WORKING GROUP ON APPLICATION OF GENETICS IN FISHERIES AND AQUACULTURE (WGAGFA)

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Contents

i Executive summary

The ICES Working Group on Application of Genetics in Fisheries and Aquaculture (WGAGFA) is composed of 51 members from 17 ICES Member States. There is a high level of commitment by the WGAGFA members to their group: During the WGAGFA cycle 2018 to 2020, the annual working group meetings were attended in average by 26 delegates from 11 ICES Member States.

The expertise of the working group members covers a broad range within the field of genetics and also with respect to the marine realm and activities, from marine ecology to applied science in fisheries and aquaculture. WGAGFA members are affiliated to academic as well as to governmental and policy institutions.

From 2018 to 2020 the WGAGFA tackled four Terms of Reference (ToRs) focusing on policy and management advice and advice needs of the industry. The ToRs discuss farmed and wild salmon interactions (ToRa), genomic selection of aquaculture species (ToRb), genetics underpinning fisheries management (ToRc) and environmental DNA (ToRd) in support of ocean governance. These ToRs covered equally wild capture fisheries and aquaculture, and touched upon ICES strategy priorities as well as global objectives, such as the Sustainable Development Goals. They have purposely been tailored to address imminent policy needs, for example under the European Union Common Fisheries Policy (ToRc), industry relevant state-of-the-art approaches (ToRb) and most recent developments that can efficiently underpin marine conservation strategies and biodiversity preservation (ToRs a and e). During the reporting period, impact has been assured through:

- Interaction with policy stakeholders, from the European Commission and the European Parliament (ToRc);
- Dissemination through the production of a WGAGFA leaflet;
- Interaction with other working groups through the organization of an Annual Science Conference Theme Session (2018);
- The provision of a highly successful training course;
- Peer reviewed publications (ToR a and e);
- A topic sheet on environmental DNA (eDNA, ToR e), which will be an ICES science highlight.

ii Expert group information

Expert group name	Working Group on Application of Genetics in Fisheries and Aquaculture (WGAGFA)
Expert group cycle	Multiannual fixed term
Year cycle started	2018
Reporting year in cycle	3/3
Chair(s)	Jann Th Martinsohn, European Union
Meeting venue(s) and dates	15-17 May 2018, Brest, France (23 participants)
	14-16 May 2019, Ispra, Italy (23 participants)
	12-15 May 2020, By Correspondence (34 participants)

Obituary

It was with great sadness we received the news about the passing of Professor Jarle Mork in the summer of 2019.

Jarle was highly respected in the field of population genetics, not only as a scientist but also as a tutor and supervisor for many master- and PhD-students. His office and laboratory was adjacent to the Trondheim fjord, his chief study area with instant access to a survey vessel. As a professor in population genetics at the Norwegian university of science and technology he lectured both in population genetics and fisheries biology, throughout his career. He was also involved in many research projects both nationally and internationally and was one of the pioneers to recognise the value of long-term repeat sampling of fish population genetics. Correspondingly, Jarle was a prominent and effective advocate in the translation of genetic advice into policy, a core objective of the current WGAGFA.

Jarle was known for his strong opinions, particularly in the area of marine fish population discrimination where he argued the importance of establishing the neutrality of observed genetic differences. However, he was always open for any discussion or collaboration. His constructive criticisms demonstrated a selfless and considered approach to his science, an approach that stimulated many colleagues and early career researchers.

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 Jarle was asked to Chair the new group. He served as Chair for six years and was very influential in how the group is functioning today. He will be sadly missed.

Contents

1 Scope and Remit

Under the remit of the ICES Aquaculture Steering Group, the Working Group on Application of Genetics in Fisheries and Aquaculture (WGAGFA) provides advice on methods to describe, conserve, and manage intraspecific biodiversity, focusing on the application of genetic and genomic analyses.

WGAGFA (formerly the Working Group on Application of Genetics in Fisheries and Mariculture – WGAGFM) works on management themes spanning from commercial fisheries to ecosystems, recreational exploitation, and aquaculture.

This ICES Working Group also looks at a number of applications for genetic methods. Examples include identifying populations, tracing the origin of fish and fish products, tracing things like migratory behaviours and habitat use, determining the dynamics of non-indigenous species, and evaluating the effects of aquaculture escapees. Technological developments that have enabled genomic bar-coding are also considered, with advice given on application in species and ecosystem management.

Advice focuses on knowledge generated from applications of molecular genetic and genomic tools to identify, trace, restore, and manage local populations of fish and shellfish. The group also hindcasts and forecasts how drivers – for example physical, climatic, and fisheries ones – affect distributions.

During this fixed-term cycle, from 2018 to 2020, in line with its objectives, the WGAGFA addressed through four Terms of Reference:

1. the quantification of indirect genetic impacts of farmed salmon on wild salmon populations;
2. principles and prospects for genomic selection in aquaculture;
3. the value of genetic and genomic tools for fisheries management;
4. environmental DNA used to support fisheries management and the monitoring of marine ecosystem monitoring.

Results, conclusions and prospects emerging from these Terms of Reference are further delineated in this report.

2 The Terms of Reference: Description

2.1 ToRa – Review and report on genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations

Atlantic salmon, *Salmo salar*, is of considerable socio-economic value in culture, and the process of domestication has resulted in significant phenotypic (i.e. physiological, Handeland et al. (2003); behavioural, Fleming et al. (1996); morphological, Fleming, Jonsson, and Gross (1994)), and genetic-based (Cross and King (1983); Karlsson et al. (2011)) differences from wild populations. Escape events from Atlantic salmon net-pen aquaculture are a regular occurrence (Keyser et al. 2018), and the number of escapees can equate to an appreciable fraction of, or exceed wild Atlantic salmon census size (Morris et al. 2008; Skilbrei, Heino, and Svåsand 2015). There is substantial evidence that direct genetic interactions, defined as interbreeding between wild Atlantic salmon and escaped domestic individuals occurs (Glover et al. 2017) and can alter wild salmon and reduce the long-term viability of wild populations (McGinnity et al. 2003; Bourret et al. 2011; Glover et al. 2013; Bolstad et al. 2017). However, indirect genetic interactions may also occur and are defined as genetic changes in wild populations resulting from ecological changes that either (1) alter the selective landscape experienced by native fish and thus change gene frequencies or (2) reduce their abundance resulting in a loss of genetic diversity (Figure 1). As indirect effects do not involve reproductive interactions, they can impact wild populations of any native species and can arise whether domestic animals escape or remain in containment. Despite the potential broad reaching impacts of indirect genetic interactions on wild Atlantic salmon and other species, the ability to assess their presence and quantify their magnitude has been limited to date (Verspoor et al. 2015). Internationally, there is continuing interest in expanding Atlantic salmon aquaculture, and although practices to limit direct genetic interactions have been implemented in many areas through the use of triploids (Verspoor et al. 2015), exotic species, and improvement in containment strategies, these do not prevent indirect genetic effects. Currently, a large expansion in the production of cultured salmon has been approved in North America, involving the production of 7 million triploid Norwegian salmon annually (DFO 2016). While the use of all female triploid salmon will reduce the likelihood of direct genetic interactions, the actual magnitude of direct and indirect genetic interactions from this planned expansion remains unknown (Verspoor et al. 2015). In Iceland there is a similar significant expansion of the industry underway (MAST 2017), also including sterile triploid Atlantic salmon (Ramsden 2018). Similarly, in other species such as brown trout or Pacific salmon species, indirect genetic interactions with Atlantic salmon aquaculture remain an ongoing concern (e.g. Coughlan et al. 2006; Ford and Myers 2008). Improved understanding of the indirect genetic effects, i.e. those less obvious impacts, from aquaculture will help to inform regulatory and policy decisions related to the long-term sustainability of the industry. The overall goals of this review are to (1) highlight the potential for indirect genetic interactions associated with Atlantic salmon net-pen aquaculture through a review of examples of changes in abundance or the environment experienced by wild populations, and (2) discuss the opportunity recent advances in population genomic approaches present for the assessment of these indirect genetic impacts.

2.2 ToRb - Review and report on principles of and prospects for 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 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.

2.3 ToRc - Assess and report on the value of genetic and genomic tools for identifying species in mixed landings, fish products and by-products.

Mixed-species landings and the use of a mix of species in fish products continues to pose a formidable challenge to fisheries control and enforcement as well as traceability along the supply chain. In light of the difficulties in monitoring mixed species landings and identifying species in fish products and by-products we aim to elaborate whether genetic and genomic tools can provide robust and cost-efficient support to determine species composition, also quantitatively, and directly supporting fisheries management and policy needs. A timely and relevant example is the global attempt to develop and implement rules that lead to the reduction of discards. Discarding is the rather common practice of returning unwanted catches to the sea, either dead or alive, because they are undersized, due to market demand, the fisher has no quota or because catch composition rules impose this. In Europe, 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 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 while 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 fishoil 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 commercial 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. Focusing on, but not dealing with exclusively, we will elaborate whether genetic methods might efficiently support the implementation of rules designed to reduce discards and related control, monitoring and enforcement measures

2.4 ToRd - 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 utilized 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 utilized 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 summarize the research to date, identify areas where tools are already available for use and examine future developments while 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 nontechnical summary of the state of the field for direct dissemination to fishery managers with little or no genetic background.

3 The Terms of Reference: Documentation

3.1 ToRa - Genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations

Contributors: **I.R. Bradbury**, Burgetz I., Coulson M.W., Verspoor, E., Gilbey, J. Lehnert S.J., Kess T., Cross T. F., Vasemägi A., Solberg, M., McGinnity P.

3.1.1 Summary

Cultured Atlantic Salmon, Salmo salar, are of considerable socio-economic value, and the process of domestication has resulted in significant behavioural, morphological, and allelic differences from wild populations. Substantial evidence now indicates that direct genetic interactions or interbreeding between wild and escaped farmed Atlantic Salmon occurs, genetically altering wild salmon and reducing population viability. However, theoretically indirect genetic non-reproductive interactions may still occur through ecological mechanisms (e.g. disease, parasites, predation, competition) both in conjunction with, and in the absence of interbreeding. Here we explore the existing evidence of indirect genetic interactions between domestic Atlantic Salmon and wild populations, and the potential use of genetic and genomic tools to resolve these impacts. Our literature review identified examples of genetic changes (i.e. indirect genetic interactions) resulting from ecological processes, predominately through pathogen or parasite transmission. In addition, many examples were identified where aquaculture activities have either altered the selective landscape experienced by wild populations or resulted in reductions in population abundance, both of which are consistent with the widespread occurrence of indirect genetic changes. We further identify opportunities for genetic or genomic methods to quantify these impacts, though careful experimental design and pre-impact comparisons are often needed to accurately attribute genetic change to aquaculture activities. Our review indicates indirect genetic interactions occur, likely impact population and persistence, and that further study is urgently needed to support an integrated understanding of aquaculture-ecosystem interactions, their implications for ecosystem stability, and the development of potential mitigation and management strategies.

3.1.2 Introduction

Atlantic Salmon, *Salmo salar*, aquaculture is of considerable socio-economic importance, and the process of domestication has resulted in significant phenotypic (i.e. physiology, Handeland et al. (2003); behavioural, Fleming et al. (1996); and morphological, Fleming et al. (1994); and allelic (Cross and King, 1983; Karlsson et al., 2011; Wringe et al., 2018a) differences from wild populations. Escape events from Atlantic Salmon net-pen aquaculture are a regular occurrence (Keyser et al., 2018), and the number of escapees equate to an appreciable fraction of, or exceed, wild Atlantic Salmon census size (Morris et al., 2008; Skilbrei et al., 2015; Wringe et al., 2018b). There is substantial evidence that direct genetic interactions, defined as interbreeding between wild Atlantic Salmon and escaped domestic individuals occurs (Karlsson et al., 2016; Glover et al., 2017; Wringe et al., 2018b) and can genetically alter wild salmon and reduce population viability (McGinnity et al., 2003; Bourret et al., 2011; Glover et al., 2013; Bolstad et al., 2017; Bradbury et

al., 2020). Both in Canada and Norway, recent evidence suggests hybridization may be extensive following escape events (Karlsson et al., 2016; Wringe et al., 2018b), and account for substantial proportion of production in smaller rivers (Sylvester et al., 2018b). Accordingly, escaped farmed salmon and direct genetic interactions have been identified as a major threat to the persistence and stability of wild salmon across the North Atlantic (Forseth et al., 2017; Bradbury et al., 2020).

However, indirect genetic interactions may also occur, either in concert with or in the absence of hybridization (Verspoor et al., 2015), due to ecological interactions such as competition, predation, and disease or parasite transfer. These can be defined as genetic changes in wild populations resulting from ecological changes that either (1) alter the selective landscape experienced by native fish and thus change allele frequencies of loci linked to fitness and/or (2) reduce population abundance resulting in a loss of genetic diversity (Figure 1). As indirect effects do not involve reproductive interactions, they can arise whether domestic animals escape or remain in containment and impact wild populations of any native species. Although practices to limit direct genetic interactions with wild Atlantic Salmon have been implemented in many areas through the use of sterilization (Verspoor et al., 2015), exotic species, and improved containment strategies (Diserud et al., 2019), these efforts do not prevent indirect genetic effects. In other species such as Brown Trout or Pacific salmon species where hybridization with escapees is not common or possible, ecological and indirect genetic interactions with Atlantic Salmon aquaculture remain an ongoing concern (e.g. Coughlan et al., 2006; Ford and Myers, 2008). Moreover, given recent trends in industry expansion (e.g. DFO, 2016), and growing concerns regarding the amplification of pests and pathogens such as sea lice through net pen aquaculture, the potential for indirect genetic interactions is likely to increase. Nonetheless, despite the potentially broad reaching and significant impacts of indirect genetic interactions on wild Atlantic Salmon and other species, the evidence of their presence and our ability to quantify their magnitude has been limited to date (Verspoor et al., 2015).

The overall goal of this review is to highlight the potential for indirect genetic interactions and to review the evidence pertaining to the potential for indirect genetic interactions between domestic Atlantic Salmon and wild populations. Specifically, our objectives are to (1) highlight the potential for indirect genetic interactions associated with Atlantic Salmon net-pen aquaculture through a review of examples of genetic changes in wild populations resulting from ecological interactions, or likely more common, evidence of changes in abundance of or the environment experienced by wild populations; (2) discuss the opportunity recent advances in population genomic approaches present for the assessment of these indirect genetic impacts. Through our review, we aim to highlight opportunities for the further study of indirect genetic impacts of Atlantic Salmon aquaculture on wild populations. We directly build on previous reviews and empirical studies focusing on direct effects (e.g. Karlsson et al., 2016; Glover et al., 2017; Bradbury et al., 2020) and on risk assessments considering both direct and indirect genetic effects (e.g. Verspoor et al., 2015). Ultimately, we suggest that indirect genetic interactions are likely ubiquitous wherever salmon farming occurs, and that further research is urgently required to better understand the magnitude of these interactions and provide advice regarding impact management and mitigation.

3.1.3 Evidence of indirect genetic impacts

Atlantic Salmon net pen aquaculture represents a substantial change to the natural environment (Garcia de Leaniz et al., 2007) and thus the adaptive landscape experienced by wild individuals. As such it can alter the stability and future evolutionary trajectories of wild populations. Furthermore, it might be expected that adjustments to a new adaptive landscape will result in reductions in productivity through increased maladaptation predicted by theoretical demo-

graphic-evolutionary models (Burger and Lynch, 1995; Gomulkiewicz and Holt, 1995; Kirkpatrick and Barton, 1997). Existing studies address genetic changes on naïve populations through disease transmission, the potential for recovery of disease resistance through natural selection, observations on genetic changes in co-occurring congener species, and impacts of the farming of non-native species. Examples of the later are the farming of European origin salmon on both the east and west coasts of North America as well as in western South America or Australia. Below we review the literature related to indirect genetic interactions associated with disease and parasite transfer, increased predation pressure, and finally increased competition (See Table 1). In each case we first highlight examples of genetic change resulting from these interactions, and then set out evidence of demographic decline or the potential for selection consistent with indirect genetic interactions. In practice, for examples related to wild Atlantic Salmon it can be difficult to distinguish the impacts of direct and indirect genetic interactions, so we focus on instances where mechanisms have been identified which are clearly non-reproductive.

A) Indirect genetic changes through disease transmission

Indirect genetic interactions via disease transmission may result in both alterations to the selective landscape potentially impacting immune associated genetic variation as well as reductions in overall genetic diversity due to demographic decline. To date, few studies have examined the presence of genetic changes due to disease transfer (Table 1A). However, de Eyto et al. (2007) ; and de Eyto et al. (2011) present evidence of genetic impacts due to novel disease exposure associated with aquaculture activities. In this study the progeny of Atlantic Salmon from a river without previous exposure to aquaculture were transferred to a river with a long history of associated farming and captive breeding that was expected to have acquired novel micro and macro-parasitic communities. This experimental design was enabled to expose animals to novel dis-ease challenges associated with escapes or inadvertent or deliberate introductions. By comparing observed and expected genotype frequencies at a marker locus for the MHC class II alpha gene and control neutral microsatellite loci at parr and migrant stages in the wild it was concluded that genetic change had occurred, and that selection was likely a result of disease-mediated natural selection, rather than any demographic event.

A significant and growing body of research exists supporting the hypothesis that wild salmon populations are adapted to local pathogen communities both in space and time (Dionne et al., 2007; Tonteri et al., 2010; Consuegra et al., 2011; Kjaerner-Semb et al., 2016; Pritchard et al., 2018; Zueva et al., 2018). For example, in studies of wild Atlantic Salmon, Dionne et al. (2009) report significant changes in myxozoan resistance associated MHC alleles likely linked with an infection-related mortality event, again supporting the potential for pathogen associated genetic impacts in wild populations. This suggests a genetic basis to differences in population immunity and that the introduction of new pathogens into susceptible populations could both impose novel selection pressures and reduce genetic diversity through demographic decline.

The possibility that pathogen transfer from domestic to wild salmon domestic individuals could drive genetic change in wild populations is supported by several recent findings documenting the potential for exposure and supporting pathogen transfer as mechanisms for indirect genetic interactions (Table 1A). First, Madhun et al. (2015) report the detection of virus infected escaped farmed salmon entering rivers near cage sites, suggesting clear evidence of exposure of freshwater rearing juvenile salmon populations to aquaculture associated pathogens. Second, Madhun et al. (2018) document the presence of piscine orthoreovirus (PRV) in returning wild adult Atlantic Salmon in Norway, and that the frequency of infection increased with body size and displayed no geographic signal suggesting infection was occurring between escapees and wild salmon at marine feeding areas. Finally, Nylund et al. (2019) report that infectious salmon anemia virus (ISAV) variants seen in farmed salmon are increasing in prevalence in the wild consistent with horizontal transmission from farmed salmon to wild populations. Taken together,

these findings indicate that indirect genetic impacts on wild salmon populations associated with disease transmission from aquaculture populations is highly likely. However, both the magnitude of new selection pressures and demographic impacts are uncertain and likely case specific.

Diseases, introduced or increased in incidence by salmon aquaculture activities, could also have an impact on co-occurring wild species such as anadromous Brown Trout (Salmo trutta L.), as implied by the steep decline in anadromous trout numbers in many Irish, Scottish, and Norwegian rivers since the late 1980s, which may be linked to sea lice infestations (see below) associated with marine salmonid farming. A study by Coughlan et al. (2006) in some Irish rivers suggested that salmon farming and ocean ranching could indirectly affect, most likely mediated by disease, the genetics of cohabiting anadromous Brown Trout by reducing variability at major histocompatibility class I genes. A significant decline in allelic richness and gene diversity at the Satr-UBA marker locus, observed since aquaculture started and which may indicate a selective response, was not reflected by similar reductions at neutral loci. Subsequent recovery of variability at the Satr-UBA marker, seen among later samples, may reflect an increased contribution by resident Brown Trout to the remaining anadromous population. Similarly, Miller et al. (2011) link genomic profiles consistent with viral infection with increased likelihood of mortality prior to spawning in Fraser River Sockeye Salmon (*Oncorhynchus nerka*).

B) Indirect genetic effects through parasites

Like disease transfer, the introduction of novel parasites could both impose new selection pressures and drive demographic decline. Although no examples of genetic change attributable to parasite transfer from salmon aquaculture were identified, substantial research has demonstrated the (1) transfer of parasites from aquaculture salmon to wild populations, (2) significant demographic impacts resulting, and (3) a genetic basis to resistance, all of which support the presence of indirect genetic change occurring as a result. Examples to date have most notably been via infections of sea lice or the monogenetic trematode Gyrodactylus salaris (Table 1B). Declines in wild stocks attributed to sea lice outbreaks in farm-intensive areas have been documented in Ireland, Scotland and Norway. Thorstad and Finstad (2018) reviewed the literature related to sea lice impacts on wild stocks and documenting 12-29% fewer returning adult spawners due to lice-induced mortality from fish farms. In one of the most extreme cases documented to date, Shephard and Gargan (2017) suggested that 1 sea-winter salmon returns on the River Erriff were more than 50% lower in years following high lice levels on nearby farms. This increased mortality was in addition to decreased returns due to poorer marine survival. Similarly, Bøhn et al. (2020) tagged and released salmon smolts both with a prophylactic treatment against lice, and without such treatment, and recaptured survivors returning to freshwater after spending 1-4 years at sea. They report that the mortality of untreated smolts was as much as 50 times higher compared to treated smolts during sea lice outbreaks.

Evidence also exists that show the transfer of sea lice from farmed Atlantic Salmon to Pacific salmon species (Nekouei et al., 2018), again consistent with the potential indirect genetic interactions. For example, out-migrating juvenile Pink Salmon (*O. gorbuscha*) and Chum Salmon (*O. keta*), are estimated to experience four times greater sea lice infection pressure near salmon farms compared to background infection levels (Krkošek et al., 2005) and in juvenile Sockeye Salmon (*O. nerka*), infection rates were elevated after migration past salmon farms (Krkošek et al., 2005; Price et al., 2011). For coho Salmon (*O. kisutch*), ecological interactions with infected species as well directly with salmon farms can result in higher infection levels (Connors et al., 2010). These lice infections in Pacific salmon species have also been associated with population declines. Krkošek et al. (2007) found that sea lice infestation from salmon farms on out-migrating Pink Salmon smolts have led to declines in wild populations in the Broughton Archipelago, with forecasting models suggesting that local extinction was imminent. For these Pink Salmon populations exposed to salmon farms, mortality rate caused by sea lice was estimated to range from 16

to 97% (Krkošek et al., 2007). Similar population declines were also observed in coho Salmon populations (Connors et al. 2010). Nonetheless, changes in parasite management on salmon farms can help reduce infection rates on wild salmon and have a positive effect on wild population productivity (Peacock et al., 2013), supporting this linkage and suggesting mitigation could be possible.

Given evidence of significant sea lice associated demographic declines, it seems likely that sea lice induced mortality could drive reductions in genetic diversity. However, beyond that, a large body of research suggests resistance to sea lice may have a genetic basis and be heritable (Tsai et al., 2016; Correa et al., 2017; Robledo et al., 2019), making it highly likely that wild populations would change in response to new selection pressures. It is worth noting that these estimates of lice-induced mortality among Atlantic Salmon should be considered as minimum estimates for species such as anadromous Brown Trout, which are more coastal, thus increasing their exposure to net pen sites (Thorstad and Finstad, 2018).

For *Gyrodactylus salaris*, the first appearance in Norway has been linked to the introduction of salmon from Baltic catchments, resulting in high levels of mortality among wild populations (Johnsen and Jensen, 1991). Notably, for *G. salaris* very high rates of mortality in naïve populations strongly supports the potential for significant demographic decline, losses of genetic diversity, and parasite driven selection. For example, following several independent introductions of *G. salaris* into Norway, exposed wild populations decreased in abundance by an average of 85% and smolt numbers decreased by as much as 98% (Denholm et al., 2016). Several studies suggest a genetic basis to *G. salaris* resistance among wild salmon populations in Europe. Gilbey et al. (2006) identified 10 genomic regions associated with heterogeneity in both innate and acquired resistance using crosses of resistant Baltic and susceptible Atlantic populations. Zueva et al. (2014) compared Baltic and Atlantic Salmon populations characterized by different levels of resistance to *G. salaris* and identified three genomic regions potentially experiencing parasite-associated adaptation in the wild. More recently, Zueva et al. (2018) compared salmon populations from northern Europe classified as extremely susceptible or resistant to *G. salaris*. They identify 57 candidate genes potentially under resistance associated selection and this set of loci were shown to be enriched for genes associated with both innate and acquired immunity. These findings suggest that indirect genetic impacts on wild salmon populations associated with para-site transmission such as sea lice from aquaculture populations are highly likely both because of the potential for substantial mortality to occur through exposure and for it to be selective through a clear genetic basis to population differences in resistance.

C) Indirect genetic effects through predation

Increased predation associated with salmon aquaculture activities could result in both declines in abundance and selective mortality. Although direct estimates of impact are lacking, some evidence exists to support the possibility of such a link, most likely it seems through predators being attracted to aquaculture activities (Table 1C). Aquaculture sites have been shown to attract wild fish, invertebrates, marine mammals, and birds, likely due to the addition of food (see review in Callier et al., 2017) and the end result may be increased predation on wild individuals in the vicinity. For example, Kennedy and Greer (1988) reported heavy predation on hatchery smolts and wild Atlantic Salmon and Brown Trout from the river Bush in Northern Ireland by the cormorant *Phalacrocorax carbo*. This suggested a link between the release of captive bred smolts (a proxy for farm escapes), the attraction of increased numbers of these predatory birds to the river and increased predation on river's wild Atlantic Salmon and Brown Trout. Similarly, Hamoutene et al. (2018) conducted experimental releases and tracking of aquaculture Atlantic Salmon near cage sites in southern Newfoundland, Canada. They found that most released fish were not detected beyond a few weeks of release, with temperature and movement data supporting predation as a cause. Increased predation of wild salmon smolts or adults near sea cages

could therefore drive demographic decline or potentially act as a selective agent if predators cued on size, or behaviour or other traits.

D) Indirect genetic effects through competitive interactions

Indirect genetic effects have also been suggested via evidence of competitive interactions among farm and wild salmon. Given the clear overlap in habitat use, and evidence of density-dependence these seem most likely to take place in freshwater during the juvenile stage (Table 1D). This has been illustrated by the work of Fleming et al. (2000) who released sexually mature farm and wild Atlantic Salmon into the River Imsa in Norway. Despite the farm fish achieving less than one third of the breeding success compared to wild fish, there was evidence of resource competition and competitive displacement, as the productivity of the wild fish was depressed by more than 30%. They concluded that invasions of farm fish have the potential for impacting wild population productivity both via changes to locally adaptive traits as well as reductions in genetic diversity. Skaala et al. (2012) documented similar effects in another natural system in Norway. They compared the performance of farm, wild and hybrid salmon and suggested that overlap in diets and competitions can impact wild productivity, an impact that could reduce genetic variation in wild populations. However, the potential for increased competition to result in changes to the selective landscape experienced by wild individuals remains unclear.

3.1.4 Quantifying indirect genetic impacts

The studies reviewed above demonstrate strong potential for indirect genetic interactions to occur in wild populations, however, quantifying indirect genetic interactions between wild and domes-tic populations remains a major challenge, particularly when hybridization is occurring (i.e. direct genetic interactions). Dramatic increases in DNA sequencing capacity over the last decade present new opportunities for the use of genomic tools to quantify the impacts of net pen aquaculture on wild populations. Indirect genetic interactions represent a special more complex challenge and the utility of genetic and genomic tools to resolve indirect genetic interactions will de-pend on the route and genomic scale of impact. That said, a large body of literature has been produced in recent years on the use of genetic/genomic tools to quantify both adaptive diversity and neutral diversity or effective population size or changes therein in wild populations. As such, a clear opportunity exists to apply genetic and genomic methods to quantify these impacts.

Detecting changes in adaptive diversity

In the context of impacts due to changes in the selective landscape driven by ecological change, genomic change could be associated with a single gene, or many genes (i.e. polygenic). Genetic and genomic tools are increasingly being used to quantify the magnitude of natural selection in the wild (Vitti et al., 2013) and many approaches have been developed (Table 2A). One of the best approaches to quantify the presence of selection associated with indirect interactions is the comparison of representative pre- and post-impact genetic samples in the absence of hybridization, or with the capacity to quantify and correct for signatures of recent or current hybridization (Leitwein et al., 2019). For time-series analysis of changes in allele frequency associated with selection, differentiation measures such as the fixation index (FST) are commonly used, and several tests have been recently proposed using bi-allelic loci including the empirical likelihood ratio test (ELRT), and the frequency increment test (FIT) (Feder et al., 2014). Recent temporal comparisons of natural selection in ecological, climate adaptation, and fishery-impact studies have revealed detectable increases in genomic differentiation over even short timeframes (e.g. one to four generations, Bitter et al., 2019; Leitwein et al., 2019; Therkildsen et al., 2019), indicating genomic tools show high power to detect changes in natural selection when recent pre-impact baselines are available. Where replicate temporal comparisons across sites can be made, this may allow uncovering parallel patterns and non-parallel signatures of adaptation. Knowledge of pre-

impact genomic variation across replicates can quantify the source and magnitude of indirect genetic impacts; sites with similar starting genomic variation are more likely to show parallel responses, unless source or strength of selection differs.

In the absence of pre-impact samples, traditional tests for the presence of outliers (e.g. Foll and Gaggiotti, 2008; Luu et al., 2016), trait associations, or selective sweeps (e.g. Nielsen et al., 2005) may be applied using genome-wide polymorphism data, though the ability to attribute a given impact to these loci may be problematic. Similar to pre and post-impact temporal comparisons, tests for genomic differentiation using metrics such as FST between sites with differing levels of exposure to stressors can be used to detect the magnitude and location of genomic change between these impacted and pristine sites (e.g. Dayan et al., 2019; Oziolor et al., 2019). Genome-wide association and genome-environment association methods also show promise in measuring aquaculture impacts but have traditionally been used to estimate correlations between genomic variants and trait or environmental variation (Rellstab et al., 2015; Santure and Garant, 2018). A recent genomic study by Lehnert et al. (2019) instead used decline status as the compared trait in genome-wide association, and uncovered polygenic associations with population decline and variation in immune and developmental genes. This approach could be further refined in future studies by incorporating continuous measures of aquaculture exposure such as magnitude of escape, site proximity, or pathogen load. Rapid evolutionary change is often associated with selection on standing genetic variation ("soft sweeps") rather than new mutations (Messer et al., 2016; Hermisson and Pennings, 2017).

Methods that utilize differences in frequency and diversity of haplotypes such as integrated haplotype score (iHS, Voight et al., 2006), extended cross population haplotype homozygosity (XP-EHH, Sabeti et al., 2007) and number of segregating sites by length (nSL, Ferrer-Admetlla et al., 2014) can identify signatures of soft selective sweeps. Identification of these sweep signatures exclusive to aquaculture-impacted populations may provide an additional way of validating genomic changes induced by indirect genetic impacts and uncover implicated target genes. Machine learning approaches have also shown promise in identifying subtle signatures of environment (Sylvester et al., 2018a) and trait associations (Brieuc et al., 2015) and selective sweep signatures (Kern and Schrider, 2018), and provide additional research areas for future research in measuring genetic impacts of aquaculture exposure that may not be detected by traditional statistical approaches. Lastly, gene ontology (Rivals et al., 2007) and gene set (Daub et al., 2017) enrichment methods can be used to characterize functional impacts and parallel responses at biological levels above changes at individual genes (Jacobs et al., 2020), and can help clarify potential targets of selection from aquaculture interactions.

Detecting changes in neutral diversity or effective population size

Genomic approaches can also be applied in the context resolving a loss of diversity due to demographic declines associated with indirect genetic impacts, genomic approaches can be applied to quantify genome-wide trends in diversity over time or estimate trends in the effective population size (Table 2B, Waples and Do, 2010). Large genomic datasets offer new opportunities for enhanced estimates of effective population size (Waples et al., 2016) as well as retrospective estimates of changes in effective population size over time (e.g. Hollenbeck et al., 2016). For example, Watson et al. (in prep) evaluated the performance of estimates of effective population size (Ne) using large genomic datasets, to assess and approximate population declines and establish a genomic baseline to detect indirect genetic interactions in southern Newfoundland Atlantic Salmon populations following the use of largely sterile salmon in aquaculture. Their results suggest that large genomic datasets (≥1000 SNPs) were able to detect population declines significantly earlier and with increased accuracy than small genetic or genomic datasets (25 microsatellites or 100 SNPs). The authors observed that the power for early detection of population decline in-creased more rapidly with the number of individuals sampled than with the number of loci genotyped.

However, monitoring using effective size requires temporal sampling which is not al-ways possible. As an alternative, Hollenbeck et al. (2016) present a method that uses linkage information to bin loci by rates of recombination and reconstruct trends in Ne decades into the past. Lehnert et al. (2019) applied this method to Atlantic Salmon across the North Atlantic and estimated that 60% of all populations have declined in recent decades. Such approaches could be used in to quantify population trends in effective size in the absence of assessment data and monitor for indirect genetic interactions in future.

3.1.5 Summary and Next Steps

Ultimately, despite much relevant and informative research, the relative importance of direct and indirect genetic interactions between domestic individuals and wild populations re-mains largely unresolved. Nonetheless, the literature reviewed suggests that ecological interactions arising from salmon aquaculture has the realistic potential to result in substantial genetic change in wild salmon populations as well as other species. Fortunately, recent advances in genetic and genomic methods present new scope for quantifying these impacts. However careful experimental design and pre-impact comparisons will in most cases be needed to accurately attribute any genetic change to indirect genetic interactions with salmon aquaculture activities. Future research should explore the sensitivities and power of these approaches to detect changes in genetic diversity and character over time. Given that both direct and indirect interactions may co-occur within the native range of Atlantic Salmon, there may be benefit to focus study on instances where interbreeding is unlikely or impossible. This could involve the study of indirect impacts in other species such as Pacific salmon species, or in Atlantic Salmon in regions where sterility is employed as a containment or mitigation measure. Alternatively, genomic approaches could be used to disentangle direct interactions from indirect interactions based on the identification of hybrids, introgressed ancestry blocks, or signatures of selection. Our review suggests that indirect genetic interactions represent both a broad reaching and largely unresolved source of genetic impact on wild populations exposed to Atlantic Salmon aquaculture activities. This further study is urgently needed to support an integrated understanding of aqua-culture-ecosystem interactions, their implications for ecosystem stability, and the identification of potential pathways of effect. This information will be essential to the development of potential mitigation and management strategies.

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Table 1. Summary of papers presenting evidence of or consistent with the potential for indirect genetic interactions among Atlantic Salmon aquaculture and wild populations.

Interaction	Primary observation	Evidence (direct or supportive)	Selection / demographic	Species impacted	Reference
A) Disease transfer					
Common garden experiment (naïve non local wild population introduced into different river system as eggs)	Evidence of allele frequency change at Major Histocompatibility (MH) genes during first six months in introduced population; no change in local population)	Supportive	Selection	Atlantic Salmon (*S. salar*)	(de Eyto et al., 2007)
Common garden experiment (naïve non local wild population introduced into different river as eggs)	Evidence of different allele frequency change at Major Histocompatibility (MH) genes in introduced population from six months to 18 months; no change in local population)	Supportive	Selection	Atlantic Salmon (*S. salar*)	(de Eyto et al., 2011)
Genetic survey of natural populations (not associated with aquaculture)	Evidence of clinal geographical response in Major Histocompatibility (MH) genes in response to water temperature variation)	Supportive	Selection	Atlantic Salmon (*S. salar*)	(Dionne et al., 2007)
Genetic survey of natural populations potentially sensitive and tolerant of *G. salaris*	Evidence of clinal geographical response in Major Histocompatibility (MH) & other immune relevant genes in response to water temperature variation)	Supportive (possible direct link to *G. salaris* parasite)	Selection	Atlantic Salmon (*S. salar*)	(Tonteri et al., 2010)
Genetic survey of natural populations in areas with and without aquaculture activity	Evidence of spatial allele variation at Major Histocompatibility (MH) genes	Supportive (possible direct link to viral pathogens)	Selection	Atlantic Salmon (*S. salar*)	(Consuegra et al., 2011)
Genetic survey of natural populations in region of significant aquaculture activity	Evidence of snp variation associated with selective sweeps of immune response genes	Supportive (source of selective agent unknown)	Selection	Atlantic Salmon (*S. salar*)	(Kjaerner-Semb et al., 2016)
Genetic survey of natural populations within single large river complex (not associated with aquaculture)	Evidence of snp variation associated with Major Histocompatibility (MH) genes	Supportive	Selection	Atlantic Salmon (*S. salar*)	(Pritchard et al., 2018)

Disease screening of escaped farmed Atlantic Salmon in a wild river	Virus infected escaped farmed salmon entering rivers near cage sites	Supportive	Both	Atlantic Salmon (*S. salar*)	(Madhun et al., 2015)
Disease screening of returning wild Atlantic Salmon in Norway at six sites	Evidence of the infection of wild salmon from escaped farmed salmon at marine feeding areas	Supportive	Both	Atlantic Salmon (*S. salar*)	(Madhun et al., 2018)
Genetic screening of ISAV variants in wild and farmed salmon in Norway	Evidence of the horizontal transmission of ISAV variants seen in farmed salmon to wild populations	Supportive	Both	Atlantic Salmon (*S. salar*)	(Nylund et al., 2019)
B) Parasite transfer					
Statistical modelling of the effect on return rates of sea lice levels (low/med/high) over a 26 year period for 1SW Erriff salmon	Wild salmon returns were strongly reduced (>50%) following years with high lice levels during smolt outmigration (farms located at the mouth of the estuary	Supportive	Both	Atlantic Salmon (*S. salar*)	(Shephard and Gargan, 2017)
Tag/recapture experiment of prophylactically treated smolts exposed to different farm-origin sea lice pressure	Recapture rate of untreated adult salmon following exposure to high sea lice density was 0.03% compared to treated salmon (1.86%)	Direct	Both	Atlantic Salmon (*S. salar*)	(Bøhn et al., 2020)
Association between sea lice counts on farmed Atlantic salmon and wild out-migrating Chum salmon	Significant positive association between the sea lice abundance on farms and the likelihood that juvenile Chum would be infested. Increased abundance of lice on farms was not significantly associated with the levels of infestation observed on juvenile Chum salmon	Supportive		Chum salmon (*Onchorynchus keta*)	(Nekouei et al., 2018),
Review paper: integrating laboratory and field observational studies of lice on out-migrating *S. salar* and *S. trutta*	Sea lice loads on out-migrating sea trout in areas with aquaculture commonly exceed threshold levels that are known to induce physiological compromise or mortality in laboratory experiments	Supportive	Both	Sea trout (*S. trutta*)	(Thorstad and Finstad, 2018)
Review paper: integrating laboratory and field observational studies of lice on out-migrating *S. salar* and *S. trutta*	Premature migratory return	Direct	Demographic	Sea trout (*S. trutta*)	(Thorstad and Finstad, 2018)

Review paper: integrating laboratory and field observational studies of lice on out-migrating *S. salar* and *S. trutta*	Summary of meta-analysis and tagged treated smolt survival to returning adults experiment	Supportive	Both	Atlantic Salmon (*S. salar*)	(Thorstad and Finstad, 2018)
Sea lice abundance on out-migrating Pink Salmon and Chum Salmon differences pre- and post-exposure to Atlantic Salmon farms	Quantitative estimate of transmission rates from farm to out-migrating Pink and Chum salmon, including subsequent transmission dynamics of lice within the wild population	Supportive	Demographic	Pink Salmon (*Oncorhynchus gorbuscha*) and Chum Salmon (*Oncorhynchus keta*)	(Krkošek et al., 2005)
Hierarchical model of stock-recruit dynamics of coho salmon with differential sea lice infestation	Coho salmon population productivity in an area of intensive salmon aquaculture was depressed approximately sevenfold during a period of salmon louse infestations compared to unexposed populations.	Supportive	Demographic	Coho salmon (*Onchorhynchus kisutch*)	(Connors et al., 2010)
Modelling effect of sea lice infections on population abundance of Pink Salmon	Pink Salmon populations exposed to salmon farms; mortality rate caused by sea lice was estimated to range from 16 to 97%	Supportive	Demographic	Pink Salmon (*Oncorynchus gorbuscha)*	(Krkošek et al., 2007)
	Pink Salmon population abundance increased following reduction of farm-origin sea lice during out-migraiton	Supportive	Demographic	Pink salmon (*Oncorhynchus gorbuscha*)	(Peacock et al., 2013),
***Gyrodactylus salaris* infection associated with wild salmon population decline**	Wild stocks decreased in size by an average of 85% and smolt numbers decreased by as much as 98% following introduction of *G. salaris* into Norway	Supportive	Demographic	Atlantic Salmon (*S. salar*)	(Denholm et al., 2016).
Genomic basis of resistance to *Gyrodactylus salaris*	Identified three genomic regions associated with adaptation to parasite resistance in wild salmon	Supportive	N/A	Atlantic Salmon (*S. salar*)	(Zueva et al., 2014)
Genomic basis of resistance to *Gyrodactylus salaris*	Identified 57 candidate genes potentially under positive selection associated with *G. salaris* resistance and enriched for lymph node development, focal adhesion genes and anti-viral responses	Supportive	N/A	Atlantic Salmon (*S. salar*)	(Zueva et al., 2018)
C) Predation					

Increased predation on wild species	Increased avian predation on wild salmon and brown trout following the release of captive bred smolts	Supportive	Demographic / selective?	Brown Trout	(Kennedy and Greer, 1988)
Predation on released farmed escapes	High levels of predation on released farmed Atlantic Salmon near cage sites	Supportive	Demographic / selective?	Atlantic Salmon	(Hamoutene et al., 2018)
D) Competition					
Competition between wild and farmed juvenile Atlantic Salmon in freshwater	30% reduction in wild population productivity in the presence of farmed fish	Supportive	Demographic	Atlantic Salmon	(Fleming et al., 2000)
Competition between wild and farmed juvenile Atlantic Salmon in freshwater	Overlap in diet among types of crosses demonstrates competition	Supportive	Demographic	Atlantic Salmon	(Skaala et al., 2012)

Table 2. Summary of available genetic and genomic methods to evaluate indirect genetic interactions.

Method	Comparison	Statistics/Tests	Reference
A) Changes in adaptive diversity			
Time-series analysis	Changes in allele frequency	Empirical likelihood ratio test (ELR)	(Feder et al., 2014)
	Changes in allele frequency	Frequency increment test (FIT)	(Feder et al., 2014)
Temporal comparisons, pre- vs. post-impact	Changes in allele frequencies	Principal component analysis, outlier detection, genetic differentiation (F_{ST})	(Bitter et al., 2019)
Temporal comparisons : **Pre- vs. post-impact**	Changes in allele frequencies in response to size-selection gradients	% polymorphism, nucleotide diversity, & allele frequency shifts (controls vs. experimental samples)	(Therkildsen et al., 2019)
Domestic ancestry estimation under different stocking intensities	Relationship between domestic ancestry and recombination rate at different genomic scales		(Leitwein et al., 2019)
Outlier detection	Locus-specific comparison of posterior probabilities of models with and without selection	F_{ST} coefficient & and Bayes factor scores	(Foll and Gaggiotti, 2008)
Outlier detection	Tests of neutrality based on principal components analysis	Mahalanobis distance	(Luu et al., 2016)

Impacted vs. non-impacted	Signatures of selection that co-vary with environmental stressor (e.g. pollution)	F_{ST}, population branch statistic, differences in nucleotide diversity along 20-kilobase sliding window	(Oziolor et al., 2019)
Impacted vs. non-impacted	Signatures of selection associated with environmental stressor	F_{ST} outlier (FDIST2)	(Dayan et al., 2019)
Genome-wide association studies	Polygenic associations with population decline involving genomic regions related to metabolism, developmental & physiological processes	Change in μ (signature of selective sweeps) between declining and non-declining population status of Atlantic salmon; Redundancy analysis (RDA) for detection of outliers, polygenic risk scores	(Lehnert et al., 2019)
Soft selective sweeps	Identification of new alleles to intermediate frequency against a background of unusually long haplotypes of low nucleotide diversity	Integrated haplotype scores (iHS)	(Voight et al., 2006)
Soft selective sweeps	Identification of selected alleles nearing or having achieved fixation in one population but that remains polymorphic in the wider group of populations.	Extended cross population haplotype homozygosity (XP-EHH)	(Sabeti et al., 2007)
Soft selective sweeps	Detection of positive selection acting to increase haplotype homozygosity; combines distribution of fragment lengths between mutations and number of segregating sites between all pairs of chromosomes; ratio of haplotype homozygosity for derived & ancestral alleles.	Number of segregating sites by length (nSL); similar to iHS but 1) a genetic map is not required and 2) more robust to recombination and/or mutation rate variation	(Ferrer-Admetlla et al., 2014)
Machine-learning	Correlates of habitat/environmental variables with observed genetic structure	Random Forest; PCA loadings; outlier detection	(Sylvester et al., 2018a)
Machine-learning	Detection of loci of small phenotypic effect on a key life-history variable (e.g. run timing) across multiple populations	Random forest; outlier detection; PCA	(Brieuc et al., 2015)
B) Changes in neutral diversity or effective population size			
Effective population size	Single-sample method based on linkage disequilibrium to estimate effective populations size	Contemporary *Ne*	(Waples and Do, 2010; Waples et al., 2016)
Effective population size	Single-sample method to estimate changes in contemporary *Ne* by comparing linkage disequilibrium estimates with recombination rates estimated from physical linkage or genomic position	Contemporary *Ne* estimates at various times in the past	(Hollenbeck et al., 2016)
Effective population size	Application of Hollenbeck et al. (2016) for range-wide populations of Atlantic salmon and associations of genomic regions to decline status	Contemporary *Ne* estimates over time	(Lehnert et al., 2019)

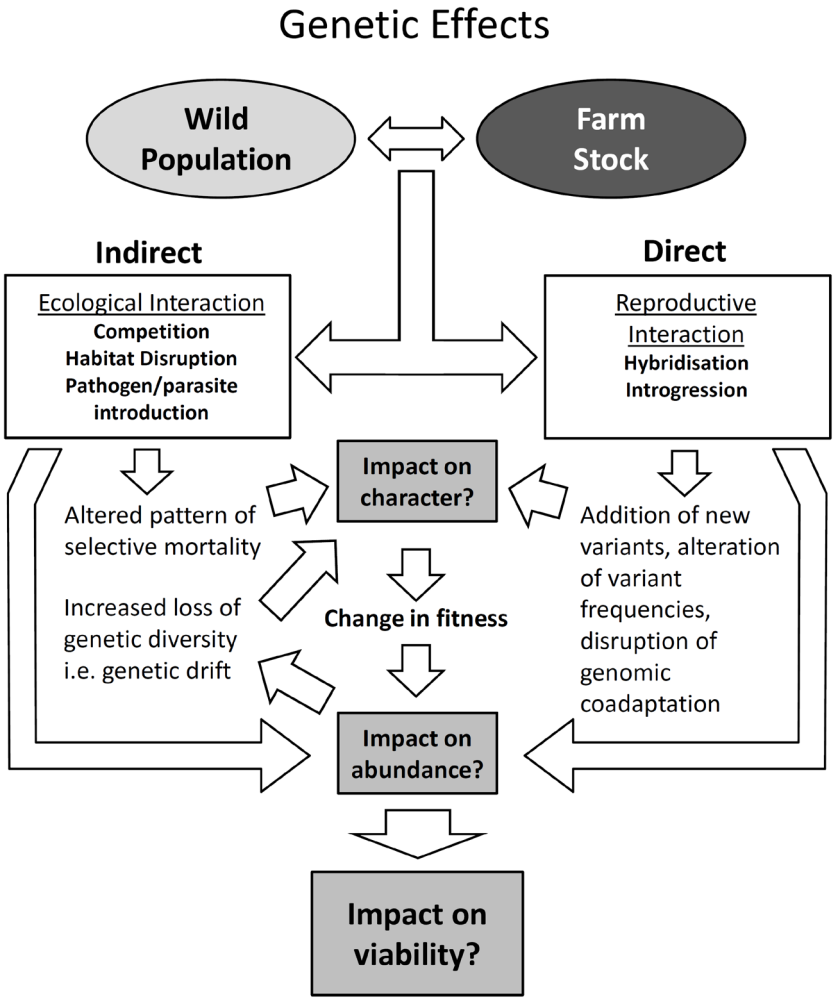

Figure 1. Schematic of direct and indirect genetic interactions between wild and domestic Atlantic salmon.

3.2 ToR b: Genomic selection applied to aquaculture species

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3.2.1 Introduction

Genomic selection (GS), first proposed in 2001 (Meuwissen et al., 2001), is a genome-wide marker-assisted selection (MAS) method dedicated to improve quantitative traits. GS is now successively implemented in an increasing number of terrestrial farmed species, in particular dairy cattle (Boichard et al., 2016) and crops (Desta & Ortiz, 2014, Heslot et al., 2014), resulting in an increase in prediction accuracy and of subsequent genetic gain.

Unlike QTL-based MAS, where the genetic fraction explained by each QTL is first tested for its statistical significance, GS omits significance testing and estimates the effect of all markers simultaneously through a prediction equation. GS aims to predict the breeding value of individuals based on their genotype at a very large number of markers spread over the genome; it is most commonly performed using SNP arrays. GS consists in two steps. The prediction equation is first established in a training population in which individuals are phenotyped and genotyped. The number of markers being much higher than the number of individuals, classical statistics cannot be applied, and the use of alternative methods is required (De los Campos et al., 2013): GBLUP - an extension of polygenic BLUP, where all markers have the same weight - or Bayesian estimates, which allows variance of allelic effects of each marker and assumes that only a small number of them have a non-zero effect. Once the prediction equation is established, breeding candidates can then be selected on the basis of their estimated genomic value with or without phenotyping. GS is of particular interest in the case of lethal traits (i.e. traits that cannot be recorded on live individuals; e.g. disease and parasite resistance, thermal and salinity tolerance), where phenotypes are recorded on relatives of the candidate breeders. In most cases, it is more efficient than "sib selection", which is classically used in such cases, because sib selection gives the same breeding value to all animals in a family, while GS allows the identification of the best candidates within each family. In terms of its limitations, GS is very demanding in terms of number of individuals genotyped and the number of markers employed. Its potential is likely to vary according to the life cycle characteristics of each species and the ability of breeding companies to invest in sophisticated and potentially resource-intensive (e.g. funding, infrastructure and training) selection programs.

3.2.2 Genotyping technology: practical information and needed investments

GS requires the availability of genome-wide SNP datasets. A number of aquaculture species already have commercially available SNP arrays (See Table 1). In addition, SNP panels can be produced de novo by reduced-representation Next Generation Sequencing (NGS) approaches, such as restriction site-associated DNA (RAD) sequencing or genotyping-by-sequencing (GBS) (Robledo et al., 2017). NGS can identify thousands of SNPs that provide a genome-wide coverage. If a large population or set of populations of a target species is genotyped, NGS could be used to develop a rich genome-wide SNP panel, which may capture the effects of a large number of genes (mostly due to linkage). Moreover, developing SNPs and subsequent SNP panels on the targeted population(s) helps to minimize both ascertainment bias and the number of potentially uninformative markers. The limitations of NGS are, however, first and foremost, that training and breeding populations should be genotyped together to have the best opportunity to discover the maximum number of shared markers. In addition, the initial NGS output is very dependent on the quality of the template DNA and of the amplification of the fragments; therefore, it may yield substantially fewer high-quality, reliable SNPs compared to a commercial SNP array. Initial development of a genome-wide array of highly polymorphic, robust SNPs can be costly and time consuming, but such an array might quickly prove cost-effective compared to NGS SNP discovery approaches because it provides a standardized genotyping platform.

Genetic maps and reference genomes are not strictly needed for the use of GS, but they can provide greater understanding of the distribution of markers around the genome and whether any areas of the genome are underrepresented or not uniformly covered. In particular, genomic maps are not needed for the GBLUP approach, although they are useful in Bayesian approaches that identify markers close to genes relevant in the selection process. The creation of genetic maps and reference genomes is a relatively costly and time-consuming enterprise, however, and cost/benefit analysis might not support the investment of resources.

Table 1: Aquaculture species for which commercial SNP chips have been recently developed.

Species	References
Salmo salar	Yanez *et al.*(2016), Houston *et al.*(2014)
Oncorhynchus mykiss	Palti *et al.*(2015)
Cyprinus carpio	Xu *et al.*(2014)
Ictalurus punctatus; Ictalurus furcatus; Ameiurus nebulosus; Ameiurus catus	Liu *et al.*(2014)
Crassostrea gigas	Gutierrez *et al.*(2017), Qi *et al.*(2017)
Ostrea edulis	Gutierrez *et al.*(2017)
Gadus morhua	Pocwierz-Kotus *et al.*(2015)
Litopenaeus vannamei	Jones *et al.*(2017)
Dicentrarchus labrax	Faggion *et al.*(2018), Vandeputte *et al.*(2019)

Specificities of aquaculture species with regards of GS

The overall consensus is that GS will enhance the rate of genetic gain both by increasing the accuracy for genetic value predictions and shortening generation intervals. Resulting GS information may also facilitate the discovery of genomic regions that contribute to the underlying genetic variation of complex traits. While the benefits of GS are undeniable, it is also important to consider and to evaluate potential challenges and pitfalls of the approach for different species and distinct breeding programs (Ibañez-Escriche & Gonzales-Recio, 2011).

GS developments in aquaculture species have been recently reviewed by several authors (Hosoya et al., 2017; Palaiokostas & Houston, 2018; Zenger et al., 2018, You et al., 2020, Houston et al., 2020). The main practical concern for the use of GS in aquaculture it is whether GS is a cost-effective selection strategy compared to individual selection, which is still widely used, or pedigree-based methods. As noted above, using commercial SNP arrays or developing 'de novo' SNP arrays and producing training and breeding populations can be expensive. For GS to benefit these aquaculture sectors, more cost-efficient genotyping is necessary as recently proposed by using low density arrays (Kriaridou et al., 2020). Despite these potential financial costs, GS has shown to be both effective and cost-effective in many common livestock species, especially those species that are costly to breed and/or phenotype or where a commercially valuable trait (for instance, milk production) expressed by only one sex is actually influenced by the genetics of both parents. Yet, considerations for the use of GS for dairy cattle and other terrestrial livestock or agricultural crops are necessarily different from those required for aquaculture (see Robledo et al. for a review), given obvious differences in life histories (e.g. generation time, fecundity, prior pedigree information, age at commercial size relative to puberty, sex-reversal...).

In aquaculture, breeding programs have only been limited to a few species, such as salmonids, shrimps, tilapia, carp, sea bream, sea bass, oysters, scallops, clams, catfish, and moronids. Many of these programs started with simple mass selection for growth and appearance, but an increasing number now use family information to improve genetic gain and enable selection on traits not easily measured on breeding candidates (e.g. disease resistance, processing yields, flesh quality). However, when information from siblings is used to select candidates on such traits, within-family variance is not exploited, and this limits the potential genetic gain. Thus, the use of GS could be especially beneficial for improving these highly desirable traits. Of particular relevance to aquaculture is that GS may be used to overcome problems related to a lack of pedigree information and inbreeding, two of the main hindrances linked to traditional selective breeding programs in finfish and shellfish. Luckily, in many new and developing breeding programs, pedigree information can be or has been reconstructed through microsatellite and/or SNP genotyping. Because the infrastructure for DNA collection and fish individual tagging is already available, these programs are good candidates for easier implementation of GS.

Moreover, compared with selective breeding programs for terrestrial species, the use of GS in both finfish and shellfish has also been limited by the lack of dense marker maps and/or high-throughput genotyping platforms. These circumstances, however, are beginning to change as advances in genomic methodologies accompanied by reduced costs for analyses are enabling the increased use of GS in aquaculture. Results from recent empirical GS studies in farmed aquatic species are confirming those from early simulations and suggest an increase in the accuracy of selection for both continuous and categorical traits (Vallejo et al., 2017; Nielsen et al., 2009; Sonesson & Meuwissen, 2009; Daetwyler et al., 2010). In addition to facilitating the increase of genetic gains, GS can also be used to introgress advantageous polymorphisms into a potential target population. For instance, Ødegård et al. (2019) demonstrated that simulated backcross breeding programs using GS provided a faster approach to developing a disease-resistant line of commercial value.

Other points for consideration are:

- the large variety of species / numerous "minor" species / high selection intensities possible / recent domestication and breeding (potential of classical breeding),
- the short generation time (not necessarily compared to poultry and pigs, but most traits are recorded before maturity, so GS cannot shorten generation time),
- the high fecundity of aquaculture species,
- the low individual value of breeders.

3.2.3 Towards phenomic selection?

Phenomic selection (PS) was recently proposed as an alternative (or complement) to GS (Rincent et al. 2018). The proposed method is based on the use of near-infrared (NIR) vibrational spectroscopy. Vibrational spectroscopy allows to characterize the fundamental absorption bands of the functional groups of bio-chemical substances that make up a sample under study and are therefore specific to an individual (i.e. chemical fingerprint or "super-phenotype"). A large number of vibration studies have been carried out to evaluate the feasibility of prediction for a number of biochemical molecules. NIR spectroscopy first became widely used in the food industry with pioneering analyses in cereals and fruits.

In their proof of concept article, Rincent et al. (2018) carried out work in wheat and poplar, showing that it is possible to estimate genetic values that are as precise (or even more precise) in PS as in GS. The advantages attributed to this spectroscopic technique are the speed and simplicity of measurement, the absence of solvent use, the low cost of implementation and the repeatability of measurements. The transfer of this principle to aquatic species presents several scientific and

technical challenges. The results presented by Rincent et al. (2018) were acquired from NIR spectra of lignified tissues whereas biological samples of aquaculture species are very rich in water, which might be problematic. An alternative to NIR spectroscopy could be the use of Raman scattering spectrometry. Feasibility and potential of PS in sea bream and Pacific oyster is currently explored in the project "Phénomix" coordinated by SYSAAF.

The following sections present current status and developments of GS in different aquaculture species.

3.2.4 Atlantic cod *(Gadus morhua)*

General Context

Atlantic cod is a marine species of great commercial interest, whose distribution ranges from the East coast of the USA to Greenland, Iceland, Norway and along the west coast of Europe. Juvenile production of Atlantic cod started in the 1980's in Norway, resulting to a few 100,000 fish per year in the late 1990's. Production then was extensive, with no breeding, and not profitable, resulting in closure of all companies. New attempts of Atlantic cod production started in the early 2000 with the first successful intensive hatcheries and production of Atlantic cod. Structured breeding programs showed potential for improvement of cultured stocks of Atlantic cod, and major improvements were made both in rearing practices as well as genetic improvements on growth traits. Production peaked at around 60 million juveniles overall. Yet, biological challenges, such as early maturation, juvenile deformities, high mortality rates in sea cages, and the financial crises of 2008 greatly affected the industry. In 2014, commercial aquaculture of Atlantic cod was effectively shut down. Two main actors in Norway continued their breeding programs and commercial production resumed in 2018 with improved growth rate as the result of selective breeding, improved rearing practices, diets and economics. The reduction in fishing quotas from natural populations of Atlantic cod also drove the interest for cod farming in Norway. To date there are still only a few producers, but interest for cod aquaculture is on the rise.

Past and current status of selective breeding in Atlantic Cod

There are two main actors of Atlantic cod breeding nowadays, both of which are located in Norway: a national program run NOFIMA, with the aim of making cod aquaculture profitable by selective breeding based on the model of Atlantic salmon, and currently produces around 400,000 juveniles per year but with the, and a private breeding program Havlandet Marin Yngel that currently produces around 3 million juveniles per year. The main traits selected for in Atlantic cod have been growth rate, morphology (absence of deformity, condition factor), as well as disease resistance. The latter have not been successfully addressed through selective breeding and disease challenges, but is now relatively well managed with vaccines and prophylactic measures. Several selective breeding strategies have been used to date: phenotypic selection and breeding value estimation. Phenotypic selection relies on selecting the best individuals based on their phenotypes, without pedigree information. In contrast, breeding value estimates are calculated using Best Linear Unbiased Prediction (BLUP) based on pedigree information and phenotypic observations from all family members and breeding candidates. In both approaches, special care is taken to limit inbreeding, either through Optimal Contribution Selection (OCS) or through producing very large number of families.

Current/future implementation of GS in Atlantic Cod

There has been no genomic selection implemented in Atlantic cod aquaculture to date. Atlantic cod aquaculture is still in its infancy, and optimal rearing techniques are now just being developed. However, Atlantic cod is in a unique position to be starting aquaculture programs at a time where many genomic resources are already available for the species. Most of these resources

have been developed in the context of wild Atlantic cod, but are directly relevant to aquaculture. In particular, the genome of Atlantic cod has been fully sequenced and is publicly available (Tørresen *et al.* 2017) and SNP chips and linkage maps are also available (Hubert *et al*. 2010, Pocwierz-Kotus *et al.* 2015). These resources could be directly used for implementation of genomic selection in Atlantic cod aquaculture for traits of interest, such as sexual maturation – which is currently the biggest bottleneck in cod aquaculture –, feed efficiency, skin health, overall immune system and muscle mass. Family based breeding for several generation combined with the genomic resources for Atlantic cod will provide the ideal set up for implementing genomic selection in this species.

Challenges for GS in Atlantic Cod

The main challenges for genomic selection in Atlantic cod aquaculture rests in the fact that this is a young industry whose rearing techniques and economic profitability still need to be validated. However, although costly, implementing genomic selection at such an early stage might be easier than it would be for other more established aquaculture species. Additionally, the large amount of genomic resources and the technical and scientific expertise of the actors in Atlantic cod aquaculture and Atlantic cod research in general might facilitate the implementation of GS.

3.2.5 American catfish

(channel catfish: *Ictalurus punctatus* and blue catfish: *Ictalurus furcatus*)

General Context

The closely related Ictalurid catfish species *Ictalurus punctatus* (channel catfish) and *Ictalurus furcatus* (blue catfish) are native to North America and have long been used as a source of dietary protein in the United States. Catfish farming represents the largest segment of aquaculture in the U.S. with approximately 340 million pounds of catfish processed in 2018 (Hanson 2019). The farm-raised catfish industry accounts for more than half of all U.S. aquaculture production. The regional economic impact exceeds $4 billion and the industry employs more than 10,000 people with an annual payroll well over $150 million in the Deep South, the most economically underdeveloped region of the United States. The 2013 Census of Aquaculture reported 605 catfish farms involved in the sale of food-size fish primarily in the states of Alabama, Arkansas, and Mississippi and having sales valued at $354,337,000. The success of the catfish aquaculture industry depends on a consistent supply of a high-quality product that meets consumer expectations for flavor, color, texture, and firmness.

Past and Current Status of Selective Breeding in American catfish

The first catfish genetics and breeding programs started at Auburn University in the 1950s and 1960s (Dunham 2006). In the decades since, breeding programs have developed and dissolved at various institutions (e.g. University of Georgia, Mississippi State University, U.S. Fish and Wildlife Service) however, these programs were able to narrow down candidate Ictalurid aquaculture species, to the blue and channel catfish that are the current focus of catfish aquaculture in the U.S (Dunham 2006). More recently, breeding programs have started generating F1 channel-blue hybrids (female channel x male blue) for faster growth, improved disease resistance, and larger fillet yields (Geng et al. 2016; Dunham et al. 2008), and hybrids now comprise 60-70% of the industry (Abdelrahman et al. 2017). Genetic improvement endeavors have primarily been conducted by public entities (Abdelrahman et al. 2017). At present, the only two institutions with major involvement in genetic enhancement are Auburn University and the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Warmwater Aquaculture Research Unit (WARU) in Stoneville, Mississippi (Dunham 2006). Researchers at the University of Georgia have also recently collaborated with WARU to implement genomic selection (Garcia et al. 2018).

Genomic resources for these species include a high-quality reference genome for the channel catfish; 98% of the 783 Mb genome is captured in 594 scaffolds (scaffold N50 = 7.73 Mb), genetic mapping of over 250,000 SNPs has validated the assembly, and 99.1% of the reference genome has been anchored to chromosomes (Zeng et al. 2017; Liu et al. 2016). Genomic sequencing on the Blue catfish has also been conducted (reported in Abdelrahman et al. 2017). Currently, four commercial catfish Affymetrix Axiom arrays are available, a 250K array (Liu et al. 2014), a 690 K array (Zeng et al. 2017), a 660K array and a 57K arrays (Waldbieser, unpublished). Several studies have also identified QTL for several important traits in catfish culture (e.g. disease resistance, hypoxia tolerance, heat stress).

Current and Future Implementation of GS in American catfish

To support the long-term sustainability of catfish aquaculture, the USDA, ARS WARU is conducting a genomic selection program for channel and blue catfish. A synthetic line of channel catfish, "Delta Select", was produced from a base population of fish obtained from ten commercial farms. Microsatellite markers were used to determine spawn parentage, and the pedigreed population underwent two generations of selection for increased growth and carcass yield using estimated breeding values derived from standard animal breeding approaches. Early in the genomic selection program, preliminary research revealed that existing SNP genotyping platforms showed an ascertainment bias in SNP polymorphism. Therefore, genomic DNA was re-sequenced from 49 founder animals to a depth of 5X genome coverage, the sequences were mapped to the channel catfish reference genome (Liu et al. 2016), and 7.4 million putative SNP loci were identified in silico. After screening 660,056 SNP loci for polymorphism and Mendelian transmission, a subset of 57,354 Delta Select SNPs were arrayed that were separated by an average distance of 13.3 kb. The 2015 year-class Delta Select broodfish were selected based on the same index for growth and carcass yield, except that EBVs were replaced with genomic estimated breeding values (GEBVs). The GEBVs were derived based on growth and carcass yield phenotypes, pedigree information and SNP genotypes using the single-step methodology developed at the University of Georgia (Misztal et al. 2016). The analysis indicated that whole genome selection based on GEBVs would increase accuracy of breeding value estimates for growth by 28% and carcass yield by 36% (Garcia et al. 2018). Comparison of the Delta Select line to an unselected control line, developed from the same base population, indicated response to selection after 3 generations of selection, and led to a 25% increase in growth rate and 0.9% increase in carcass yield (Bosworth et al., 2020). It is estimated that an increased growth rate of 14-20% and an increased filet yield of 0.3-0.6% over two generations of channel catfish would add $7-12 million annual profit to the catfish industry above current production costs. Additional phenotypic data has been collected on body composition and reproductive traits; heritabilities and genetic correlations for these traits will be estimated to determine if they warrant inclusion in a selection index. The WARU released Delta Select germplasm to U.S. catfish producers in February 2020.

A GS program for blue catfish was recently initiated. Preliminary performance trials of a diverse collection of blue catfish germplasm has revealed founder broodstock. A team from WARU and Auburn produced a chromosome-level blue catfish reference genome (Waldbieser and Liu, unpublished), identified 2.1 million putative SNP loci in the blue catfish breeding population, and placed 660,000 SNP loci onto an array for genotype validation (Waldbieser and Bsoworth, unpublished). Blue catfish will be selected with a focus on improving the performance of F1 hybrid offspring of blue catfish sires and channel catfish dams, because the hybrid fish are valued by catfish producers for their superior performance in commercial culture. The WARU released blue catfish germplasm to U.S. catfish producers in April 2020.

Challenges for genomic selection in American catfish

Challenges to implementing genomic selection include the costs associated with developing the genotyping array and the costs of genotyping a sufficient number of individuals. It is also necessary to collaborate with other institutions to identify scientists with the skillset to implement genomic selection on the data (e.g. to test and apply appropriate models).

3.2.6 Pacific oyster *(Crassostrea gigas)*

General Context

Pacific oyster is the primary farmed mollusc species in many regions of the world, due to its fast growth and robustness to diverse environments (FAO). Originating in the Northwest Pacific, it has been widely introduced to North America (since 1920s) in Australia and New Zealand and Europe (since 1960s), either to replace depleted native stocks, unintentionally, or to instigate new industry. Since then, further introductions and distribution across countries has resulted in the species being one of the most farmed aquaculture species globally, with 574K tonnes produced in 2016 (FAO). Pacific oyster is also been listed as invasive in an increasing number of countries (FAO). While initial culture methods in Japan, Korea and China were typically entirely reliant on settlement of wild spat, which remains the main source of juveniles in numerous countries, control of reproduction has allowed the development of hatcheries, allowing the production of seed outside optimal environmental conditions and increasingly from selective breeding programs (reviewed by Hollenbeck and Johnston, 2018), and/or using polyploids.

Historically, European broodstock originated either directly from Japanese populations, or populations sourced from British Columbia, Canada (Troost, 2010). However, during the following years there was substantial movement and sharing of stock between European nations to the extent that direct tracing of broodstock origin has become impractical, although population genetic studies clearly distinguish two main clusters (Lallias et al., 2015). Contemporary hatchery practice involves ownership of unique broodstock, and as such it is now possible to identify northern and southern hatchery populations, reflecting the historical introduction routes of the species in Europe. However, there continues to be mixing of stocks throughout Europe, between both hatchery and naturalized populations, alongside additional smaller scale introductions from Japan (Vendrami et al., 2019). In Australia and New Zealand, more direct links can be made between original broodstock introductions and source populations in Japan (Kijas et al., 2019).

Past and current status of selective breeding in Pacific oyster

A primary focal trait for oyster selective breeding programs has been increased growth rate, which is straightforward to measure on selection candidates themselves. In oysters, growth rate and weight traits can refer to the animal including the shell, but the weight of the oyster without the shell ('wet weight'), or meat to shell ratio, is also a target for improvement. Superior growth of triploid oysters is one of the main reasons why they have been increasingly produced since the 1900's.

Disease resistance became the key target trait for improvement in Pacific oyster, primarily due to the global disease outbreak caused by ostreid herpesvirus 1 (OsHV-1) µVar, which severely affected the industry in most oyster producing countries (Pernet et al., 2016). Promisingly, host resistance to OsHV-1 is heritable and over 60 % improvement in survival was observed with mass selection vs. unselected controls in response to OsHV-1 exposure after four generations (Dégremont et al., 2015). Since then most oyster producing nations have rolled out successful programs breeding to improve resistance to OsHV-1; either via family based or mass selection techniques. One of the reasons that genetic improvement of disease resistance is so important in

oysters is that often alternative means of disease prevention are lacking, and traditional vaccination approaches are impossible in molluscan aquaculture due to the lack of an adaptive immune system (Wang et al., 2013).

Genotype by environment interaction (GxE) is an important consideration for target traits in oyster breeding. Since individuals from a breeding nucleus are likely to be distributed from hatcheries and breeding programs to multiple, diverse environments, the robustness of their performance for traits of interest across these environments is an important consideration (reviewed by Hollenbeck and Johnston, 2018). However, most studies report limited GxE effects.

Mass selection has been performed in Pacific oyster (as highlighted above for resistance to OsHV-1), but while effective in the short term it is unlikely to be sustainable due to a lack of control of inbreeding. Therefore, several countries have established well-managed family based breeding programs, including in Australia, New Zealand, the USA, and France (reviewed by Hollenbeck and Johnston, 2018). Family based selection enables the incorporation of multiple traits into the breeding goal (in contrast to mass selection), and also to include traits that are not measurable on the selection candidates themselves. This is particularly relevant to Pacific oyster breeding because disease resistance is a key trait, and often such traits are measured on relatives of selection candidates. However, in some cases (e.g. in New Zealand) breeding from survivors has been successfully practiced (Gutierrez et al., 2020).

Major stakeholders (companies, public bodies, etc.) and countries involved in breeding

Almost all breeding programs were initially publicly funded. Some programs, for example in France, USA, New Zealand and Australia, have now been taken on by industry-led bodies or private companies. There are also genetic services companies that provide breeding program support and management to hatcheries and producers.

Current/future implementation of GS in Pacific oyster

One prerequisite for genomic selection is the availability of genotyping technology for reliable genome-wide typing of large numbers of individuals. Two medium-high density SNP arrays have been developed for Pacific oyster (Gutierrez et al., 2017; Qi et al., 2017) which are suitable tools for testing genomic selection. While it is unclear whether genomic selection is operational in oyster selective breeding currently, there are studies highlighting its potential. For example, the accuracy of prediction of breeding values for growth-related traits was shown to be 25-30% higher using genomic prediction than using pedigree-based prediction in a UK oyster population (Gutierrez et al., 2018). Furthermore, the advantages of genomic prediction were also highlighted for disease resistance, with approximately 19 % higher accuracy compared to pedigree methods (Gutierrez et al. 2020). Interestingly, in both studies, the marker density required to achieve this increase in accuracy over pedigree methods was only approximately 1,000 SNPs. This is likely to be due to the fact that most of the benefit comes from capturing the within-family component of genetic variation for large full sibling families, and therefore the training and reference populations share long genomic segments captured effectively by few markers. However, further testing of this theory would require additional studies, including in larger populations under selection.

GS is particularly useful for traits that are expensive or difficult to measure on the selection candidates themselves. In family–based selective breeding programs, routine testing of siblings is performed. This is usually the case in oysters, although sometimes breeding populations themselves are phenotyped directly (Symonds et al., 2019). Genomic selection enables breeding values to be estimated more accurately, as described above, by capturing the within-family component of genetic variation. Therefore, such traits may include disease resistance (field trials and experimental challenges) and invasive traits such as meat quantity or quality.

Pros: improved accuracy of selection, especially for traits measured on sibs, due to capturing both within and between family genetic variation in the traits. The higher accuracy leads to equivalent improvement in genetic gain in the breeding programs. Possibility of predicting breeding values across generations without additional phenotyping, although the genomic diversity of haplotypes that segregates in this species may rapidly blur the relationship between phenotypes and genotypes.

Cons: To fully capitalize on the benefits of genomic selection in oyster breeding it is necessary to genotype many selection candidates and test populations (e.g. siblings), and this is very expensive using currently available genotyping technologies (SNP arrays or genotyping by sequencing). Very cost-effective genotyping and phenotyping solutions are needed. The use of polyploids complicates applications of genomic selection.

Challenges for genomic selection in Pacific oyster

An economic assessment of the benefits offered by genomic selection relative to the extra costs of genotyping needs to be undertaken. This is particularly the case for highly fecund species like Pacific oyster which can produce tens of millions of offspring per single cross, and the value of any individual offspring is very low. New genotyping techniques such as genotype imputation, where parents are genotyped at high density and offspring are genotyped at low density and imputed to high density, may be more cost-effective. Optimized molecular protocols: standard molecular biology techniques such as obtaining high quality DNA and genotyping are more challenging in oysters than for other species, and the process of reliable sampling and processing for genotyping from commercial operations will need optimized. This will be particularly the case for high-throughput sequencing (e.g. if genotyping by sequencing is used rather than SNP arrays).

Detailed understanding of how hatchery, practices impact inheritance, larval survival and in particular the potential on introducing artificial selective bias (see (Plough, 2016) that may later be a cause of GxE and reduce the field accuracy of GS is needed.

Shellfish farming has historically been an industry made of many small businesses. This model previously left minimal capital for speculation, and as such all the breeding programs to date had to be initiated with public funding. Some of the contemporary larger hatchery companies may be in a position to test GS methods but it is likely that the initial application of industry-scale GS in oysters will have to be centrally funded. Adaptation of genomic selection methods for improvement of triploid or tetraploid performance is needed, since current studies and theory are largely based on diploids.

3.2.7 European sea bass (*Dicentrarchus labrax*)

General Context

Aquaculture of European sea bass has been traditional in Valli in Italy, but the onset of large-scale production came when controlled reproduction, hatchery and cage on growing methods were developed in the early 1980's. Cultured sea bass production exceeded capture for the first time in 1991, and now represents 96% of the total production of this species, which reached 221,000 t in 2017 (FAO).

The first captive broodstock of European sea bass were founded from West-Mediterranean and Adriatic stocks in France and Italy in the 1990's. Since then, other broodstock populations have

been established from both Eastern Mediterranean and Atlantic populations. The oldest domesticated stocks had been bred in captivity for 8 generations without input from wild stocks in 2016 (Chavanne et al., 2016).

Past and current status of selective breeding in European sea bass

The first trait of interest has been growth rate, similar to other fish selective breeding programs (for a review, see Vandeputte et al. (2019)). Avoidance of deformities, which can reach a high incidence as in many marine species, have also been a trait of interest. Disease resistance is also a key trait, with the main disease targeted being viral nervous necrosis as it is the primary disease problem for Mediterranean aquaculture. Other important diseases for which selective breeding is now investigated as a possible solution are vibriosis and diseases caused by parasites such as *Diplectanum spp*. and isopods. Recent traits of interest for genetic improvement include feed efficiency and processing yields.

Individual selection has been and remains the main selection method used in sea bass breeding programs. However, family selection, including BLUP using molecular pedigrees or separate rearing of families is used in several programs, in some cases including testing of full siblings of the selection candidates for disease resistance traits. Genomic selection has been trialed (see below), and the fist sea bass selected using genomic selection are expected to be on the market in 2019.

Companies with breeding programs for sea bass are located in France, Greece, Italy and Turkey. They are all private companies. There are also genetic services companies that provide breeding program support and management to hatcheries and producers.

Current/future implementation of GS in European sea bass

Initially, genome-wide genotyping studies in sea bass have been conducted using a genotyping by sequencing method known as RAD-sequencing as part of the European Union FP7 project FISHBOOST (Palaiokostas et al., 2018). However, SNP arrays are likely to be the standard genotyping method for commercial application of genomic selection. In 2017, a 3K Illumina SNP Chip has been developed (Faggion et al., 2018), and in 2018 a 57K Thermofisher SNP-Chip has been developed by a French consortium (Vandeputte et al., 2019). Two EU projects, MedAid and Performfish have also developed a combined-species (European sea bass, gilthead sea bream) with 35K SNPs of each species included.

Genomic selection is most suitable for traits that are difficult or expensive to measure directly on the selection candidates themselves, such as disease resistance, feed efficiency, or fillet traits. Genomic selection has been shown to improve the accuracy of prediction of VNN resistant and susceptible sea bass by approximately 13% (Palaiokostas et al., 2018), and is thus a suitable technique to improve genetic gain for this trait. A new technique to evaluate individual feed efficiency in individual aquaria has recently been developed in sea bass, and it was shown that the reliability of EBVs was 10 to 125% better with genomic selection, with a reference population of limited size (<350 individuals), which is of special interest as individual phenotyping of fish for feed efficiency is costly and tedious (Besson et al., 2019). For this trait, GS could be an attractive option, as an important selection pressure could be applied on candidates not genotyped for feed efficiency, using a prediction equation established on a limited number of phenotyped sibs.

3.2.8 Gilthead sea bream (*Sparus aurata*)

General Context

The gilthead sea bream is an important demersal commercial species, highly appreciated as food fish for its flesh. It prefers warm coastal euryhaline waters and its life cycle is determined by

protandrous hermaphroditism. It has been traditionally cultured in Mediterranean coastal lagoons for centuries, and it is now reared intensively both in sea cages and in land-based farms. Global production has reached 185,980 metric tonnes in 2016, primarily from aquaculture. It is the main premium marine aquaculture species in the Mediterranean region.

Gilthead sea bream has been cultured in Mediterranean coastal lagoons and brackish/saltwater ponds for centuries, especially confined areas, such as the northern Adriatic valli in Italy and the Egyptian hosha. These extensive fish rearing systems act as natural fish traps, taking advantage of the natural trophic migration of juveniles from the sea, though often restocking has been performed with wild fry and juveniles to enhance production. However, by the late 1970s the reduced and irregular availability of wild fry and the increasing demand of juveniles for intensive culture accelerated the development and the implementation of induced spawning techniques. The mass production of gilthead sea bream, based on a reliable and consistent supply of hatchery fry and juveniles, started in the late 1980s. Broodstocks were established independently in various hatcheries in several countries, often mixing up fish from different geographic origins. A population genetic survey based on a medium SNP panel (approximately 1,500 loci) was carried out within the framework of the EU-funded project AquaTrace revealed limited genetic differentiation between natural populations across the entire distribution range of the species. Likewise, most broodstock populations were genetically similar to wild ones, although those putatively being subject to genetic selection for several generations showed higher divergence (F. Maroso personal communication).

Past and current status of selective breeding in gilthead sea bream

The first trait of interest has been growth rate, as in all fish species (for review see Vandeputte et al. 2019). The first trials on selective breeding of sea bream were carried out in the mid-1990's and it was only in the early 2000's that the first commercial breeding programs of sea bream were initiated (Chauvanne et al. 2016, Janssen et al. 2017). Deformities, which can reach a high incidence as in many marine species, have also been a trait of interest. Disease resistance is also a key trait, with the main disease targeted being pasteurellosis (photobacteriosis). Heritability for resistance to this bacterial infection was reported to be moderate (0.18-0.45) (Antonello et al. 2009). (Other important diseases for which selective breeding is now investigated as a possible solution are those caused by parasites such as *Sparicotyle chrysophrii*. Recent traits of interest are feed efficiency and processing yields.

Individual selection has been and remains the main selection method used in sea bass breeding programs. However, family selection including BLUP using molecular pedigrees or separate rearing of families is used in several programs. Artificial fertilization is less well established in the gilthead sea bream compared to other marine species, while its sequential hermaphroditism represents an addition issue to be considered in any breeding program. Genomic selection has been shown to be potentially effective in controlled experiments, but it remains to be implemented in an industrial context.

Companies with breeding programs for sea bream are located in France, Greece, Italy, Spain, Croatia, Israel, and Turkey. They are all private companies. There are also genetic services companies that provide breeding program support and management to hatcheries and producers.

Current/future implementation of GS in gilthead sea bream

Initially, genome-wide genotyping studies in European sea bass have been conducted using a genotyping by sequencing method (Robledo et al. 2018) known as 2bRAD-sequencing (Palaiokostas et al., 2016, Aslam et al. 2018). However, SNP arrays are likely to be the standard genotyping method for commercial application of genomic selection. In 2019, a 57K Thermofisher SNP-Chip has been developed by a French consortium. Two EU projects, MedAid and

Performfish have also developed a combined-species (European sea bass, Gilthead sea bream) with 35K SNPs of each species included.

Genomic selection is most suitable for traits that are difficult or expensive to measure directly on the selection candidates themselves, such as disease resistance, feed efficiency, or fillet traits. Genomic selection has been shown to improve the accuracy of prediction of pasteurellosis resistant and susceptible sea bream up to 24% (Palaiokostas et al., 2016, Aslam et al. 2018), and is thus a suitable technique to improve genetic gain for this trait.

3.2.9 Atlantic salmon (*Salmo salar*)

General Context

Modern farming of Atlantic salmon started in Norway at the beginning of the 1970's. The main producers of Atlantic salmon (*Salmo salar*) are currently based in in Norway, Chile, UK, Canada and Australia. The A. salmon start their life cycle in freshwater, where they are raised in recirculating hatcheries and/or freshwater net pens, before undergoing smoltification and transfer to seawater for growing on to harvest size They are slaughtered at around 4 kg. The fillets are red and contain high levels of fat (~13-18%), which contains omega-3 fatty acids that are known to have beneficial human health effects.

Selective breeding activity has been an integral part of the farming of salmon since the beginning of the modern farming practices in Norway. The first major trials of family based breeding programs were in the early 1970s (Gjedrem et al., 2012). These trials involved collection of populations from Atlantic salmon originating in ~40 Norwegian rivers, which were used to estimate robust genetic parameters for important production traits. This led to the first commercial breeding program (Gjøen and Bentsen, 1997). Subsequent initiatives have resulted in the establishment of strains such as the Mowi, the Rauma, the Jakta and the Bolaks originating in various sampling events and locations (Glover et al., 2017). The vast majority of global salmon production is still derived from these strains, after a series of crossing and international export events. The exceptions are the North American-derived Atlantic salmon aquaculture strains (predominantly farmed in the Australian (primarily Tasmanian) and Canadian industries) which are genetically quite distinct from the European Atlantic salmon, with a distinct karyoptype (Brenna-Hansen et al., 2012). There is also a small amount of production in Scotland using Scottish origin strains (Munro, 2019)
Most breeding programs of Atlantic salmon are selling fertilized eggs to multipliers, which in turn sell fry to producers. There are also fully integrated companies that include their own breeding programs and manage the fish until slaughter.

Past and current status of selective breeding in Atlantic salmon

The first traits included in the breeding goals were mainly those that could be measured on the selection candidates themselves. This included increased growth rate, because that results in shorter production times, and has a medium-high heritability. Reduced incidence of precocious sexual maturity was also a major target, because this causes negative effects on growth, flesh quality and fish health. As breeding programs have advanced, they have included multiple additional traits into the breeding goals, including those which can only be measured on relatives of selection candidates. These include product quality traits, e.g. fat content, pigmentation and spine deformities, and Resistance to different diseases, e.g. IPN, PD and salmon lice. These traits often have medium-high heritabilities, meaning genetic gain can be relatively rapid, although it is limited by the long generation interval of salmon (3-4 years).

There are two major designs of breeding programs for salmon. One is where families (200-800) are kept separately until individual tagging can take place (using some kind of Passive Integrated Transponder (PIT)- tag). This system gives accurate pedigree and data for the genetic evaluation. However, it requires significant investment in hatchery infrastructure, and its size depend on the number of families. Genomic selection (Nielsen et al., 2009; Sonesson & Meuwissen, 2009) and mating (Sonesson & Odegard, 2016) designs for these programs are available, as is also design for optimum contribution selection (Nielsen et al., 2010).

The second design is where fish from different families are merged at an early stage and DNA markers are used to identify a number of preselected individuals (in combination with individual PIT-tags). This system requires less investment in hatchery facilities, but has less control of family contributions in different batches of fish, which may result in loss of whole or parts of families. This may lead to unbalanced data for the genetic analysis, and ultimately lower selection intensity for certain traits, and higher risks of inbreeding accumulation. Often, larger number of families are produced to reduce the risk of getting a too small population. Examples of genomic selection designs are available for these programs are available (Sonesson et al., 2010).
Since the beginning of the modern salmon breeding programs, the pedigree and trait data collected have been used to calculate Best Linear Unbiased Prediction (BLUP) breeding values for selection candidates (Henderson, 1973). BLUP is a technique used to estimate breeding values for individuals by making use of the additive genetic relationship between individuals and thus seeks to allow crossings to be performed which attempt to maximize the gains obtained from a selection program (Harville 1990). BLUP has been extensively utilized in selection programs of salmon, however, since the development of the first high density SNP arrays (e.g. Houston et al. 2014, Yanez et al. 2016), genomic selection has become more commonplace. The advantages of genomic selection have been shown in several studies, in terms of improved prediction accuracies compared to pedigree methods, such as growth (Tsai et al. 2015), fatty acid composition traits (Horn et al. 2020), fillet pigmentation (Odegard et al. 2014), resistance to sea lice (Odegard et al., 2014; Tsai et al., 2016; Correra et al. 2017; Kjetsa et al., 2020), resistance to Amoebic Gill disease (Robledo et al. 2018; Aslam et al. 2020), resistance to salmon rickettsial syndrome (Bangera et al. 2017).

The large breeding programs of salmon build-up in-house R&D groups to manage data and perform the genetic evaluation, and many also collaborate with academic and private partners to develop and apply genomic tools and techniques. There are <10 breeding companies of salmon that have global activity. They are in Norway, Chile, Canada, UK and Australia. They are privately owned.

Current/future implementation of GS in Atlantic salmon

In salmon, GS is now routine. Traits that are not measurable on the selection candidates themselves benefit most from GS compared to pedigree selection.

All the breeding companies have developed their own SNP chips and use them for GS in Atlantic salmon. Some of these SNP chips have already been refined several times for the quality of the SNPs, e.g. density, polymorphism rate, trait effects etc. There has been substantial interest in optimizing SNP density to reduce genotyping costs. Due to the large full-sibling families used in salmon breeding, the reference population normally contains very close relatives to the validation population. This close relationship means that relatively sparse markers can be used to accurately define genomic relationships, and much of the benefit of genomic selection is due to more accurate estimation of the within-family component of genetic variation. However, most programs routinely use a ~50-70k SNP chip, partly due to the high volume of samples resulting in competitive prices per chip. Imputation from low to high density has also investigated (e.g.

Yoshida et al., 2018; Tsairidou et al., 2020) with high prediction accuracy shown even with just several hundred markers but imputation to sequence data has not been tested.

Challenges for genomic selection in Atlantic salmon

Genomic selection accuracy and performance is high in the context of sib-testing schemes in salmon, due to the aforementioned close relationships between reference and validation populations. However, as that relationship becomes more distant, the accuracy drops off rapidly. For example, prediction accuracy in a specific year group of a breeding program was shown to be near zero when another year group was used as the training population (Tsai et al. 2016). Therefore, a major challenge is to improve prediction accuracy in distant relatives, which may reduce the need for routine phenotyping. To meet this challenge, identification of functional variants impacting the trait may be key, and employing a suite of modern genomic and genome editing tools will assist with that process (Houston et al. 2020). The value of enrichment for functional variants in increasing prediction accuracy, and in the persistency of that prediction accuracy across distant relatives can then be evaluated more thoroughly, in conjunction with population-scale whole genome sequence data on the populations.

3.2.10 Rainbow trout *(Oncorhynchus mykiss)*

General Context

The rainbow trout (also known as steelhead trout) Oncorhynchus mykiss (Walbaum, 1792) is a salmonid fish species native to cold waters of the Pacific Ocean in Asia and North America. Given its popularity for both recreational angling and aquaculture, since the end of the 19th century, the species has been widely introduced to suitable waters around the world. Rainbow trout aquaculture started to substantially expand from the 1950s with the development of pelleted feeds, and it is now one of the main species cultivated in cold freshwater habitats around the word, with particular focus in Europe, the Americas and Asia (Janssen et al., 2017; D'Ambrosio et al., 2019). As a result of ongoing aquaculture efforts, several local domesticated strains have been developed, while others have been produced through mass selection and crossbreeding for improved cultural qualities (Cowx, 2019). On a country basis, Chile (currently the largest producer), Peru, Japan, Australia, Iran, and USA are among the largest producers. In Europe, the main produces are Norway, France, Italy, Denmark, Germany, UK and Spain (Cowx, 2019). On a world scale the rainbow trout aquaculture is currently worth over USD 3.8 billion with Europe (USD ≈ 1.39 billion), Asia (USD ≈ 1.97 billion) and the Americas (USD ≈ 1.24 billion) as the major producers (source FAOSTAT database 2018).

Past and Current Status of Selective Breeding in rainbow trout

Rainbow trout selective breeding programs date back from the end of the 19th century with earlier efforts orientated towards improving fecundity, delaying time to sexual maturation, and off-season spawning (Janssen et al., 2017). Following the substantial expansion of the rainbow aquaculture industry in the 1950s, hatcheries started to further develop selective breeding programs aiming at the improvement of additional traits relevant to aquaculture including improved growth performance and bodyweight, fillet quality and disease resistance (D'Ambrosio et al., 2019). Recent advances in genomics resources for the species, including access to the full genome sequence information (Berthelot et a. 2014), detailed genetic maps (Guyomard et al., 2012, Gonzalez-Pena et al., 2016, Fraslin et al., 2018) and species-specific SNP chips (Palti et al., 2015) are now providing the means to new and more powerful approaches to the further development and monitoring of rainbow trout breeding programs (e.g. Reis et al., 2018).

Current and Future Implementation of GS in rainbow trout

Current rainbow trout selective breeding programs are predominantly based on mass selection for growth or and/or a combination of marker selection on growth and sib selection to improve other desirable traits for aquaculture (e.g. Palti et al., 2015b; Liu et al., 2015; D'Ambrosio et al., 2020). The difficult logistics associated with family based breeding programs and, the often, complex genetic architecture of many traits of interest (e.g. disease, slaughter traits, female reproduction traits) makes these selection approaches challenging (Vallejo et al., 2017). The two breeding companies in Norway use family based selection combined with genomic selection. From decades, these companies have been using family based selection mainly for growth, sexual maturity, skeletal deformities, and other slaughter traits. Additionally, selection for disease resistance (infectious pancreatic necrosis and/or flavobacteriosis) is also performed which is based on detected quantitative traits loci-based information. While global implementation of genomic selection in commercial aquaculture is still limited, some early studies have been showing promising results. Vallejo et al (2017) have shown that the accuracy of genomic prediction is significantly higher than estimates generated from traditional pedigree-based methods for bacterial cold-water resistance in rainbow trout. In a comparison involving traditional pedigree-based approaches and genomic prediction, Yoshida et al. (2018) suggested that the latter method could be used to improve the accuracy of breeding values for resistance against infectious pancreatic necrosis virus in rainbow trout. Silva et al. (2019), examining the genetic architecture of columnaris disease in rainbow trout, argued that genomic-wide selection is better to predict future performance compared with pedigree-based selection. D'Ambrosio et al. (2020) suggested that genomic prediction would allow significant gains of accuracy compared with pedigree-based approach for predicting female reproduction traits (body weight, spawning date, fecundity, and egg size).

3.2.11 Conclusions: potential and challenges for further implementation and optimization of GS in the aquaculture breeding industry

Compared to most terrestrial farmed species, the advantage for aquaculture is the possibility to produce very large families. This can increase the accuracy of the within-family component significantly. Within-family GS is a special case that can utilize these large family sizes effectively, while using very low genetic marker densities (Lillehammer et al., 2013). Genotyping costs is however an important limitation for implementing GS, because in addition to genotyping a large number of selection candidates, representatives from all families must be genotyped for all traits that are measured on the sibs instead of on the candidates. In the case of disease traits, this means one group per trait. In the case of slaughter traits, one group can be used for recording several traits. Genotyping of pooled DNA sib groups is one way to reduce genotyping costs, albeit with a reduced selection accuracy compared to a full GS program.

Many of the current challenges to the widespread implementing genomic selection-based approaches are common among aquaculture species. Among these, are the costs associated with the development of informative genotyping arrays and the subsequent genotyping of many individuals. The genotyping costs are much variable depending on bulk of samples genotyped annually, and therefore causing relatively more challenges for the small and medium-sized enterprises (SMEs) in terms of adoptability of genomic selection than the bigger companies. Most of the breeding companies are undertaking the development of their own SNP arrays for genomic prediction due to privacy/IP issues. The huge number of arrays used/bought by bigger companies cause significant reduction in genotyping cost per samples compared to relatively small-scale operators. As cost of array depends on bulk of samples genotyped, and therefore

feasibility with higher production costs for SMEs. So, the SMEs must wait for the technology to become cheaper or when the low-cost innovative technology becomes available. Hence, they will be left behind in getting timely advantage from the state-of-the-art technologies and ultimately difference in product. One of the solutions could be the development of multispecies genotyping arrays which could be used by multiple companies with joint agreement. This will increase per annum purchase of arrays jointly, and ultimately reduction in cost. Other possible ways which can make SMEs to stay competitive include smart genotyping and application of within family genomic selection (Lillehammer et al., 2013), using imputations (Tsairidou et al., 2020), and/or applying combined relationship matrix which could link genotyped and ungenotyped individuals (Legarra et al. 2009). As suggested by Yoshida et al. (2018), genomic prediction could provide an alternative approach to improve the accuracy of breeding values for complex traits for which more traditional methods have not been effective. Moreover, there are smarter methods and strategies to possible run the breeding programs sustainably with the use of genomics and ultimately beneficial outcomes (Houston et al. 2020).

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3.3 ToR c: Assess and report on the value of genetic and genomic tools for identifying species in mixed landings, fish products and by-products.

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3.3.1 Introduction

To set a context, we introduce briefly the legal framework in relation to mixed landings, fish products and by-products. Importantly, we consider potential applications especially in relation to the Landing Obligation. The landing obligation requires all catches of regulated commercial species on-board to be landed and counted against quota, starting from 2015, and with full implementation from January 2019. Our focus will consider the extent to which genetic and genomic tools can assist in the enforcement and detection of infringements to the Landing Obligation. Compliance with such measures faces not only economic but also social and regional challenges (Soto-Onate & Lemos-Nobre, 2020), requiring the development and implementation of concerted and robust measures. However, it is necessary to ensure a mutual understanding between scientists and end-users to identify end-user needs and the most critical issues to be addressed, and to clarify which issues relevant to fisheries management can and should be tackled by genetic approaches and also to render limits of such approaches evident. This includes a discussion on the design of statistically robust sampling protocols and the production of evidence to be used in an enforcement context. Based on an initial assessment, carried out by this working group (WGAGFA) in May 2018 and May 2019, and first documented feedback by stakeholders, the stakeholder workshop WKGenoTools helped to clarify to what extent genetic and genomic approaches can support the aforementioned key components of fisheries management. Two application examples were selected to be presented in this final ToR report: one about DNA-based methodologies to identify and quantify species present in fish silage, the other about the DNA-based identification of species at sea and during landing.

3.3.2 Rationale

Discarding is the practice of returning unwanted catches to sea, either dead or alive, because they are undersized, due to market demand, because the fisher has no quota, or because catch composition rules impose it. Discarding is a major contributor to overexploitation. Estimates for the impact of such actions vary from local, where discards may account for up to 80% of the catch (Guillen et al., 2018), to global of up to around 30 million tons, representing 23% of global catches (Nellemann et al., 2009), for a global review of discards see (Zeller et al., 2018).

Under the remit of the Common Fisheries Policy (CFP), the 28 Member States of the European Union (EU) strive to eradicate the wasteful practice of discarding unwanted catches at sea. This fisheries management objective, already pursued by some countries, such as Norway, Iceland, Chile and New Zealand [ToR C ANNEX], should be especially supported by technical measures that lead to improvement in fishing selectivity. It is generally acknowledged that the implementation of the EU Landing Obligation (LO; in phases from 2015 to 2019) is a highly challenging and complex endeavour, and there is a need to ensure monitoring and control. However, the complexity inherent to present fishing practices confronts both the industry and authorities that are mandated with monitoring and control with unprecedented challenges. As a consequence, the fishing industry should be supported in every way to be able to implement the LO, and efficient monitoring and control measures must be developed and applied. Monitoring is needed because non-compliance and infringements are a serious possibility, leading to unfair and distorted fishing practices, and undermining the objectives of the LO. The ongoing impact of illegal, unreported and unregulated fishing practices within European waters and globally (ref to add), reinforces the need for collective effort. A monitoring example could be species substitution identification: how can it be assured that no protected species have been landed with legal catch, or that undersized fish are in fact the officially reported species; considering that in both cases the landed biomass tends to be immediately processed for products that are not for direct human

consumption? The species composition of processed mixed species fisheries products is extremely difficult to discern, especially when considering highly processed products like fishoil and gelatine. Additionally, how can we enhance both the robustness and reliability of tools to support enforcement, as well as optimize efficiency in practice? For example, can tools be developed that assess species composition on board without the need for time-consuming analyses of subsamples of many individual fish? In these situations, recent advances in genetic and genomic technology and analysis offer new and complementary opportunities to address such issues.

3.3.3 Reasons for discard, circumvention strategies and the role of genetics

Discarding occurs for both legal and economic reasons (see (Guillen et al., 2018)). ToR C ANNEX summarizes those reasons and the underlying problems leading to the current discard of catches. Mixed-species landings and the use of a mix of species in fish products continues to pose a formidable challenge to fisheries control and enforcement as well as traceability along the supply chain.

In light of the difficulties in monitoring mixed species landings and identifying species in fish products and by-products we assess the utility of genetic and genomic tools to yield robust and cost-efficient support to determine species composition, also quantitatively, and directly supporting fisheries management and policy needs. Basic principles will be illustrated based on critically considered scenarios in the context of mixed-species landings and the landing obligation.

A timely and relevant example is the global attempt to develop and implement rules that lead to the reduction of discards. Discarding is the rather common practice of returning unwanted catches to the sea, either dead or alive, because they are undersized, due to market demand, the fisher has no quota or because catch composition rules impose this. In Europe, 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 while 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 fishoil and gelatine. Genetic and genomic methods can address 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 pro-cessed as "undersized, animal-by-products". Moreover, with recent advances in genetically determined population assignment, there is potential also, to detect infringements where fish are landed from fisheries that are closed, comprise under-sized individuals, or are otherwise restricted (Nielsen et al., 2012).

If undersized commercial species need to be processed separated from bycatch species, genetic tools might further help to test if this is in fact the case in a given situation or if for example commercial 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. Focusing on, but not dealing with exclusively, we will elaborate whether genetic methods might efficiently support the implementation of rules designed to reduce discards and related control, monitoring and enforcement measures.

In addition, it highlights potential strategies to comply with the EU Landing Obligation but also strategies used to circumvent economic disadvantage, i.e. "strategies to cheat", which involve mislabelling of some sort, including false declaration of species identification and origin of catch. In the following we clarify and define DNA-analytical applications and applications for identification of species and origin to enable a discussion on needs arising for the implementation of the Landing Obligation and the potential value of DNA-based analysis to tackle those needs. Details on the range of salient genomic tools, alongside recent advances, are presented in ToR C ANNEX.

3.3.4 Stakeholder feedback

To identify end-user needs and the most critical issues to be addressed, and to clarify which issues relevant to fisheries management can and should be tackled by genetic approaches, including their limitations, a stakeholder workshop (SWS) was organized in February 2020 in Brussels. WKGenoTools "On the value of genetic and genomic tools for identifying species in mixed landings, fish products and by-products" convened policy-makers and internationally renowned experts to clarify which are the most pressing needs in the field and how to best enable a successful technology and knowledge transfer.

Participants to the workshop (in alphabetical order): Jurgen Batsleer, Wageningen University, Netherlands; John Hederman, European Commission DG MARE; Torild Johansen, Institute of Marine Research, Norway; Claudia Junge, Institute of Marine Research, Norway; Jann Martinsohn, European Commission JRC; Einar Eg Nielsen, National Institute of Aquatic Resources, Denmark; Irina Popescu, European Parliament Research Service; Cristina Ribeiro, European Commission DG MARE; Aronne Spezzani, European Commission DG MARE; Antonella Zanzi, European Commission JRC.

The first day an intense discussion focused on support needs of the EU Landing Obligation (the Discard Ban), the legislation that intends to abolish the practice of throwing fish that is caught back into the sea.

John Hederman introduced the legal framework, explained control challenges and presented typical examples of infringement. The Landing Obligation described in the reformed Common Fisheries Policy requires fishers to land all catches of specified species so that they count against their quota and are fully documented and accounted for. John Hederman emphasized that the Landing Obligation is not a straightforward discard ban and that controlling the Landing Obligation is complicated by several exemptions such as predator damaged fish, prohibited species, high survivability, etc. He emphasized also that any evidence of infringement must be robust enough to withstand scrutiny by the defence during court cases. He concluded his presentation mentioning the Remote Electronic Monitoring (REM) used in fisheries management. REM equipped with video technology (CCTV) and sensors has been widely recognized as the best way to effectively control the Landing Obligation at sea and is increasingly used for control purposes in fisheries management around the world.

Einar Eg Nielsen presented the state-of-the-art in the genetic and genomic methodologies that can be potentially used for identifying species in mixed landings, fish products and by-products. He presented two approaches for mixed species samples:

- The next generation sequencing and "meta-barcoding", that is a semi-quantitative approach;
- The quantitative PCR "qPCR" with species-specific primers.

Einar Eg Nielsen then introduced the environmental DNA (eDNA) methodology and the recent advancements in the technology allowing to monitor eDNA *in situ*. He finally presented some interesting results on fish silage DNA analyses carried out in the context of the DiscardLess project (Horizon 2020 - the Framework Programme for Research and Innovation 2014-2020).

Torild Johansen, on behalf of Åse Ingvill Berge, presented the experiences in Norwegian fisheries on the use of genetic tools for control or other purposes. Genetic tools are not applied regularly in Norwegian fisheries control, but more regularly in fisheries management. The following are examples where samples were taken on board or in factories, and analysed at the Norwegian Institute of Marine Research:

- Coastal cod and northeast Arctic cod to protect coastal cod spawning grounds;
- Shrimp catches: Coastal cod and golden redfish juveniles in the catches (the Directorate for Fisheries will close area for fishing);
- Identify if a catch consists of greater argentine (*Argentina silus*) or lesser argentine (*Argentina sphyraena*).

At the end of the first workshop day, from the discussion among the workshop participants, some scenarios, such as false labelling of fish, were identified as being resolvable by DNA-technology. Also new approaches such as eDNA might help to create valuable evidence, perhaps also the identification of species composition of catches or processed products and the amount of fish caught.

During the second day, Cristina Ribeiro and Aronne Spezzani presented a case study commissioned by DG MARE that explored the usage of genetics for control and enforcement in the North Atlantic Fisheries Organisation (NAFO), that is one of the Regional Fisheries Management Organisations (RFMO). Objective of the case study was to developed a protocol and a manual to guide the collection and the chain of custody process of the samples to ensure the integrity and reliability of the results. The EU has commissioned this case study via the framework contract (EASME/EMFF/2016/008 "Scientific advice for fisheries beyond EU waters), namely under the specific contract No. 15: "Study to produce an International Manual of Procedures to be used in the NAFO Regulatory Area to guide the collection of samples from fisheries products for genetic analysis". DG MARE submitted the study outcomes, a literature review report and a manual ("Fish Products Sampling for DNA Testing") developed as a guideline for best practices with respect to genetic sampling and analysis, to the WKGenoTools workshop participants and will also submit both report and manual to the International Council for the Exploration of the Sea (ICES) for review.

Following the workshop presentations and discussion among workshop participants a few recommendations were compiled, and two examples were selected, where we consider the application of genetics/genomics in support of composition analysis of catches and landings feasible. Those are presented in the current report of the ICES Working Group on Application of Genetics in Fisheries and Aquaculture (WGAGFA): one about DNA-based methodologies to identify and quantify species present in fish silage, and the other about the DNA-based identification of species at sea and during landing. More details on both can be found in the section "application examples".

3.3.5 Sampling procedures

This summary is based on a recent report documenting a study tendered by the European Commission (2019). The study examined the potential usage of genetics for control and enforcement in the North Atlantic Fisheries Organisation (NAFO) and underpins an International Manual of Procedures (IMP; (see previous section).

- What type of material should be sampled and how?

For DNA identification of individual fish, the above mentioned International Manual of Procedures (IMP) on "Fish products sampling for DNA testing" recommends that samples of muscle or fin are collected as these were considered the most appropriate and convenient types of tissue due to the ease of collection, minimum handling, DNA stability and ability to better control for potential contamination, compared to other biological material (teeth, scales, skin, blood or viscera) (Bandarra et al., 2019). The IMP acknowledges that filleting and partitioning of fish encourages fraud, which is why their recommendation is for tissue collection of fresh whole products, prior to any transformation. This might not always be possible as sometime fileting occurs directly after the catch. In that case, it might be necessary to collect tissue samples from the filets, especially if the purpose is to control for correct species identification and labelling. The sampling procedure included in the IMP recommends the use of a standardized ready-to-use sampling kit to ensure reliability and reproducibility, containing all reagents and consumables needed to sample a single fish, specific forms for registration of sample collection, transport and delivery of fisheries products, in addition to a photo-camera for recording of the sampling process.

- How many samples/what proportion of the lot need to be taken for a "representative sample" of a catch?

As the lot dimension can range widely, it is essential to determine the number of boxes needed to efficiently represent the lot. The IMP proposes that the number of boxes to collect is estimated by the Cochran (1963) formula, taking into account the probability of a species (as a variability coefficient) being detected in a given fishery products' lot (function of the fisheries knowledge in the NAFO RA) (minimum sample size: three boxes per lot) (see IMP 2019 for details). The IMP collection procedure suggests sampling a single fish from each box, by clipping a portion (3 cm × 3 cm portion with 0.5 cm thickness, whenever possible) of tissue using disposable equipment and subdividing into three vials containing ethanol or RNAlater, enclosed in a labelled safety casing. Safety casings should be placed in a thermal box to prevent potential DNA degradation during transport to laboratory. The IMP currently does not consider how to effectively sample processed or mixed fish products.

- How to cope with contamination and ensure chain of custody?

To prevent cross-contaminations during sampling procedures, the IMP recommends not only the use of pre-prepared individual, disposable sampling kit, but also the immediate segregation and packaging of sample groups into separate bags, storing bulk and tracing samples separately at all times. To ensure sample chain of custody, specific registration forms are recommended by the IMP including: name and number of the certified inspectors handling sample collection; location, date, and time of collection; type of sample collected (muscle/fin); and mission references. The maximum time between sample collection and delivery to laboratory should not, ideally, exceed five working day to prevent sample degradation.

Suitable training and certification of the fishery inspectors for sampling of fishery products for DNA analysis are to be prepared by the Fisheries Control body of each country.

3.3.6 Application examples

3.3.6.1 DNA-based species detection in the field

The first example addresses the problem to identify fish species directly on board or at port that are not easily identifiable by a visual inspection. Several portable solutions, which can facilitate the species barcoding on board of the fishing vessels or at port, have been developed in recent years. This so-called "genetic barcoding" sequences a short fragment of the DNA using universal primers and comparing them with available genetic databases to identify species (more details in ToR C ANNEX).

The minION portable nanopore sequencer has revolutionized species detection in the field and it has potential applications in fisheries for detecting rare and prohibited species. With this hand-held sequencer, an entirely portable genomics lab can be assembled for as little as 7000 USD and it can be used in tough environmental conditions in the field (Maestri et al., 2019)(Figure 1). In fact, a portable genomics lab and minION sequencer have been used to rapidly survey biodiversity in remote rainforests in Ecuador (Pomerantz et al., 2018), Madagascar (Blanco et al., 2019), and Tanzania (Menegon et al., 2017). Despite this technology being both fast and cost-effective, these hand-held sequencers and portable genomics labs have not been widely implemented for marine species detection, for instance by fisheries managers in the field/at ports. Outside the field however, minION technology has been used to identify CITES-listed sharks in Indian markets (Johri et al., 2019) and mislabelled fish in markets in Singapore (Ho et al., 2020).

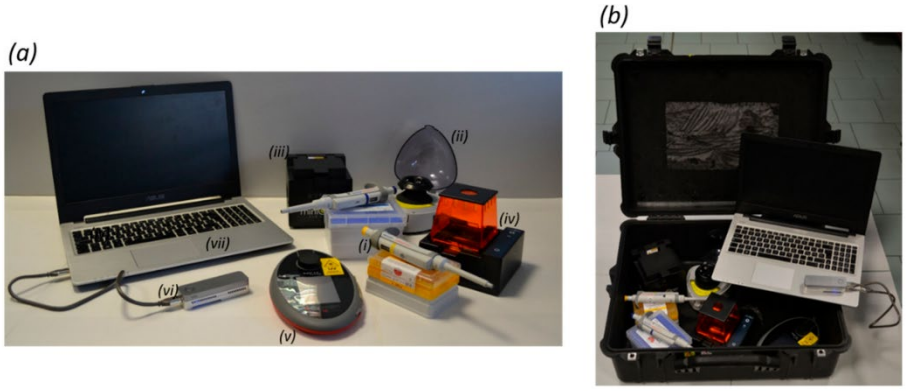

Figure 1. The portable genomics laboratory. Panel (a) shows the equipment comprising the portable genomics laboratory, namely (i) micropipettes, (ii) a mini-microcentrifuge, (iii) a thermal cycler, (iv) an electrophoresis system, (v) a fluorometer, (vi) the nanopore sequencer MinION, and (vii) a laptop. Panel (b) shows how the laboratory is transported. Source: Maestri *et al.*(2019), ©creative commons.

A more recently developed device is the MIC (Magnetic Induction Cycler, © Thermagenix, Inc.), a portable qPCR device (quantitative PCR), which works with the already optimized FASTFISH-IDTM technology. This device allows the reliable identification of fish species in about 2 hours using three steps (see www.thermagenix.com/how-to-use.html). The device produces a curve whose shape is different for every species, known and stored in an accessible reference database.

The reference database is mostly dominated by North American species, but work is being done to extend it to northeast Atlantic species too.

3.3.6.2 Fish silage

The second example is motivated by the possibility, under the EU Landing Obligation, to use fish that is not commercially viable to land as whole fish to produce fish silage directly on board (Viðarsson et al. 2019). The fish used for silage should still be counted against the allocated quota and hence methods to control the content of fish silage are needed for successful implementation.

However, control measures based on visual inspection of content are not possible once the fish have been digested by acid in the silage tanks. Here, DNA based methodology presents one potential solution to identify and quantify species present in fish silage. In principle, DNA based analyses would be applicable throughout the supply chain from when the fish are initially placed into tanks to when highly degraded silage is landed even days after catch.

DNA based methods have been applied for food authenticity testing and analyses of species composition in mixed meat (Ballin et al. 2009), dairy (Agrimonti et al. 2015) and processed fish (Sánchez et al. 2019) products, but have so far not been applied to fish silage where tissue is highly degraded by acidic treatment (Viðarsson et al. 2019). Pilot studies to investigate the feasibility of analysing DNA from silage were initiated in the EU H2020 funded project Discardless (www.discardless.eu). These studies presented as proof-of-concept under controlled laboratory conditions to evaluate the potential of the approach. Here, silage was produced by mixing known proportions of four different fish species by their weight. . Subsequently, DNA was extracted from the silage up to 21 days after the initiation of the experiment. Results are very promising as known proportions of codfish could be reproduced relatively accurately with DNA based quantification even up to 21 days after the fish were initially placed in the tanks (Jacobsen et al. 2019, Hansen et al. 2020). The study also showed that different groups of species (here codfish vs. wolffish) may have different DNA to biomass relationships, and hence that technical calibration based on expected species composition is an important part of an implementation process for obtaining reliable quantification.

Both targeted (e.g. quantitative real time PCR - qPCR) and more broad scale screening (e.g. metabarcoding) could be applied to monitor and quantify silage composition. Targeted approaches appear at present to be more reliable for quantification but require prior knowledge of the species expected to be present in the silage. Broader scale screening can be an alternative in situations where species composition is unknown or if the presence of a range of rare/threated species in silage is of concern. Importantly, recent developments into portable sequencing devices, which are relatively easy to operate, represent a real potential for this application to be carried out directly in the field and by non-specialists in future.

3.3.7 Summary and Outlook

Motivated by the challenges inherent to the implementation of discard bans and the analysis of mixed catches, we identified situations in which genetic methods could aid monitoring as well as control. After identifying relevant stakeholders, we held a stakeholder consultation workshop to ensure that we are addressing the most crucial issues of interest to policy-makers and other end-users charged to ensure implementation and compliance of the landing obligation. Based on the feedback of various stakeholders, we documented examples where genetic methods have successfully been used to aid management with respect to species and stock identification based on whole fish, filets and processed products. We believe and have shown that genetic tools constitute a valuable component in support of the implementation of the (European Union) Landing Obligation, with special emphasis on mixed species catches and the identification of their composition. Moving forward, we refer to our recommendation to address ICES advice provision clients, such as DG MARE and the European Parliament.

In how far genetic methods can be used in future in conjunction with other monitoring and control tools such as e.g. REM, will need to be tested in the field and in the lab to ensure reliability, cost-effectivity and feasibility.

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3.3.9 ToR c: ANNEX

3.3.9.1 ANNEX The Norwegian discard Ban

In Norway, a ban on discard was initiated in 1984 (Gullestad et al., 2015). The discard ban of dead or dying cod and haddock came into force in 1987, and by 2008, a total of 18 fish species were covered by the ban. In 2009, the old act relating to seawater fisheries was replaced and an obligation to land all catch of fish ('discard ban') was made in general form (Marine Resources Act[1]). After some adjustments the following years, in 2014 the discard ban comprised approximately 55 fish species. The regulation related to seawaters fisheries lists the species for which the discard ban applies (for details see (Gullestad et al., 2015)).

In Norwegian discard ban:

- The ban applies to dead or dying fish, viable fish can be released back to the sea.
- All catches of commercial species (with some exceptions) are landed and can be sold through ordinary market outlets.
- Presence and surveillance at sea is carried out by the Norwegian Coast Guard.
- In the case of contravention of provisions, both the master of the vessel and the owner may be fined (in extreme cases, the fishing licence may be withdrawn for a period) and catches may be confiscated.
- The discard ban was preceded by a program of real time closures (RTC) of fishing areas which was developed from 1984 onwards.
- The RTC system involves the continuous monitoring of fishing grounds by trained inspectors on board chartered vessels: areas are closed when inspectors register that catches of juvenile fish exceed a certain limit. In addition, fishers are obliged to move fishing grounds if they observe excessive juvenile bycatch in a haul.

[1] Act no. 37 of 6 June 2008 relating to the management of wild living marine resources. http://app.uio.no/ub/ujur/oversatte-lover/data/lov-20080606-037-eng.pdf

ToR C ANNEX - Potential strategies for non-compliance and genetic testing

Reason	Problem	Potential Strategies	Genetics useful?
Legal	Catches exceed a quota	- labelled as different species which has: 1) not fulfilled its quota yet, or 2) does not have a quota	- YES, DNA barcoding
		- processed (from simply beheading to fish filet) and labelled as different species	- YES, DNA barcoding
		- processed (or highly processed), mixed with other species and species ID hidden	- YES*, DNA metabarcoding, ddPCR (quantification)
		- different catch area reported	- YES, SNPs/ microsatellites
Legal	Catches are below a minimum legal landing size	- labelled as different species (unprocessed or fileted)	- YES, DNA barcoding
		- different catch area with larger minimum landing size reported	- YES, SNPs/microsatellites
		- processed and legal size pretended	- NO
Legal	Catches do not meet catch composition rules cannot be retained on board and must be discarded	- if too much bycatch: processed all together and larger proportion of target species claimed	- YES, DNA (meta)-barcoding, ddPCR
		- if only or mostly juveniles of the target species: processed and size hidden	- NO
Economic	Catches comprise small individuals of commercial species that command low prices	- processed and size hidden	- NO
		- processed and different species claimed	- YES, DNA barcoding
Economic	Catches are of poor quality (e.g. damaged, diseased, or not so fresh)	- processed and quality issues hidden	- NO
		- if visibly diseased: obvious signs of disease (e.g. parasites) removed and hidden	- YES
Economic	Catches include species of low market value	- labelled as different species	- YES, DNA barcoding
		- processed and labelled as different species	- YES, DNA barcoding

		- processed and mixed with other species	- YES, DNA meta-barcoding, ddPCR
Economic	Catches are of non-commercial species	- labelled as different species	- YES, DNA barcoding
		- processed and labelled as different species	- YES, DNA barcoding
		- processed and mixed with other species	- YES, DNA meta-barcoding, ddPCR

List of reasons for discarding and the underlying problems (adapted from Guillen et al. 2018), as well as "strategies to cheat", and an indication if and which genetic tools could be successfully applied. * For highly processed products like fishoil validation studies will have to be carried out for species identification and quantification.

3.3.9.2 ANNEX Genetic tools and applications

1. Tools

- DNA extraction
  - The ability to extract and purify DNA is the key starting point for a variety of downstream molecular procedures.
  - DNA can be extracted from a variety of materials including muscle and fin tissue, blood, slime, and other bodily fluids, as well as from processed products like food products, pellets, and oil, and environmental samples.
  - DNA extraction processes require careful handling of biological material to prevent sample contamination and crossover.
- DNA barcoding and DNA meta-barcoding
  - It is possible to correctly identify most fish species with genetic methods by sequencing DNA fragments using universal primers, based on mitochondrial (e.g. Cytochrome Oxidase I, Cytochrome b) or nuclear markers (e.g. 18S rDNA), and comparing them with available genetic databases (Genbank: www.ncbi.nlm.nih.gov, BOLD: www.barcodeoflife.org, EMBL-EBI: www.ebi.ac.uk/ena). This approach is referred to as "genetic barcoding". This approach takes advantage of a large species database and the availability of off-the-shelf kits.
  - DNA metabarcoding combines this classic barcoding with next-generation sequencing (NGS) approaches (e.g. Illumina and IonTorrent sequencing platforms).
- qPCR
  - Real-time PCR or qPCR (quantitative Polymerase Chain Reaction) is a technique that allows identification and quantification of individual species or a group of species DNA, in a sample in which the amplification of DNA in a PCR is monitored in real-time, as the reaction progresses.
- Microsatellite and SNP genotyping

- Microsatellites consist of highly variables stretches of repeated elements, while nuclear SNPs are sites in the genome with single base changes in a DNA sequence. SNPs are very abundant and widespread in most genomes, often every 200–500 bp.
- The rapid progress of DNA analysis technologies will have significant effects on the development of population analysis and traceability tools relevant to implementation of the landing obligation. High throughput sequencing has declined dramatically in cost, while speed and quality of analysis has increased by orders of magnitude, allowing high throughput analysis of individuals.

2. Applications for identification of species and origin

- Species ID confirmation on whole fish (e.g. without head, fins, etc.) or filet
  - The correct identification of commercial fish species is challenging in many cases by conventional methods since common practices include animals dismantled on board, keeping only parts of the animal such as fillets, gill plates and fins. DNA barcoding fish parts/whole individuals to correctly assign them to a taxonomic category can therefore be particularly useful, e.g. to avoid trade of endangered species (Steinke et al., 2017) or to identify cryptic species with different conservation status (e.g. (Castilho et al., 2007).
- Highly processed mixed products: Species composition
  - Analysing highly processed samples is more difficult due to typically small amounts of DNA which can also be highly degraded, making DNA extraction as well as amplification more challenging. ToR C ANNEX_3.3 in ICES (2018)* provides an overview of processed products, the genetic analysis opportunities including studies where they have been successfully used, as well as prospects which should be investigated further to evaluate their applicability to highly processed fish products.
  - Nothing has been done so far on fishoil. However, molecular approaches have been developed to ensure the traceability on other oil products, such as olive oil, for at least a decade (e.g. researchgate project: https://bit.ly/2LahlDm). For instance, a recent article reports the development of a genetic database to allow the use microsatellite-based approaches for the traceability of olive oil (Ben Ayed et al. 2016). The applicability of such approaches on fishoil should be investigated.
- Catch composition in mixed fisheries or with respect to bycatch
  - Accurately assessing the catch composition is crucial to the management of mixed fisheries. However, this task is very challenging when catches include species that are morphologically very similar or different populations of the same species.
  - Genetic tools have proven very useful for estimating the catch composition in several fisheries, like redfish (Sebastes sp) (Cadrin et al.; Saha et al., 2017), cod (Gadus morhua) (Dahle et al., 2018; Johansen et al., 2017) and salmon (Bradbury et al., 2015, 2016).
- Identification of origin
  - In relation to the LO, traceability tools should be available throughout the food supply chain from capture to a customer's plate (from ocean to fork) (Helyar et al., 2014; Leal et al., 2015) and should be amenable to forensic validation for use in a court of law if required. While there have been a plethora of genetic tools for identifying and monitoring the identity of fish stocks (Hauser Lorenz and Carvalho Gary R, 2008),

the most informative and objective contemporary approach that is amenable to high throughput cost-effective analysis is the use of "SNPs".

* *ICES. 2018. Interim Report of the Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA), 15–17 May 2018, Brest, France. ICES CM 2018/ASG:03. 39 pp.*

3.4 ToR d: eDNA in Fisheries Management and Ecosystem Monitoring.

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3.4.1 Introduction

Recent rapid developments in the field of environmental DNA (eDNA) means it is timely to review the state-of-the-art in the field. Managers and policy-makers see such developments and are very interested in how this new tool can be applied to management and monitoring of the marine environment. It is difficult, however for a non-specialist to disentangle approaches to fishery management and ecosystem monitoring which are well developed from those which are still in the research phase. This ToR seeks to critically analyse the field and at the same time produce a non-technical advice summary for decision-makers.

The focus for this ToR was to perform a high-level evidence synthesis of the field with particular emphasis on the identification of areas in which eDNA approaches are already available and being used and which might be of more general usefulness to fishery managers, aquaculture, and related ecosystem monitoring. We identified useful and well-developed approaches and provided a non-technical summary of such techniques. At the same time, we described approaches which have the potential to provide useful information but for which further research is required before they are available for practical use outside research applications.

The following activities have been pursued during the 2018-2020 WGAGFA cycle:

Year 1
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 utilized in the marine sphere. Produce a glossary or commonly used terms in the field.
Year 2
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
Finalize 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.

3.4.2 Overview

Developments in the field of genetics have transformed our under-standing of the natural world. In a fisheries context among other things it has helped us identify species, de-fine 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. eDNA approaches promise to revolutionise the examination of biodiversity in the wild by allowing the detection of 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 utilized 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 utilized 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 examined the use of eDNA approaches in the marine environment to date, identified areas where tools are already available for practical use and identified areas where the use of the new approaches are still at the research phase.

3.4.3 Progress made

3.4.3.1 Literature review

An extensive literature review was undertaken with the identification of a rapidly expanding number of papers focusing both on specific applications of the use of eDNA to answer management objectives, and also on the development of the technology and approaches. A reference database was created containing 364 indexed PDFs of the papers identified. This database was uploaded to an online depository and can be downloaded and imported to any reference manager software.

3.4.3.2 Glossary of technical terms relating to eDNA

A glossary was produced in order to provide non-technical definitions of common scientific terms in use when utilizing eDNA.

DNA Term	Definition
Amplicon	A piece of DNA that is the source and/or product of amplification or PCR replication events.
Barcodes	Specific gene fragments targeted for amplification and for which there are databases which allow matches of individual sequences to species identifiers.
Barcoding	The taxonomic identification of a species based on single specimen sequencing of diagnostic barcoding markers
Benthic	Benthic refers to the lowest region of a water body, including the surface and the first layers of the seabed.
Biodiversity	The makeup of all organisms (number and types) that exist in a particular ecosystem.
Bioinformatic pipeline	The combining of processes/functions to go from raw sequence reads to quality filtered final data for analysis (e.g. list of species present).

Biomonitoring	The monitoring of the biological composition and/or characteristics of a particular area.
Cryptic species	A group of closely related species that are very similar in appearance to the point that the boundaries between them are often unclear and hard to identify using traditional methods.
ddPCR	Digital Droplet PCR refers to a technique that allows identification and quantification of species-specific DNA in a sample
DNA Amplification	The copying millions of times of a specific area of interest within the genome.
DNA library	A collection of DNA fragments to be sequenced
DNA Sequence	The succession of letters that indicate the order of nucleotides within a DNA molecule (composed of ATCG).
DNA Sequencing	The process of reading a sequence of DNA such that its genetic sequence is determined.
Environmental DNA (eDNA)	eDNA refers to DNA deposited in the environment through substances such as faeces, mucus, gametes, shed skin, carcasses and hair etc. This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from.
False negative	Missed detection of a species when it is in reality present in the sample
False positive	Incorrect detection of a species when it is in reality absent
High Throughput Sequencing	Techniques which allow simultaneous sequencing of thousands/millions of sequences.
Metabarcoding	Metabarcoding is a rapid method of biodiversity assessment that combines two technologies: DNA based identification and high-throughput DNA sequencing. It uses universal PCR primers to mass-amplify DNA Barcode genes from eDNA. The PCR product is sequenced using a next generation sequencer with the resulting amplicon sequences being matched to databases to allow multiple species identification.
Molecular Operational Taxonomic Unit (MOTU)	Groups of sequences identified and grouped using certain similarity thresholds. MOTUs are thus proxies for "species" in the absence of traditional systems of biological classification.
Molecular tag	A short DNA sequence (~6–8 bp) joined to amplicons that individually labels the sample to allow for multiplexing (may be referred to as an index barcode)
Multiplexing	The procedure by which individual samples are tagged with unique identifiers to allow them to be combined in a single sequencing run.
Next Generation Sequencing (NGS)	Technology developed in the 2000s that produces millions of DNA sequences in parallel at the same time. Various different technologies exist to do this. Also known as high-throughput or parallel sequencing.
PCR Primers	Short sections of DNA which the researcher adds to the PCR reaction and which attach at either end of a DNA section of interest providing templates for the PCR amplification of this region.
Pelagic	The water column of an open water body.
Pipeline	A bioinformatics procedure consisting of multiple steps to clean, quality control and convert raw high-throughput DNA sequencing data into a format such that analysis can be performed.
Polymerase Chain Reaction (PCR)	A process by which millions of copies of a particular DNA segment are produced through a series of heating and cooling steps and the utilization of the DNA replication enzyme DNA polymerase (e.g. Taq polymerase).

Quantitative PCR (qPCR)	A PCR reaction incorporating a coloured dye that fluoresces during amplification, allowing a machine to track the progress of the reaction in real-time. Often used with species-specific Primers where detection of amplification is used to infer presence of the target species' DNA in the sample. The degree of fluorescence can also be used to quantify the abundance of DNA in the sample. Sometimes also known as Real-time PCR.
Sequencing reads	The sequence of basepairs that is obtained after the sequencing process and corresponding to a section of a unique DNA fragment

3.4.3.3 The eDNA Topic Sheet

The next two pages present a non-technical two-page topic sheet was produced introducing non-specialists to eDNA and applications relevant to fishery management and ecosystem monitoring.

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FACT SHEET

Environmental DNA (eDNA) for the management of marine living resources

Managment and monitoring

Ecosystem-based management increasingly demands regular monitoring of the marine environment and its living resources. Progress with recent genetic technologies has provided powerful and practical tools to address a diversity of issues spanning biosurveillance, biosecurity, conservation, ecosystem monitoring, and aquaculture and fishery management.

Many approaches are routinely employed and provide important contributions to fishery management and ecosystem monitoring. One such approach is the use of environmental DNA (eDNA). This information sheet aims at providing a non-technical overview of the rapidly developing eDNA field and relevant applications.

What is eDNA?

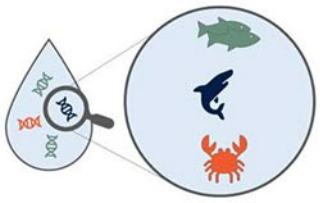
Environmental DNA is the genetic material released from an organism into its environment through physiological processes. This DNA persists in the environment for some time and can be collected for analysis. For fish, eDNA traces can come from cells in blood, mucus, faeces, scales, gametes and carcasses. DNA from microorganisms and benthic communities can also be collected from water and sediment samples.

Collection and analysis

eDNA can be studied by simply collecting water samples in sterile containers that are then filtered through sterile filters, with a mesh so fine they can retain genetic material. The DNA retained in the filters is then extracted in the laboratory. Depending on the scope of the survey, these samples can be used to target a specific organism or the community as a whole, or both. In either case, extracted DNA is processed to produce a catalogue of different "DNA-types", that can be matched to the species they come from using a reference database.

eDNA and traditional surveys

Environmental DNA and traditional survey methods complement, rather than replace each other. While many studies have shown that eDNA is faster and more cost-effective in assessing biodiversity and community composition, it cannot replace traditional methods when investigating size, age class distribution, and, for now, abundance. Occasional taxonomic validation, especially in understudied systems remains crucial.



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Working Group on Application of Genetics in Fisheries and Aquaculture

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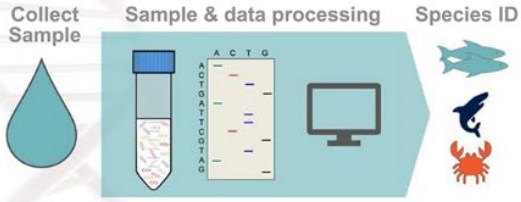
Environmental DNA (eDNA)

Fisheries Management

Management of fisheries relies on accurate estimates of both stock distribution and abundance. eDNA can aid the former, while more research is needed to reliably use this tool for the latter. Using eDNA in fisheries management has the potential to enable non-invasive, less costly and faster monitoring of stocks, and may identify a greater diversity of the species by capturing organisms which can be under-represented in traditional surveys. At present, eDNA sampling based approaches typically provide information on species diversity and the presence of spawning activity. Research efforts are now focusing on improved precision of species abundance estimates.



How to Apply?

eDNA sampling can be readily integrated into existing monitoring programs. Particular attention to avoid sample contamination with DNA from other sources is a prerequisite. The samples themselves are analysed in dedicated molecular labs although a number of portable and automated solutions have been developed. The results provide novel complementary information to traditional surveys from a sample of seawater/sediment.

Environmental Monitoring

eDNA analysis has many cost-effective applications for environmental monitoring. eDNA is particularly well suited for rapid detection of invasive species and those species that are under-estimated/ under-represented using traditional sampling approaches. Given its relatively short life-span eDNA is useful in giving a reliable snapshot of species present in a habitat at the time of sampling. That means that it is also useful for assessing small scale migration of species of interest, and in quantifying trophic food-webs. eDNA focusing on the identification of multiple species (called eDNA metabarcoding) can characterise the majority of species composition of a benthic or pelagic location. Impacts due to anthropogenic influences such as fishery or aquaculture activities can thus be estimated and addressed.

Conclusions

The benefits of using eDNA based approaches are numerous and can prove invaluable in monitoring marine environments. The field is relatively new, but developing at an incredibly fast pace, with new applications continuously being optimised, including the deployment of autonomous underwater vehicles (AUV).

At a time when the marine environment is under threat, eDNA techniques are a cost-effective resource to inform managers and policy-makers, hence aiding the sustainable exploitation of aquatic living resources.

More information at
www.ices.dk/community/groups/Pages/WGAGFA.aspx

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Working Group on Application of Genetics in Fisheries and Aquaculture

A review paper was produced covering the application of eDNA to fishery management and ecosystem modelling. This review gives a non-technical summary of different approaches in the field of eDNA analysis, with value for the governance and management of aquatic ecosystems. The paper focuses on disentangling those tools which are readily applicable and those which show promise but are currently in development. The paper focused on three high level applications for eDNA: targeted species detection, community characterization, and species abundance estimation.

Targeted species detection from eDNA involves the development of sets of probes explicitly designed to identify the presence of a species, or a group of species, from a known list of those potentially present using eDNA collected from filtered water samples. The eDNA is amplified through quantitative PCR (qPCR) using those specific primers, allowing determination of presence/absence and potential quantification of the abundance of the species under investigation. Examples of this application are covered in the paper and include: detection and mapping of the spread of invasive or non-native species; parasite detection; identification and monitoring of rare/endangered species; detection of cryptic species; detection of "invisible" species (e.g. planktonic life stages); investigating spawning activity; monitoring of high diversity (multispecies) environments; monitoring of hard to access environments; and monitoring of pathogens in aquaculture.

Community characterization, often referred to as community metabarcoding, is a technique used to characterize either the full species composition, or a selected subset of species, whose eDNA is represented in a water sample using a conserved region of DNA amplified from all representative sequences within the water sample which is then compared to reference sequences within a database. Examples of this application are covered in the paper and include: defining fish diversity; identification of new species; connection of life stages; clarification of feeding behaviour; ecosystem foodweb structure and dynamics; the impact of aquaculture on benthic communities; identification of non-indigenous species in ballast/harbour water; monitoring of marine vertebrates; habitat preference; characterization of non-indigenous species; and biodiversity assessment - marine sanctuaries.

Together with the identification of both individual species and ecosystem species biodiversity, eDNA can be used to attempt to estimate either the relative abundance of multiple species, or the absolute abundance of individual species. At its simplest such approaches involve quantifying the amount of eDNA from a species represented in a sample and using that as a simple proxy for abundance. In the marine environment abundance estimates using eDNA are developing rapidly, and while at present robust relationships between abundance quantification using eDNA and more traditional methods are sometimes weak, in others the approach seems to be comparable to that of other quantitative methods. Examples of this application are covered in the paper and include: seasonal fish abundance; marine vertebrate abundance; monitoring pathogen abundance in aquaculture; monitoring deep-water species; invasive species abundance; stock assessment.

We conclude that rapid developments in the field of environmental DNA analysis have been providing a range of new tools for research scientists and fishery and ecosystem managers. We have attempted to provide a topic-based overview which goes some way to inform managers of the strengths and weaknesses of the various approaches available.

Title: Life in a drop: sampling environmental DNA for fishery management and ecosystem monitoring

The manuscript has meanwhile (june2020) been submitted to the journal Marine Policy.

Abstract: Science-based management of marine fisheries and effective ecosystem monitoring both require the collection of large amounts of often difficult to collect information on which decisions

can be based and management policies developed. Legislation also increasingly requires the attainment of good environmental status, which again demands collection of data to enable efficient monitoring and management of biodiversity. Traditionally such information is obtained as a result of research surveys through the capture and/or visual identification of organisms in water or sediment samples. Recent years have seen significant advances in the utilization of environmental DNA (eDNA) in the marine environment to try to address the information needs in a cost-effective manner. Such approaches attempt to identify and/or quantify the species present at a location through the use of extra-organismal DNA shed into the environment. These new eDNA based approaches have the potential to revolutionise data collection in the marine environment. However, the rapid developments in the field provide an oftentimes bewildering suit of novel tools which have the potential to be utilized to examine issues of relevance to managers, monitors and policy-makers and it is difficult for a non-specialist to be able to make informed decisions as to the utility of such approaches to answer questions of interest. In order to bridge this information gap, here we present a non-technical summary of different approaches in the field of eDNA analysis, with value for the governance and management of marine aquatic ecosystems. The paper focuses on disentangling those tools, which are readily applicable, and those, which show promise but are currently in development.

4 Additional results during the reporting period

4.1 Additional Output

WGAGFA Leaflet: During the reporting period, a leaflet has been developed by the WGAGFA, depicting the scope and expertise and activities under the ICES remit. This leaflet is downloadable from the ICES web page and part of the WGAGFA dissemination strategy.

ICES Training Report "Genomics in support of fisheries and aquaculture management": In September 2019 the WGAGFA provided the first ICES training course on genomic approaches and their integration into fisheries and aquaculture management. This successful ICES training activity was documented in a report that has been submitted to ICES.

ToRa publication: The Terms of Reference group dealing with farmed and wild salmon interactions under the lead of Ian Bradbury (DFO Canada) is currently preparing a manuscript that is to be submitted as a peer reviewed article.

ToRd publication: The Term of Reference group dealing with environmental DNA under the lead of John Gilbey (Marine Science Scotland) has prepared a manuscript that has been submitted to Marine Policy.

ToRd eDNA topic sheet: The Term of Reference group dealing with environmental DNA under the lead of John Gilbey (Marine Science Scotland) has submitted a draft for a topic sheet on the use of eDNA for ocean governance to be published under the ICES remit.

WKGENOTOOLS report: an account, documenting the objectives scope, results and remit of the "Stakeholder Workshop on the Value of Genetic and Genomic Tools for identifying species in mixed landings, fish products and by-products", has been prepared and been submitted to ICES.

ICES ASC Theme Session 2018 publication: a manuscript for a peer-reviewed publication emerging from the Theme Session that the chairs of WGSEDA, WGPDMO, and WGAGFA organized, has been prepared.

4.2 Outreach Activities

ICES Training Genomics in Support of Fisheries and Aquaculture Management: In September 2019 the WGAGFA provided a 3-Day training course on genomic approaches and their integration into fisheries and aquaculture management. This successful ICES training activity that received exceptionally positive feedback from the participants, is thoroughly documented in a report that has been submitted to ICES.

WKGENOTOOLS: In February 2020, in support of ToRc, the WGAGFA, with financial support from the European Commission Joint Research Centre (JRC) and resources from the JRC and the Norwegian Marine Research Institute (IMR), organized a two-day stakeholder workshop in Brussels on ToRc. The workshop resulted in a valuable exchange between WGAGFA scientists and stakeholders from the European Commission Directorate General MARE, the European Parliament, as well as the industry. Policy and legislative needs, with a focus on the EU landing obligations were identified, followed by an assessment of the value of state-of-the-art genetics and genomics for the implementation of relevant legislation. Results and outcomes of this workshop are documented in a report that will be submitted to ICES together with the final WGAGFA report.

ICES ASC Theme Session 2018: Under the remit of the Aquaculture Steering Group, the chairs of the ICES Working Groups on Social and Economic Dimensions of Aquaculture (WGSEDA), Pathology and Diseases of Marine Organisms (WGPDMO), and on the Application of Genetics in Fisheries and Aquaculture (WGAGFA) co-organized the Theme Session O - Working toward an ecosystem approach to North Atlantic marine aquaculture. A report documenting the output of this successful session has been submitted to ICES and a manuscript for a peer-reviewed publication is being prepared.

World Fisheries Congress 2020 (postponed to 2021): Members of the WGAGFA are involved in the organization of sessions during the next world fisheries Congress.

FishGenome: Following a specific request by the European Commission Directorate General MARE (DG MARE), the WGAGFA embarked on the dialogue with the FishGenome project consortium. The project FishGenome, "Improving Cost-Efficiency of Fisheries Research Surveys and Fish Stocks Assessments using Next-Generation Genetic Sequencing Methods" is based on the tender issued by DG MARE, and managed by the European Agency for Small and Medium-Sized Enterprises (EASME; https://ted.europa.eu/udl?uri=TED:NOTICE:264865-2018:TEXT:EN:HTML). With Gary Carvalho, a WGAGFA member and former working group chair, is a member of the FishGenome scientific advisory group. The FishGenome consortium presented FishGenome, its objectives and scope, just before the WGAGFA annual meeting 2020. Moreover, a number of WGAGFA members participated upon invitation in the dedicated FishGenome stakeholder workshop in May 2020. Further common activities are under discussion, with the objective to maintain the established link between ICES and FishGenome, and as to ensure the best possible outcome in line with the policy-oriented objectives of the project.

GECKA: A research project that emerged from WGAGFA 2015-18 ToRd. GECKA is led by AZTI, financed by the Joint Research Centre and a number of WGAGFA members contribute. GECKA assesses whether genetic close-kin mark recapture (CKMR) method can be employed for obtaining fisheries-independent abundance estimates for highly valuable and exploited deep-sea stocks.

Annex 1: List of participants

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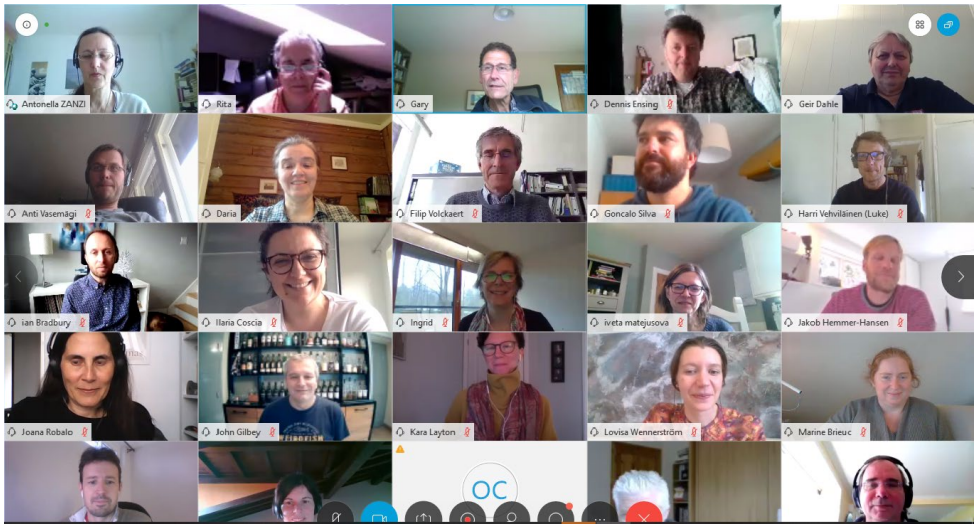
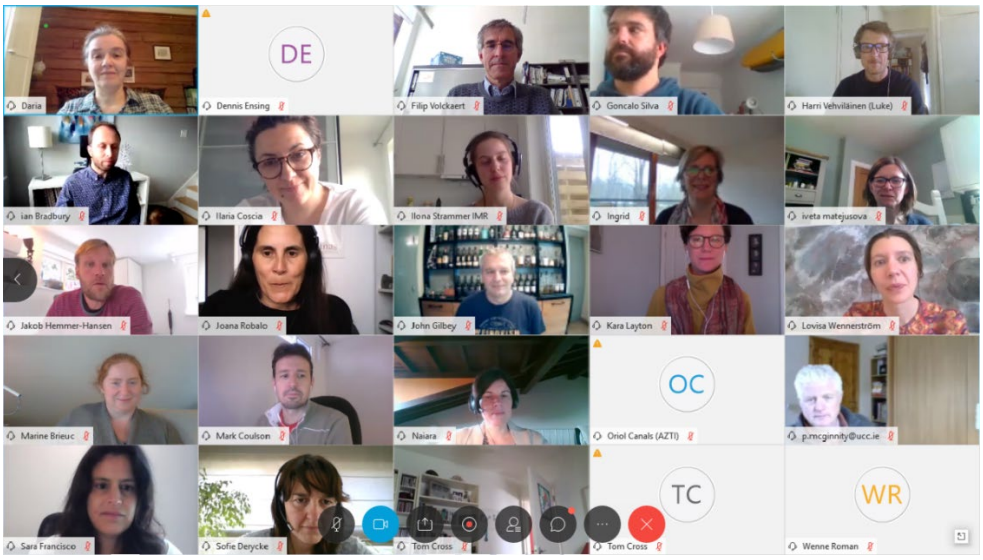
Annex 2: Group Pictures

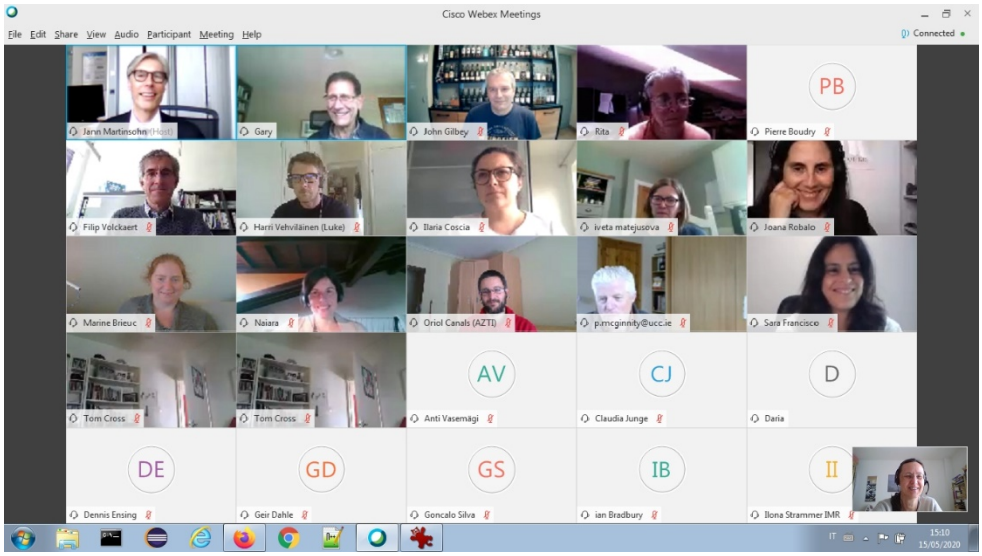
WGAGFA 2018 - Institut Universitaire Européen de la Mer (l'IUEM), Brest, France, 15-17 May

WGAGFA 2019 – European Commission Joint Research Centre (JRC), Ispra, Italy, 14-17 May

WGAGFA 2020 – Worldwide Web, 12-14 May 2020

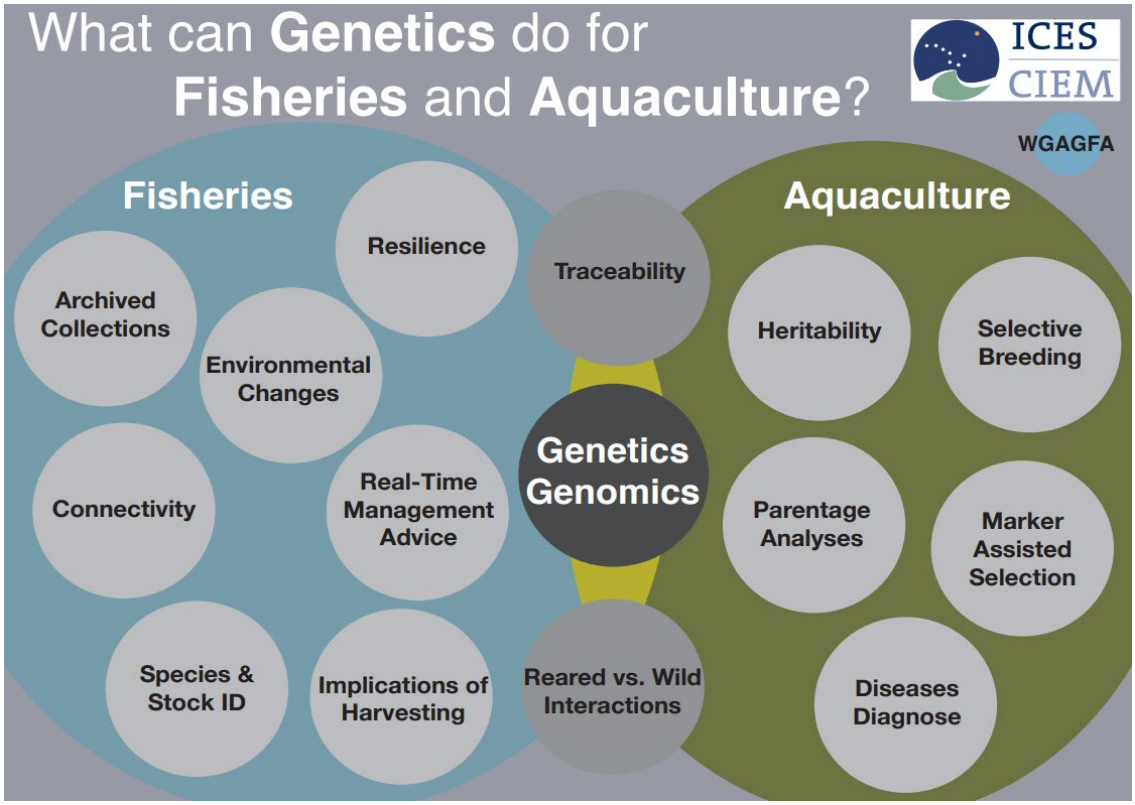


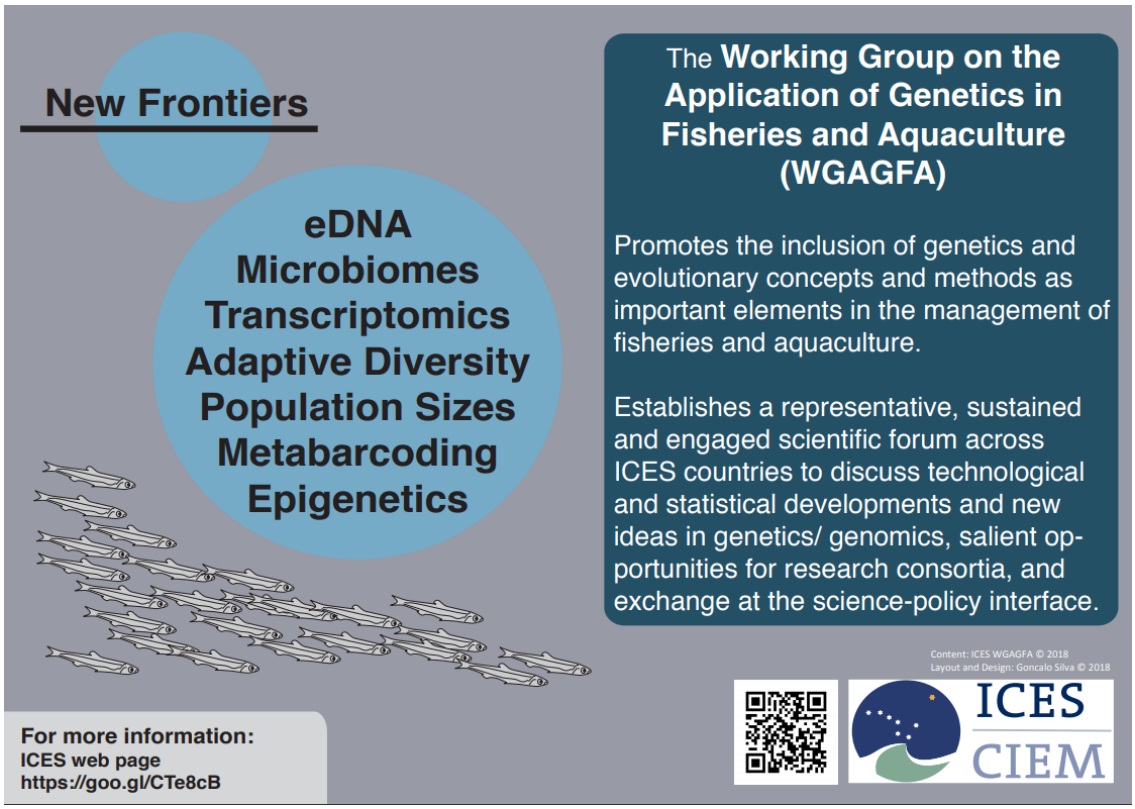




Annex 3: The WGAGFA Leaflet

For a download see also the ICES WGAGFA website.





Annex 4: Resolutions

Proposed under the remit of the Aquaculture Steering Group Expert Group Resolutions. Agreed upon by the ICES Council, the Advisory Committee (ACOM), and the Science Committee (SCICOM).

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	
Year 2019	13–17 May	Ispra, Italy	Interim report by 30 June	
Year 2020	11-15 May	By correspondence	Final report by 12 June to ACOM and SCICOM	

ToR descriptors

ToR	Description	Background	Science Plan codes	Duration	Expected Deliverables
a	Review and report on 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.	2.7, 5.6, 6.1	3 years	Review paper and metrics for measures of indurect genetic impacts
b	Review and report on principles of and prospects for	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,	4.1, 4.5, 5.5	2-3 years*	(a) Review Paper (b) seafood production brief (c) Publication

	genomic selection applied to aquaculture species	such as acceleration of breeding cycle, increase of accuracy of prediction of multi-trait 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	Assess and report on the value of genetic and genomic tools for identifying species in mixed landings, fish products and by-products.	Mixed-species landings and the use of a mix of species in fish products continues to pose a formidable challenge to fisheries control and enforcement as well as traceability along the supply chain. In light of the difficulties in monitoring mixed species landings and identifying species in fish products and by-products we aim to elaborate whether genetic and genomic tools can provide robust and cost-efficient support to determine species composition, also quantitatively, and directly supporting fisheries management and policy needs. A timely and relevant example is the global attempt to develop and implement rules that lead to the reduction of discards. Discarding is the rather common practice of returning unwanted catches to the sea, either dead or alive, because they are undersized, due to market demand, the fisher has no quota or because catch composition rules impose this. In Europe, 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	1.6, 2.7, 6.3	3 years	a) Review Paper; b) ICES Viewpoint.

		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 fishoil 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 commercial 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. Focusing on, but not dealing with exclusively, we will elaborate whether genetic methods might efficiently support the implementation of rules designed to reduce discards and related control, monitoring and enforcement measures.			
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.	1.6, 4.1, 4.4	3 years	(a) Review paper (b) Non-technical review topic sheet.

	A number of approaches using eDNA have been utilized 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 utilized 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 summarize 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.

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 recommeandations 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 utilized 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; topic summaries; publication; implications and recommendations. **ToR d)** Finalize 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 assessing the species composition of fish products and by-products. 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 EPDSG, EOSG and EPISG. Additionally, several EGs, including WGITMO, WGBIODIV, WGBOSV.
Linkages to other organizations	European Commission, Ifremer, NOAA, DFO