ICES WGAGFA REPORT 2018

AQUACULTURE STEERING GROUP

ICES CM 2018/ASG:03

REF. ACOM, SCICOM

Interim Report of the Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA)

15–17 May 2018

Brest, France

ICES
International Council for the Exploration of the Sea

CIEM
Conseil International pour l'Exploration de la Mer

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

Recommended format for purposes of citation:

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.

Contents

Executive summary

The Working Group on the Application of Genetics in Fisheries and Aquaculture (WGAGFA) convened at the Pôle Numérique Brest Iroise (PNBI, Université Bretagne Loire) in Plouzané, Brest, France, 15–17 May 2018. The meeting was hosted by Pierre Boudry (LEMAR, Ifremer) and Grégory Charrier (LEMAR UBO) and was attended by 24 participants from 11 countries.

The ToRs are highly relevant to current challenges inherent to the management and conservation of marine living resources (ToR A, C, D) and the benefit to the aquaculture industry (ToR B). All ToRs do not only consider state-of-the-art science but also strive to maximise impact through peer-reviewed publications and active engagement with stakeholders.

The WGAGFA members reflected on state-of-the-art of genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations (ToR A). The magnitude of escape events of farmed Atlantic salmon raise concern about indirect genetic interactions between wild Atlantic salmon and escaped domestic individuals, that could lead to significant loss of fitness of wild salmon populations. An understanding of indirect genetic effects will inform policy related to the sustainable aquaculture. Different forms of indirect genetic effects are discussed. A literature review suggests that ecological interactions resulting from aquaculture can result in significant genetic change in wild salmon populations and other species and that novel genetic and genomic approaches can help to quantify impacts. Future work will scrutinize the power of novel approaches to detect changes in genetic diversity and character over time.

Genomic selection (GS), a genetic marker-assisted selection method, applied for many terrestrial farmed species, is discussed for aquaculture species in ToR B. GS can efficiently support breeding strategies in aquaculture. The approach is described and advantages as well as limitations are delineated. In summary GS will enhance the rate of genetic gain and GS information may also facilitate the discovery of genomic regions that contribute to the genetic variation of complex traits. Importantly, challenges of the approach for different species and breeding programmes have considered. The main practical concern GS in aquaculture it is whether it is a cost-effective selection strategy. Advances in genomic methodologies accompanied by reduced costs for analyses are enabling the increased use of GS in aquaculture. This will be monitored and discussed in future work, along with recommendations its introduction in aquaculture activities.

The reduction of discards is a high priority on fishery policy agendas worldwide, but legislation tends to be difficult to implement. The WGAGFA discussed how genetic approaches could help to facilitate discard avoidance strategies with a focus on the EU landing obligation but by also tapping into experiences and practices from ICES Member States in general (ToR C). It is important to enable the industry to comply with established rules and to ensure that efficient monitoring, control and enforcement measures are in place. Through literature and discussion with experts, reasons for discarding and non-compliance strategies are depicted and genetic applications delineated that can help to support efforts against discards. This compilation of information and an analysis will be exposed to interested parties and a stakeholder consultation workshop in year 2 is proposed (see recommendations) to ensure that ToR C will address the most crucial is-

sues of interest to policy makers and stakeholders charged to ensure the implementation and compliance of the landing obligation.

The field of environmental DNA (eDNA) is quickly evolving, and raises high interest in the scientific, marine management and policy world. As this bears great opportunities but also the risk of exaggerated expectations, a critical review of the field with the aim to ultimately produce a non-technical advice summary for decision makers was deemed important (ToR D). An evidence synthesis with emphasis on the identification of areas in which eDNA tools are already available and used and which might be valuable to fishery, aquaculture, and ecosystem monitoring was focussed on. An online reference database was created containing the electronic versions of relevant literature, which will be regularly updated and freely shared with interested parties. In summary the synthesis led to the conclusion that eDNA can be used to detect the presence of targeted species and/or to produce an ecosystem biodiversity inventory. In fact, eDNA is already used to aid fisheries management marine ecosystem monitoring and a number of areas where the approach provides invaluable insight in specific situations are delineated. However, challenges with respect to the feasibility and robustness of eDNA analysis persist and need to be tackled. Complied information and material will be incorporated into an evidence synthesis paper and a non-technical review topic sheet to be produced in year 3.

1 Administrative details

Working Group name

Working Group on Application of Genetics in Fisheries and Aquaculture (WGAGFA)

Year of Appointment within current cycle

2018

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

1

Chair(s)

Jann Th. Martinsohn, European Commission

Meeting dates

15–17 May 2018

Meeting venue

Brest, France

2 Terms of Reference a) – z)

ToR a - Review and report on genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations

Duration: 3 years.
Deliverables: Review paper and metrics for measures of indirect genetic impacts.
Rational: See Annex.

ToR b - Review and report on principles of and prospects for genomic selection applied to aquaculture species

Duration: 2–3 years.
Deliverables: (a) Review Paper; (b) Sea-Food Production Brief; (c) Publication.
Rational: See Annex.

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

Duration: 3 years.
Deliverables: a) Review Paper; b) ICES Viewpoint.
Rational: See Annex.

ToR d - eDNA in Fisheries Management and Ecosystem Monitoring

Duration: 3 years.
Deliverables: (a) Review paper; (b) Non-technical review topic sheet.
Rational: See Annex.

3 Summary of Work plan

Year 1

ToR a) Review the literature on indirect genetic interactions among aquaculture salmon and wild populations.

ToR b) Review of the basic principles of genomic selection and the key steps of its implementation in breeding programs, focus on current progresses and prospects for aquaculture species and propose recommendations to facilitate its future developments in these species.

ToR c) Review the legal framework and supporting information, such as reports on the Landing Obligation by the Scientific, Technical and Economic Committee for Fisheries (STECF); identify the stakeholders; develop a work flow chart to work up mixed species samples, with decision points; develop theoretical scenarios/cases where genetic testing would be helpful and how the implications would be for a given outcome.

ToR d) Review of the literature on the use of eDNA in the aquatic environment. Together with an overview of the field, particular focus will be to identify where eDNA techniques have/are being used at present in the marine environment and on other techniques used in freshwater that may be utilised in the marine sphere. Produce a glossary or commonly used terms in the field.

Year 2

ToR a) Identify approaches to quantify indirect genetic impacts and explore their sensitivity and power.

ToR b) Develop cases where genomic selection would be helpful and how its implementation would benefit selective breeding programs.

ToR c) Real-life scenario test based on developed work flow chart (from year 1) using real product samples; report results and discuss; report on feasibility and cost issues; recommendations to adjust methods/work flow developed in year 1 if needed.

ToR d) Continuation of the literature review and identification of key studies describing the use of eDNA in the marine environment where the techniques used have significant potential for novel species and/or situations. Produce a flowchart of the critical steps needed from sampling to biodiversity assessment. Start to formulate review paper manuscript.

Year 3

ToR a) Complete review paper, and develop recommendations.

ToR b) Develop a knowledge transfer plan; industry briefs; publication; implications, advice and final recommendations.

ToR c) Develop a knowledge transfer plan; topic summaries; publication; implications and recommendations.

ToR d) Finalise and update review: detail key studies, identify areas where novel techniques show particular promise, and identify problematic areas requiring future research. Finish review paper and non-technical review topic sheet.

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

ToR A: Genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations

- Review paper assessing the occurrence and magnitude of indirect impacts of salmon on wild populations - ongoing
- Recommendations on metrics for assessing indirect genetic impacts and assessment of their respective power – ongoing

ToR B: Genomic selection applied to aquaculture species

- Review of the basic principles of genomic selection. -ongoing
- Online database of literature relating to genomic selection in aquaculture. - ongoing

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

- Review the legal framework and supporting information, such as reports on the Landing Obligation by the Scientific, Technical and Economic Committee for Fisheries (STECF). - Ongoing.
- Identify the stakeholders; develop theoretical scenarios/cases where genetic testing would be helpful and how the implications would be for a given outcome. – Ongoing.

5 Progress report on ToRs and workplan

5.1 ToR A: Genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations

Contributors: Ian Bradbury, John Gilbey, Mark Coulson, Ingrid Burgetz, Paulo Prodohl, Phil McGinnity

The Atlantic salmon, Salmo salar, is of considerable socioeconomic 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.

Farm escapees and the progeny of farm escapees represent substantial change to the environment (Garcia de Leaniz, 2008) and thus the adaptive landscape, and hence are likely to modify future evolutionary trajectories of wild populations. Furthermore, it might be expected that adjustments to a new adaptive landscape will be consistent with reductions in productivity predicted by theoretical demographic-evolutionary models (Burger & Lynch 1995; Gomulkiewicz & Holt 1995; Kirkpatrick & Barton 1997). Evidence of indirect genetic changes in natural salmonid populations resulting from salmon farming is fairly sparse; more information exists on pathogen or parasite transmission, less so on changes mediated through ecological interactions. Those studies address genetic changes in 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 farming of non-native species in increasingly remote, including oceanic scale, trans-locations, for example the farming of European origin salmon on both east and west coasts of North America.

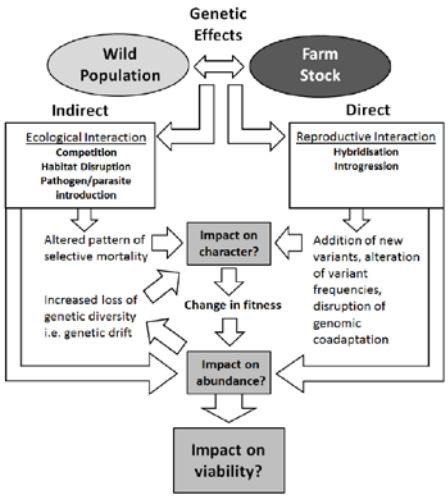

Figure 1. Overview of indirect and direct genetic interactions of escaped farmed fish with wild populations. Verspoor *et al.* (2015).

Indirect genetic changes on naïve populations through pathogen and parasite introduction and transmission. The introduction of parasites or pathogens from the use of salmon from a different region has previously occurred, and while practices and regulations have changed, the impact from the introduction of parasites and pathogens is still of concern. Historically in Norway, the first appearance of *Gyrodactylus salaris* and *Aeromonas salmonicida* has been linked to the introduction of salmon from other regions, which has led to high levels of mortality among wild stocks (Johnsen and Jensen 1991; Bakke and Harris 1998). Pathogen transmission may both impose selection and reduce overall genetic diversity. de Eyto *et al.* (2007) and de Eyto *et al.* (2011) transferred progeny of Atlantic salmon from a river without previous exposure to aquaculture to one with a long history of associated farming and captive breeding that was expected to have acquired a novel micro and macro-parasitic community. This experimental design was a way of exposing animals to novel disease challenges associated with escapes or introductions. By comparing observed and expected genotype frequencies at MH class II alpha locus and control micro-satellite loci at parr and migrant stages in the wild, it was concluded that genetic change had occurred, and that selection was a result of disease-mediated natural selection, rather than any demographic event. The effect of an increase in naturally occurring parasite populations, such as sea lice (*Lepeophtheirus salmonis*) that infest culture salmon on wild populations may also result in an indirect genetic effects. Declines in wild stocks from sea lice in farm intensive areas have been documented in Ireland, Scotland & Norway. Thorstad and Finstad (2018) summarised sea lice impacts on wild stocks, documenting 12–29% fewer returning adult spawners due to lice induced

mortality from fish farms. In the most extreme case documented to date, Shephard and Gargan (2017) suggested that 1 SW returns on the River Erriff were >50% lower in years following high lice levels on nearby farms. This increased mortality was on top of the increased mortality due to poorer marine survival. It has also been suggested that these estimates of, lice-induced mortality among salmon should be considered as minimum estimates for sea trout, which remain in more coastal areas, thus increasing their exposure (Thorstad and Finstad 2018). While there are no studies to date relating changes in genetic diversity due to disease-related effects from farms, it is expected that in some cases, depending on populations size, that increased lice levels could result in too few spawners to reach conservation limits and loss of genetic diversity.

Indirect genetic effects through predation. Increased predation associated with salmon aquaculture activities could impose both selective mortality and declines in abundance. For example, Kennedy and Greer (1988) reported heavy predation on hatchery smolts and wild salmon and brown trout from the river Bush in Northern Ireland by the cormorant Phalacrocorax carbo, suggesting a link between the release of captive bred smolts (a possible proxy for farm escapes), the attraction of increased numbers of these predatory birds to the river and increased predation on river's wild salmon and brown trout.

Indirect genetic effects through competitive interactions. Indirect genetic effects have also been suggested via evidence for competitive interactions among farm and wild salmon. For example, Fleming *et al.* (2000) released sexually mature farm and wild salmon into the River Imsa. Despite the farm fish achieving less than 1/3 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 such invasions have the potential for impacting wild population productivity both via changes to locally adaptive traits as well as reducing genetic diversity. Skaala *et al.* (2012) documented similar effects in a natural system in Norway comparing the performance of farm, wild and hybrid salmon and suggest that overlap in diets and competitions can impact wild productivity.

Indirect genetic effects on local congeners. Diseases, introduced or increased in incidence by salmon aquaculture activities, have an impact on co-occurring wild sea trout (*Salmo trutta L.*), as implied by the steep decline in sea trout numbers in many Irish, Scottish, and Norwegian rivers since the late 1980s, which in some cases may be linked to sea lice infestations 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 sea 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 sea trout stock.

Genetic and Genomic Methods. The utility of genetic and genomic tools to resolve indirect genetic interactions will depend on the route and genomic scale of impact. For example, in the context of impacts due to selective landscape and ecological changes, genomic change could be associated with single genes, or many genes (i.e., polygenic). Genetic and genomic tools are increasingly being used to quantify the magnitude of nat-

ural selection in the wild (Vitti, Grossman, and Sabeti 2013). One of the best approaches to quantify the presence of selection associated with indirect interactions would be the comparison of representative pre- and post-impact genetic samples in the absence of hybridization. For time series analysis of changes in allele frequency associated with selection, several tests have been recently proposed using bi-allelic loci including the empirical likelihood ratio test (ELRT), and the frequency increment test (FIT); (Feder, Kryazhimskiy, and Plotkin 2014). In the absence of pre-impact samples, traditional tests for the presence of outliers (e.g., Foll and Gaggiotti 2008; Luu, Bazin, and Blum 2016) 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 with these loci may be problematic. 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 (Waples and Do 2010). Large genomic datasets offer new opportunities for enhanced estimates of effective population size (Waples, Larson, and Waples 2016) as well as retrospective estimates of changes in effective population size over time (e.g., Hollenbeck, Portnoy, and Gold 2016). Again, in the absence of pre-impact samples for comparison, it may be difficult to attribute observed genetic changes to indirect interactions with high certainty.

Summary and Next Steps: Ultimately, the relative importance of direct and indirect genetic interactions between domestic individuals and wild populations remains largely unresolved. Nonetheless, our review of the literature here suggests that ecological interactions resulting from salmon aquaculture can result in significant genetic change in wild salmon populations as well as other species. Recent advances in genetic and genomic methods present new opportunities for quantifying these impacts but careful experimental design and pre-impact comparisons are often needed to accurately attribute genetic change to indirect genetic interactions with salmon aquaculture activities. Future work should explore the sensitivities and power of these approaches to detect changes in genetic diversity and character over time.

References: see Annex 3

5.2 ToR B: Genomic selection applied to aquaculture species

Contributors: Pierre Boudry, Federico Calboli, Daria Zelenina, Kristen Gruenthal, François Allal, Marc Vandeputte

Introduction

Genomic selection (GS), first published in 2001 [1], is a 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 [2] and plants [3,4], 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 [5]: 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 (e.g. destructive traits). 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. 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 expertise) selection programs.

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 annex 3). 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) [6]. 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 selec-

tion 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.

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 programmes [7].

GS developments in aquaculture species have been recently reviewed [8–10]. 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. 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 [7] 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, selection programmes have only been limited to a few species, such as salmonids, shrimps, tilapia, carp, seabream, seabass, oysters, scallops, 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 selection programmes 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, in comparison to selective breeding programmes 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 cate-

gorical traits [11–14]. In addition to facilitating the increase of genetic gains, GS can also be used to introgress advantageous genes into a potential target population. For instance, [15] demonstrated that simulated backcross breeding programmes using GS provided a faster approach to developing a disease-resistant line of commercial value.

Other points for consideration are (1) the large variety of species / numerous "minor" species / high selection intensities possible / recent domestication and breeding (potential of classical breeding) , (2) the short generation time (not necessarily compared to poultry and pigs, but most traits are recorded before maturity, so GS cannot shorten generation time), (3) the high fecundity of aquaculture species and (4) the low individual value of breeders.

References: see Annex 3

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

Contributors: Claudia Junge, Jann Th. Martinsohn, Sara Vandamme, Gonçalo Silva, Torild Johansen, Geir Dahle, Rita Castilho, Antonella Zanzi, Steven Holmes, Gary Carvalho, Grégory Charrier, Ilaria Coscia, Federico Calboli, William Handal, Joana Robalo, Nils Chr. Stenseth

In this first year of the ToR we have identified the legal framework and supporting information as well as the possible genetic tools and applications. There already exist several examples of applications, which can be found in the Annex 3.

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 fisherman 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 [see ToR C ANNEX_3.1], 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. Consequently, 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. A monitoring example could be species substitution identification: how can it be assured that no pro-

tected 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 products like fish oil and gelatine. In these situations the recent progresses in genetic and genomic technology and analyis offer the best opportunities to address these issues.

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_3.2 summarises those reasons and the underlying problems leading to the current discard of catches. 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 implemnation of the Landing Obligation and the potential value of DNA-based analysis to tackle those needs.

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 (for examples see Annex 3)
    - 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. Annex 3 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 fish oil. 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 fish oil should be investigated.
    - Catch composition in mixed fisheries or with respect to bycatch
        - Accurately assessing the catch composition is crucial for 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).

o 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".

Summary and Outlook

We identified issues related to the implementation of the landing obligation and respective situations in which genetic methods could aid monitoring as well as control. We documented case studies 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. After identifying the stakeholders, we propose to hold a stakeholder consultation workshop in year 2 (see recommendations) to ensure that we are addressing the most crucial issues of interest to policy makers charged to ensure implementation and compliance of the landing obligation.

References: see Annex 3

5.4 ToR D: eDNA in Fisheries Management and Ecosystem Monitoring

Contributors: John Gilbey, Pierre Boudry, Ian Bradbury, Ingrid Burgetz, Frederico Calboli, Gary Carvalho, Rita Castilho, Grégory Charrier, Ilaria Coscia, Mark Coulson, Geir Dahle, Torild Johansen, Claudia Junge, Jann Martinsohn, Philip McGinnity, Paulo Prodohl, Joana Robalo, Naiara Rodríguez-Ezpeleta, Goncalo Silva, Daria Zelenina

Rational

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. The ToR seeks to critically analyse the field and at the same time produce a non-technical advice summary for decision makers.

eDNA can be defined as a complex mixture of extra organismal DNA originating from faeces, mucus, gametes, shed skin/cells/scales and carcasses and found in an environmental sample such as soil, seawater, or even air (Ficetola *et al.*, 2008). Alternatively, environmental DNA can also be defined from a different perspective where it corresponds to any DNA extracted from an environmental sample, including whole organisms (e.g. al-

gae, bacteria, micro-invertebrates). In this case, eDNA would include both extra organismal DNA but also DNA from organisms collected within the environmental sample (Levy-Booth *et al.*, 2007; Pietramellara *et al.*, 2009). In the current overview, we shall adopt the latter of these descriptions and will critically review the application of techniques utilizing sources of eDNA for both fishery management, aquaculture monitoring and ecosystem surveillance purposes, as eDNA from both origins has the potential to provide useful tools in these situations.

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

Progress made

- 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 specific management objectives, and also on the development of the technology and approaches. An online reference database was created which will contain PDFs of the papers identified, and which will be freely shared with interested parties. We will update this database through the three years of the ToR.

- Technical approaches

eDNA can be used to detect the presence of a single or a few targeted species and/or to produce an inventory of the biodiversity of an ecosystem.

  - Targeted species detection. Targeted species detection from eDNA involves the development of sets of primers explicitly designed to identify the presence of a species, or a group of species, from a known list of those potentially present. 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 (Shaw *et al.*, 2017). Such an approach is often used when examining specific invasive species (e.g. Clusa *et al.*, 2017; Sousa *et al.*, 2014), the presence of specific pathogens in the ecosystem (e.g. Bass *et al.*, 2015; Huver *et al.*, 2015) (both for "wild" and aquaculture) and relative quantification of specific targeted species complexes (e.g. Davy *et al.*, 2015; Baldigo *et al.*, 2017).
  - Community Metabarcoding. Metabarcoding aims to detect all species with a representation of their eDNA in a sample. Using this procedure, specific regions of the genome are sequenced (e.g. 12S, Cytochrome B, Cytochrome Oxidase, etc) with resulting unique sequences queried against reference databases in an attempt to identify the various species present in the sample (e.g. Peters *et al.*, 2017; Hänfling *et al.*, 2016). Such an approach thus provides a powerful tool for biomonitoring as it can

simultaneously investigate whole ecosystem communities (Stat *et al.*, 2017). This technique involves PCR amplification of bulk extracts of target genes (and taxa) on eDNA, combined with Next Generation Sequencing (NGS) of the resulting amplicons to provide high-throughput information on the taxonomic diversity present (Taberlet *et al.*, 2012).

- Applications

Analysis of eDNA is already being used to aid management of fisheries and monitor ecosystems (fisheries and aquaculture), and the literature review carried out together with the expert input from the working group participants identified a number of areas where the techniques are providing invaluable insights in specific situations, where the tools available might prove useful for novel species and/or situations, or where significant research progress has been made in specific areas. Such applications include:

  - Biotic indices (e.g. within Europe, the Water Framework Directive and the Marine Strategy Framework Directive)
    - phytoplankton
    - macroinvertebrates
    - fish
  - Early detection of non-indigenous species, including invaders and indicators of climate change
  - Early detection of parasites/pathogens
  - Identification and monitoring of rare/endangered species
  - Benthic monitoring of ecosystem health in relation to aquaculture sites
  - Food webs (through the analysis of stomach/gut contents and faecal analysis of fish predators such as seals and birds)
  - Definition of spawning periods/areas through the analysis of shed gametes
  - Analysis of by-catch from deck water
  - Detection of "invisible" species (e.g. planktonic life stages)
  - Monitoring of Marine Protected Areas

- Challenges

Together with management situations where eDNA tools are already providing invaluable insights there are other promising areas which are under active research but where challenges still exist before robust and user-friendly tools will become available. Such challenges include:

  - Relative and absolute abundance. Substantial effort is being directed into technical and statistical methods to investigate species abundance
  - Contamination. This represents a two-fold challenge: prevention of contamination during sampling, and checking for contamination during the sample processing in the lab (detection of false positives by using PCR blanks etc.)
  - Persistence (how long the DNA stays in the environment). A recent review by Hansen et al. (2018) addressed the possibilities and limitations (when compared with traditional methods) of the use of eDNA for stock assessment studies for marine fisheries. This is a particularly important and urgent analysis because in the near future, governmental and private

entities will have to decide whether or not use these studies as an additional approach to traditional methods, or as the unique way to assess biodiversity (qualitatively or quantitatively). The authors identified the following 5 major challenges:

- Can we find what we are looking for?;
- What is the spatial origin of the DNA?;
- Relationship between eDNA and biomass/numbers;
- Application to fisheries management;
- Other sources of eDNA in fisheries applications
    - Species-specific shedding rates (how much tissue/cells/DNA each organisms releases into the environment)
    - Sampling protocols. The influence of when/where/how the sample is taken and how often (number of replicates)
    - Development of bespoke tools/technology for specific sampling situations/environments (e.g. hydrothermal vents, deep-sea)
    - Databases. The development and quality control of high quality reference databases for metabarcoding
    - Infective agents. Detection and particularly quantification of the abundance of infective agents (e.g. viral infections in mollusc aquaculture and pathogens associated with fin fish farms) and the ability to identify active v inactive forms
    - Standardisation and cross-laboratory calibration of procedures and protocols such that robust replication can be achieved, especially when applied in a management framework
    - Novel marker development to include more groups of organisms
    - Technology transfer. The transfer of techniques out of a research context into a management tool which can be used in the field by 'non-experts' (e.g. https://www.smith-root.com/edna/ande).

Over the remaining period of the ToR we will continue to review the applications and challenges associated with the approaches such that the most up to date situations will be presented in the final text and thus be available for decision makers.

- Glossary

WGAGFA created a glossary of technical terms relating the use of Edna, which is included in Annex 3. We will expand this glossary through the progress of this ToR with an eventual goal of inclusion in the non-technical review topic sheet in year three.

- Evidence synthesis paper

The outline of the evidence synthesis paper was agreed. The aims of the paper will be to try to summarise the approaches of using eDNA in fishery management and ecosystem monitoring (fisheries and aquaculture) contexts such that tools that are readily available to managers are identified, and areas requiring further research outlined. The review will seek to provide illustrations clearly outlining the sources of eDNA into the environment, the methods to sample this material and the various techniques used in the laboratory to analyse the DNA. We will also produce a flow-chart focused on decision makers such that a clear decision tree will be produced showing interested parties which approaches might be best suited to particular situations, and where in other cases particular management objectives might still not be tractable using currently available tools. In the text,

we will expand on this and try to clearly outline the successful use of such tools, and also the challenges still outstanding.

Summary

During the first year of this ToR we have achieved our stated aims of undertaking a literature review of the field and the production of a glossary of technical terms. We have also defined the structure of our evidence synthesis paper and began to formulate various text and illustrations relating to this paper. These materials will also be incorporated into the final non-technical review topic sheet to be produced in year 3.

References: see Annex 3

6 Revisions to the work plan and justification

Not applicable.

7 Next meetings

WGAGFA will meet in Ispra; Italy, on 13–17 May 2019.

Annex 1: List of participants

NAME	AFFILIATION	COUNTRY (of Institute)	E-MAIL
BOUDRY, Pierre	Ifremer	FR	pboudry@Ifremer.fr
BRADBURY, Ian	Fisheries and Oceans Canada	CA	ian.bradbury@dfo-mpo.gc.ca
BURGETZ, Ingrid	Fisheries and Oceans Canada	CA	ingrid.burgetz@dfo-mpo.gc.ca
CALBOLI, Federico	KU Leuven	BE	federico.calboli@kuleuven.be
CARVALHO, Gary	Bangor University, UK	UK	g.r.carvalho@bangor.ac.uk
CASTILHO, Rita*	CCMAR	PT	rcastil@ualg.pt
CHARRIER, Grégory	LEMAR UBO	FR	gregory.charrier@univ-brest.fr
COSCIA, Ilaria	University of Salford	UK	I.Coscia@salford.ac.uk
COULSON, Mark	University of the Highlands and Islands	UK	Mark.Coulson.ic@uhi.ac.uk
DAHLE, Geir	Institute of Marine Research	NO	geir.dahle@imr.no
GILBEY, John	Marine Scotland Science	UK	gilbeyj@marlab.ac.uk
GRUENTHAL, Kristen*	NOAA	US	kristen.gruenthal@noaa.gov
HANDAL, William	LEMAR UBO	FR	william.handal@univ-brest.fr
JOHANSEN, Torild	Institute of marine research Norway	NO	torild.johansen@imr.no
JUNGE, Claudia	Institute of Marine Research, Norway	NO	claudia.junge@hi.no
MARTINSOHN, Jann	European Commission Joint Research Centre	EU	jann.martinsohn@ec.europa.eu
MCGINNITY, Philip	University College Cork	IE	p.mcginnity@ucc.ie
PRODOHL, Paulo	Queen's University Belfast	UK	p.prodohl@qub.ac.uk
ROBALO, Joana Isabel*	MARE/ISPA	PT	jrobalo@ispa.pt
RODRIGUEZ-EZPELETA, Naiara	AZTI Tecnalia	ES	nrodriguez@azti.es
SILVA, Gonçalo	MARE-ISPA	PT	gsilva@ispa.pt
ZANZI, Antonella*	European Commission Joint Research Centre	EU	antonella.zanzi@ec.europa.eu
ZELENINA, Daria	VNIRO	RU	zelenina@vniro.ru

***Remote Participation**

Annex 2: Recommendations

RECOMMENDATION	ADRESSED TO
1. We recommend that ICES endorses and supports a stakeholder workshop on the value of genetic tools for supporting the European Union Landing Obligation, convened by the Working Group on the Application of Genetics for Fisheries and Aquaculture (WGAGFA) – See ToR C and background below.	ICES Secretariat

Recommendation – Background

Topic: We recommend that ICES endorses and supports a stakeholder workshop on the value of genetic tools for supporting the European Union Landing Obligation, convened by the Working Group on the Application of Genetics for Fisheries and Aquaculture (WGAGFA).

When: First Trimester 2019

Where: Brussels, BE

Duration: 1 Day

Number of contributors: 15 to max. 20.

Rational:

It is generally acknowledged that discarding is a wasteful practice, impacting the endeavour of moving towards sustainable fisheries. This is why a number of countries and the European Union attempt to tackle the issue of discarding through dedicated fisheries management measures. To this end, the European Union is currently implementing the Landing Obligation. However, the complexity inherent to the present fishing practices confronts both the industry and authorities that are mandated with monitoring and controlling with unprecedented challenges.

To support the implementation of the Landing Obligation, opportunities offered through the recent progress in genetic and genomic technological and analytical applications should be tapped into.

However, it is necessary to ensure a mutual understanding between scientists and end-users to identify end-user needs and to clarify which issues relevant for the Landing Obligation can be tackled and also to render limits evident.

Based on an initial assessment, carried out by the WGAGFA and first documented feedback by stakeholders, this workshop will help to clarify to what extent genetic and genomic approaches can support the Landing Obligation implementation, and which are the necessary steps to enable a successful technology and knowledge transfer.

Deliverable:

A concise documentation of discussions and results emerging from the workshop, in a format suitable to be further disseminated among concerned parties. This documentation will also help to steer further activities under the remit of WGAGFA ToRc (2018–20).

Please note: A similar workshop was held in 2007 by the European Commission in the frame of the consultation process accompanying the Common Fisheries Policy Control Regulation reform. – It was highly successful and contributed to the introduction of genetics under Article 13 of (Council Regulation (EC) No 1224/2009.

Envisioned participants:

ICES WGAGFA members, European Commission DG MARE, The Norwegian Directorate of Fisheries, Representatives of Regional Advisory Councils (Commission Delegated Regulation (EU) 2017/1575).

Annex 3: Additional information

ANNEX_3 ToR A: Review and report on genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations

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

Duration: 3 years

Deliverables: Review paper and metrics for measures of indirect genetic impacts

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.

References

Bakke, T. A. and P. D. Harris. 1998. Diseases and parasites in wild Atlantic salmon (Salmo salar) populations. Canadian Journal of Fisheries and Aquatic Sciences 55:247–266.

Bolstad, G.H., K. Hindar, G. Robertsen, B. Jonsson, H. Sægrov, O.H. Diserud, P. Fiske, A.J. Jensen, K. Urdal, T.F. Næsje, B.T. Barlaup, B. Florø-Larsen, H. Lo, E. Niemelä, and S. Karlsson. 2017. Gene flow from domesticated escapes alters the life history of wild Atlantic salmon. *Nature Ecology & Evolution* 1:0124.

Bourret, V., P. T. O'Reilly, J. W. Carr, P. R. Berg, and L. Bernatchez. 2011. Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (Salmo salar) population following introgression by farmed escapees. *Heredity* 106 (3):500–510.

Coughlan, James, Philip McGinnity, Brian O'Farrell, Eileen Dillane, Ola Diserud, Elvira de Eyto, Killian Farrell, Ken Whelan, René J. M. Stet, and Thomas F. Cross. 2006. Temporal variation in an immune response gene (MHC I) in anadromous Salmo trutta in an Irish river before and during aquaculture activities. *ICES Journal of Marine Science* 63 (7):1248–1255.

Cross, T. F. and J. King. 1983. Genetic effects of hatchery rearing in Atlantic salmon. *Aquaculture* 33 (1–4):33–40.

de Eyto, E., P. McGinnity, S. Consuegra, J. Coughlan, J. Tufto, K. Farrell, H. Megens, W. C. Jordan, T. Cross, and R.J.M Stet. 2007. Natural selection acts on Atlantic salmon major histocompatibility (MH) variability in the wild. *Proceedings of the Royal Society B: Biological Sciences* 274:861–869.

de Eyto, E., P. McGinnity, J. Huisman, J. Coughlan, S. Consuegra, K. Farrell, C. O'Toole, J. Tufto, H. Megens, W. C. Jordan, T. Cross, and Rene J. M. Stet. 2011. Varying disease-mediated selection at different life-history stages of Atlantic salmon in fresh water. *Evolutionary Applications* 4 (6):749–762.

DFO. 2016. Proposed Use of European-Strain Triploid Atlantic Salmon in Marine Cage Aquaculture in Placentia Bay, NL. *DFO Can. Sci. Advis. Sec. Sci. Resp.* 2016/034.

Feder, A.F., S. Kryazhimskiy, and J.B. Plotkin. 2014. Identifying Signatures of Selection in Genetic Time Series. *Genetics* 196:509–522.

Fleming, I. A., K. Hindar, I.B. Mjølnerød, B. Jonsson, T. Balstad, and A. Lamberg. 2000. Lifetime success and interactions of farm salmon invading a native population. *Proceedings of the Royal Society Biological Sciences Series B* 267:1517–1524.

Fleming, I. A., B. Jonsson, and M. R. Gross. 1994. Phenotypic divergence of sea-ranched, farmed, and wild salmon. *Canadian Journal of Fisheries and Aquatic Sciences* 51 (12):2808–2824.

Fleming, I. A., B. Jonsson, M. R. Gross, and A. Lamberg. 1996. An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). *Journal of Applied Ecology* 33 (4):893–905.

Foll, M. and O. Gaggiotti. 2008. A Genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics* 180:977 - 993.

Ford, J.S. and R.A. Myers. 2008. A Global Assessment of Salmon Aquaculture Impacts on Wild Salmonids. *PLoS Biology* 6 (2):e33.

Glover, K.A., C. Pertoldi, F. Besnier, V. Wennevik, M.P. Kent, and Ø. Skaala. 2013. Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. *BMC Genetics* 14:74.

Glover, K.A., M.F. Solberg, P. McGinnity, K. Hindar, E. Verspoor, M.W. Coulson, M.M. Hansen, H. Araki, Ø. Skaala, and T. Svåsand. 2017. Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. *Fish and Fisheries* 18:890–927.

Handeland, S. O., B. T. Björnsson, A. M. Arnesen, and S. O. Stefansson. 2003. Seawater adaptation and growth of post-smolt Atlantic salmon (*Salmo salar*) of wild and farmed strains. *Aquaculture* 220 (1–4):367–384.

Hollenbeck, C. M., D. S. Portnoy, and J. R. Gold. 2016. A method for detecting recent changes in contemporary effective population size from linkage disequilibrium at linked and unlinked loci. *Heredity* 117:207.

Johnsen, B. O. and A. J. Jensen. 1991. The Gyrodactylus story in Norway. Aquaculture 98:289–302.

Karlsson, S., T. Moen, S. Lien, K. A. Glover, and K. Hindar. 2011. Generic genetic differences between farmed and

wild Atlantic salmon identified from a 7K SNP-chip. *Molecular Ecology Resources* 11 Suppl 1:247–53.

Kennedy, G. J. A. and J.E. Greer. 1988. Predation by cormorants Phalacrocorax carbo (L.), on an Irish river. *Aquaculture and Fisheries Management* 19:159 - 170.

Keyser, F., B.F. Wringe, N.W. Jeffery, J. B. Dempson, S. Duffy, and I.R. Bradbury. 2018. Predicting the impacts of escaped farmed Atlantic salmon on wild salmon populations. *Canadian Journal of Fisheries and Aquatic Sciences*:1–7.

Luu, K., E. Bazin, and M.G.B. Blum. 2016. pcadapt: an R package to perform genome scans for selection based on principal component analysis. *bioRxiv*.

MAST. Annual Report of Veterinary Fisheries 2017, Reykjavik, Iceland 2017 [cited. Available from

http://www.mast.is/library/Sk%C3%BDrslur/Arsskyrsla-dyralaeknis-fisksjukdoma-2017.pdf.

McGinnity, P., P. Prodöhl, A. Ferguson, R.A. Hynes, N. Ó Maoiléidigh, N. Baker, D. Cotter, B. O'Hea, D. Cooke, G. Rogan, J. B. Taggart, and T. Cross. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society Biological Sciences Series B* 270:2443–2450.

Morris, M. R. J., D. J. Fraser, A. J. Heggelin, F. G. Whoriskey, J. W. Carr, S. F. O'Neil, and J. A. Hutchings. 2008. Prevalence and recurrence of escaped farmed Atlantic salmon (*Salmo salar*) in eastern North American rivers. *Canadian Journal of Fisheries and Aquatic Sciences* 65 (12):2807–2826.

Nielsen, R., S.H. Williamson, Yuseob Kim, M.J. Hubisz, A.G. Clark, and C. D. Bustamante. 2005. Genomic scans for selective sweeps using SNP data. *Genome Research* 15 (11):1566–1575.

Ramsden, N. Iceland salmon farmer applies for huge expansion, eyes sterility tech. 2018 [cited 22/5/18.

Available from https://www.undercurrentnews.com/2018/01/19/iceland-salmon-farmer-applies-for-10000t-expansion-eyes-sterility-tech/.

Shephard, S. and P. Gargan. 2017. Quantifying the contribution of sea lice from aquaculture to declining annual returns in a wild Atlantic salmon population. *Aquaculture Environment Interactions* 9:181–192.

Skaala, Ø., K.A. Glover, B.T. Barlaup, T. Svåsand, F. Besnier, M.M. Hansen, and R. Borgstrøm. 2012. Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. . *Canadian Journal of Fisheries and Aquatic Sciences* 69 (12):1994–2006.

Skilbrei, O. T., M. Heino, and T. Svåsand. 2015. Using simulated escape events to assess the annual numbers and destinies of escaped farmed Atlantic salmon of different life stages from farm sites in Norway. *ICES Journal of Marine Science* 72 (2):670–685.

Thorstad, E.B. and B. Finstad. 2018. Impacts of salmon lice emanating from salmon farms on wild Atlantic salmon and sea trout. *NINA Report* 1449:1–22.

Verspoor, E., P. McGinnity, I. Bradbury, and B. Glebe. 2015. The potential direct and indirect genetic consequences for native Newfoundland Atlantic Salmon from interbreeding with European-origin farm escapes. *DFO Can. Sci. Advis. Sec. Res. Doc* 2015/030:viii + 36.

Vitti, J.V., S.R. Grossman, and P.C. Sabeti. 2013. Detecting Natural Selection in Genomic Data. *Annual Review of Genetics* 47 (1):97–120.

Waples, R. K., W. A. Larson, and R. S. Waples. 2016. Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity* 117:233.

Waples, R.S. and C. Do. 2010. Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* 3 (3):244–262.

ANNEX_3 ToR B: Genomic selection applied to aquaculture species

ToR b – Review and report on principles of and prospects for genomic selection applied to aquaculture species

Duration: 2–3 years

Deliverables: (a) Review Paper; (b) Sea-Food Production Brief; (c) Publication.

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.

References

[1] Meuwissen THE, Hayes BJ, Goddard ME (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819–1829.

[2] Boichard D, Ducrocq V, Croiseau P, Fritz S (2016). Genomic selection in domestic animals: Principles, applications and perspectives. Comptes Rendus Biologies 339(7–8): 274–277. DOI: 10.1016/j.crvi.2016.04.007

[3] Desta ZA, Ortiz R (2014). Genomic selection: genome-wide prediction in plant improvement.r eTnds in Plant Science 19:592–601. doi: 10.1016/j.tplants.2014.05.006

[4] Heslot N, Jannink J-L, Sorrells ME (2015). Perspectives for Genomic Selection Applications and Research in Plants. Crop Science 55:1–12. DOI: 10.2135/cropsci2014.03.0249

[5] De los Campos G, Hickey JM, Pong-Wong R, Daetwyler, HD, Calus MPL (2013) Whole-Genome Regression and Prediction Methods Applied to Plant and Animal Breeding. Genetics 193:327–345. DOI: 10.1534/genetics.112.143313

[6] Robledo D, Palaiokostas C, Bargelloni L, Martínez P, and Houston R (2017). Applications of genotyping by sequencing in aquaculture breeding and genetics. Reviews in Aquaculture 1–13. doi:10.1111/raq.12193

[7] Ibañez-Escriche N and Gonzales-Recio O (2011) Review. Promises, pitfalls and challenges of genomic selection in breeding programs. Spanish Journal of Agricultural Research 9(2): 404–413.

[8] Hosoya S, Kikichi K, Nagashima H, Onodera J, Sugimoto K, Satoh K, Matsuzaki K, Yasugi M, Nagano AJ, Kamagayi A, Ueda K, Kutokawa T (2017). Genomic selection in aquaculture. Bull. Jap. Fish. Res. Edu. Agen. 45: 35—39.

[9] Palaiokostas C, Houston, RD (2018). Genome-wide approaches to understanding and improving complex traits in aquaculture species. CAB Reviews. DOI: 10.1079/PAVSNNR201712055.

[10] Zenger KR, Khatkar MS, Jerry DR, Raasdsma HW (2018). The next wave in selective breeding: implementing genomic selection in aquaculture. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 22: 105–112.

[11] Vallejo RL, Leeds TD, Gao G,

Parsons JE, Martin KE, Evenhuis JP, Fragomeni BO, Wiens, GD, Palti, Y (2017) Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. Genetics Selection Evolution 49:17. doi:10.1186/s12711–017–0293–6.

[12] Nielsen HM, Sonesson AK, Yazdi H, Meuwissen THE (2009). Comparison of accuracy of genome-wide and BLUP breeding value estimatesin sib based aquaculture breeding schemes. Aquaculture 289:259–264. doi:10.1016/j.aquaculture.2009.01.027

[13] Sonesson AK and Meuwissen THE (2009). Testing strategies for genomic selection in aquaculture breeding programs. Genetics Selection Evolution 41:37. DOI:10.1186/1297–9686–41–37

[14] Daetwyler HD, Pong-Wong R, Villanueva B, Woolliams JA (2010). The impact of genetic architecture on genome-wide evaluation methods. Genetics 185(3):1021–31. DOI: 10.1534/genetics.110.116855.

[15] Ødegård J, Yazdi MH, Sonesson AK, Meuwissen THE (2019) Incorporating Desirable Genetic Characteristics From an Inferior Into a Superior Population Using Genomic Selection. Genetics 181(2):737–745. DOI:10.1534/genetics.108.098160.

Aquaculture species for which is currently available a commercial SNP chip

Species	**Reference**
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	Illumina (reference in preparation)
Litopenaeus vannamei	Jones *et al.* 2017
Dicentrarchus labrax	Faggion *et al.* (in prep), Illumina iselect 3K Allal *et al.* (in prep), Affymetrix Axiom 57K

References

Gutierrez, A. P., Turner, F., Gharbi, K., Talbot, R., Lowe, N. R., Peñaloza, C., *et al.* (2017). Development of a Medium Density Combined-Species SNP Array for Pacific and European Oysters (Crassostrea gigas and Ostrea edulis). G3: Genes|Genomes|Genetics, 7(7), 2209–2218. http://doi.org/10.1534/g3.117.041780

Houston R. D., Taggart J. B., Cézard T., Bekaert M., Lowe N. R., *et al.* (2014) Development and validation of a high density SNP genotyping array for Atlantic salmon (Salmo salar). BMC Genomics 15: 90.

Jones, D. B., Jerry, D. R., Khatkar, M. S., Raadsma, H. W., Steen, H. van der, Prochaska, J., *et al.* (2017). A comparative integrated gene-based linkage and locus ordering by linkage disequilibrium map for the Pacific white shrimp, Litopenaeus vannamei. Scientific Reports, 7, 10360. http://doi.org/10.1038/s41598–017–10515–7

Liu, S., Sun, L., Li, Y., Sun, F., Jiang, Y., Zhang, Y., *et al.* (2014). Development of the catfish 250K SNP array for genome-wide association studies. BMC Research Notes, 7, 135. http://doi.org/10.1186/1756–0500–7-135

Palti Y., Gao G., Liu S., Kent M. P., Lien S., Miller M. R., *et al.* (2015). The development and characterization of a 57K single nucleotide polymorphism array for rainbow trout. Mol. Ecol. Resour. 15, 662–672. 10.1111/1755–0998.12337

Qi, H., Song, K., Li, C., Wang, W., Li, B., Li, L., & Zhang, G. (2017). Construction and evaluation of a high-density SNP array for the Pacific oyster (*Crassostrea gigas*). PLoS ONE, 12(3), e0174007. http://doi.org/10.1371/journal.pone.0174007

Xu J., Zhao Z., Zhang X., Zheng X., Li J, Jiang Y. *et al.* (2014) Development and evaluation of the first high-throughput SNP array for common carp (*Cyprinus carpio*). BMC Genomics 15: 307.

Yáñez J.M., Naswa S., López M.E. *et al.* (2016) Genome-wide single nucleotide polymorphism (SNP) discovery in Atlantic salmon (*Salmo salar*): validation in wild and farmed American and European populations. Molecular Ecology Resources 16, 1002–11.

ANNEX_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 (Supporting the EU landing obligation)

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

Duration: 3 years.

Deliverables: a) Review Paper; b) ICES Viewpoint.

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 fisherman 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 con-sumption purposes whilst prohibited species cannot be retained on board and must be returned to the sea. The discarding of prohibited species should be recorded in the logbook and forms an important part of the science base for the monitoring of these species. (https://ec.europa.eu/fisheries/cfp/fishing_rules).

It is generally acknowledged that the implementation of the landing obligation is a highly challenging and complex endeavour. For example, how can it be assured that no prohibited species have been landed and that undersized fish are in fact from the officially reported species, given that in both cases the landed biomass tends to be immediately processed for products that are not for direct human consumption? These potentially mixed species samples are very difficult to identify once they have been processed, especially when considering products like fish oil and gelatine. Genetic and genomic methods might help with the challenge of ensuring that these "by-products" only contain the undersized catches (or potentially non- commercial bycatch spe-cies) but no other, illegal-to-land, species that 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. Focussing 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.

References

Ben Ayed, R., Ben Hassen, H., Ennouri, K., Ben Marzoug, R., and Rebai, A. (2016). OGDD (Olive Genetic Diversity Database): a microsatellite markers' genotypes database of worldwide olive trees for cultivar identification and virgin olive oil traceability. Database *2016*, bav090.

Bradbury, I.R., Hamilton, L.C., Rafferty, S., Meerburg, D., Poole, R., Dempson, J.B., Robertson, M.J., Reddin, D.G., Bourret, V., Dionne, M., *et al.* (2015). Genetic evidence of local exploitation of Atlantic salmon in a coastal subsistence fishery in the Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences *72*, 83–95.

Bradbury, I.R., Hamilton, L.C., Chaput, G., Robertson, M.J., Goraguer, H., Walsh, A., Morris, V., Reddin, D., Dempson, J.B., Sheehan, T.F., *et al.* (2016). Genetic mixed stock analysis of an interceptory Atlantic salmon fishery in the Northwest Atlantic. Fisheries Research *174*, 234–244.

Cadrin, S.X., Bernreuther, M., Danıelsdottir, A.K., Hjorleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., *et al.* Population structure of beaked redfish, Sebastes mentella: evidence of divergence associated with different habitats. 14.

Castilho, R., Freitas, M., Silva, G., Fernandez-Carvalho, J., and Coelho, R. (2007). Morphological and mitochondrial DNA divergence validates blackmouth, Galeus melastomus, and Atlantic sawtail catsharks, Galeus atlanticus, as separate species. Journal of Fish Biology *70*, 346–358.

Dahle, G., Johansen, T., Westgaard, J.-I., Aglen, A., and Glover, K.A. (2018). Genetic management of mixed-stock fisheries "real-time": The case of the largest remaining cod fishery operating in the Atlantic in 2007–2017. Fisheries Research *205*, 77–85.

Guillen, J., Holmes, S.J., Carvalho, N., Casey, J., Dörner, H., Gibin, M., Mannini, A., Vasilakopoulos, P., and Zanzi, A. (2018). A Review of the European Union Landing Obligation Focusing on Its Implications for Fisheries and the Environment. Sustainability *10*, 900.

Gullestad, P., Blom, G., Bakke, G., and Bogstad, B. (2015). The "Discard Ban Package": Experiences in efforts to improve the exploitation patterns in Norwegian fisheries. Marine Policy *54*, 1–9.

Hauser Lorenz, and Carvalho Gary R (2008). Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish and Fisheries *9*, 333–362.

Helyar, S.J., Lloyd, H. ap D., de Bruyn, M., Leake, J., Bennett, N., and Carvalho, G.R. (2014). Fish Product Mislabelling: Failings of Traceability in the Production Chain and Implications for Illegal, Unreported and Unregulated (IUU) Fishing. PLoS ONE *9*, e98691.

Johansen, T., Westgaard, J.-I., Seliussen, B.B., Nedreaas, K., Dahle, G., Glover, K.A., Kvalsund, R., Aglen, A., and Handling editor: Stewart Grant (2017). "Real-time" genetic monitoring of a commercial fishery on the doorstep of an MPA reveals unique insights into the interaction between coastal and migratory forms of the Atlantic cod. ICES Journal of Marine Science.

Leal, M.C., Pimentel, T., Ricardo, F., Rosa, R., and Calado, R. (2015). Seafood traceability: current needs, available tools, and biotechnological challenges for origin certification. Trends in Biotechnology *33*, 331–336.

Nellemann, C., MacDevette, M., Manders, T., Eickhout, B., Svihus, B., Prins, A.., and Kaltenborn, B.. (2009). The environmental food crisis: the environment's role in averting future food crises: a UNEP rapid response assessment (Arendal, Norway: UNEP).

Saha, A., Hauser, L., Hedeholm, R., Planque, B., Fevolden, S.-E., Boje, J., Johansen, T., and Handling editor: W. Stewart Grant (2017). Cryptic Sebastes norvegicus species in Greenland waters revealed by microsatellites. ICES Journal of Marine Science *74*, 2148–2158.

Steinke, D., Bernard, A.M., Horn, R.L., Hilton, P., Hanner, R., and Shivji, M.S. (2017). DNA analysis of traded shark fins and mobulid gill plates reveals a high proportion of species of conservation concern. Scientific Reports *7*.

Zeller, D., Cashion, T., Palomares, M., and Pauly, D. (2018). Global marine fisheries discards: A synthesis of reconstructed data. Fish and Fisheries *19*, 30–39.

ToR C. ANNEX_3.1 – 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 sea waters fisheries lists the species for which the discard ban applies (for details see (Gullestad *et al.*, 2015)).

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_3.2 – Potential strategies for non-compliance and genetic testing

Reason	Problem	Potential Strategies	Genetics useful?
Legal	Catches exceed a quota	- labeled 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 labeled as different species	- YES, DNA barcoding
		- processed (or highly processed), mixed with other species and species ID hidden	- YES*, DNA meta-barcoding, ddPCR (quantification)
		- different catch area reported	- YES, SNPs/ microsatellites
Legal	Catches are below a	- labeled as different species (unprocessed	- YES, DNA barcoding

	minimum legal landing size	or fileted)	
		- 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 higher 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 hiden	- 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	- labeled as different species	- YES, DNA barcoding
		- processed and labeled as different species	- YES, DNA barcoding
		- processed and mixed with other species	- YES, DNA meta-barcoding, ddPCR
Economic	Catches are of non-commercial species	- labeled as different species	- YES, DNA barcoding
		- processed and labeled 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 fish oil validation studies will have to be carried out for species identification and quantification.

ToR C. ANNEX_3.3 – Highly processed products and their analysis

Product	Species ID	Quantification	Future Prospects
Fish fingers, fish cakes, surimi	YES, DNA metabarcoding	YES	Reference to be established.
Fish feed	YES, DNA metabarcoding	YES	Reference to be established.
Fish oil	MOST LIKELY, DNA metabarcoding	MAYBE, has not been done /	genetic database to allow the use of microsatellite-based approaches for the traceability of olive oil (Ben Ayed

		published	*et al.*, 2016)
Canned fish	YES, DNA metabarcoding	YES	Reference to be established.
Dried/salted fish	YES, DNA metabarcoding	YES	Reference to be established.

ANNEX_3 ToR D: eDNA in Fisheries Management and Ecosystem Monitoring

ToR d – eDNA in Fisheries Management and Ecosystem Monitoring

Duration: 3 years.

Deliverables: (a) Review paper; (b) Non-technical review topic sheet.

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, define population structures, begin to understand the genetic basis of adaptive traits and monitor adaptive population changes. Typically, such insights have been gained from analysis of DNA obtained from tissue samples collected directly from individuals across a study area. Additionally, the analysis of DNA through metabarcoding from a bulk sample composed of a mixture of individuals of different zooplankton and/or macroinvertebrate species has enabled more cost-effective biodiversity assessments. Recently however, a new source of DNA has begun to be used for analysis of macro species, so-called "environmental DNA" (eDNA), which relies on collection of DNA sloughed off from tissue (e.g. skin, blood, faeces, mucous, eggs) into the natural environment. This eDNA promises to revolutionise the examination of biodiversity in the wild by allowing the detection larger organisms without needing to sample them and may be of particular usefulness in the marine environment where traditional sampling is difficult to carry out.

A number of approaches using eDNA have been utilised already and/or are under development. These include species identification (especially useful for rare/cryptic/small individuals), community com-position, ecosystem monitoring, relative species abundance and even attempts at absolute species abundance. In the aquatic environment such techniques have often been developed in freshwater ecosystems but are now beginning to be utilised in the marine environment. As such there is a growing recognition that the use of eDNA in the marine sphere may in the near future bring powerful new tools to the arsenal of the fishery manager and also allow new approaches to ecosystem monitoring. However, there are also numerous caveats associated with eDNA approaches linked to sampling strategies, DNA stability in different environments, analytical approaches etc. that require expert attention to enable proper interpretation of study data. This ToR will summarise the research to date, identify areas where tools are already available for use and examine future developments whilst crucially seeking to also identify areas where the use of the new approaches should be undertaken with care if at all. The ToR will also try to produce a non-technical summary of the state of the field for direct dissemination to fishery managers with little or no genetic background.

References

Baldigo, B. P., L. A. Sporn, S. D. George, and J. A. Ball. 2017. Efficacy of Environmental DNA to Detect and Quantify Brook Trout Populations in Headwater Streams of the Adirondack Mountains, New York. Transactions of the American Fisheries Society 146:99–111.

Bass, D., G. D. Stentiford, D. T. J. Littlewood, and H. Hartikainen. 2015. Diverse Applications of Environmental DNA Methods in Parasitology. Trends in Parasitology 31:499–513.

Clusa, L., L. Miralles, A. Basanta, C. Escot, and E. García-Vázquez. 2017. eDNA for detection of five highly invasive molluscs. A case study in urban rivers from the Iberian Peninsula. PLoS ONE 12:e0188126.

Davy, C. M., A. G. Kidd, and C. C. Wilson. 2015. Development and Validation of Environmental DNA (eDNA) Markers for Detection of Freshwater Turtles. PLoS ONE 10:e0130965.

Ficetola, G. F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. Species detection using environmental DNA from water samples. Biology Letters 4:423–425.

Hänfling, B., L. Lawson Handley, S. Read Daniel, C. Hahn, J. Li, P. Nichols, C. Blackman Rosetta, A. Oliver, and J. Winfield Ian. 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Molecular Ecology 25:3101–3119.

Hansen, B. K., Bekkevold, D., Clausen, L., W., and Nielsen, E. E. 2018. The sceptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries. Fish and Fisheries, Online Early Access,

https://onlinelibrary.wiley.com/doi/abs/10.1111/faf.12286.

Huver, J. R., J. Koprivnikar, P. T. J. Johnson, and S. Whyard. 2015. Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. Ecological Applications 25:991–1002.

Levy-Booth, D. J., R. G. Campbell, R. H. Gulden, M. M. Hart, J. R. Powell, J. N. Klironomos, K. P. Pauls, C. J. Swanton, J. T. Trevors, and K. E. Dunfield. 2007. Cycling of extracellular DNA in the soil environment. Soil Biology and Biochemistry 39:2977–2991.

Peters, L., S. Spatharis, M. A. Dario, I. J. T. Roca, A. Kintner, O. Kanstad-Hanssen, M. S. Llewellyn, and K. Praebel. 2017. Environmental DNA: a new low-cost monitoring tool for pathogens in salmonid aquaculture. bioRxiv.

Pietramellara, G., J. Ascher, F. Borgogni, M. T. Ceccherini, G. Guerri, and P. Nannipieri. 2009. Extracellular DNA in soil and sediment: fate and ecological relevance. Biology and Fertility of Soils 45:219–235.

Shaw, J. L. A., L. Weyrich, and A. Cooper. 2017. Using environmental (e)DNA sequencing for aquatic biodiversity surveys: a beginner's guide. Marine & freshwater research 68:pp. 20–33–2017 v.2068 no.2011.

Sousa, R., A. Novais, R. Costa, and D. L. Strayer. 2014. Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. Hydrobiologia 735:233–251.

Stat, M., M. J. Huggett, R. Bernasconi, J. D. DiBattista, T. E. Berry, S. J. Newman, E. S. Harvey, and M. Bunce. 2017. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. Scientific Reports 7:12240.

Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology 21:2045–2050.

Glossary of technical terms relating to eDNA

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) of 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 sent to 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" at different taxonomic levels, 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.
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 utilisation 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 a cluster that is obtained after the end of the sequencing process which is ultimately the sequence of a section of a unique fragment

Annex 4: WGAGFA 2018 meeting agenda

Cover Photo: Credit – BMO; IFREMER Logo

Working Group on the Application of Genetics in Fisheries and Aquaculture - WGAGFA

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

Terms of Reference (Lead)

a) Review and report on genetic and genomic approaches for quantifying indirect genetics of salmon aquaculture on wild salmon populations (Ian Bradbury);
b) Review and report on principles of and prospects for genomic selection applied to aquaculture species (Pierre Boudry);
c) Assess and report on the value of genetic and genomic tools for identifying species in mixed landings, fish products and byproducts. - Supporting the Landing Obligation (Claudia Junge/Jann Martinsohn).
d) eDNA in Fisheries Management and Ecosystem Monitoring (John Gilbey)

Monday 14 May 2018

ARRIVAL

Tuesday 15 May 2018

09:00-09:30 **Arrival L'UIEM // Welcome & Housekeeping (Jann, Pierre, Greg) // Introduction and planning - Reporting guidelines and timeline (Jann)**

09:30-10:00 **ICES News; Fisheries and Aquaculture worldwide: Relevant events and developments (Jann)**

10:00-10:30 **Presentation (15' max.) of ToRa: Background; Scope; Aims (Ian)**

10:30-11:00 **Presentation (15' max.) of ToRb: Background; Scope; Aims (Pierre)**

11:00-11:30 **Presentation (15' max.) of ToRc: Background; Scope; Aims (Claudia)**

11:30-12:00 **Presentation (15' max.) of ToRd: Background; Scope; Aims (John)**

12:00-12:15 **Formation of ToR Subgroups**

12:15-13:15 **Lunch**

13:00-16:30 **Work on ToR's**

16:30-17:30 **Plenary: Summary of progress and observations (ToR leaders) / Conclusions (Jann)/ Wrap-up**

Evening: TBD

Wednesday 16 May 2018

09:00-09:30 **Arrival at L´UIEM // Welcome & Housekeeping (Jann, Pierre, Greg) // Introduction and planning (Jann) //**

09:30-10:30 **Work on ToR´s**

10:30-11:00 **Coffee Break**

11:00-12:30 **Work on ToR´s**

12:30-13:30 **LUNCH**

13:30-14:00 **ICES Requests and AOB**

14:00-16:30 **Work on ToR´s**

16:30-17:30 **Plenary: Summary of progress and observations (ToR leaders) / Conclusions (Jann)/ Wrap-up**

Evening: TBD

Thursday 17 May 2018

09:00-09:30 **Arrival at L´UIEM // Welcome & Housekeeping (Jann, Pierre, Greg) // Introduction and planning (Jann) //**

09:30-10:30 **Work on ToR´s**

10:30-11:00 **Coffee Break**

11:00-12:30 **Work on ToR´s**

12:30-13:30 **LUNCH**

13:30-16:00 **Work on ToR´s**

16:00-17:30 **Plenary: Summary of progress, leftovers and timeline, next meeting (ToR leaders/All) / Conclusions (Jann)/ Wrap-up**

Friday 18 May 2018

09:00-12:00 **TBD: Arrival at L´UIEM // PLENARY Conclusions and Planning ahead**

Departure and travel home

ANNEX: Practical Information