *et al.,* 2013), a hybridisation 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 modelling 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 (Kibenge *et al.,* 2009).

5.1.4.2 Non cultivable pathogens

Non-cultivable pathogens dominate in the marine realm. Therefore, 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, and sample concentration and DNA extraction suffice. Identification of the operational taxonomic units (OTUs) relies on genomic data banks. For example, Lohan *et al.,* (2016) used high throughput sequencing to successfully characterize the protistan community in ship's ballast water.

5.1.4.3 Detecting pathogen presence does not mean disease

The detection of a pathogen does not confirm 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.

5.1.4.4 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.

5.1.4.5 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 is also material which is 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	Microsporidians	eDNA Metabarcoding	Virus/pathogen can be present in the absence of disease
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, etc.)	Molecular epidemiology – ancestry reconstruction – e.g. ISAV – identify origin	Multiple approaches	
Processed seafood	*Listeria* in smoked salmon	RNA or DNA-based; high-throguhput NGS amplicon sequencing	Processing of the seafood increases the challenge to detect the primary pathogen

5.1.4.6 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.

5.1.5 Molecular tools for screening pathogens in seafood

The armoury of molecular tools available to both research scientist and regulatory bodies alike has undergone a major expansion in recent years. Some molecular approaches may be tailored towards low throughput basic research and target discovery (e.g. whole genome sequencing, transcriptomics, metagenomics); perhaps not immediately appropriate in a surveillance context in term of cost and analytical complexity. Conversely other molecular approaches (e.g. Sanger sequencing, SNP arrays, qPCR) may me more appropriate for high throughput screening, potentially in situ (e.g. Xia *et al.* 2014) for the purpose of defining pathogen prevalence and distribution. It is important to recognise the link between the two types of approach; whereby novel targets identifying pathogen species, strains, virulence factors are first identified via genome-wide approaches and later developed in to rapid screening tools. Furthermore, both cost and efficiency of deployment are major considerations (Biswas *et al.* 2014). As such, 'classic' molecular tools (e.g. PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) may be preferable over more technologically advanced approaches in many circumstances (Su *et al.* 2015). Furthermore, novel methodologies like loop-mediated isothermal amplification (LAMP), PCR-dipstick tests (Carrasco *et al.,* 2012) as well as several commercially available portable qPCR machines means that screening can be carried out and acted upon on-site. In Figure 2 we supply a diagram to assist in the appropriate deployments of different tests.

In short: the first step is generally the identification or verification of the targeted pathogen(s) which may occur with or without a priori knowledge leading to different starting points, with an additional step focussed on the identification through (meta)barcoding if no a priori information is available. Once the identity of the targeted pathogen or pathogens is resolved, the objectives will be on either i) detection, ii) quantification, and/or iii) viability assessment. Important is here to make a distinction between pathogens from pure extracts/cultures and mixed (multiple) pathogen samples, e.g. from environmental or swap samples, as the tools that can be used vary accordingly. For all three objectives and units of analysis (i.e. single pathogen vs multiple pathogens), different molecular methods are available as illustrated in Figure 2 and explained in more detail in the main text, separated into DNA-based and RNA-based methods.

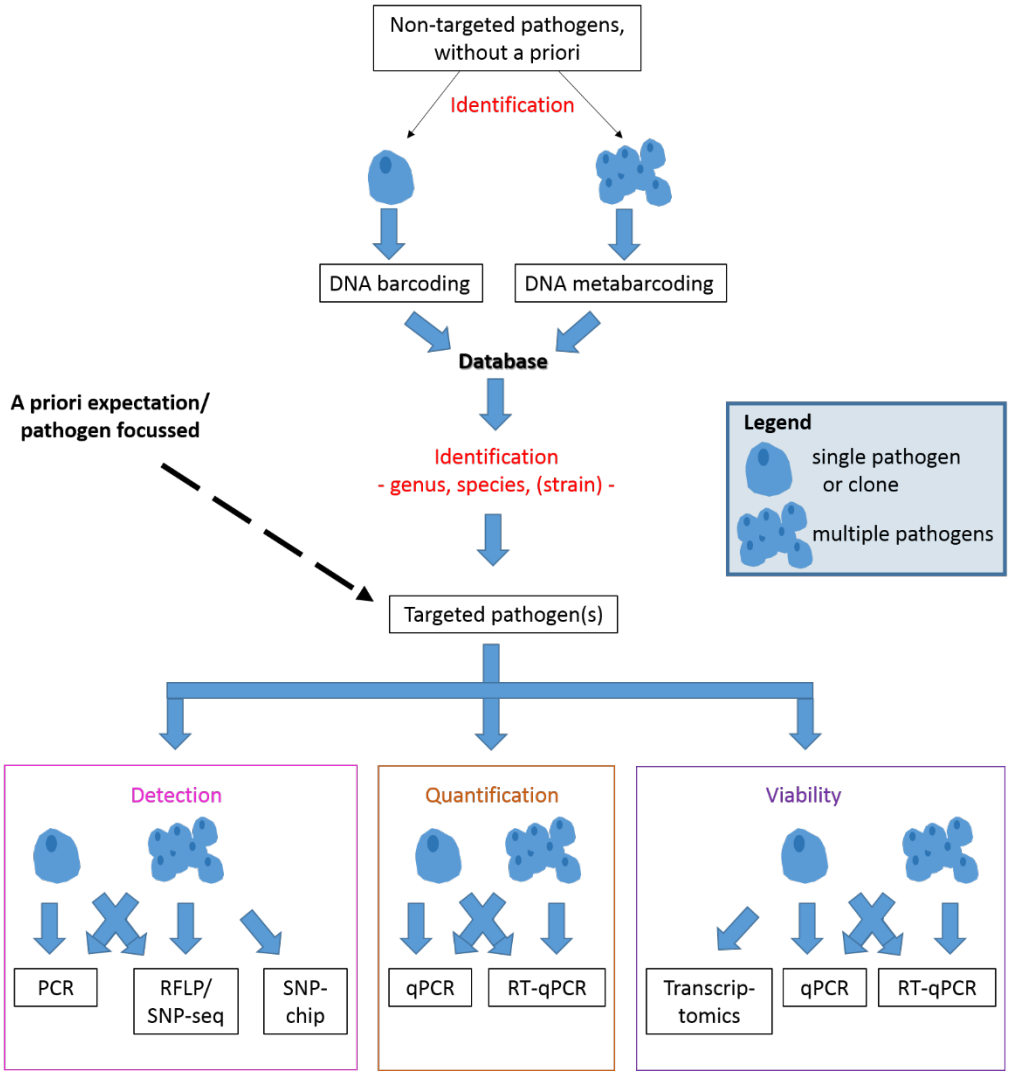

Figure 2. Workflow to aid decision-making when analysing pathogen samples from seafood. Decisions are based on i) a priori knowledge of the pathogen Y/N, and ii) application, i.e. identification, detection, quantification, or viability.

In the following section, we provide an overview of the molecular marker system available to detect pathogens transferred via seafood.

5.1.5.1 DNA based methods

DNA-based identification methods rely on the positive identification of a unique (set of) marker(s) linked to the identity of a pathogen. There is no clear consensus on which markers should be chosen to provide taxonomic information, principally because the taxonomic level required will vary between studies. Species-level assignment may be appropriate for some pathogens, strain-level assignments maybe appropriate for others. Molecular approaches can be categorised very broadly as: taxon-specific (where a priori information is available - perhaps idiopathic histological evidence – to guide a researcher toward a potential agent) or universal (where the molecular marker is shared among many species, each which display idiosyncratic sequence diversity at that locus). The

latter are often termed barcoding loci where specific markers are targeted. Pathogen genotyping systems are not restricted to single locus analyses. Multi-locus Sequence Typing (MLST) has been a mainstay of many bacterial (Maiden 2006) and eukaryotic (e.g. Odds 2008) molecular identification drives. MLST has now largely been superseded by whole genome sequencing which can provide an insight into single nucleotide polymorphism between individuals, genome-wide. Such information can then be exploited to generate high throughput SNP arrays (e.g. Illumina, Affimetrix), as well as targeted SNP assays which can be used to target particular strains or species. For the purposes of pathogen detection and identification in live seafood, we will focus on a number of novel approaches which show promise in terms of low cost and scalability.

DNA Barcoding: For multicellular eukaryotes barcoding loci include mitochondrial (e.g. Cytochrome Oxidase I, Cytochrome b) or nuclear markers (e.g. 18S rDNA), all of which are extensively validated and supported large online reference datasets (Silva (Quast *et al.* 2013), Barcodesforlife (Ratnasingham and Hebert 2007) and RDP (Cole *et al.* 2014). The most extensive bacterial and archael datasets are supported for the 16S rDNA locus (e.g. Greengenes, RDP, Silva). Although generally described as 'universal' barcoding loci, the choice of marker used, and which variable domain one might consider using can depend on the group of organisms of interest (e.g. COI for insects, Cyt b for mammals). Synthetic oligonucleotide sequences used to prime the PCR reactions that target barcoding regions are often degenerate and target conserved domains to achieve amplification of multiple species. PCR products are then DNA sequenced and compared to online databases to identify the pathogen in question. DNA barcoding is highly sensitive to contamination from non-target species, including host DNA and commensal microorganisms.

DNA Metabarcoding: DNA metabarcoding combines classic barcoding (above) and next generation single molecule amplicon sequencing approaches (e.g., Illumina and IonTorrent sequencing platforms). Metabarcoding is employed when the DNA template is suspected to consist of sequences from two or more different species origins. Tens of thousands of sequence reads are generated per sample, wherein the diversity and abundance of different sequence types is associated with the diversity and abundance of the organisms contained within the sample (Staats *et al.* 2016). As with DNA barcoding, contamination is a major consideration. Also, short read lengths (ca. 300 bp) generated by most next generated sequencing methodologies means that metabarcoding is not currently used as a reliable means of unequivocal pathogen detection. However, as read lengths continually improve so will their ability to resolve species-level taxonomic assignments.

Single locus SNP genotyping and SNP arrays: Multiple approaches target specific pathogen sequences for the purposes of molecular identification, including 'classic' genotyping approached (PCR-RFLP etc.). Next generation tools are now available to improve on the performance and repeatability of such approaches. In principal, a priori information is required to guide the researcher toward marker choice. In practice, arrays of probes corresponding to particular pathogen sequences can be generated to screen for multiple pathogens in a targeted fashion, circumventing contamination issues. Scaling has historically been a problem for SNP arrays, however, while set-up costs remain very high, the technologies are now in place to screen in the 10s (Pieprzyk and High 2009) and the 100s (Tobler *et al.* 2005) of target sequences, not simply the 1000s (Perkel 2008).

Quantitative approaches: Quantitative PCR exploits the binding of fluorescent dies or fluorescently tagged probes and resultant photonic emissions under UV light to reveal

the rate of accumulation of replicant DNA molecules in a PCR reaction. This information can be used to infer the abundance of a target sequence in a sample. Taxon specific DNA primers circumvent contamination from non-target sequences. qPCR Reagent and equipment costs are declining rapidly, and several companies offer 12-volt portable machines, meaning the pathogen identity and abundance can be determined in situ. Microfluidic technology (e.g. Fluidigm Biomark (Pieprzyk and High, 2009)) provides the opportunity for highly multiplex qPCR screening, enabling 96 (target) x 96 (sample) quantitative screening – although currently at very high cost.

Emerging technologies: In recent years several low-cost, portable, easy-to-use assays have been developed and applied for pathogen identification, mostly in the clinical field (Renner *et al.* 2017). Yet these assays can be easily adapted to be used in the seafood control context. Among the most promising techniques are the isothermal PCR techniques, which do not rely on the use of high temperatures for DNA denaturation. Low tech, point of care (POC) DNA-based pathogen detection: Although PCR-RFLP and Amplified Fragment Length polymorphism are the widely used traditional "go to" low-tech methodologies for pathogen detection in seafood, a new generation of low-tech approaches are emerging. Such approaches aim to reduce or obviate the necessity for specialist equipment involved. LAMP achieves amplification of target sequences without the need for a thermocycler, for example (Biswas *et al.* 2014). As such, a short DNA fragment can be amplified in the field with the only need of a thermoblock and in a less than 90 min (Notomi, Mori *et al.* 2015). Meanwhile lateral flow assays and PCR dipsticks, antibody or bead-based can enable specific detection of the target fragment rapidly, without any requirement for gel electrophoresis equipment (Tian *et al.* 2016), or dyes (SYBR Green, EvaGreen) can be added directly to tubes (Centeno-Cuadros, Abbasi *et al.* 2017). Several studies have applied LAMP to search for parasites in food products. In particular attempts have been performed for detecting Vibrio species in oysters and shrimps (e.g. (Han, Wang *et al.* 2011; Yamazaki, Kumeda *et al.* 2011)).

DNA isolation bottlenecks: Highly multiplex, high throughput approaches can now process large datasets in record time. A major consideration in terms of cost and effort is DNA isolation from sample material. Several commercial extraction kit producers offer robotic solutions. However, costs can be prohibitive and sample purity in excess of that required. Several simple and low cost lysis and DNA stabilisation approaches are under development, and this is an area in which we expect considerable innovation and interest in coming years.

5.1.5.2 RNA based methods

RNA-based methods focus on either the transcripts of genes, or genomic sequence in the case of RNA viruses. 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). The application of RNA based detection approaches in screening seafood pathogens is limited to the detection of RNA viruses. Transcriptional variation is not a likely target of study in screening studies. As with DNA based approaches microfluidic qPCR can offer multiplex screening of RNA viruses. RNA extrac-

tion methodologies are at present both costly and cumbersome, especially as mRNA is highly sensitive to spontaneous and enzyme mediated degradation.

5.1.6 Cases of successful application of molecular monitoring

5.1.6.1 Piscine Reovirus

Piscine Reovirus (PRV) causing Heart and Skeletal Muscle Inflammation (HSMI) that affects Atlantic salmon and other species, which can result in up to 20% mortality in affected farms (Hoffman, 1990). Although virus-like particles were observed in sick animals, traditional approaches such as culture, subtractive cloning and consensus polymerase chain reaction for detection of other viruses, failed to identify the disease agent. Yet, high-throughput sequencing of RNA from the cardiac tissue of infected salmon predicted amino acid sequences with 49% similarity to a mammalian orthoreovirus protein. Moreover, subsequent reverse transcriptase qPCR (RT-qPCR) was able to target the associated DNA sequence and unequivocally establish the association of the viral sequence with diseased individuals.

5.1.6.2 Bonamiosis affecting flat oysters

Over 40 species have been described in the *protist phylum Haplosporidia*, comprising species infecting a range of molluscs, including several ecologically and economically significant pathogens such as *Haplosporidium nelsoni* and *Bonamia ostreae*. In recent years, molecular phylogenetic studies added ten new species in this phylum. The genus *Bonamia* currently comprises at least three valid species (*B. ostreae*, *B. exitiosa*, *B. perspora*). The status of *B. roughleyi* is controversial (Engelsma *et al.* 2014). An addition Bonamia sp. might remain to be named (Arzul and Carnegie, 2016). Bonamia infects a variety of flat oyster species including *Ostrea edulis*, *Ostrea chilensis*, *Ostrea stentina*, among others, across the globe (see Engelsma *et al.* 2014). *B. ostreae* was first reported in Europe in Brittany, France by Pichot *et al.* (1979; Peeler *et al.,* 2011). Since then, it has been reported in association with oyster mortality in an increasing number of European countries and, more recently for the first time in the southern hemisphere in New Zealand (Lane *et al.,* 2016). It is considered to have been introduced into Europe by a trans-shipment of infected oysters from its putative endemic area of eastern USA (Elston *et al.* 1986). The source of *B. ostreae* infection in New Zealand is currently unclear, but the introduction of flat oysters seems likely. Conversely, *B. exitiosa*, was first described in New Zealand (Hine *et al.* 2001) and was detected for the first time in Europe in 2007 in the Atlantic coast of Spain (Abollo *et al.* 2008) and since then in several other European countries (Batista *et al.,* 2016), in USA in 2012 (Dungan *et al.,* 2012) and in Australia (Carnegie *et al.,* 2014).

Screening for Bonamia relies primarily on histological approaches (European Union Reference Laboratory, www.eurl-mollusc.eu). Molecular diagnoses are increasingly based on PCR amplification of the 18S/ITS1/5.8S region, using different combinations of primer pairs allowing distinguishing *B. ostreae* from *B. exitiosa* (Ramilo *et al.* 2013), PCR-RFLP and DNA sequencing. Absence of PCR inhibitors can be tested using primers amplifying COI and 28S of oysters (Batista *et al.,* 2016). In situ hybridization is also performed to visualize Bonamia cells in host tissues (Engelsma *et al.,* 2014). Both PCR amplification and ISH were used to demonstrate that *B. ostreae* can infect oyster larvae (Arzul *et al.,* 2011;

Flannery *et al.*, 2016). Viability of Bonamia can be assessed using flow cytometry (Arzul *et al.*, 2009).

5.1.6.3 Myxobolus cerebralis

Whirling disease is caused by the myxozoan parasite (Cnidaria), *Myxobolus cerebralis*, and affects wild and farmed freshwater and anadromous salmonids (Hoffman, 1990). It requires a freshwater oligochaete worm – *Tubifex tubifex*, for the completion of its lifecycle. Pathology is focused on the skeletal and central nervous system, resulting in skeletal deformations and nervous abnormalities that result in the classic 'whirling' swimming behaviour. The disease is thought to have spread into North America via transfer of salmonids, and *M. cerebralis* transfer via frozen fish material poses particular threats, as the T. tubifex infective stage is very resilient, surviving several months at -20 °C. Several diagnostic assays have been developed and tested, including histological and molecular approaches (standard PCR, qPCR, LAMP) (ref). For PCR approaches, at least, sensitivity and specificity is high. In European terms, high-throughput screening approaches would be helpful for both intra-European transfer as well as export transfers of potentially infected fishes.

5.1.7 Conclusions

With increasing chances for (and cases of) zoonoses, parasite spillovers and spillbacks through the trade and transfer of seafood products, the need to shift from response to prevention in biosecurity is obvious. This requires regular monitoring not only for pathogens related to food safety, but also pathogens affecting the ecosystem. Thus, there is a need for coordinated approaches between seafood pathologists and genomics experts as chances for zoonoses are steadily increasing, and this requires the combination of fundamental, applied and operational research. Genomic approaches offer powerful cost-effective approaches for seafood pathogen control, but prior to their introduction in regular surveys, evaluation of their utility and likelihood of uptake, potential and drawbacks is required. In the end the paradox of advanced diagnostics has to be avoided through integration in a broader community context combining PCR screening and classic microscopic surveys (Carnegie *et al.* 2016).

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

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

5.2.1 Introduction

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

The role of WGAGFM can be summarised as:

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

2) To establish a representative, sustained and engaged scientific forum across ICES countries to i) discuss technological and statistical developments and innovative ideas in genetics/genomics, ii) salient opportunities for research consortia, and iii) promote 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 important for economic development, global food safety and security. Many major breeding companies for fish and invertebrates recognise the vital role of genetics for their development, whereas the role of genetics in relation to capture fisheries appears to be less recognised, though there are notable exceptions as described below.

Another emerging issue of common concern is the interaction between captive-bred individuals, either 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, which was initiated by the sequencing of the human genome. Such genome-wide approaches are particularly important in fisheries and aquaculture, especially with the current availability of entire genome sequences 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 is a corresponding bourgeoning of applications pertinent to fisheries and aquaculture, some of which we highlight below. Successful 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.*, 2013) 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 historical changes in 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) Genetic methods 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 conspecifics (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) Outputs from the EU-funded FishPopTrace project (https://fishpoptrace.jrc.ec.europa.eu/) focused on population structure and traceability of marine fish species and products (cod, herring, sole, hake), and directly influenced the incorporation of genetic approaches into traceability within the European Common Fisheries Policy (CFP).

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 sizes, 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 which, include:

- **Metabarcoding**: it is now possible using high throughput sequencing 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 to promoting sustainability and conservation of natural resources.
- **Environmental DNA (eD)**: rapid advances in the retrieval of free-floating DNA taken directly from water samples allow the detection of single and multiple species without the need to sample organisms directly (Bohmann *et al.,* 2014). The integration of metabarcoding and eDNA analyses enables further 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, down to one or a few molecules, so enabling earlier diagnosis. Such developments are the subject of a current ToR (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 now being 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 health and disease, nutrition, immunity, development, behaviour and life histories.
- **Quantitative genetics of wild populations** (see 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 of specific functional genes** underpinning key physiological processes including immune competence (MHC) and growth maturity axes (VGLL3); (Hemmer-Hansen *et al.,* 2014), is now available.

- **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 practice

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 often perceived 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 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 raise questions or solve problems in a milieu with a broad 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 (see annex 2). The Expert Group report, including the specific recommendations, is presented to SCICOM (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 con-

servation 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, the coherent and routine integration of such information 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). As stated above, the WGAGFM was structured such that relevant research topics could be discussed in the context of key technical and conceptual advances, as well as exercising a clear focus on implications for marine conservation and management issues. Such recommendations were addressed at a variety of ICES structural units, such as ACOM, SCICOM and various working groups (see ANNEX – Summary of WGAGFM Recommendations). 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 out-reaching 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 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 requirements, 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 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 Data Collecting Framework (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 a 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 'database' ToR 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, like 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.

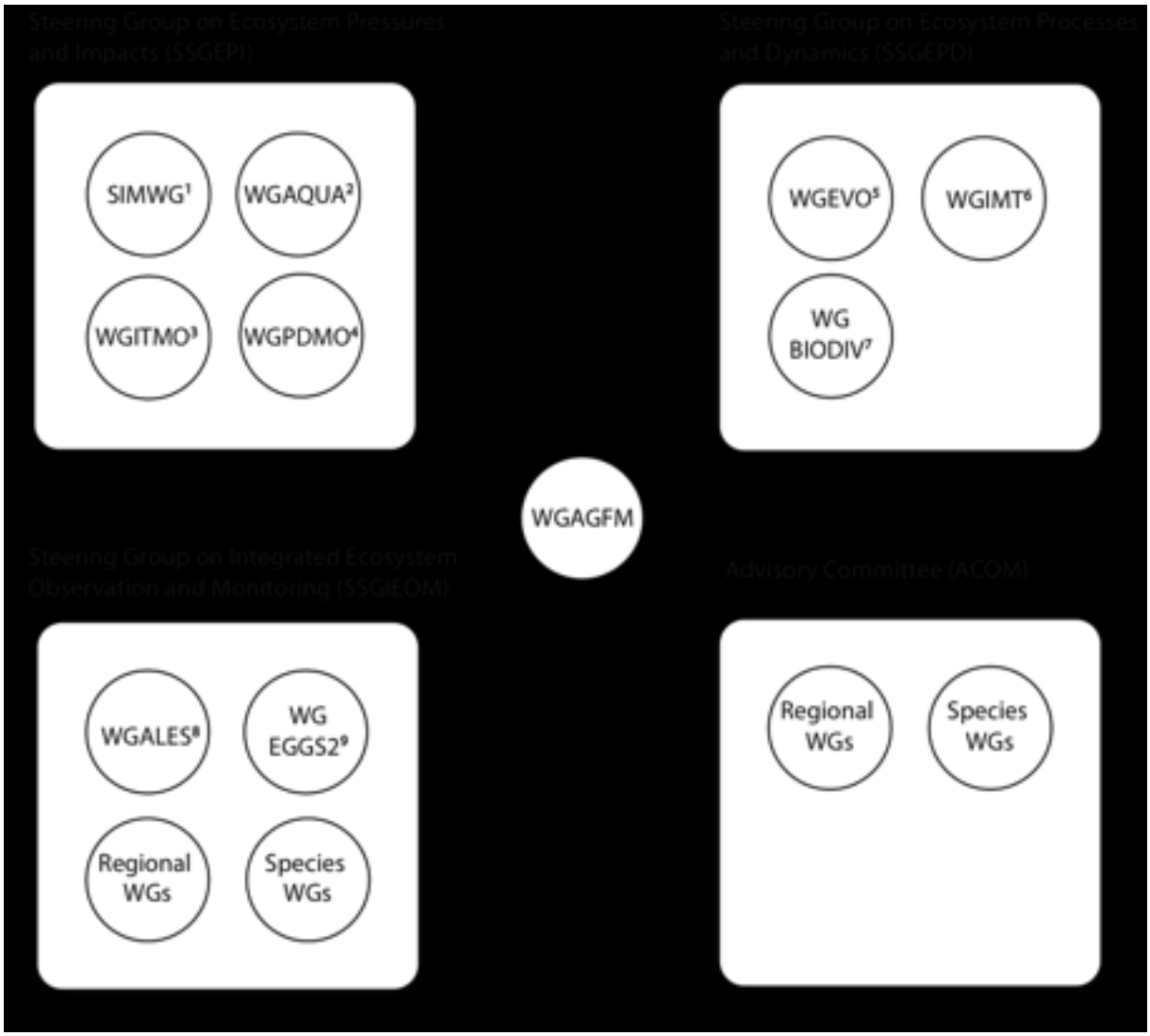


Figure 1. Expert Groups with potential complementarity in interest with WGAGFM or past/future interactions with WGAGFM (see text for brief details).

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) **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.

3) **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 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'.

4) **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.

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

6) **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.

7) **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.

8) **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.

It should be emphasised at this stage that the approach was exploratory only. Due to the unrepresentative number of responses received, we do not provide further details here. We also examined the various Benchmark meetings, and very quickly identified two were the WGAGFM would contribute to the discussions and recommendations:

WKCOSTBEN 2016 - Workshop on cost benefit analysis of data collection in support of stock assessment and fishery management. A long-standing quest for WGAGFM has been to promote involvement in the planning of data collection – and based on parts of the planned activities this is an important Benchmark meeting for the WGAGFM and its expertise; "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".

WKIrish2 - Benchmark Workshop on sharing information on the Irish Sea Ecosystem, stock assessments, and fisheries issues, and scoping needs for assessment and management advice. In 2015, the WKIrish1 meeting concluded among several priorities, for actions 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, a topic that is within the expertise of the WGAGFM.

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.

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:

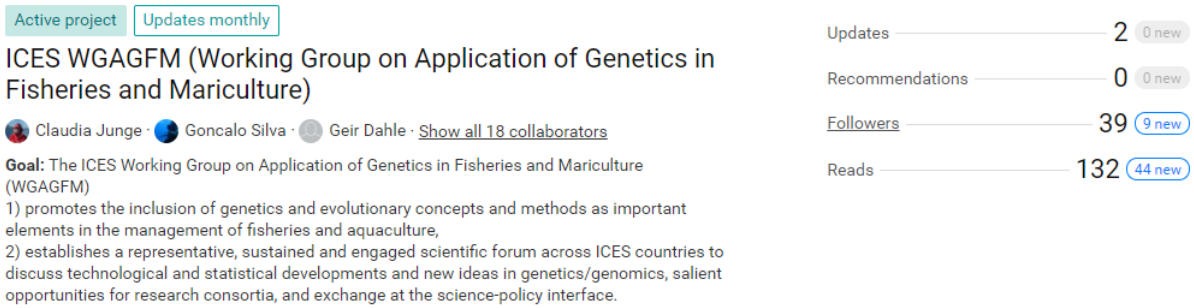
5.2.7 The way forward

(Progress in Year 3, Olhao, Portugal)

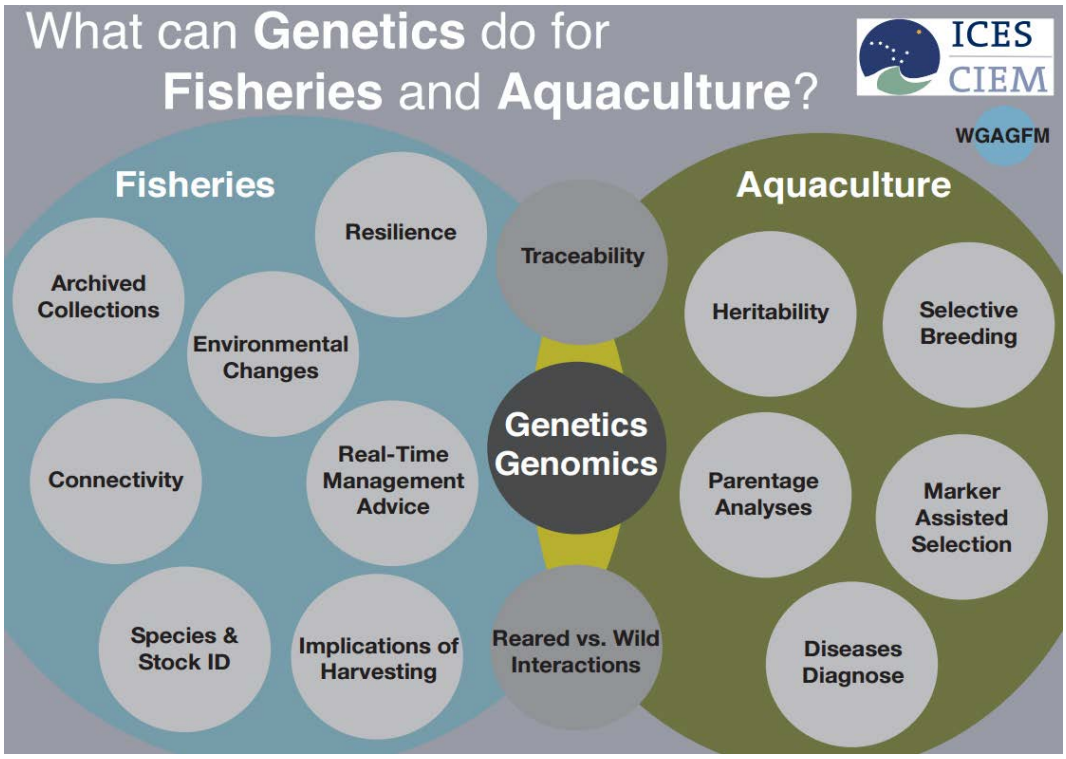
The WGAGFM recognizes that as an Expert Group, we must deliver a three-year report to SCICOM on the work performed on the different ToRs as well as recommendations emerging from these ToRs. We are however concerned about the extent of dissemination of the recommendations or expert advice to the scientific community, stakeholders; industry, management, "decision makers" (National governments, EU, FAO), research councils etc.

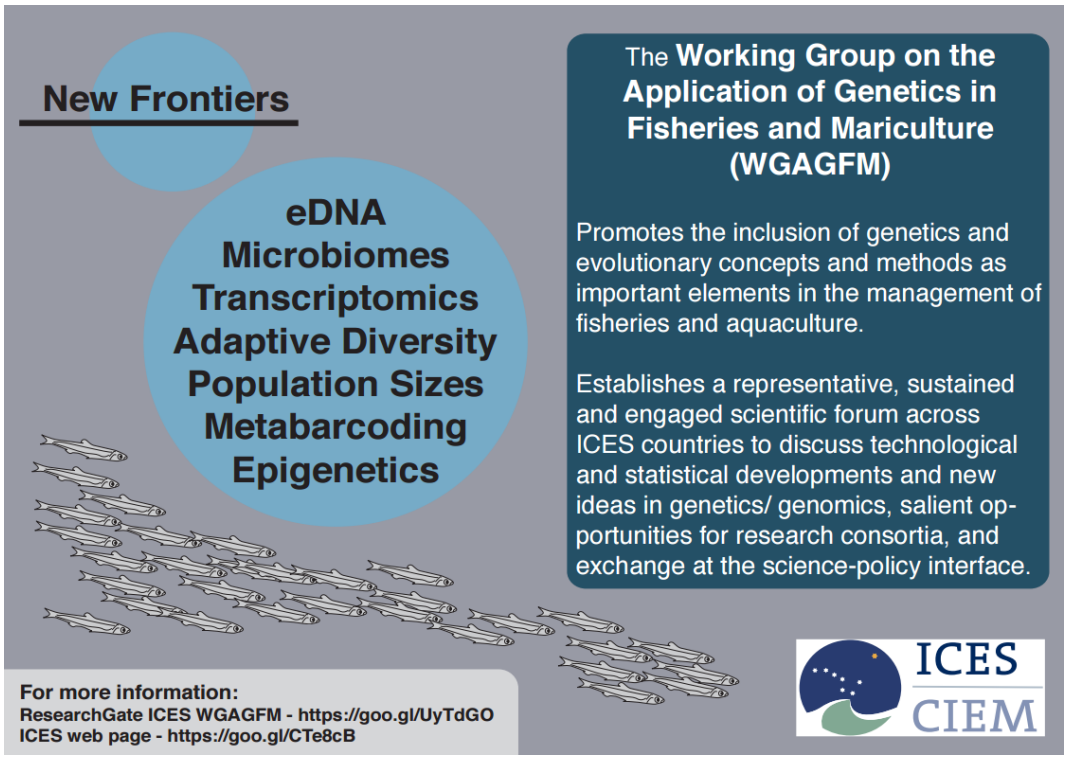
To accommodate these needs, the WG decided that we will use two additional information approaches "outside" of the normal ICES channels:

First, we have created a "Project" in "Researchgate.com" to reach the scientific community in a more targeted way (https://www.researchgate.net/project/ICES-WGAGFM-Working-Group-on-Application-of-Genetics-in-Fisheries-and-Mariculture). This will allow us to link to the ICES webpage (WGAGFM), where in addition to the background for the group, the annual reports and list of group members also would be available. Within less than 1 month of set-up, we have over 30 followers and over 130 reads, which indicates a wider interest and the fact that we are in fact reaching a broader audience of researchers now (see below).



Second, we have also created a 2-page leaflet for targeting industry, management, national governments, EU, FAO, research councils etc. (see design draft below) The leaflet includes a schematic presentation of the scope of expertise and services the WG can offer to these groups. To profile the target users, a database will be constructed.


What can Genetics do for Fisheries and Aquaculture?
ICES CIEM
WGAGFM
Fisheries
Aquaculture
Resilience
Traceability
Archived Collections
Environmental Changes
Heritability
Selective Breeding
Genetics Genomics
Connectivity
Real-Time Management Advice
Parentage Analyses
Marker Assisted Selection
Species & Stock ID
Implications of Harvesting
Reared vs. Wild Interactions
Diseases Diagnose


New Frontiers
eDNA
Microbiomes
Transcriptomics
Adaptive Diversity
Population Sizes
Metabarcoding
Epigenetics
The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM)
Promotes the inclusion of genetics and evolutionary concepts and methods as important elements in the management of fisheries and aquaculture.
Establishes 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.
For more information:
ResearchGate ICES WGAGFM - https://goo.gl/UyTdGO
ICES web page - https://goo.gl/CTe8cB
ICES CIEM

Recommendations

R1. **Identification of mechanisms to promote dialogue among Expert Groups** to capitalise on shared objectives in management of aquatic resources. For example: (a) the inclusion of a forum at the Annual ICES Science Conferences for selected Expert Group Chairs to maximize efficiencies in addressing the ICES Science Plan goals and supporting activities; (b) the directed contribution/participation of WGAGFM to salient benchmark exercises.

R2. **The promotion of the WGAGFM as an interdisciplinary knowledge base within ICES** to respond to key management questions that could greatly benefit from genetic and genomic information. For example, contribution to studies of connectivity, stock discrimination, impact of environmental change, conservation of genetic resources, best practice in breeding programmes, quantification of stock abundance, predator-prey interactions, community diversity and resilience, etc.

R3. **A more effective dissemination of accessible information** on resources, opportunities, and mechanisms for implementation offered by genetic and genomic tools. Initially to include the production of post-note flyers describing WGAGFM relevance in relation to ICES (and affiliated bodies) science priorities, scope and activities, with and selected case studies demonstrating implementation and impact.

R4. **An increased presence and opportunity for scientific exchange of WGAGFM activities** through considered deployment of social media, such as Research Gate.

R5. **The establishment of an archive compiling salient illustrative case studies** that demonstrate: (a) effective integration of approaches, uptake and implementation of genetics/genomics into management, and (b) contributions to ICES (and beyond where applicable) science priorities.

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

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5.3.1 Introduction

Understanding the genetic foundation of phenotypic variation is important in exploited marine species, as stocks are often identified based on traits subject to functional genetic variation, and stock assessment models incorporate estimators of life-history parameters. Climate change, intraspecific hybridisation, harvest and management actions can all drive rapid evolutionary change (e.g., Conover and Munch, 2002; Naish and Hard, 2008; Sgrò *et al.*, 2011) potentially impacting stock productivity and persistence (e.g., Schindler *et al.*, 2010). It is the recognition in recent years that evolutionary change can take place much more quickly than hitherto thought that renders a consideration of the genetic basis of key traits such as growth, recruitment, behaviour and maturation schedules, as a priority consideration for environmental managers. Such considerations are of direct relevance to issues relating to resilience, recovery and sustainability of exploited resources that are exposed to rapid environmental change.

Individuals from natural populations will respond to environmental variability such as habitat change through, for example, altered behaviour and differential expression of life history traits (i.e. phenotypic plasticity). At the population level, responses to environmental change are also likely to include distributional shifts and altered ecosystem interactions, but importantly will also occur through genetic (i.e. evolutionary) responses. It is a stated objective of the ICES Science Plan to contribute research that allows us to understand and forecast impacts of environmental changes in ecosystem processes (ICES, 2013). In the plan, there is specific emphasis on the development of methods enabling greater understanding of the influence of both aquaculture-wild interactions and the impacts of climate change on fisheries, and to identify indicators of such impacts and how they drive ecosystem changes. Knowledge of a species adaptive potential will be a prerequisite for forecasting trajectories of change and for assessing the vulnerability of (key) species and ecosystems.

5.3.2 Variation in phenotypic traits and the rate of evolutionary change

Adaptive potential in its purest definition concerns Darwinian (i.e. purely genetic) evolution in response to natural selection. There is already evidence for rapid genetic evolution in response to climate change in several short-lived species (Reusch & Wood, 2007; Merila & Hendry 2014), and for fisheries induced evolution in several species (Kuparinen & Merilä, 2007; Heino *et al.,* 2015), suggesting that many organisms have adaptive genetic variation and the capacity for an evolutionary response to environmental change within a time frame of tens of years (Bradshaw & Holzapfel, 2008). However, often as the result of the difficulties of quantifying such adaptive responses, managers tend to ignore evolutionary processes when devising management criteria. The concept of evolutionary resilience is a way of incorporating and articulating knowledge of evolutionary processes in marine management and marine spatial planning.

In order to determine the complex basis of these genetic changes, quantitative genetic techniques are required. The basic principles of quantitative genetics have been understood since the early twentieth century (Sax, 1923), but a lack of molecular markers and effective statistical tools restricted advances in the field until the landmark study by Lander & Botstein (1989), provided the first statistical framework for QTL analyses. Since this time, there have been revolutions both in the technical ability to provide genetic data (i.e.: Next Generation Sequencing, and associated protocols such as RAD-sequencing and whole genome resequencing) and in the accompanying bioinformatics and statistical tools.

5.3.3 The quantitative genetic approach

The basic theory behind these statistical methods is that the genetic architecture of complex traits is due to many (possibly interacting) genes, and this requires a complex analytical framework to disentangle. Two methods are primarily used: Quantitative Trait Loci (QTL) analysis and Genome Wide Association Studies (GWAS).

In QTL analysis, traits are mapped using linkage analysis. This is frequently applied in animal breeding and aquaculture to study economically important traits (de Koning *et al.,* 1999; Liu & Cordes, 2004; Lee *et al.,* 2005). Traditional QTL approaches required data on crosses and pedigrees (e.g. Slate *et al.,* 2002), however, new multi-marker techniques ena-

ble the study of a traits underlying genetic architecture in the wild without such restrictions (Robinson *et al.*, 2013; Bérénos *et al.*, 2014).

GWAS has evolved over the last ten years into a powerful tool for investigating genetic architecture. The methods are based on linear mixed models and were initially applied in human epidemiological studies, where thousands of markers have been identified as associated with diseases (e.g. affecting immune response, metabolism, or cardiovascular performance (Casto & Feldman, 2011; Stranger *et al.*, 2011)). These techniques are increasingly being applied to wild animals (e.g. Johnston *et al.*, 2011; Santure *et al.*, 2013), including to some fish (predominantly salmonid) species (e.g. Johnston *et al.*, 2014; Gutierrez *et al.*, 2015). Additionally novel methods such as multivariate random forest (RF) algorithms are being used to conduct association mapping (Brieuc *et al.*, 2105; Hess *et al.*, 2016).

Increasingly the wide availability of genome wide genotyping approaches and data presents new opportunities for the estimation of heritability and additive genetic variance in large exploited marine species and populations previously beyond the reach of traditional pedigree approaches. Some of these approaches (such as Yang *et al.*, 2010), may be particularly well suited to marine species as they are designed for large populations with low levels of relatedness, although it is likely that large numbers of loci and individuals will be required (Gagnaire and Gaggiotti, 2016). Taken together these approaches in conjunction with genome wide datasets present obvious potential, allowing research to address questions hitherto impossible, including the magnitude of potential adaptive responses to pressures such as climate change and fisheries. However to date, none of these approaches have been applied to marine species, and the ultimate applicability in a fishery or marine management context remains to be demonstrated.

5.3.4 The key contributions of quantitative genetic analyses in fisheries management

In summary, quantitative genetic analyses can be tailored to address three main issues directly relevant to fisheries management. **First**, analyses may allow determination of the genetic basis of a specific phenotype and how it varies spatially across a species (intraspecific biodiversity) and has varied over time (retrospective analysis) in response to environmental drivers. **Second**, such detailed understanding of the genetic basis of phenotypes permits the genetic monitoring and prediction of the direction and rate of quantitative trait changes in the wild. **Third**, identification of adaptive differences among population sub-units allow for improved robustness of stock assessment models incorporating estimators of local demographic parameters, hence leading to improved management. Traditionally, quantitative genetic examinations of phenotype variation and heritability have required either controlled laboratory experiments or the production of experimental lines with detailed pedigree information. However the integration of traditional quantitative genetic analysis approaches with molecular genomics, and particularly advances in sequencing technology, have opened new opportunities that may also directly contribute to fisheries management.

Within this ToR, we have produced an overview of the scope of quantitative genetic based methods, of pedigree- and pedigree-free genomic mapping approaches, and how they can be applied in planning for evolutionary resilience, sustainable stock exploitation at MSY and in predictions for stock recovery. Such approaches can be integrated with the

goal to improve biological information of direct relevance for assessing the dynamics of exploited fish stocks in time and space, with emphasis on dynamics under anthropogenic change for incorporation into policy and management of marine resources in general. We aim to publish the full report in a peer reviewed journal in the coming months, where additional detail and illustrative examples will be available.

Recommendation

- The application of novel quantitative genetic and genomic approaches to estimate changes in key life history traits of exploited marine populations, to enable the impact of evolutionary change to be incorporated into population modelling for management advice. (PGDATA/ WGBIOP/SCICOM).
- That Assessment groups should take into more explicit consideration that key life history traits are subject to adaptive change over generations and between populations, and that these changes may have a genetic basis and can be rapid. (PGDATA/ WGBIOP).
- The routine collection of appropriate tissue samples for DNA coupled with phenotype measures on the same individuals, and associated environmental data to enable monitoring of quantitative genetic change.

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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

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In 2015/16 a JRC Technical report was produced, offering a reflection and review of the close-kin approach suggested by the Commonwealth Scientific and Industrial Research Organisation (CSIRO – 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 the ICES WGAGFM interim report (2016). In June 2016 a web-conference meeting was convened to establish a strategy that allows to move closer to a practical project type of approach, which was extended in July 2016 with Robin Waples of the US National Oceanic and Atmospheric Administration (NOAA). Outcomes of these activities are documented together with recommendations in this final report.

Summary

In ToR d we aimed to explore the feasibility of applying genetic close-kin mark-recapture (CKMR) analysis, as suggested by Bravington *et al.* (2014), to estimate abundance of commercially exploited deep-sea fish. Abundance estimates of commercially exploited fish (stock assessments) are a key and challenging component of any fisheries management approach. As acknowledged in the recently implemented EU Regulation 2016/2336 on deep sea stocks, and further emphasised at the international level by General Assembly resolutions of the United Nations, the sustainable exploitation of deep sea fish is still greatly impeded by a lack of scientific knowledge and data, which renders stock assessments difficult. In this context, the genetic close-kin approach to abundance estimates could provide a valuable mechanism to inform the management of deep-sea fisheries, thus promoting greater environmental and socio-economic sustainability.

For the purpose of evaluating the feasibility of the close-kin approach in support of deep-sea fisheries management it was decided to focus initially on white anglerfish (*Lophius piscatorius*), as an illustrative species and fishery. While the white anglerfish, strictly speaking, is not a deep-sea species, it shares many characteristics of fish occurring in deep-sea waters, a reasonable amount of biological and stock-relevant information is available, and access to samples is granted. Thus, the intention here is to provide generic information that can be applied to other such species sharing similar life history, demographic and distributional characteristics.

As documented in the ICES WGAGFM Midterm Report 2016, basic simulations using white anglerfish fishing data covering ICES divisions VIIIc and IX (Iberian region), indicate that, assuming a coefficient of variation (CV) of 10% (Bravington *et al.* 2014), a sample size of about 17 000 individuals, 8500 adults and 8500 juveniles, would be required to obtain reliable estimates of abundance (i.e. breeding population that in this case, based

on existing stock assessments, is believed to be ~1.5 million individuals) based on the close-kin method (see ICES WGAGFM Midterm Report 2016). Using the same estimate of abundance (i.e. 1.5 million individuals), further simulations were subsequently carried out to investigate sampling requirements under distinct CV levels and, more specifically, the minimum number of parent-offspring pairs (POPs) that would have to be identified to obtain reliable estimates of abundance (i.e. close to "real" value) based on the close-kin approach. Variation of CV levels had a considerable impact on sample size requirement. For instance, a CV of 12% reduced sampling requirements to about 14 500 individuals (7250 adults and 7250 juveniles). The most important parameter, however, was found to be the number of POPs that need to be identified to obtain reliable estimates of abundance. Assuming a breeding population of ~1.5 million individuals, and a CV of 10%, 70 POPs need to be identified among samples to truly reflect "real" abundance. Under this criteria, a lower number of identified POPs will result in greatly inflated measures of abundance while larger numbers will result in major overestimations. A simple Excel based model was developed to guide users in choosing the best possible sampling design. It is anticipated that the use of additional biological data will assist to reduce model uncertainty.

We conclude in the same report that the genetic close-kin abundance estimate approach has not been sufficiently tested under fully controlled conditions. This prompted us to suggest pursuing a conservative approach by carrying out a pilot study in an experimental framework using a well described and assessed species. This would allow to ground truth the approach under coherent and stringent conditions. However it is evident that compared Southern Bluefin Tuna, the target species of the study carried out by Bravington *et al.*, the information on the biology of white anglerfish is limited.

During the final year of ToR d, further evaluation was carried out to assess the potential utility of close-kin approaches for estimating marine fish stock abundance. In particular, the assessment was extended by considering the biological information required, as well as the 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) or microsatellites utilising recent developments (Farrell *et al.* 2016) in genotyping by sequencing of microsatellites or a combination of SNPs and microsatellites. Notwithstanding the precise choice of molecular markers, it is vital to choose markers with sufficient statistical power, as discussed below, for parentage analysis.

5.4.1 Background

Deep-sea fisheries target species beyond the main fishing grounds on the continental shelves, at depths of greater than 400 metres and down to 1500 metres. In the North-East Atlantic, deep-sea fisheries operate in EU waters, including the outermost regions of Portugal and Spain, and in international waters, governed by conservation measures adopted within the North East Atlantic Fisheries Commission (NEAFC), which includes EU and non-EU countries fishing in the area. For ICES, the working group on biology and assessment of deep-sea fisheries resources (WGDEEP) provides scientific advice on deep-water stocks, including those on shelf areas and deep waters.

The deep-sea is a fragile environment and deep-sea fish stocks are easily overfished and difficult to recover due to their low reproduction rates. Indeed, catches of deep sea fisher-

ies, and consequently jobs depending on his sector, have been declining for years, due to the depletion of targeted stocks. This, together with a lack of scientific data on deep sea stocks, has led the European Commission request for an improved management framework for deep-sea fisheries, as highlighted in a communication of the European Commission to the European Parliament and the Council on deep sea fisheries management in 2007 (COM(2007) 30 final), according to which deep-water fisheries have developed and expanded before sufficient scientific information was available on which to base management advice. The fact that landings and fishing effort data are poor, and discards largely unreported, even though they may well be significant, has impeded ICES to advise on sustainable exploitation levels, and has emphasized that most exploited deep-water species were harvested outside safe biological limits, and that there should be fishing effort reductions.

In response to such concerns, Regulation (EU) 2016/2336 of the European Parliament and of the Council, implemented since January 2017, seeks to ensure the sustainable exploitation of deep-sea stocks while reducing the environmental impact of these fisheries and improving the information base for scientific assessment through data collection. According to article 1 (objectives), this regulation must contribute for deep-sea species to the achievement of the objectives of the Common Fisheries basic Regulation (EU) No 1380/2013 laid down in Article 2 that is e.g. to ensure that exploitation of living marine biological resources restores and maintains populations of harvested species above levels which can produce the maximum sustainable yield (MSY). Moreover, Regulation (EU) 2016/2336 aims at improving scientific knowledge on deep-sea species and their habitats, preventing significant adverse impacts on vulnerable marine ecosystems (VMEs) within the framework of deep-sea fishing and ensuring the long- term conservation of deep-sea fish stocks. It also strives to ensure that EU measures for the purpose of sustainable management of deep-sea fish stocks are consistent with Resolutions 61/105 and 64/72, adopted by the General Assembly of the United Nations (UNGA, 2006, 2007).

Fishery independent abundance estimates are especially relevant for deep sea fisheries management. Recently, a method that derives absolute abundance from the number of parent offspring pairs encountered during the sampling of juveniles and adults has been described and successfully applied to several species. The method, known as the close-kin-mark-recapture (CKMR), is described below.

5.4.2 The principle of genetic close-kin mark-recapture (CKMR) analysis

The CKMR 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 given 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 juveniles and the genotyping of a sufficiently high number of genetic markers in order to conduct parentage analysis (27 microsatellites, in the specific case of Bravington *et al.*, 2014).

The genetic DNA profile (i.e. multilocus genotype) of the offspring (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 have 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)

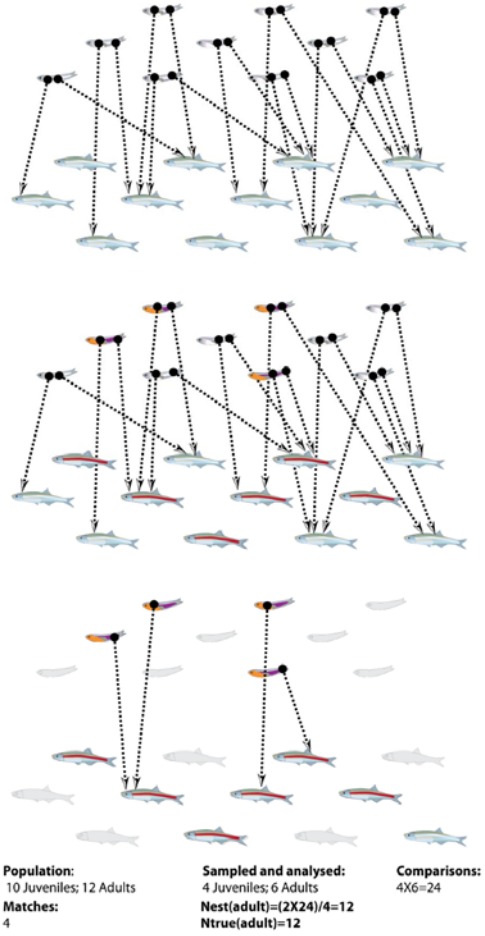

Figure 1. Close-kin abundance estimate: The principle (see text for explanation).

5.4.3 The application of close-kin abundance estimates on tuna

The CKMR method has been successfully applied to Southern Bluefin Tuna, *Thunnus maccoyii* (SBT), to estimate fishery independent absolute spawning stock biomass. This species consists of a single population, which is a huge asset in that there are no analytical complications due to stock structure, and has only one known spawning ground. Moreover, a single fishery of adults that spans the whole spawning season and region allows collection of key biological data including length, age, and sex and well as sampling for genetic analysis of thousands of both adults and juvenile individuals. Such a scenario is ideal for the application of the CKMR method, and does not apply to most other marine species. Thus, in order to avoid a miss-application of the method which can result in erroneous derived estimation of abundance, the applicability of method should be assessed taking in consideration the level of biological information (including stock structure) available for each species in any given region.

To accommodate possible limitations inherent to the analysis of marine fish species, Bravington *et al.* (2015; 2016) have i) developed a model that uses Half-Sibling-Pairs so that for a given individual, the number of kin-pairs is larger and are thus a smaller sample size is required, ii) assessed suitability of Single Nucleotide Polymorphisms instead of

microsatellites as informative markers, iii) developed a more general theoretical framework for the CKMR that accounts for different sampling and alternative demography so that the method can be applied to other species. These advances enabled the application of the method to other species such as Australian river sharks (*Glyphis spp*.), school sharks (*Galeorhinus galeus*) and white sharks (*Carcharodon carcharias*). Moreover, the method is under exploration for its applicability to Pacific Bluefin Tuna (*Thunnus orientalis*) and Atlantic Bluefin Tuna (*Thunnus thynnus*); recently a thorough assessment on the applicability of this method for the latter has been produced (Davies *et al.,* 2017).

There are notable features of the Atlantic Bluefin Tuna that should be considered before envisaging application of the CKMR method to this species: the presence of (at least) two potentially genetically differentiated spawning components (the Gulf or Mexico and the Western Mediterranean, with a potential third spawning ground in the Eastern Mediterranean) that mix throughout the Atlantic Ocean. This, together with a more limited biological background knowledge and a more complex logistic/operational environment for sampling, calls for a more sophisticated version of the CKMR. According to Davies *et al.* (2017), the application of the CKMR method to the Atlantic Bluefin Tuna would be feasible but would require:

(i) increasing the annual sample size of tissue, otolith and length samples obtained from within Mediterranean and eastern/central Atlantic sampling programs;

(ii) distinguishing between individuals of eastern and western origin with a high probability

(iii) implementing high quality sample, processing and data management programs to minimize the likelihood of genotyping errors

5.4.4 The choice of the white anglerfish as a study target species

The prevailing lack of data, knowledge and information on deep-sea fish species is unfavourable for testing the genetic close-kin approach. Therefore, it was decided to evaluate the feasibility of the method in a species with background information and more availability of samples such as the white anglerfish, *Lophius piscatorius*.

The white anglerfish is distributed in the Mediterranean Sea up to the Barents Sea, although it is more common in northern European waters, between 20 and 1000 m depth (Caruso, 1986). Spawning behaviour is not well known, but it has been suggested that it could occur in late Winter-early Spring in Faroese waters (Ofstad and Laurenson, 2007). Eggs are buoyant and larvae can live in the water column for 2–4 months before recruiting (Hislop *et al.,* 2001). Age at first maturity is 7 years for males, and 9–10 years for females (Duarte *et al.,* 2001). Once thought to be sedentary, tagging studies have revealed that adults can travel for up to 200 km (Fariña *et al.,* 2004; Laurenson *et al.,* 2005).

In the Atlantic, the white anglerfish is managed as three separate stocks (southern, in ICES divisions VIIIc and IXa; northern in ICES divisions VIIb-k-VIIIab and a northern shelf unit, in divisions IIa and IVa). Over the last decade, many studies have looked into the biological support to such subdivision, using different tools. Overall, the currently applied management units do not appear to be supported biologically, as demonstrated by genetics (Crozier, 1987; Fariña *et al.,* 2004; Charrier *et al.,* 2006; Blanco *et al.,* 2008),

morphometrics (Fariña *et al.,* 2004), and otolith shape analysis (Cañás *et al.,* 2012). Overall, the Atlantic population of *Lophius piscatorius* seems to be genetically homogeneous, with high levels of gene flow which is likely to be maintained by this species' life-history that include a long pelagic larval phase.

5.4.5 The exploitation of white anglerfish

The following summary is mainly derived from ICES advice (2017) and a documentation by Papakonstantinou *et al.* (2011).

L. piscatorius is among the most valuable and sought after fish targeted in western and southern European waters and is targeted in bottom trawl fisheries and gill net fisheries off Portugal and Spain where the landings peaked between 1980 and 1986/1987 and have generally decreased thereafter. However, from 2000 onwards, the number of juveniles caught in the Spanish Northeast Atlantic trawl fisheries increased in most areas. The North Atlantic trawl fishery for *L. piscatorius* expanded rapidly in the late 1980s and early 1990s. Landings peaked at almost 35 000 tonnes (considering also the black anglerfish, *L. budegassa,* which constitutes a small percentage of the catches) in 1996 and significant subsequent decline thereafter. Total allowable catch limits were set in 1999, however, some misreporting is apparently suspected. Declines of *Lophius spp.* catches in the ICES Divisions Vllb,c and j,k in Irish waters since the mid-1980s have also been reported. Stock assessments are impeded by a significant lack of data (see also next section).

In ICES Divisions VIIb-k and VIIIa,b,d the 2011 and 2012 TACs were 40 950 tonnes and 38 900 tonnes respectively for each species, while the 2010 TAC for both species combined was 41 400 tonnes and estimated landings 28 880 tonnes. Spain and France account for about 80% of total landings. Since 2007 the assessment has been based on commercial landing per unit effort (LPUE) as well as data from four surveys that gather data on biomass, abundance indices and length distributions.

In ICES Divisions VIIIc and IXa landings of both species combined were 2331 tonnes in 2010, which was 58% above the set TAC of 1496 tonnes. The TAC in 2011 and 2012 was 1571 tonnes and 3300 tonnes respectively. The two species are assessed separately (but managed together) using a surplus-production model (software ASPIC), tuned with commercial LPUE series for *L. budegassa* and a length based SS3 implementation for *L. piscatorius*.

L. piscatorus has occurred as bycatch in North Atlantic fisheries for at least the past century. They began to be specifically targeted in the 1980s by bottom trawl and gill net fisheries. *L. budegassa* and *Lophius piscatorius* are lumped together in the fishery off Portugal and Spain where total allowable catch limits were set at high levels from 1987 to 1999 (10 000 to 13 000 tonnes), and did not restrict the fishery. Since 2005, the TAC has been set at 2000 tonnes, while ICES has recommended a full moratorium (Azevedo *et al.* 2008). In the eastern Mediterranean, *L. budegassa* may be experiencing growth overfishing, as the stock was mostly composed of 3-4 year old juveniles and spawners were scarce (Carlucci *et al.* 2009).

5.4.6 Current scientific advice to the management of white anglerfish

In the European waters of the Northeast Atlantic (Area 27) the data collection framework Regulation (EC) 199/2008 includes anglerfish species, both *L. budegassa* and *L. piscatorius*.

These species are important for commercial fleets in this area, contributing with a total income of approximately 50 ME, which represents about 5% of the total value of landings in FAO 27. Due to its commercial value, national marine research institutes maintain sampling programmes covering also anglerfish. The collection of data is carried out both in the auction markets, where commercial fleets land their catches, as well as during scientific research surveys. In some cases, anglerfish is also sampled on-board of commercial vessels. Each sample contributes information about the length frequency of the catch (or haul) and, when possible, biological information. It is, for example, common practice to collect otoliths and gonads to study growth and reproduction. The number of individuals sampled by the sampling programs is not easily established. Clearly, however the programs facilitate access to biological material, including historical samples, since national laboratories keep reference collections of otholits. This might enable the establishment of a genetic analytical time-series and constitutes an additional valuable asset in favour of white anglerfish as a target species (Nielsen and Hansen, 2008).

For assessment purposes ICES considers three areas: subareas 4 and 6 and Division 3.a; divisions 7.b–k, 8.a–b, and 8.d; and divisions 8.c and 9.a; and two species: *Lophius budegassa* and *Lophius piscatorius*; in a total of six stocks. These stocks are assessed with survey trends, surplus production models (ASPIC and SPiCT) or statistical catch-at-age models (SS3). One of the major problems with anglerfish assessments is related to the estimation of individual growth because of the large uncertainties linked to the available ageing methods (ICES, 2012). This severely limits stock assessment models and the quality of current abundance estimates resulting from their application.

Close-kin mark-recapture estimates of number of breeders could be a valuable complement current assessment models. Resulting information could be used to increase the precision of current stock assessments by allowing better scaling of spawning stock biomass (SSB) and/or abundance estimates, which are elements greatly needed to understand population dynamics, like stock productivity and resilience to exploitation. As precise age readings are not available, close-kin analysis can constitute an important added value to current stock assessment approaches, since adults and juveniles are easily distinguishable through the determination of the gonadal maturation stage (Afonso-Dias and Hislop, 1996; Quinoces *et al.* 1998a,b).

5.4.7 Simulations, feasibility, resource needs and outlook

In our previous report (ICES WGAGFM Midterm Report 2016), we summarised the results of basic simulations in evaluating the potential of the close-kin analytical framework to estimate anglerfish abundance. Thus, based upon white anglerfish fishing data covering ICES divisions VIIIc and IX (Iberian region), which indicate a breeding population of ~1.5 million individuals, the simulations suggested that a sample size of ~17 000 individuals (equally split between adults and juveniles i.e. 8500 each) would be required to obtain reliable estimates of abundance (assuming a coefficient of variation CV =10%). This sample size represents ~1% of the total breeding population. Using the same estimate of abundance, here we have extended the assessment of the close-kin method by carrying out further simulations with the aim to investigate sampling requirements under distinct CV levels and, more specifically, to determine the minimum number of parent-offspring pairs (POPs) that need to be identified to obtain reliable estimates of abundance. Variation on both parameters were found to significantly affect the outcome of the simulations.

The simulation was first run fixing the CV at 12% (following Bravington *et al.* 2014), and allowing the number of POPs to vary in order to identify the ideal POP number. Results of this simulation are displayed in Table SI. It is clear that this method is highly sensitive to the number of POPs identified among samples. Smaller or larger numbers will substantially underestimate or overestimate "true" abundance values.

In the second simulation, the number of POPs was fixed at 70 (presumed optimum number of POPs from Simulation 1) while the CV was allowed to vary from 5% to 20%. Results of this simulation are displayed in Table SII. Similar to the first simulation, it is clear that the method is also sensitive to the choice of CV. It important to emphasise, however, that all simulations carried out here are over simplistic in nature. Indeed, Bravington *et al.* (2014), have argued that the main purpose of the model is to assist with sample-size calculation for design purposes only. The achieved CV can be different for a number of reasons, in particular because the true abundance may be very different from the guess estimate. Bravington *et al.* (2014) also emphasised the importance of added biological information (length, age, size of maturity, etc.) and due consideration to associated logistic complications including: multi-year sampling; age dependent sampling probability and non-equilibrium conditions in the spawning population. All these issues will have to be considered in studies willing to avail of the usefulness of the close-kin method. It is anticipated that the use of additional biological data will assist to reduce model uncertainty.

No. POPs	N=2mjmA/h
5	20,833,333
10	10,416,667
15	6,944,444
20	5,208,333
25	4,166,667
30	3,472,222
35	2,976,190
40	2,604,167
45	2,314,815
50	2,083,333
55	1,893,939
60	1,736,111
65	1,602,564
70	1,488,095
75	1,388,889
80	1,302,083
85	1,225,490
90	1,157,407
95	1,096,491
100	1,041,667
105	992,063
110	946,970
115	905,797
120	868,056
125	833,333
130	801,282
135	771,605
140	744,048
145	718,391
150	694,444

Table SI. Summary results of simulations with CV fixed at 12%. Under this scenario, the recommended number of samples (i.e. juveniles + adults) is 14 434 (i.e. 7217 adults and 7217 juveniles). The "best" minimum number of samples that would have to be identified among the samples (i.e. leading to an estimate close to "real" value) is 70 (highlighted in grey).

mJ	mA	m	CV	N=2mJmA/h
17,321	17,321	34,641	5%	8,571,429
7,217	7,217	14,434	12%	1,488,095
4,558	4,558	9,116	19%	593,589
3,331	3,331	6,662	26%	316,991
4,811	4,811	9,623	18%	661,376
4,330	4,330	8,660	20%	535,714
3,936	3,936	7,873	22%	442,739
3,608	3,608	7,217	24%	372,024

Table SII. Summary results of simulations with No. POPs fixed to 70. 'mJ' and 'mA' represent the number of juveniles and adults respectively, 'm' = (mJ + mA), 'N=2mJmA' = estimated abundance. The "best" CV value is highlighted in grey.

Resulting from the exercise underlying ToR d is the ability to estimate requirements and resources that would be needed to support a study on abundance estimates of white anglerfish based on close-kin analysis. Using the same general principles, such elements of design and costing can be extended to other species of known biology.

i) Sampling;

ii) DNA extraction and marker identification;

iii) Statistical evaluation to identify parent-offspring pairs allowing to deduce an abundance estimate.

Approach and resource needs are estimated as follows:

i) Sampling

Number of samples: 17 000 (8500 adults; 8500 juveniles, but see also simulations)
Source: National fisheries institutes; Processing industry.
Geography: ICES divisions VIIIc and IX (Iberian region),
Time needed: 12 months
Infrastructure needed: Sampling equipment
Human resource needs: 6PM
Costs: Sampling equipment and Salary

ii) DNA extraction/analysis:

Approach: a) DNA extraction: kits; b) DNA-analysis: High throughput sequencing; Genotyping.
Infrastructure needed: DNA Laboratory.
Time needed: 6 Months
Human resource needs: 4PM (marker selection: 3PM; genotyping: 0.5PM)
Costs: ca. 10 000E. Marker Selection (only laboratory consumables) and 3000E genotyping (only laboratory consumables).

iii) Statistical analysis/ Abundance estimate

Approach: Bravington *et al.* (2016)
Infrastructure needed: Computing facility.
Time needed: 6 Months
Human Resource needs: 12PM
Costs: Salary.

Based on the review of what is known about white anglerfish, its biology, and commercial exploitation, scientific advice, on one hand and about the genetic close-kin mark-recapture (CKMR) analysis on the other hand, it is reasonable to propose that CKMR is potentially a powerful means to support the stock assessment of this species and of deep sea species more generally. We have also examined other recent suggestions for abundance estimates based on genetics and genomics (e.g. Thomsen *et al.* 2016; Ovenden *et al.* 2016), but could not confirm that those are currently as robust and feasible as CKMR.

In summary, as also reflected in the recommendations emerging from this ToR, we suggest that the application of CKMR to white anglerfish and, by extension, to deep seas fish in general, should be comprehensively assessed in an experimental study.

Supporting Literature

General

A better future for the EU deep sea (https://ec.europa.eu/fisheries/better-future-eu-deep-sea_en)

See Deep-sea fishing opportunities for 2017-2018: unanimous agreement on deep sea quotas for the next two years (https://ec.europa.eu/fisheries/deep-sea-fishing-opportunities-2017-2018-unanimous-agreement-deep-sea-quotas-next-two-years_en)

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Relevant EU Legislation

Council Regulation (EU) No 1367/2014 of 15 December 2014 fixing for 2015 and 2016 the fishing opportunities for Union fishing vessels for certain deep-sea fish stocks (OJ L 366, 20.12.2014, pp. 1-14).

Council Regulation (EU) 2016/72 of 22 January 2016 fixing for 2016 the fishing opportunities for certain fish stocks and groups of fish stocks, applicable in Union waters and, for Union fishing vessels, in certain non-Union waters, and amending Regulation (EU) 2015/104 (OJ L 22, 28.1.2016, pp. 1-165)

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Recommendations

R1. In light of the potential value of the close-kin analysis for deep sea fisheries management, we recommend that members of WGDEEP assess/review/consider ToR d so that they can 1) identify priority target species and 2) provide feedback on already available biological and stock structure information which might underpin abundance estimates.

R2. Accurate delineation of stock structure of deep sea fish species is essential to a sustainable management of resources. Stock structure is highly dependent on scientific knowledge which has been recognised in Regulation (EU) 2016/2336 as insufficient. Filling in these gaps are required to employ genetic stock identification techniques and abundance estimates approaches such as close-kin analysis. We therefore recommend ICES to endorse genetic stock identification approach to deep sea species listed in Annex I of Regulation (EU) 2016/2336.

6 Cooperation

- **Cooperation with other WGs**

  i) Information/Advisory requests: While interactions among potential complimentary ICES working groups is not extensive, several information requests between 2015-2017, (e.g. from the Working Group on Integrated Morphological and Molecular Taxonomy (WGMIT), Bench-mark Workshop on Northern Haddock Stocks (WKHAD) and the Stock Identification Methods Working Group (SIMWG), indicates active complementarity.

  ii) ToR (a) The final 3-year ToR a will be communicated to WGPDMO. Contact has been established during year 2 of this ToR and further communication is planned for exchanging knowledge and for seeking potential synergies among both groups.

  iii) ToR (b): 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.

  iv) ToR (d): ICES WGDEEP was 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.

7 Summary of Working Group self-evaluation and conclusions

A copy of the full Working Group evaluation is available in Annex 4.

It was agreed unanimously that the WGAGFM, would wish to be affiliated to the new Aquaculture Steering Group (ASG), while retaining collective interest in capture fisheries. We propose that the name of the WGAGFM, be modified to: Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA), in line with our remit and contemporary terminology.

It was further agreed that the WGAGFM would submit an application to coordinate an ICES training course broadly on the "Application of Genetics and Genomics to Fisheries and Aquaculture Science". Further details will be communicated following the formal submission of the template proposal form for proposing a new ICES training course.

In accordance with the multi-annual three-year cycle, a new WGAGFM Chair was proposed and approved, to start in January 2018: Dr Jann Martinsohn, current Head of Sector, Fisheries and Aquaculture, at the Directorate-General Joint Research Centre, European Commission, Ispra, Italy.

Finally, it was agreed to propose the continuation of WGAGFM, 2018–2020, and accordingly, 4 new ToRs were proposed.

It is proposed to hold the next meeting of the WGAGFM at the French Research Institute for Exploitation of the Sea, IFREMER, Direction du Centre de Brest, Plouzané, France, 15-17 May 2018, and hosted by Dr Pierre Boudry.

Annex 1: List of participants

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Annex 2: Recommendations

	RECOMMENDATION	ADRESSED TO
1.	Review of the ToR a to: i) suggest areas for potential collaboration and synergy and ii) provide a priority list of pathogens/zoonoses to be screened.	WGPDMO
2.	Promotion of the integration of molecular monitoring into decision making and risk assessment of pathogen transfer from seafood to wild populations, including applicability in a legal/regulatory context.	SCICOM
3.	Identification of mechanisms to promote dialogue among Expert Groups.	SCICOM
4.	The application of novel quantitative genetic and genomic approaches to estimate changes in key life history traits of exploited marine populations, to enable the impact of evolutionary change to be incorporated into population modelling for management advice.	PGDATA/ WGBIOP / SCICOM
5.	We recommend that members of WGDEEP as sess/review/consider ToR d so that they can 1) identify priority target species and 2) provide feedback on already available biological and stock structure information which might underpin abundance estimates.	WGDEEP
6.	Change of the EG name in line with the establishment of the Science Steering Group for Aquaculture, from WGAGFM to Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA).	SCICOM

Annex 3: WGAGFM draft resolution 2018–2020

2017/MA2/ASG01 The **Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM)** will be renamed the **Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA)**, chaired by Jann Martinsohn, Italy/European Commission, will work on ToRs and generate deliverables as listed in the Table below.

	Meeting dates	Venue	Reporting details	Comments (change in Chair, etc.)
Year 2018	15–17 May	Brest, France	Interim report by 30 June to ASG, SCICOM and ACOM	
Year 2019			Interim report by DATE to ASG, SCICOM and ACOM	
Year 2020			Final report by DATE to ASG, SCICOM and ACOM	

ToR descriptors

ToR	Description	Background	Science Plan topics addressed	Duration	Expected Deliverables
a	Genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations	There is substantial existing evidence that interbreeding between wild Atlantic salmon and escaped domestic individuals occurs, and alters the nature and reduces the viability of wild populations. However, indirect genetic interactions may also occur. Caged or escaped farm fish can change the environment, so as to alter selective pressures and long-term fitness in wild populations even in the absence of direct interbreeding. This can lead to changes in the life history traits of wild populations, decreased survival, and reductions in population size. The production of all-female sterile triploids is seen as an approach to reduce the likelihood of effects on wild fish populations. In North America a large expansion has been approved involving the production of 7 million triploid Norwegian salmon annually. The use of triploid all female salmon is expected to reduce direct genetic interactions though the actual magnitude of direct and indirect genetic interactions remains unknown). This ToR will review the literature and explore the potential for genetic and genomic tools to quantify indirect interactions with wild salmon populations. This will involve the assessment of genomic tools to allow quantification of changes in wild populations due to changes in the selective landscape (i.e. disease, parasite, competition); as well as the estimation of effective population size of wild populations to allow declines in wild population size due to indirect effects to be quantified.	11, 17	3 years	Review paper and metrics for measures of indurect genetic impacts
b	Genomic selection applied to aquaculture species	Genomic selection is a genome-wide marker-assisted selection method that caused a revolution in terrestrial animal and plant breeding in the last decade. Expected gains, such as acceleration of breeding cycle, increase of accuracy of prediction of multi-trait	17, 28	2-3 years*	(a) Scientific advice for policy brief (b) seafood production brief

		performance, are particularly high for long-lived species. The development of high-throughput SNP arrays for an increasing number of species now allows the potential implementation of genomic selection in aquaculture. However, biological characteristics of most aquaculture species request specific optimization of genomic selection studied prior to their application for these species, as clearly demonstrated by simulation studies. Results are promising as recent genome-wide association studies in different salmonid species have concluded that genomic selection could efficiently contribute to improve disease resistance. The present ToR will introduce basic principles of genomic selection and the key steps of its implementation in breeding programs. It will focus on current progresses and prospects for aquaculture species and propose recommendations to facilitate its future developments in these species.			(c) Publication
c	Assessing the value of genetic and genomic tools in support of the implementation of the EU landing obligation.	Discarding is the practice of returning unwanted catches to the sea, either dead or alive, because they are undersized, due to market demand, the fisherman has no quota or because catch composition rules impose this. The reform of the Common Fisheries Policy (CFP) of 2013 aims at gradually eliminating this wasteful practice and seeks to phase in the implementation of the landing obligation ("the discard ban") from 2015 through to 2019 for all commercial fisheries (species under TACs, or under minimum sizes) in European waters and for European vessels fishing in the high seas. The landing obligation requires all catches of regulated commercial species on-board to be landed and counted against quota. These are species under TAC (Total Allowance Catch, and so-called quotas) or, in the Mediterranean, species which have a minimum landing size (MLS – under the Landing Obligation: minimum conservation reference sizes (MCRS)). Undersized fish cannot be marketed for direct human consumption purposes whilst prohibited species cannot be retained on board and must be returned to the sea. The discarding of prohibited species should be recorded in the logbook and forms an important part of the science base for the monitoring of these species. (https://ec.europa.eu/fisheries/cfp/fishing_rules) It is generally acknowledged that the implementation of the landing obligation is a highly challenging and complex endeavour. For example, how can it be assured that no prohibited species have been landed and that undersized fish are in fact from the officially reported species, given that in both cases the landed biomass tends to be immediately processed for products that are not for direct human consumption? These potentially mixed species samples are very difficult to identify once they have been processed, especially when considering products like fish oil and gelatine. Genetic and genomic methods might help with the challenge of ensuring that these "by-products" only contain the undersized catches (or potentially non- commercial bycatch species) but no other, illegal-to-land, species which might have been processed as "undersized, animal-by-products". If undersized commercial species need to be processed separated from bycatch species, genetics tools might further help to test if this is in fact the case in a given situation or if for example com-	27. 28	3 years	(a) Advice for policy brief; (b) Fisheries industry brief; (c) seafood product industry brief; (d) scientific publication; (e) Recommendations to SCICOM

		mercial species are being processed as "bycatch" to avoid overstepping a quota. If both do not need to be processed separately, the relative proportion of them within a product should be roughly according to their reported catch proportions. Genetic methods might here help to determine product composition, also quantitatively, which is either in line, or not, with the reported landing numbers or accepted production purity thresholds. In light of the apprehended difficulties for the monitoring of the implementation of and compliance with the landing obligation we aim to elaborate how genetic and genomic tools can provide robust and cost-efficient support.			
D	eDNA in Fisheries Management and Ecosystem Monitoring	Developments in the field of genetics have transformed our understanding of the natural world. In a fisheries context among other things it has helped us identify species, define population structures, begin to understand the genetic basis of adaptive traits and monitor adaptive population changes. Typically such insights have been gained from analysis of DNA obtained from tissue samples collected directly from individuals across a study area. Additionally, the analysis of DNA through metabarcoding from a bulk sample composed of a mixture of individuals of different zooplankton and/or macroinvertebrate species has enabled more cost-effective biodiversity assessments. Recently however, a new source of DNA has begun to be used for analysis of macro species, so-called "environmental DNA" (eDNA), which relies on collection of DNA sloughed off from tissue (e.g. skin, blood, faeces, mucous, eggs) into the natural environment. This eDNA promises to revolutionise the examination of biodiversity in the wild by allowing the detection larger organisms without needing to sample them and may be of particular usefulness in the marine environment where traditional sampling is difficult to carry out. A number of approaches using eDNA have been utilised already and/or are under development. These include species identification (especially useful for rare/cryptic/small individuals), community composition, ecosystem monitoring, relative species abundance and even attempts at absolute species abundance. In the aquatic environment such techniques have often been developed in freshwater ecosystems but are now beginning to be utilised in the marine environment. As such there is a growing recognition that the use of eDNA in the marine sphere may in the near future bring powerful new tools to the arsenal of the fishery manager and also allow new approaches to ecosystem monitoring. However, there are also numerous caveats associated with eDNA approaches linked to sampling strategies, DNA stability in different environments, analytical approaches etc. that require expert attention to enable proper interpretation of study data. This ToR will summarise the research to date, identify areas where tools are already available for use and examine future developments whilst crucially seeking to also identify areas where the use of the new approaches should be undertaken with care if at all. The ToR will also try to produce a non-technical summary of the state of the field for direct dissemination to fishery managers with little or no genetic background.	1, 2, 4, 6, 9, 10, 11, 13, 17, 22, 27, 28	3 years	(a) Review paper (b) Non-technical review topic sheet.

Summary of the Work Plan

Year 1	**ToR a)** Review the literature on indirect genetic interactions among aquaculture salmon and wild populations. **ToR b)** Review of the basic principles of genomic selection and the key steps of its implementation in breeding programs, focus on current progresses and prospects for aquaculture species and propose recommendations to facilitate its future developments in these species. **ToR c)** Review the legal framework and supporting information, such as reports on the Landing Obligation by the Scientific, Technical and Economic Committee for Fisheries (STECF); identify the stakeholders; develop a work flow chart to work up mixed species samples, with decision points; develop theoretical scenarios/cases where genetic testing would be helpful and how the implications would be for a given outcome. **ToR d)** Review of the literature on the use of eDNA in the aquatic environment. Together with an overview of the field, particular focus will be to identify where eDNA techniques have/are being used at present in the marine environment and on other techniques used in freshwater that may be utilised in the marine sphere. Produce a glossary or commonly used terms in the field.
Year 2	**ToR a)** Identify approaches to quantify indirect genetic impacts and explore their sensitivity and power. **ToR b)** Develop cases where genomic selection would be helpful and how its implementation would benefit selective breeding programs. **ToR c)** Real-life scenario test based on developed work flow chart (from year 1) using real product samples; report results and discuss; report on feasibility and cost issues; recommendations to adjust methods/work flow developed in year 1 if needed. **ToR d)** Continuation of the literature review and identification of key studies describing the use of eDNA in the marine environment where the techniques used have significant potential for novel species and/or situations. Produce a flowchart of the critical steps needed from sampling to biodiversity assessment. Start to formulate review paper manuscript.
Year 3	**ToR a)** Complete review paper, and develop recommendations. **ToR b)** Develop a knowledge transfer plan; industry briefs; publication; implications, advice and final recommendations. **ToR c)** Develop a knowledge transfer plan; policy brief; industry briefs; publication; implications, advice and final recommendations. **ToR d)** Finalise and update review: detail key studies, identify areas where novel techniques show particular promise, and identify problematic areas requiring future research. Finish review paper and non-technical review topic sheet.

Supporting information

Priority	The current activities of this Group will lead ICES into issues related to the sustainable management of fisheries and aquaculture practices, monitoring of marine biodiversity and ecosystem function, and the impacts of discards in relation to Landing Obligations. Consequently, these activities are considered to have a very high priority.
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants	The Group is normally attended by some 15-20 members and guests.
Secretariat facilities	None.
Financial	No financial implications.

Linkages to ACOM and groups under ACOM	Joint SCICOM/ACOM group.
Linkages to other committees or groups	There is a very close working relationship with SSGEPD, SSGIEOM and SSGEPI. Additionally, several EGs, including WGITMO, WGBIODIV, WGBOSV.
Linkages to other organizations	European Commission, IFREMER, NOAA, DFO

Annex 4: Copy of Working Group evaluation

1) Working Group name. Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM)

2) Year of appointment.2015

3) Current Chairs. Gary R Carvalho (2015-2017), Jann Martinsohn (2018-2020)

4) Venues, dates and number of participants per meeting.

(**2015**) Joint Research Centre of the European Commission (JRC), Ispra, Italy, 6-8 May 2015, 16 delegates

(**2016**) School of Biological Sciences, Queens University, Belfast UK, 11-13 May 2015. 13 delegates

(**2017**) Olhão, Portugal, Centre for Marine Sciences (CCMAR), Faro, and the University of Algarve, Portugal, 2-5 May 2017, 19 delegates

WG Evaluation

5) If applicable, please indicate the research priorities (and sub priorities) of the Science Plan to which the WG make a significant contribution. These include research priorities 1, 2, 4, 6, 9, 10, 11, 13, 17, 22, 27, 28.

6) In bullet form, list the main outcomes and achievements of the WG since their last evaluation. Outcomes including publications, advisory products, modelling outputs, methodological developments, etc. *

Coordination of Theme Session at the ICES Annual Science Conference (21-25 September, 2015, Copenhagen): *A holistic ecosystem approach for marine management and conservation: Opportunities through the application of genetic and genomic approaches.* The theme session was well attended and included 14 oral presentations and 6 flash talks, and 10 posters. The aim was to raise awareness across researchers and stakeholders and to facilitate the integration of genetics and genomics into a holistic approach to an ecosystem based marine resource management. The session considered the current status of the application of genetic and genomic approaches to marine management, their benefits as well as obstacles to their routine application. We included a broad spectrum of natural renewable resource management targets and aspects, including aquaculture. Studies spanned the integration of genetic analyses with approaches such as habitat mapping and fisheries modelling, as well as linking genetic diversity with ecosystem function and resilience of harvested species, and also cost-benefit estimates.

- **Tool to assess the infection risk of a range of scenarios of host-pathogen infection (ToR a), Figure 1).** A scheme was developed in which several host-pathogen scenarios leading to different infection risks were characterized. We consider this a simple but useful tool to highlight the highest priority situations. It is based on successive parameters characterizing the state of seafood and its eventual pathogenic agents, such as live versus dead, fresh versus frozen, cooked or processed and symptomatic versus asymptomatic. Each case was associated with an infection potential arbitrary ranging from a value of 0 to 5 and a trans-

mission potential ranging from 1 to 2, leading to an infection risk on a scale from 0 to 10, calculated by multiplying their infectious potential with their transmission potential. The highest infection risk (value 10) corresponds to symptomatic live seafood carrying live pathogens. Special attention must be paid to the international trade of live seafood which is common for numerous species that can be consumed alive (e.g., oysters), or alive before cooking (e.g., lobsters and most fishes in Asia). In that case, non-local seafood is often maintained in recirculating-systems for which seawater must be sterilized (using U.V., chlorine, ozone…) prior to release into the wild in order to prevent the transfer of pathogens.

- **Workflow design to aid decision-making when analysing pathogen samples from seafood using molecular tools (ToR a), Figure 2).** The workflow is designed to assist in the choice of the most effective molecular approaches to employ in analysing pathogen samples from seafood, highlighting detection, quantification and viability assessment. It is important to recognise the link between the two types of approach; whereby novel targets identifying pathogen species, strains, virulence factors are first identified via genome-wide approaches and later developed in to rapid screening tools. Furthermore, both cost and efficiency of deployment are major considerations. As such, 'classic' molecular tools (e.g. PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) may be preferable over more technologically advanced approaches in many circumstances. Furthermore, novel methodologies like loop-mediated isothermal amplification (LAMP), PCR-dipstick tests as well as several commercially available portable qPCR machines means that screening can be carried out and acted upon on-site. In Figure 2 we supply a diagram to assist in the appropriate deployments of different tests.
- **Establishment of a WGAGFM Project in "Researchgate.com" (ToR b)** (https://www.researchgate.net/project/ICES-WGAGFM-Working-Group-on-Application-of-Genetics-in-Fisheries-and-Mariculture).) In addition to existing methods for ICES dissemination of expertise and activities of the WGAGFM, a project using social media based on the Researchgate.com site was established with the aim of extending our reach. The new site will allow linking to the ICES webpage (WGAGFM), where in addition to the background for the group, the annual reports and list of group members would be available. Within less than 1 month of set-up, we have over 30 followers and over 130 reads, which indicates a wider interest and the fact that we are now reaching a broader audience of researchers.
- **Design of a 2-page flyer summarising the objectives, interests, activities and expertise of the WGAGFM (ToR b)**. WGAGFM has created a 2-page leaflet for targeting industry, management, national governments, EU, FAO, research councils etc. (see design draft). The leaflet includes a schematic presentation of the scope of expertise and services the EG offers, designed around a key question: "what can genetics do for fisheries and aquaculture?". New frontiers of application in fisheries and aquaculture are highlighted, including environmental DNA (eDNA), microbiomes, transcriptomics, adaptive diversity, population sizes, meta-barcoding, and epigenetics. The key objectives of the WGAGFM in the context of ICES, is highlighted. To profile the target users, a database will be constructed, for electronic dissemination, and potential production of hard copies for distribution.
- **Simulations using white anglerfish (*Lophius piscatorius*) to identify appropriate sampling design for estimating reliable estimates of abundance using close-kin mark recap-**

ture approaches (ToR d). Basic simulations using white anglerfish fishing data covering ICES divisions VIIIc and IX (Iberian region), indicate that, assuming a coefficient of variation (CV) of 10%, a sample size of about 17 000 individuals, 8500 adults and 8500 juveniles, would be required to obtain reliable estimates of abundance (i.e. breeding population that in this case, based on existing stock assessments, is believed to be ~1.5 million individuals) based on the close-kin method. Using the same estimate of abundance (i.e. 1.5 million individuals), further simulations were subsequently carried out to investigate sampling requirements under distinct CV levels and, more specifically, the minimum number of parent-offspring pairs (POPs) that would have to be identified to obtain reliable estimates of abundance (i.e. close to "real" value) based on the close-kin approach. Variation of CV levels had a considerable impact on sample size requirement. For instance, a CV of 12% reduced sampling requirements to about 14 500 individuals (7250 adults and 7250 juveniles). The most important parameter, however, was found to be the number of POPs that need to be identified to obtain reliable estimates of abundance. Assuming a breeding population of ~1.5 million individuals, and a CV of 10%, 70 POPs need to be identified among samples to truly reflect "real" abundance. Under this criteria, a lower number of identified POPs will result in greatly inflated measures of abundance while larger numbers will result in major overestimations.

- **A quantitative assessment of the optimal sampling design and resource requirements for implementation of the close-kin mark recapture method for extension to deep sea fishes (ToR d).** It is recognised that a crucial component of the utility and implementation of molecular tools, is the transfer of quantifiable information on optimal design and resource requirements. A detailed outline considering sampling, DNA extraction and marker identification, and the statistical evaluation to identify parent-offspring pairs allowed to deduce an abundance estimate, is presented. In addition to precise estimates of the number of samples, capture locations, time, personnel and infrastructure requirements, specific suggestions are provided on the choice of DNA extraction methods and statistical analyses. Overall, analysis of approximately 17 000 individuals, would require an estimated 24 person months for execution.
- **Agreement to submit a proposal to coordinate an ICES training course:** Following correspondence with the ICES Secretariat, it was agreed to submit a proposal in advance of September 2017, to coordinate a training course, broadly on the Applications of Genetics/Genomics to Management of Fisheries and Aquaculture. While the detail is yet to be can finalised, several guiding principles were agreed: 1.to focus on clear practical questions of importance to the ICES mission and beyond; 2.to consider critically, with a full cost-benefit analysis the optimal design and choice of tools to tackle key questions; 3.to consider underpinning concepts relating to environmental change and harvesting, and consequences for stock resilience, recovery and sustainability; 4.to focus on opportunities for integration of molecular tools with traditional estimates of stock status and abundance, including population modelling, oceanography, biomonitoring; and non-genetic methods of stock ID; 5.to illustrate the above principles and applications with reference to salient case studies demonstrating utility, impact and constraints; 6. The science : policy interface - mechanisms to promote impact and uptake of genetic/genomic tools.

7) Has the WG contributed to Advisory needs? If so, please list when, to whom, and what was the essence of the advice.

WGAGFM has contributed with its available expertise to advise on the need to sustain traceability schemes in the fisheries and aquaculture sector, and notably contributed to the revision of the Common Fisheries Policy Control scheme revision (Regulation (EC) 1224/2009)

WGAGFM Members have been invited speakers at the World Fisheries Congress 2016 in Busan during a session organised by the OECD on the Role of Genomics in Fisheries Management.

WGAGFM has responded on an ad hoc basis as indicated within the Final Report 2017 to various EG and ICES requests.

8) Please list any specific outreach activities of the WG outside the ICES network (unless listed in question 6). For example, EC projects directly emanating from the WG discussions, representation of the WG in meetings of outside organizations, contributions to other agencies' activities.

Various European Commission- funded projects, including FishPopTrace, AquaTrace, AquaGen, MerSNiP, and science and advisory bodies including JRC at DG MARE, DEFRA (UK, NOAA

FAO Fisheries and Aquaculture Technical Paper No. 585 Fish identification tools for biodiversity and fisheries assessments Review and guidance for decision-makers.

9) Please indicate what difficulties, if any, have been encountered in achieving the workplan.

A major challenge is the provision of sufficient financial resources (Travel, accommodation) costs to fulfil the work plan and especially attendance at the Annual WGAGFM meetings, as the activities tap entirely into voluntary support.

Future plans

10) Does the group think that a continuation of the WG beyond its current term is required? (If yes, please list the reasons)

Yes. Here is a need to maintain and enhance the awareness of opportunities for fisheries and aquaculture management emerging from genetic and (increasingly) genomic approaches.

11) If you are not requesting an extension, does the group consider that a new WG is required to further develop the science previously addressed by the existing WG.

We suggest a renaming of the group to better acknowledge for the increasing significance of aquaculture activity. We propose a change of the EG title in line with the establishment of the Science Steering Group for Aquaculture, from WGAGFM to Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA)

12) What additional expertise would improve the ability of the new (or in case of renewal, existing) WG to fulfil its ToR?

An increase in WGAGFM member representation in aquaculture, and expertise in the use of genetic data in population modelling of spatially-resolved fish stocks.

13) Which conclusions/or knowledge acquired of the WG do you think should be used in the Advisory process, if not already used? (please be specific)

- Integration of molecular monitoring into decision making and risk assessment of pathogen transfer from seafood to wild populations, including applicability in a legal/regulatory context
- The application of novel quantitative genetic and genomic approaches to estimate changes in key life history traits of exploited marine populations, to enable the impact of evolutionary change to be incorporated into population modelling for management advice.
- The critical evaluation of use of close-kin mark recapture methodology to estimate fish abundance as in independent assessment of population status, resilience and potential for stock recovery, especially in deep sea fishes.