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SCICOM STEERING GROUP ON ECOSYSTEM PRESSURES AND IMPACTS

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

6–8 May 2015

Ispra, Italy



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## Executive summary

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The Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM) convened at the Joint Research Centre of the European Commission (JRC), Ispra, Italy, 6–8 May 2015. Sixteen delegates from ten nations, discussed three Terms of Reference (ToR), and associated matters. In particular, there was general discussion on the potential to enhance WGAGFM contributions to ICES within the context of the ICES Science Plan Performance Evaluation (February 2015). Noteworthy WGAGFM proposals included promotion of multi-disciplinary approaches for achieving sustainability and resilience, enhanced integration of genetics in fisheries conservation and management, and prioritising regular assessment and monitoring of marine biodiversity. Interactions with staff from the Maritime Affairs Unit Fisheries and Aquaculture Sector of the JRC, including a seminar on the Integration of Genetics into Stock Assessment (E. Jardim, JRC) provided additional opportunities for inputs and discussion.

Two multi-annual ToRs (3-year), and a 1-year ToR were considered. Members discussed the status and potential for application of molecular methods to evaluate infectious disease and parasite spread from transferred seafood into wild populations (ToR a). Existing molecular tools for routine screening of microorganismal and multicellular parasites using case studies as successful examples were reviewed, as well as consideration of potential applications of innovative high-throughput genetic techniques for early detection of harmful organisms in seafood. Techniques offering particular promise included taxonomic identification of environmental samples (metabarcoding) and high-throughput genotyping. Coincident with discussions on the wider role of WGAGFM in ICES, members considered strategies to enhance integration of genetic monitoring and assessment methods into advice and management based on ecosystem-based considerations (ToR b). Context was set by considering the history, structure and activities of WGAGFM. An archive of past WGAGFM ToRs was established and categorised according to external and WGAGFM drivers. A case study focusing on establishment of a standardised and accessible marine genetic database served to identify generic impediments to implementation, including lack of resources and limited interactions across ICES Committees. Complementary among salient Expert Groups offering most potential for interactions was recognised as a key mechanism to enhance impact and uptake of recommendations. Understanding the genetic foundation of phenotypic variation is particularly important in exploited marine species, as distinct stocks are typically associated with differences in functional genetic variation (i.e., local adaptation, (ToRc)). Quantitative genetic analyses can address questions relating to the genetic basis of trait variation. New tools, developed from humans, now enable the genome-wide and high throughput assessment of quantitative genetic variation in wild populations that is not dependent on initial pedigree data or tailored genomic resources. Examples outlined demonstrate the potential for enhanced understanding of stock structure and potential responses to climate change, harvesting, release of captive bred fish and habitat change.

Other activities included a response to recommendations submitted from the Benchmark Workshop on Northern Haddock Stocks (WKHAD), reported elsewhere, options for promoting training in fisheries and conservation genetics, and preparations for the 2016 WGAGFM, to be hosted at Queens University, Belfast, UK.

## 1 Administrative details

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

#### Year of Appointment:

2015

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

1

#### Chair:

Gary R Carvalho, UK

#### Meeting venue

Ispira, Italy

#### Meeting dates

6–8 May 2015

## 2 Terms of Reference a) – z)

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

**Contributors:** Naiara Rodriguez-Ezpeleta, Claudia Junge, Martin Llewellyn, Johann Hofherr & Filip A.M. Volckaert (2-year)

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, Torild Johansen, Martin Taylor (3-year)

It is a scientific aim to integrate genetic monitoring and assessment methods into advice and management. There is, in principle, particular potential to implement advances in salient concepts and technologies into fisheries resource management,

governance and policy formulation. The overall aim is to enhance the integration of genetic monitoring and assessment methods into advice and management. The nature and effectiveness of implementation processes, as well as a consideration of strategies to promote such integration within the context of the ICES structure and community and beyond will be considered. As such, the ToR provides an opportunity to review past and current impact of outputs generated via the annual WGAGFM meetings and associated activities.

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

**Contributors:** Sarah Helyar, Dorte Bekkevold, Ian Bradbury, John Gilbey, Phil McGinnity, Paulo Prodohl, Jochen Trautner and 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.

## **3 Summary of Work plan**

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**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:** 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; produce a final report and publish a position paper.

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

outside the ICES community in relation to management advice and policy formulation.

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

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

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Ongoing.

## 5 Progress report on ToRs and workplan

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

#### Rationale

Trade in live and frozen seafood products has augmented worldwide translating into increased opportunities for infectious diseases to cross natural biological barriers, potentially causing important economic losses, impacts on local diversity, and biosecurity issues. Thus, experts require fast, accurate, cost-effective and high-throughput diagnostic tools to identify viral, microbial and multicellular pathogens in seafood. Such needs can be achieved through DNA-based techniques, particularly, with the advent of high-throughput DNA sequencing and genotyping through next-generation sequencing (NGS) technologies. Although some examples of successful application of DNA-based methods for infectious disease agent identification exist, the potential of such techniques remains underexplored. Hence, during this two-year period, we will i) review existing and potentially applicable genetic and genomic methods for infectious disease and parasite spread in seafood and ii) assess the applicability of such tools for policy decisions on animal health and risk assessment arising from trade of indigenous populations.

#### Progress made

An extensive literature review has been performed; so far more than 200 publications have been screened and two main goals have been achieved: i) collect a series of examples of methods used for parasite screening and identify cases where high-throughput molecular techniques are already applied, ii) compile information on existing and new molecular methods used for application.

### Examples of successful application of high-throughput techniques

We have collected a series of representative cases where molecular monitoring has been or has potential for implementation, and present three well-documented examples as case studies:

- a) Picine 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. 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.
- b) *Bonamia* species parasites are a derived clade of Haplosporidians that infect a variety of oyster species. Although primers targetted to the *Bonamia ostreae* 16S rRNA gene have been available since 2000, their level of specificity is not fully validated. Through a more sensitive qPCR assay it is now possible to distinguish different *Bonamia* species, and a recent work has been undertaken to develop molecular probes for *in situ* hybridization and distinction of *B. ostreae* and *B. exitiosa*, which now occur sympatrically in certain parts of Europe. Differentiation between the two *Bonamia* ssp is of importance since both are listed under EU animal health requirements (Council Directive 2006/88/EC), but one as exotic disease (*B. exitiosa*) and the other as non-exotic disease (*B. ostreae*). However, throughput, sensitivity and specificity all require improvement, something that NGS could deliver readily.
- c) Whirling disease is caused by the myxozoan parasite, *Myxobolus cerebralis*, and affects wild and farmed freshwater and anadromous salmonids. 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). 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.

There are multiple examples, among which these three, represent compelling evidence of the potential of high-throughput molecular methods for seafood disease monitoring. Our intention is to expand the list of existing examples and highlight areas where high-throughput approaches have high applicability.



### Review of existing genetic tools

A review of the literature indicated that most methods using molecular techniques for infectious disease identification are based on amplification of one genetic marker in order to determine if a particular species or virulent gene is present. In some cases, this requires previous isolation (and culturing) of the agent. When the presence of a given agent is suspected, PCR (to detect presence/absence) or RT-PCR (if quantification is desired) using specific primers can be used. When there is no clear idea of the nature of the agent, PCR using degenerate primers followed by Sanger sequencing should be used. In order to determine a strain within a single species, more sensitive markers such as microsatellites or SNPs can be used. Limitations arise when single species/strains cannot be isolated or cultured, in which case low-throughput sequencing techniques (i.e. Sanger) require the tedious and low cost-effective task of cloning individual fragments into vectors and sequencing each in order to look for the potential agent.

### Potential of high-throughput molecular methods

To overcome the limitations of traditional molecular methods when the agent cannot be isolated/cultured, several alternatives exist, including:

- Metabarcoding, i.e., the taxonomic identification of individuals present in an environmental sample based on a small fraction of the genome (barcode) is a promising approach. The method allows sequencing the a common homologous fragments of genomes in organisms present in a complex sample using so-called universal primers (they are able to amplify a wide range of taxonomic groups); once sequenced, the fragments can be compared against a reference database to identify species present.
- Microfluidic high-throughput genotyping, based on probes of known infection agents has also proven useful for example in the identification of parasites of several salmon species. The method consists of amplifying simultaneously hundreds of samples using hundreds of different primer pairs by RT-PCR. In a single assay the presence of multiple potential infectious agents, as well as their abundance, can be determined.

### Additional avenues

Additional potential genetic methods for examining stressor responses to detect infectious agents include assays of genetic variation within hosts, as well as gene expression profiling. Additional work is required in order to determine whether these alternative approaches are potentially relevant for the current ToR.

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

**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.

### Introduction – historical narrative and role of the WGAGFM

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 81<sup>st</sup> 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 Headquarter 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 1 in qualitative genetics and subgroup 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 81<sup>st</sup> Statutory Meeting. The primary driver was the rapid growth of mariculture in marine food production that already had taken place, and its anticipated further increase. It was recognised that that a resilient and sustainable mariculture industry be founded on sound genetic management, including breeding programs to increase, for example, production efficiency and disease resistance. Broader considerations of capture fisheries were included, and it was subsequently decided to integrate the qualitative and quantitative sub-group components.

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

Typically, participants select most Terms of Reference for annual meetings, in line with advances in the field and perceived policy needs. A valuable by-product of the WGAGFM meeting format is to generate opportunities for publication of TOR topics in peer-reviewed journals. Initially, WGAGFM reported to the Mariculture Committee, though from 2008 internal reorganisation of ICES resulted in the renaming of the former Consultative Committee into the Science Committee (SCICOM), an equal partner to the already existing Advisory Committee (ACOM). The SCICOM directs the scientific programme of ICES on behalf of the Council. All former science committees (including the Mariculture committee) ceased to exist in 2008, and the concept of Expert Groups were 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.

### **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 1 for list). The Expert group report, including the specific recommendations is presented to SCICOM (SSGEPI).

### **Changes through two decades**

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

### **Enhancing impact of ToR recommendations**

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

Clearly, there is a need to change from a tendency to remain inward looking towards an outreaching attitude: This includes enhancing interactions with other relevant Working Groups and Benchmarking meetings. WGAGFM will pursue an ICES Expert Group (EG) mapping exercise that will identify and cluster EGs according to their scope and activities. The inventory will greatly facilitate the building of a timely interaction and exchange with the EGs in the contexts of specific ToRs. In line with the inventory, we aim to establish an appropriate network between WGAGFM, other EGs and stakeholders outside ICES, including policy makers and fishery/aquaculture managers, to better integrate genetic information into management and policy options. We consider developing a questionnaire as to inquire about awareness of other

EGs of the WGAGFM and to investigate the perception of fisheries and aquaculture genetics, similar to that used by Ovenden *et al.* (2013). Over the three-year duration, we will additionally review the outcome of a representative range of previous recommendations. An example is given below with a series of ToRs pursued from 2006 to 2012 on the need to centrally compile genetic data on marine species and to render that data publicly accessible. The activity will allow the WGAGFM to identify pitfalls and impediments to impact creation, as well as disclosing examples with impact.

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

#### **Impact of previous recommendations**

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.

#### **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 in nature since the topic was considered sequentially each year until 2012. In addition to the WGAGFM endeavours, complementary external drivers endorsed such needs.

Foremost among these were the Data Collection Framework (Council Regulation (EC) No 199/2008), for EU-wide collection of biological and economic fisheries data (but not genetic data), and regional Data Base FishFrame (<http://www.ices.dk/marine-data/data-portals/Pages/RDB-FishFrame.aspx>), and the set of DCF databases (<https://datacollection.jrc.ec.europa.eu>), hosted by the European Commission Joint Research Centre, FP7 project FishPopTrace (<https://fishpoptrace.jrc.ec.europa.eu>). In 2011, in the context of the reform of the DCF, a ToR was dedicated to the possibility of integration of genetic data under the remit of such 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 ToR ‘database’ was negligible: there is currently no such integrated collective framework encompassing fisheries and aquaculture genetic data at a species or geographic level. Our example is counter to the general acceptance of the value of such endeavours for the provision of scientific advice to marine and maritime governance, including the DCF and as other initiatives such as EMODNET (<http://www.emodnet.eu>). We do not know to what extent, if at all, the topic was considered within the ICES structure: feedback from SCICOM, ACOM or any other Working Group was not forthcoming. Specific obstacles undoubtedly relate to the need for dedicated resources, though scenarios can be envisaged that incorporate genetic/genomics data on an ongoing basis within existing data collection initiatives. The resource issue was recognised by WGAGFM, and as early as 2008 the WGAGFM embarked in a discussion on possible venues with the ICES data centre, and evaluated the possibility of developing a marine fish genetic database under the remit of EMODNET (<http://www.emodnet.eu>). The discussion however was inconclusive, mostly because potential resources remained unidentified. When reviewing the ToRs on databases, there was clearly a lack of interaction with other potentially relevant ICES Working Groups, an issue for priority consideration in future strategies.

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

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

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

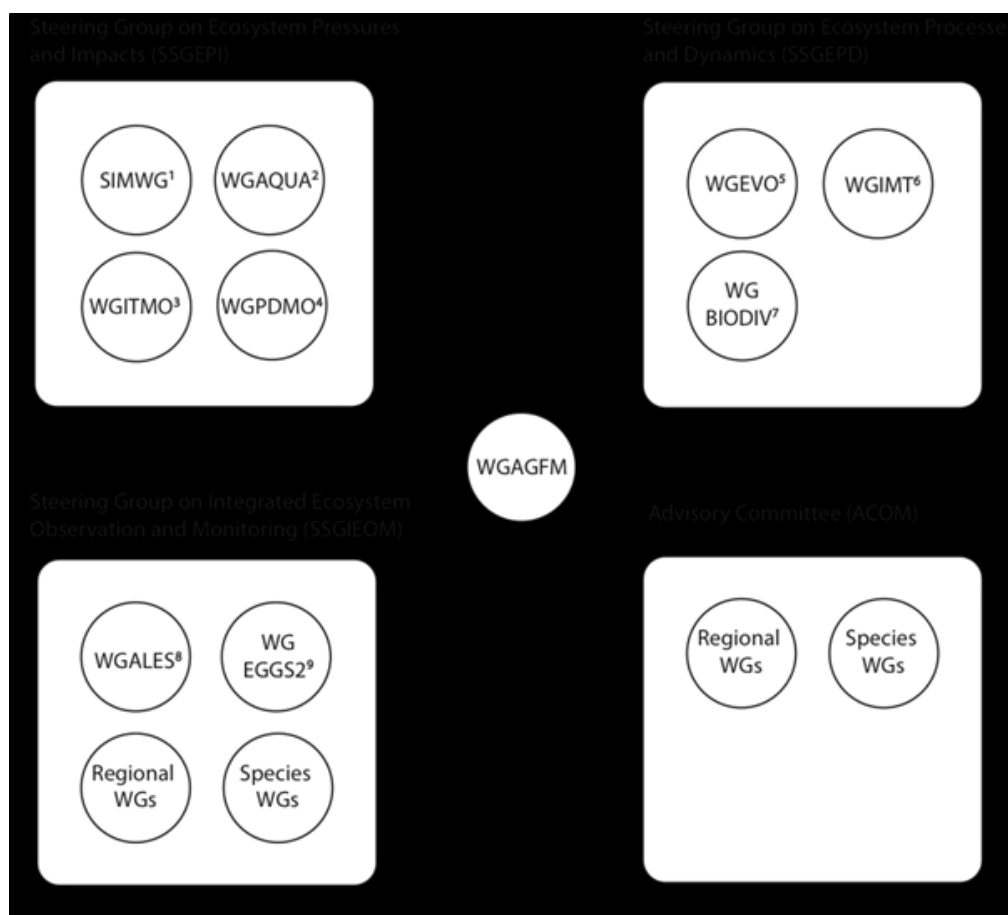


Figure 1. Expert Groups with potential overlap in interest with WGAGFM or past/future interactions with WGAGFM (see text for brief details).

- 1) **SIMWG: Stock Identification Methods Working Group.** Genetics is one of the key methods used in stock identification and thus there is considerable overlap with this EG. In particular, developments in sequencing and genotyping technology are disclosing increasingly fine-scaled population structuring.
- 2) **WGAQUA: Working Group on Aquaculture.** There are a number of genetic methods that are of interest to the WGAqua EG. Relevant areas shared with WGAqua include: Genomic selection; pedigree / parentage analysis; traceability; genetic diversity maintenance / inbreeding avoidance; disease / pathogen identification.
- 3) **WGITMO: Working Group on Introductions and Transfers of Marine Organisms.** Overlap with this group focuses on the detection and monitoring of alien / invasive species using molecular methods including eDNA.
- 4) **WGPDMO: Working Group on Pathology and Diseases of Marine Organisms.** There are several genetic methods that are used for the identification of diseases in wild and aquaculture species. Thus, there is overlap with this EG, and in particular with the current WGAGFM ToR to 'Review and assess the utility of molecular techniques to evaluate disease and parasite spread from transferred seafood into wild populations'.
- 5) **WGEVO: Working Group on Fisheries-Induced Evolution.** The basis of phenotypic changes of fish stocks associated with fishing pressure remains an on-going priority issue. Increasing evidence supports the notion that

genetic changes play a key role in the reduction in body size and size at maturity found across many fish stocks and species. Thus, information on population genetic structure and demographics, as well as a need to better assess the quantitative genetic basis of such shifts, represent two complementary fields for interaction.

- 6) **WGIMT: Working Group on Integrated Morphological and Molecular Taxonomy.** There are clear potential interactions with WGIMT in terms of molecular phylogenetics and species identification.
- 7) **WGBIODIV: Working Group on Biodiversity Science.** There are many areas where WGAGFM can interact with WGBIODIV. These include elucidation and monitoring of genetic diversity; species identification using DNA barcoding and metabarcoding; eDNA methods for biodiversity assessment; population structure and stock dynamics.
- 8) **WGALES: Working Group on Atlantic Fish Larvae and Eggs Surveys.**  
Genetic methods can identify cryptic species in ichthyoplankton surveys, such as the identification of visually indistinguishable gadoid eggs (cod, haddock and whiting). Further, genetic methods have been applied to eggs and larvae to ascertain stock structure at different life history stages and geographic origin.
- 9) **WGEGGS2: Working Group 2 on North Sea Cod and Plaice Egg Surveys in the North Sea.** Similar scope and complementarity as with the WGALES above are evident.

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

### Introduction

Within natural populations, there is an immense diversity of phenotypic variation for traits such as morphology, physiology, behaviour, and disease resistance. Typically, underlying this diversity are multiple genetic loci that interact with each other and

the environment. Understanding the scope of genetic and phenotypic diversity, and the genetic basis of adaptation is fundamental to our understanding of how marine populations will respond to changing environments, and the effective ecosystem-based management of these important natural resources (Falconer & Mackay, 1996; Lynch & Walsh, 1998).

Individuals from natural populations will respond to environmental variability such as habitat change through, for example, altered behaviour and different expression of life history traits. At the population level, these responses are likely to include distributional shifts, and altered ecosystem interactions, but importantly will also occur through genetic 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 climate and fisheries impacts and to identifying indicators of such impacts on 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. There is already evidence for rapid evolution in response to climate change in several short-lived species (Reusch & Wood, 2007) and for fisheries induced evolution in several species (Kuparinen & Merilä, 2007), suggesting that many organisms have adaptive variation and the capacity to respond to environmental change within a time frame of tens of years (Bradshaw & Holzapfel, 2008). However, 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.

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

Most recent literature in population genetics focuses on models of selection at one, or a small number of loci. This is in contrast to classical models of natural and artificial selection in quantitative genetics, where it is assumed that most traits of interest are highly polygenic, and are influenced to a small degree by standing variation at many loci (Falconer & Mackay, 1996). Classical breeding and selection experiments and occasionally field observations endorse the quantitative genetics view, together with genome-wide association studies showing that many traits are highly polygenic. Thus, an additional challenge (Pritchard & Di Rienzo, 2010) is to understand the extent and role of genetic diversity already present for polygenic traits, and the potential for adaptation (and hence evolution) in response to changing conditions. *On the theoretical side* [and empirically by experimentation and application] *there is a need to*



*bridge the gap between a classical quantitative perspective and a population genetics approach (which has focused more explicitly on quantities (allelic frequencies) that can be measured in molecular data (Pritchard & Di Rienzo, 2010).*

#### **A definition of quantitative genetics**

Quantitative genetics examines the mechanistic link between phenotypes and genomic variation (Lynch & Walsh, 1998). Phenotypic variation can be influenced by a single gene or more commonly suites of genes (i.e. polygenic traits) and these effects can be additive or non-additive in nature ([Falconer and Mackay, 1996](#)). The basic principles 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. We here summarise the methods and tools recently used in the field of quantitative genetics, focussing on approaches that are potentially of value in a marine conservation and management context. We describe how such developments can be applied in planning for evolutionary resilience, sustainable stock exploitation at MSY and in predictions for stock recovery, and suggest how they might be incorporated into policy and management of marine resources in general.

#### **Recent advances in methods**

Insights into the genomes of non-model (wild) species are rapidly increasing due to technical advances in Next Generation Sequencing (NGS), and advances in the accompanying bioinformatic and statistical tools. These technical advances in NGS improve throughput and accuracy, and decrease costs of screening many individuals at large numbers (hundreds to thousands or tens of thousands) of markers revolutionizing the opportunities for generating genetic resources in non-model organisms. In turn, this is driving a shift away from markers such as microsatellites to direct analyses of sequence variation including single nucleotide polymorphisms (SNPs), and the use of techniques such as Restriction Associated DNA (RAD) Sequencing (Baird *et al.*, 2008; Miller, Dunham, Amores, Cresko, & Johnson, 2007). The shift to high throughput genotyping offers the ability to examine both neutral variation and regions under selection and allows unparalleled scope for expansive screening of genome-wide variation in large sample sizes from natural populations.

Alongside the rapid advances and decreasing costs of NGS has come a parallel revolution in analytical capacity to accommodate the ever increasing amounts of data produced: many of these techniques have originated in the fields of human genetics and animal breeding. As the basic theory behind these methods is that the genetic architecture of complex traits is due to many (possibly interacting) genes, this requires a complex analytical framework to disentangle. Two methods are primarily used: Quantitative Trait Loci (QTL) analysis, where traits are mapped using linkage analysis, frequently applied in animal breeding and aquaculture to study economically important traits (de Koning *et al.*, 1999; Liu & Cordes, 2004); and Genome Wide Association Studies (GWAS), where using linear mixed models, hundreds of markers have been identified as associated with traits under selection, typically in human epidemiology (Klein *et al.*, 2005). While neither of these methods are new, their application to complex traits in non-model organisms remains rare (Stinchcombe & Hoekstra, 2008; Johnston *et al.*, 2011), although both techniques have known limitations (Beavis, 1994; Manolio *et al.*, 2009; J Slate, 2013). New methods are being developed allowing the partitioning of additive genetic variance across the genome in

order to accurately estimate the actual genome-wide relatedness among individuals (Bérénos, Ellis, Pilkington, & Pemberton, 2014; Visscher, 2009), to test for the effects of rare variants (Ionita-Laza, Lee, Makarov, Buxbaum, & Lin, 2013), for the analysis of polygenic traits (Goldstein, Hubbard, Cutler, & Barcellos, 2010), to correct for pedigree and population structure (Thornton & McPeck, 2010), and to reduce the impact of confounding effects (Listgarten *et al.*, 2013; Speed & Balding, 2014). These techniques have been used to great effect in studies in humans (Park *et al.*, 2010; Yang *et al.*, 2010), and are now being exploited to give insights into selection in ecologically relevant studies of non-model species (Holliday, Wang, & Aitken, 2012; Robinson, Santure, Decauwer, Sheldon, & Slate, 2013; Santure *et al.*, 2013). As fishes play a key role in marine ecosystems, understanding how evolutionary processes of adaptation affect population demography within ecological timescales is especially important to predicting response to natural and man-made environmental changes, and to the implementation of ecosystem-based management (Hendry, 2013; Schoener, 2011).

The use of novel quantitative approaches in wild organisms is opening up the ability to study changes in genes involved in polygenic traits, allowing research to address questions hitherto impossible, including the magnitude of potential adaptive responses to pressures such as climate change and fisheries. The developments in technological and statistical approaches to quantifying the genetic basis of quantitative traits have enabled investigations of terrestrial and marine non-model species. Traditional approaches required data on crosses and pedigrees, whereas new methods enable study of underlying genetic architecture of traits in the wild without such restrictions.

#### **Research in humans**

Many techniques exist for investigating the quantitative genetic basis of human traits and disease phenotype. The recent escalation in genomic studies confirms early expectations that many traits and complex diseases are influenced by many genes of small effect (Stranger, Stahl, & Raj, 2011). The last decade has witnessed a marked transition from studies estimating genetic variance, co-variance, and heritabilities to GWAS utilizing 100,000's of loci distributed across the genome. Due to low cost and high throughput of recent NGS platforms, GWAS enable swift mapping of genes associated with complex traits and human disease. The approach is unbiased to particular regions of the genome or prior knowledge, and in this way, has the potential to reveal hidden associations and new genic or non-genic causal variants. More than 2,000 trait-SNP associations have been identified in humans (Casto & Feldman, 2011); many of which are associated with such processes as immune response, metabolism, or cardiovascular performance (Stranger *et al.*, 2011). Perhaps not surprisingly, GWAS of related diseases frequently display overlapping causal variants. For instance, multiple autoimmune diseases are associated with SNPs found in MHC and other known immunity genes. Similarly, bladder, breast, colon, and ovarian cancer are strongly associated with a single gene region (Ghoussaini, Song, Koessler, & Al Olama, 2008). Also, GWAS studies of Immune related disorders show significantly greater signatures of selection than other disorders suggesting selection and immunity have in the past and continue to shape human evolution (Barreiro & Quintana-Murci, 2010).

#### **Research in non-model organisms**

For many years the majority of studies on non-model species were carried out on inbred lines, which were often removed from the wild for several generations, and

were rarely reared in their natural environment (for a review see Slate, 2005). Many studies were then carried out using the 'animal model' (a mixed effects model in which components of variance; e.g. additive genetic variance, maternal effects, environmental variance), estimated using restricted maximum-likelihood (REML) (see Kruuk, 2004 for a review), because the approach accommodates complex and uncontrolled pedigrees found in wild populations (L. E. B. Kruuk, Slate, & Wilson, 2008). A landmark study was carried out in a wild red deer population which demonstrated that QTLs could be mapped in an unmanipulated wild population (Slate *et al.*, 2002). In this study, three QTL for birth weight (a trait positively correlated with fitness components) were identified in a pedigreed population containing 365 deer. All QTL were of large effect, and interestingly, one appeared to be paternally silenced, i.e. the QTL effect was only present when inherited from the mother. However, these studies still relied on the genomic resources (usually microsatellites) developed for domesticated species such as sheep and cattle (Beraldi *et al.*, 2006; Slate *et al.*, 2002).

The revolution of NGS and the associated analytical techniques have now made it possible to develop and apply genetic tools to any organism. Within the last 5 years, the outcomes yield encouraging results studies, for example, on horn phenotypes in Soay sheep, which are associated with reduced fitness in both sexes. In contrast to many of the studies in humans, where only a small proportion of the genetic variation is often explained, this GWAS study identified a QTL which accounted for 76% of the variation of the trait (Johnston *et al.*, 2011). However, a study of clutch size and egg mass in Great tits (*Parus major*), which used chromosome partitioning, QTL mapping and GWAS, concluded that variation in both these traits is due to many loci of small effect spread across the genome (Santure *et al.*, 2013). To overcome the limitations of applying QTL or GWAS analyses to pedigree based data, two recent studies have applied multi-marker techniques to estimate the actual genome-wide relatedness among individuals (Visscher, 2009). The first of these looked at wing length in Great tits, and determined it to be polygenic (Robinson *et al.*, 2013). While this paper examined only one complex trait, another recent paper compared heritabilities estimated from these methods with those determined via pedigree based methods for a range of morphometric traits, and found the former to be highly (Bérénos *et al.*, 2014).

### Research in fishes

The majority of quantitative genetic research on fishes has derives from aquaculture and hence has primarily focused on salmonids, investigating traits such as disease resistance, growth and flesh quality. Such focus has resulted in the availability of highly developed genomic resources such as high-density SNP arrays and associated high density linkage maps, which has facilitated the application of quantitative techniques to a range of salmonids in the wild. Early such applications identified genes for which transcription levels are potentially under directional selection (Roberge, Guderley, & Bernatchez, 2007); differences in potentially adaptive traits between European and North American lineages of Atlantic salmon (Boulding *et al.*, 2008), and growth and development traits in Coho salmon (McClelland & Naish, 2010; O'Malley, McClelland, & Naish, 2010). A recent common-garden experiment to identify mechanisms of thermal adaptation and acclimation in redband trout (*Oncorhynchus mykiss gairdneri*), found differences in the heat shock response between desert and montane populations using a GWAS approach, yielding some insight into the ability of aquatic species to evolve adaptive responses (Narum, Campbell, Meyer, Miller, & Hardy, 2013). More recent research applied to wild populations has identified QTLs and associated chromosomal regions influencing migratory life-history tactics in rainbow and steelhead trout (*Oncorhynchus mykiss*) (Hecht, Campbell,

Holecek, & Narum, 2013; Hecht, Thrower, Hale, Miller, & Nichols, 2012). Migration in diadromous fish is an important component of their life-history strategy. Changes in the marine environment, such as those caused by climate change have the potential to affect their long-term survival. In Atlantic salmon, the migratory strategy incorporates a varying number of years in the marine environment before returning to its natal stream to spawn. The strategy carries an increased risk of mortality before spawning, which is highly responsive to changes in the environment such as global climate change. Johnston *et al.* (2014) identified several regions of the genome which vary in association with phenotype and/or selection between sea ages, with nearby genes having functions related to muscle development, metabolism, immune response and mate choice. Climate change also carries implicit risks of raised environmental temperature that in some cases may exceed those predicted to be lethal for a species. Everett & Seeb (2014) used high-throughput genotyping by sequencing (GBS) to identify three quantitative loci for temperature tolerance in Chinook salmon (*Oncorhynchus tshawytscha*). Given the expectations of global warming, an improved understanding of the genetic basis of temperature tolerance is especially important for the management and conservation of aquatic species.

Salmonids are not the only fish species to have benefitted from new insights into the quantitative genetic architecture of important phenotypic traits. A study of lake whitefish (*Coregonas clupeaformis*) identified QTLs for multiple ecologically important phenotypic traits between two reproductively isolated forms (Gagnaire, Normandeau, Pavey, & Bernatchez, 2013). A quantitative genetic approach to assess the resilience of a coastal marine species to changes in environmental conditions associated with ocean acidification was undertaken by Malvezzi *et al.*, (2015). Wild Atlantic silverside, (*Menidia menidia*) were reared under elevated CO<sub>2</sub> levels. Early life survival of *M. menidia* under high CO<sub>2</sub> levels had a significant additive genetic component that could elicit an evolutionary response to ocean acidification which is predicted under models of future climate change (Doney, Fabry, Feely, & Kleypas, 2009). As these new genetic and statistical methods are becoming increasingly affordable and accessible, the distinction between model- and non-model organisms is less restrictive. Recent advances yield insightful tools to address fundamental long-standing questions such the maintenance of genetic variation, limits to long term selection, phenotypic stability and adaptive potential in the face of a changing environment.

### Summary

Understanding the genetic foundation of phenotypic variation is particularly important in exploited marine species, as stocks are often identified based on differences in functional genetic variation (i.e., local adaptation). Moreover, global climate change, harvest, and management actions can all drive rapid evolutionary change (e.g., [Conover and Munch, 2002](#); [Naish and Hard, 2008](#); [Sgrò \*et al.\*, 2011](#)) (e.g., [Conover and Munch, 2002](#); [Naish and Hard, 2008](#); [Sgrò \*et al.\*, 2011](#)) (e.g., Conover & Munch, 2002; Naish & Hard, 2008; Sgrò, Lowe, & Hoffmann, 2011) potentially impacting stock productivity and persistence (e.g., [Schindler \*et al.\*, 2010](#)). Quantitative genetic analyses can address two main questions directly relevant to fisheries management and conservation. First, what is the genetic basis of phenotype and how does this vary spatially across a species? Second, can a detailed understanding of the genetic basis of phenotype permit the genetic monitoring of the direction and rate of quantitative trait change in the wild? Traditionally quantitative genetic examinations of phenotype have required either controlled laboratory experiments to remove environmental influences, or experimental lines and detailed pedigree information ([Falconer and Mackay, 1996](#); [Naish and Hard, 2008](#)). However

the melding of traditional quantitative genetics with molecular genetics / genomics (e.g. Stinchcombe & Hoekstra, 2008) and particularly recent advances in sequencing technology has opened new opportunities for fisheries management (e.g., [Johnston et al., 2014](#); [Malvezzi et al., 2015](#)). The examples outlined above from human, terrestrial and marine studies have demonstrated that the tools now exist which provide an enhanced understanding of stock structure and potential responses to environmental disturbances from factors such as climate change, fisheries induced evolution, release of captive bred fish into the wild and habitat change.

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## 6 Revisions to the work plan and justification

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ToR a) After careful evaluation of the work needed to complete a comprehensive review of existing and potential molecular techniques to evaluate infectious disease and parasite spread from transferred seafood into wild populations and, particularly, after considering critical the interaction with WGPDMO, we recommend extending this 1-year ToR into a 2-year ToR. Completion of ToR b will therefore be delayed until May 2016, though reported fully alongside remaining ToRs.

An additional 2-year ToR is proposed for consideration at the 2016-17 WGAGFM meetings:

- 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.

(Jann Martinsohn, Ernesto Jardim & Naiara Rodriguez-Ezpeleta)

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<sup>1</sup>, 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.

**Action Points:**

- Interaction with WGDEEP (ACOM) - Working Group on the Biology and Assessment of Deep-sea Fisheries Resources. Chairs: Gudmundur Thordarson, Pascal Lorange.
- Contacting the ICES Secretariat with the suggestion to send this ToR to DG MARE.

**Time range:** Two years – 2016/17

## **7 Next meetings**

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The 2016 meeting of the WGAGFM will take place at Queens University, Belfast, UK, 11–13 May 2016.

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<sup>1</sup> Bravington MV, Grewe PG, Davies CR (2014). Fishery-independent estimate of spawning biomass of Southern Bluefin Tuna through identification of close-kin using genetic markers. FRDC Report 2007/034. CSIRO, Australia

## Annex 1: List of participants

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## Annex 2: Agenda





**JRC Sites**

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**ICES**

Working Group On Applied Genetics  
for  
Fisheries & Mariculture

Annual Meeting 2015






**Tuesday 05 May**

Arrival

**Wednesday 06 May**

0900 Welcome by host Jann Martinsohn and housekeeping

0920 Welcome, introduction and updates by WG Chair Gary Carvalho

0945 Presentation and discussion of ToR position papers (20 minutes each + discussion):

- (a) Review and assess the utility of molecular techniques to evaluate disease and parasite spread from transferred seafood into wild populations;
- (b) Review and map decision channels for integrating WGAGFM advice into fisheries assessment and management;
- (c) Review application of quantitative genetic techniques into non-mariculture marine species.

1030 Coffee break

1045 Presentation and discussion of ToR position papers continued

12.45 Update of ToRs and procedures for drafting text

1300 Lunch (paid for individually)

1400 ToR position paper drafting in working groups

1530 Coffee break

1545 WGAGFM issues for discussion (Chair)

1615 ToR position paper drafting in working groups

1715 Working group status, and wrap up

1800 Departure to Hotel

2000 Joint Dinner (paid for individually)

**Thursday 07 May**

0900 Update on activities and housekeeping

0915 ToR position paper drafting in working groups

1030 Coffee break

1045 Seminar: Scientific advice under the remit of the Common Fisheries Policy (Ernesto Jardim)

1145 ToR position paper drafting in working groups

1300 Lunch (paid for individually)

1400 ToR position paper drafting in working groups

1530 Coffee break

1545 ToR position paper drafting in working groups

1630 Working group status, draft recommendations and wrap up

1730 Departure to Hotel

2000 Joint Dinner (paid for individually)

**Friday 08 May**

0900 Update on activities and housekeeping

0915 Presentation and discussion of ToR draft position papers and recommendations

1030 Coffee break

1200 New ToRs and WGAGFM 2016 venue

1250 Wrap up

1300 Lunch (paid for individually)

**Departure**

Working Group On Applied Genetics  
for  
Fisheries & Mariculture

**WGAGFM**

Annual Meeting 2015

06-08 May

JRC Ispira, Italy

