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

7–9 May 2014

Olhãu, Portugal

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Executive summary

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) convened in Olhãu, Portugal 7–9 May 2014. Members met to discuss and consider the five Terms of Reference (ToR) decided by the ICES Science Committee. The report contains the main issues discussed and the management recommendations for each of these ToRs. Dorte Bekkevold (Denmark) chaired the meeting, which opened at 09:00 on the 7 May and closed at 13.30 on 9 May. The meeting had 23 participants representing the European Joint Research Centre in Italy and Australia, Belgium, Canada, Denmark, France, Germany, Iceland, Norway, Portugal, Russian Federation, Spain, Sweden and UK. WGAGFM have established a three-year term for the chair, and it was the final year for the current chair's term. The members present at the meeting unanimously supported that WG member Professor Gary R. Carvalho become the next Chair for WGAGFM.

Members discussed the current status and way forward in integrating genomic methods with marine fisheries management. Fisheries biologists and managers have long acknowledged the importance of intraspecific diversity, as described for most exploited species, though management remains mostly based at the scale of large sea basins with fixed administrative boundaries and rectangular management areas. The latter geographically defined framework typically fails to match the biological structure of populations. It follows that in order to move towards sustainable fisheries, a central challenge is to incorporate spatial biological diversity into contemporary management schemes Moreover, population connectivity and dynamics must be reliably monitored to support management strategy implementation. Genomic methods provide one important tool to achieve this goal and members discussed cases incorporating such approaches with other relevant data in diverse fisheries management scenarios, showing that evolutionary thinking can add valuable information to the successful implementation of strategies to promote profitable and sustainable fisheries within an ecosystem context .Members found that the examples demonstrate the methods' relevance for a suite of management questions and recommend that ICES SCICOM and ACOM push for more standardized use of the methods as well as initiate that application of genetic methods are included in its training courses.

WGAGFM received an advice request from OSPAR (4/2014) on "Interactions be-tween wild and captive fish stocks". WGAGFM contributed information on genetic effects and potential management solutions to mitigate adverse impact. Several studies have demonstrated that the gene pools of wild populations change when hatchery produced farm fish escape (or are released) at large-scales. Several studies also report that introgression by escaped farm fish can incur a fitness cost to wild populations, causing increasing concern for the continuing health and viability of wild populations and awareness about conserving native fish gene pools. Knowledge is mainly based on salmonids fish but should be transferrable to fully marine organisms, making aquaculture escapees a general concern. Molecular quantification has proved valuable for demonstrating introgression by farm fish. However, WGAGFM reviewed studies and found that in many cases, the introgression process is complex, e.g. with respect to escape rates and genetic make-up of escapees, and impacts can therefore be difficult to assess and predict. Members concluded that in order to develop and implement reliable management strategies and advice, locally and internationally, it is of importance to consider on a case-by-case basis the different options for the analysis of genetic data to quantify level of introgression.

Following on from work initiated in 2013, members discussed the application of genetic methods in shellfish. Invertebrates such as shellfish of interest in an aquaculture context have very different life histories compared to finfish and these characteristics mean that the transfer of technology and selection approaches from the finfish industry to the shellfish one is not always simple or even possible. However, this emphatically does not mean that the general principle of identifying adaptive markers and utilizing them in the scenarios outlined above cannot result in benefits to both industry and wild populations. Recent developments in genetic screening techniques (e.g. Next-Generation Sequencing and genome sequencing) promise even greater power to identify markers linked to traits of interest and the incorporation of such techniques should be encouraged in the shellfish aquaculture context.

1 Opening of the meeting

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met in Olhão 7–9 May 2014. The Terms of Reference (ToR) were decided by ICES Science Committee in Reykjavik, Iceland, in 2013. Dorte Bekkevold (Denmark) chaired the meeting, which opened at 09:00 on Wednesday, 7 May and closed at 13.30, Friday, 9 May 2014.

The meeting had 23 participants of which 17 were members, representing the European Joint Research Centre and 12 member nations (Belgium, Canada, Denmark, France, Germany, Iceland, Norway, Portugal, Russian Federation, Spain, Sweden and UK; Annex 2).

The meeting was hosted by the University of Algarve, Centre of Marine Sciences in Faro. The meeting was very well organized and we are gratful to local host Dr Rita Castilho, whose hospitality and kind and efficient assistance never tired.

WGAGFM has an established framework for completing its ToR. Prior to the meeting, small *ad hoc* working groups, under the leadership of one or more persons, are established to prepare position papers related to specific issues in the Terms of Reference. The leader(s) of each ToR is responsible for presenting the position paper in plenary at the meeting and chairing the discussion. Thereafter, volunteers undertake the task of editing and updating position papers according to points raised in the plenary discussions. The ToR leader(s) is responsible for preparing the final report text from their sessions. Prior to the meeting the agenda is circulated to all members.

2 ToR a) Identification and use of adaptive gene markers in shellfish aquaculture and for the genetic characterization of wild populations – issues and solutions.

John Gilbey, Sara Bonanomi, Pierre Boudry

2.1 Introduction

There is an increasing pressure for sustainable aquaculture of many finfish and shellfish species in Europe and worldwide. The development of DNA-based genetic techniques has had a revolutionary effect on aquaculture of many finfish species. Genetic approaches have and are being used to examine many different questions of importance to this industry and of particular focus has been the identification and use of adaptive markers. There is evidence of local adaptation in many species of fish and shellfish and locally adapted populations are often characterized by heritable traits allowing them to survive and thrive under heterogeneous environmental conditions (Sanford and Kelly, 2011). Some such traits are often of particular interest in relation to aquaculture production (e.g. those associated with growth-rates, survival to biotic or abiotic factors). The fast developing field of genomics offers a potential to identify the necessary markers linked to such adaptive traits (Stinchcombe and Hoekstra, 2007). Once identified these markers can be utilized in Marker Assisted Selection (MAS) programmes which seek to identify individuals exhibiting beneficial genetic variants and using these as broodstock to achieve enhancement of the selected line within the aquaculture environment (Lande and Thompson, 1990; Dudley, 1992; Poompuang and Hallerman, 1997; Ribaut and Hoisington, 1998).

Identification of such adaptive markers relies on techniques which link genetic variation to heritable phenotypic variation at traits of interest. Typically this involves genome scans using one of a number of genetic markers (outlined below) of a number of individuals which differ in the trait of interest. Various statistical approaches can then be used to identify those markers linked to the trait and if data are available to position this marker on the genome of the species (Lynch and Walsh, 1998).

In theory therefore it might be expected that the same or similar techniques could be used to identify and utilize such markers in the shellfish aquaculture industry. However, there are a number of characteristics of shellfish species which have to be taken into account when performing such investigations in these species and which may mean that different approaches may have to be used than those typically employed with finfish.

First, and perhaps most importantly, many shellfish aquaculture species still reply on collection of individuals from the wild for growing on (i.e. seed collection). In many of these species therefore there will be no selected lines to work with unlike in the finfish context. Selective improvement of lines using techniques such as MAS cannot be envisaged. This does not mean however that both the identification and use of adaptive markers is also impossible. Rather than using selected lines as is often the case in the finfish context, wild individuals which still differ in the trait of interest can be examined, genotyped and adaptive markers identified. Particular populations of individuals which show beneficial phenotypes can then be targeted for the collection of individuals for growing on with such populations being identified using the available adaptive markers.

Again however the particular life-history characteristics of many shellfish species means that development of this local adaptation that may be utilized in such stock selection programmes may be different from that seen with many finfish species. Many bivalves exhibit only weak genetic structure, or even panmixia, when examined using neutral markers which indicates significant gene flow between populations and which may suggest a lack of local adaptation. Further, the high fecundity, broadcast spawning and pelagic larval phases might all act to prevent local adaptive selection occurring. However, it should also be noted that the often large shellfish populations have substantial within-population genetic variation which provides opportunities for natural selection in different ecological settings and thus has the potential to result in local adaptation. Such adaptation has already been reported in a number of invertebrate species of interest to the aquaculture industry. For example, Pespeni *et al*. (2012) reported significant differentiation at functional genes between populations of the purple sea urchin (*Strongylocentrotus purpuratus*), Riginos and Cunningham (2005) showed strong evidence of local adaption in the *Mytilus* spp. complex even at small spatial scales (Yanick *et al*. 2003), and Sanford and Worth (2010) showed local adaption in the snail *Nucella canaliculata* using reciprocal translocation experiments.

Another area where adaptive markers may prove useful in the shellfish context is in both species identification and the identification of introgression. Typically, such investigations have relied on the use of various neutral markers (i.e. those not known to be linked to adaptive traits) diagnostic of the species in question. However in a number of closely related shellfish species such markers are not available. It may be that in these closely related species markers linked to divergent adaptive traits will show the highest discriminatory power and so could be targeted in such investigations (see Twyford and Ennos, 2012 and references therein).

The differing characteristics of wild finfish and shellfish populations also mean that the potential impacts of interactions between wild and aquaculture stocks may be very different. As has previously been mentioned, unlike most finfish scenarios, shellfish production is typically based on collection of individuals from the wild. It is unclear therefore just what negative fitness impacts will arise if these same individuals later are released or 'escape' back into these same wild environments. It could be envisioned that selection may act on the collected individuals such that individuals are selected that do well in the farm context, and that a large input of these may have the potential to negatively impact their originator wild populations, but currently there is no evidence of this occurring. Indeed it may be that another feature of wild shellfish populations may also act to negate this potential influence and that is the very large effective population sizes of the wild shellfish populations.

Typically, and compared to many finfish species in aquaculture, shellfish have enormous effective population sizes often approaching or at infinity when measured using the usual population genetic techniques. Release of hundreds or even thousands on individuals from the aquaculture environment back into such large wild populations may therefore be expected to have less impact than such interactions with much smaller finfish populations (e.g. salmonids). However, modelling work has been carried out in hatchery lines of scallops which suggests that as is often the case with hatchery lines of fish and shellfish, the lower levels of genetic diversity exhibited in these stocks has the potential to reduce the effective population size of wild stocks if escapes or introductions occur (Hold *et al*. 2013). It is often difficult however to be able to measure such effects using traditional neutral markers, and again here it may be the case that the discriminatory power that can potentially be obtained using adaptive markers will help examine such issues.

As in the finfish aquaculture context therefore it can be seen that the use of adaptive markers has the potential to help in a number of different areas related to both shellfish aquaculture and cultured/wild interactions, but that the methods employed may differ from those in the finfish industry due to the particular characteristics of the shellfish species used. The WGAGFM report of 2013 (ICES 2013) suggested a two year programme should be initiated to examine the use of adaptive genetic markers in the shellfish aquaculture context:

1) Use the internal expertise of the WGAGFM members to
   - identify the full set of genomic tools and techniques available and/or under development
   - identify specific issues/situations that are being examined using the tools
   - identify particular issues/situations of concern that may benefit from research using the tools

2) Approach the ICES Working Group on Aquaculture (WGAQUA) to
   - outline the tools available and/or under development
   - identify issues of concern in the culture situation and those associated with mariculture/wild interactions which may be addressed using the tools

3) Approach researchers outside the ICES environment to
   - identify novel tools and techniques now under development
   - identify situations in which the genomic tools available and/or under development may be of use

The current report addresses part 1 of these action points. A brief review and description of the relevant genetic tool-kit available will be outlined, previous and current work being undertaken in the subject area will be collated, and finally situations discussed where the tool-kits and techniques available will be evaluated.

2.2 Genetic Tool-Kit

A number of molecular markers have been used in shellfish aquaculture, among the most popular being: allozymes, RFLP (Restriction fragment length polymorphism), mtDNA (mitochondrial DNA), SSR microsatellite polymorphism and SNP (or Single nucleotide polymorphism). Allozymes are allelic variants of proteins produced by a single gene locus, and are of interest as markers because polymorphism exists and because they represent protein products of genes which may be acted on directly by selective pressures. Several authors have reported the occurrence of selective phenomena for certain allozymes (Ben-Schlomo and Nevo 1988; Lavie and Nevo 1986). These markers have had wide applications in aquaculture genetics, including inbreeding, population analysis and hybrid identification.

Restriction fragment length polymorphisms (RFLP), were regarded as the first shot in the genome revolution (Botstein *et al*., 1980; Dodgson *et al*., 1997), marking the start of an entirely different era in the biological sciences. Digestion of DNA with restriction enzymes results in fragments whose number and size can vary among individuals, populations, and species. The major strength of RFLP markers is that they are co-dominant markers, i.e. both alleles in an individual are observed in the analysis. Because the size difference is often large, scoring is relatively easy. The major disadvantage of RFLP is the relatively low level of polymorphism. Moreover, the potential power of RFLP markers in revealing genetic variation is relatively low compared to more recently developed markers.

Mitochondrial DNA (mtDNA) represents only a tiny fraction of organismal genome size, yet it has been one of the most popular markers of molecular diversity in animals over the last three decades. Mitochondrial DNA (mtDNA) has a number of specific biological properties, which should make it an appropriate marker of molecular biodiversity (Harrison 1989; Galtier *et al*. 2009). First, its inheritance is clonal (maternal), which means that the whole genome behaves as a single, no recombining locus all sites share a common genealogy. This considerably simplifies the representation and analysis of within species variation data. Secondly, mtDNA has been supposed to evolve in a nearly neutral fashion. Finally, and not independently, the evolutionary rate of mtDNA has been frequently assumed to be clock-like in the absence of any mutations spreading through positive selection, only neutral (and slightly deleterious) mutations accumulate in time, so that mtDNA divergence levels should roughly reflect divergence times. Clonal, neutral and clock-like: mtDNA apparently stands as the ideal witness of population and species history (Birky *et al*. 1989; Moritz 1994).

Microsatellites have often become the marker of choice for application in fish and shellfish population genetic studies (Beckmann and Soller 1990; Cruz *et al*. 2005). They have multiple alleles which are highly polymorphic among individuals. The polymorphism obtained with microsatellite markers has provided powerful information to be considered in the management of fish stocks (Alam and Islam, 2005), population analysis and biodiversity conservation (Romana – Eguia *et al*., 2004). Microsatellites are preferable because they are potentially codominant and highly polymorphic. In addition these markers have a wide distribution in the genome and can be efficiently identified, which

is essential in studies about genetic variability of populations (Boris *et al*., 2011). Microsatellite markers are ideal for many types of applications in aquaculture. They give crucial information in aquaculture fish population, such as: (i) identification of genetic variability between and within stocks; (ii) monitoring genetic changes in stocks; (iii) parentage and pedigree analysis in selective breeding;(iv) genomic mapping and detection of quantitative trait loci (QTL; Hutchinson *et al*. 2001; Chistiakov *et al*.,2005, Sauvage *et al*., 2010).

Single nucleotide polymorphism (SNP) describes polymorphisms caused by point mutations that give rise to different alleles containing alternative bases at a given nucleotide position within a locus. Several approaches have been used for SNP discovery including single-strand conformational polymorphism analysis (SSCP, Reed and Wittwer 2004), heteroduplex analysis (Palais *et al*., 2005), and direct DNA sequencing. DNA sequencing has been the most accurate and most used approach for SNP discovery. These methods consist of a number of laborious steps that make SNP discovery complex and expensive. However, after detection the SNP analysis is fast, cheap and it offer unprecedented insights for tracking locally adapted populations in space and time (Stapley *et al*., 2010; Therkildsen *et al*., 2013). One of the major benefits of SNP markers is their frequency across the genome. As such a large number of markers can be investigated (i.e. often hundreds of thousands or even millions) and SNPs identified linked to traits of interest. SNP markers thus offer potentially greater power than many of the other marker types due to their linkage with adaptive traits which may be under selection.

The choice of marker for a particular application will vary and depend on a number of variables including the availability of the marker type in the species under investigation, how polymorphic is the marker, the technology available, the speed of genotyping, the ease and accuracy of scoring, the degree of genome coverage required, the level of discriminatory power required and of course cost. Table 1 summarized some of the techniques often used to examine issues in the shellfish aquaculture industry and makes some suggestions as to possible marker types that may be of most use.

Table 1. Suggested marker systems for aquaculture genetics (after Liu and Cordes, 2004).

Tasks	Recommended marker system	Other useful marker types
Species identification	mtDNA, SNPs	AFLP, allozymes
Strain identification	mtDNA, microsatellites, SNPs	RAPD, AFLP
Hybrid identification	mtDNA, microsatellites, SNPs	RAPD, AFLP
Paternity determination	Microsatellites, SNPs	
Genetic resource/diversity analysis	mtDNA, microsatellites, SNPs	RAPD, AFLP, allozymes
Genetic mapping	SNPs	Microsatellites, AFLP, RFLP
Comparative mapping	SNPs	Microsatellites, AFLP, RFLP

2.3 Adaptive Genetics and shellfish aquaculture: review

In both the reared and the wild situations adaptive genetic markers have been utilized to examine a number of questions related to shellfish aquaculture and the interaction of aquaculture with wild stocks. Such marker resources however differ greatly between the different species under culture. In some cases complete genomes have been

sequenced, while in others few if any markers are available. Table 2 summarizes genetic investigations in shellfish aquaculture situations.

Table 2. Marker and mapping resources available for shellfish species in aquaculture.

Species	Genomic resources			
	Marker types	Genome sequence	EST libraries	QTL maps
Crassostrea gigas (Pacific oyster)	- Allozyme [1] - AFLP [6] - mtDNA [7] - RFLP [8] - Microsatellite [9] - SNP [10]	Yes [2]	Yes [3]	Yes [4, 5]
Crassostrea virginica (Eastern oyster)	- AFLP [11] - mtDNA [14] - Microsatellite [11] - SNP [15]		Yes [11,12]	Yes [13]
Mytilus edulis (Blue mussel)	- AFLP [16] - mtDNA [18] - Microsatellite [19] - SNP [20]		Yes [17]	Yes [16]
Mytilus galloprovincialis (Mediterranean mussel)	- mtDNA [21] - RFLP [21] - Microsatellite [25] - SNP [26]		Yes [22, 23]	Yes [24]
Dreissena polymorpha (Freshwater mussel)	- AFLP [27] - mtDNA [30] - RFLP [30] - Microsatellite [31]		Yes [28, 29]	
Ostrea edulis (European flat oyster)	- Allozyme [32] - AFLP [34] - Microsatellite [35]			Yes [33]
Aequipecten irradians (Atlantic bay scallop)	- AFLP [36] - mtDNA [39] - RFLP [39] - Microsatellite [38]		Yes [37]	Yes [38]
Pecten maximus (King scallop)	- mtDNA [40] - RFLP [40] - Microsatellite [43]		Yes [41, 42]	
Mercenaria mercenaria (Hard clam)	- Allozyme [44, 45] - Microsatellite [47]		Yes [46]	
Solen marginatus (Razor clam)	- Allozyme [48] - RFLP [49] - Microsatellite [50]			
Aequipecten opercularis (Queen scallop)	- Allozyme [51] - mtDNA [52] - RFLP [52] - Microsatellite [53] - SNP [54]			
Venerupis senegalensis (Pullet carpet shell)	-RFLP [55] -RAPD [56]			

[1] McGoldrick & Hedgecock 1997, [2] Zhang et al. 2012, [3] de Lorgeril et al. 2011, [4] Sauvage et al. 2010, [5] Guo et al. 2012, [6] Li & Guo 2004, [7] Cordes et al. 2008, [8] Okimoto et al. 2008, [9] Hubert & Hedgecock 2004, [10] Zhong et al. 2013, [11] Yu & Guo 2003, [12] Wang et al. 2009, [13] Yu et al. 2006, [14] Milbury & Gaffney 2005, [15] Quilang et al. 2007, [16] Lallias et al. 2007a, [17] Tanguy et al. 2008, [18] Stewart et al. 1995, [19] Presa et al. 2002, [20] Zbawicka et al. 2012, [21] Ladoukakis et al. 2002, [22] Craft et al. 2010, [23] Venier et al. 2009, [24] Mizi et al. 2005, [25] Diz & Presa 2008, [26] Vera et al. 2010, [27] Rajagopal et al. 2009, [28] Xu & Faisal 2009a, [29] Xu & Faisal 2009b, [30] Baldwin et al. 1996, [31] Astanei et al. 2005, [32] Saavedra & Guerra 1996, [33] Lallias et al. 2009, [34] Lallias et al. 2007b, [35] Launey et al. 2002, [36] Wang et al. 2007, [37] Zhan et al. 2005, [38] Li et al. 2012, [39] Blake & Graves 1995, [40] Wilding et al. 1997, [41] Johnston 2006, [42] Biscotti et al. 2007, [43] Watts et al. 2005, [44] Adamkewicz et al. 1984, [45] Hadley et al. 1991, [46] Perrigault et al. 2009, [47] Wang et al. 2010, [48] Hmida et al. 2012, [49] Fernández-Tajes & Méndez 2007, [50] Francisco-Candeira et al. 2007, [51] Beaumont 1991, [52] Fernandez-Moreno et al. 2008, [53] Arias et al. 2010, [54] Arias et al. 2009, [55] Fernández et al. 2002, [56] Joaquim et al. 2010.

2.4 Scenarios of interest

2.4.1 Species identification and cryptic species

Species identification in shellfish can be challenging due to plasticity of morphometric traits (e.g. shell morphology, soft tissues), general lack of information about a very diverse group of species or due to cryptic species that are very difficult to distinguish. Some closely related species can hybridize in the wild, generating zones were species identification of pure and hybrid individuals requires the use of molecular markers.

Proper identification of species is essential to be able to describe their respective geographic range, habitats and environmental preferences. There are numerous examples where genetic markers have been proposed to help distinguishing well known species or to define/distinguish cryptic species. In some cases, the taxonomic distinction of specimens or subspecies is in some cases not in agreement with phylogenetic data or is at least matters of discussions. After initial allozyme studies, molecular data from the 16S rRNA or Cytochrome Oxydase mitochondrial genes have been widely used to identify species and is commonly known as DNA barcoding.

Table 3. Molecular markers-based studies aiming to differentiate presumed cryptic species.

Studied species	Type of marker used	References
Pecten maximus / P. jacobeus	16S rRNA mitochondrial gene sequencing ; allozymes	Canapa et al., 2000 , Rios et al.,
Mytilus edulis / M. galloprovincialis / M. trossulus	Many	See Koehn, 1991 ; Bierne et al, 2003$_{a,b}$, Riginos & Cunningham, 2005 and references within these[1].
Crassostrea gigas / *Crassostrea angulata*	mitochondrial DNA RFLP and sequencing, microsatellites	O'Foighil et al., 1998, Boudry et al., 1998, Huvet et al., 2000, 2004.
Ostrea edulis /O. stentina	Allozymes	Gonzalez-Wanguemert et al.
Cerastoderma edule / C. lamarcki	RAPD	Andre et al., 1999

[1] There are numerous studies examining *Mytilus* spp. differentiation.

2.5 General population differentiation over the species range

Global population genetic differentiation results of several factors acting in different ways (gene flow, drift, selection etc.). Studies aiming to assess population genetic differentiation over large geographic zones, potentially covering the whole range of a given species, can identify discontinuities in population clustering (e.g. the Almeria-Oran front) or continuous variation which can result from isolation by distance (e.g. the European flat oyster, Diaz-Almela *et al*. 2004). Clinal variation of markers along environmental clines can also be indicative of local adaptation. These can however result from other evolutionary phenomena as well such as secondary contacts zones leading to endogenous incompatibilities (e.g. in mussels: Boon *et al*., 2009, Bierne *et al*., 2011).

2.5.1 Local population differentiation for traits of aquaculture interest

Aquaculture production relies on supply of juveniles that can either come from natural recruitment (i.e. seed collection) or hatcheries. The geographic origin of juveniles might influence their performance in two ways: local environmental conditions can influence the physiological or disease status of individuals (i.e. phenotypic plasticity) and local genetic adaptation can lead to genotypes that are better adapted to later aquaculture conditions. Detangling environmental and genetic variation between seed collection

sites is challenging and requires common garden experiments that have seldom been performed in shellfish.

Genetic markers can contribute identifying adaptive traits thought genome scans (see for review Boudry *et al*., 2011, Rohfritsch *et al*., 2013) and genome wide association studies (GWAS). One of the major limitations of such approaches is the relatively poor genome annotation of most shellfish species. Functional genomic studies of candidate genes can also contribute to establish direct links between genes and traits (e.g. RNAi in the Pacific oyster, Fabioux *et al*., 2009, Huvet *et al*., 2012).

Table 4. Selected studies reporting presumed genetically-based differences between natural populations of cultured shellfish.

Species	Studied trait(s)	References
Ostrea edulis	Resistance to bonamiosis	Culloty et al., 2004
Mya arenaria	Resistance to saxitonin	Bricelj et al., 2005
Crassostrea gigas / Crassostrea angulata	Growth, survival, reproductive allocation	Soletchnik et al, 2002
Crassostrea virginica	Growth	Dittman et al. 1998
Mytilus edulis / Mytilus galloprovincialis	Many	See Koehn, 1991 ; Riginos & Cunningham, 2005 and references within these[1]

2.5.2 Selective breeding and stock improvement

Relative to finfish, selective breeding of shellfish is more recent and less developed. This is notably due to the use of natural recruitment as major (or even sole) source of juveniles in many cultured shellfish species. Genetic Improvement through selective breeding has been imitated in some species, for which hatchery production of juveniles is well mastered and represents a significant part of the seed supply. Targeted traits included general production traits such as growth or yield but special attention was given to disease resistance (Boudry *et al*. 1997). Resulting selected stock or lines have been used for QTL mapping studies (see for review Boudry *et al*., 2008). Validation of QTLs in different lines is needed before their eventual use in marker assisted selection programs (MAS).

2.5.3 Conservation and restoration of shellfish populations

Due to the high fecundity of most cultured shellfish species, mass production of juveniles in hatcheries can be achieved using small numbers of parental individuals. Furthermore, high variance of reproductive success has been reported in shellfish (Pacific oyster: Boudry *et al*., 2002; European flat oyster: Lallias *et al*., 2010).

The resulting progenies commonly show effective population sizes that are much lower than corresponding wild populations. Aquaculture production of these hatchery-propagated stocks is most often done in open waters were cultured stocks can reproduce in contact with wild or naturalized stocks. Individuals being cultured in bags (oysters) or cages (abalones), attached on ropes (mussels) or just placed in the natural environment (bottom culture), their likelihood to be "lost" in the wild is far for negligible. As a result, the notion of "escape" of cultured shellfish and their potential impact on wild populations is clearly different than for most finfish in aquaculture.

The first potential impact of genetic interaction between wild and hatchery-propagated stocks is a global loss of genetic diversity. Massive seeding of hatchery stocks exhibiting low genetic diversity may decrease local diversity. Gaffney (2006) reviewed the

effect of hatchery supplementation on the effective population size of a recipient wild population (Ryman-Laikre effect) is in light of the population biology of bivalve molluscs. According to simulation results, such impact appears to be minimal.

Table 5. Selected studies reporting potential impact or enhancement of hatchery-propagated shellfish on wild populations.

Species	Genetic markers		
Pecten maximus	microsatellites	Hold et al., 2013	Simulations based on a Ne estimates in one wild and one hatchery population.
Crassostrea virginica	mtDNA, microsatellites	Hare et al., 2006 Carlsson et al., 2008	Low enhancement success
Venerupis senegalensis	RAPD	Joaquim et al., 2010	Comparison between two wild populations for further restocking actions.

2.6 Summary

Invertebrates such as shellfish of interest in an aquaculture context have very different life histories compared to finfish and these characteristics mean that the transfer of technology and selection approaches from the finfish industry to the shellfish one is not always simple or even possible. However, this emphatically does not mean that the general principle of identifying adaptive markers and utilizing them in the scenarios outlined above cannot result in benefits to both industry and wild populations. Recent developments in genetic screening techniques (e.g. Next-Generation Sequencing and genome sequencing) promise even greater power to identify markers linked to traits of interest and the incorporation of such techniques should be encouraged in the shellfish aquaculture context.

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3 Term of Reference b): Review and consider methods for integrating genomic methods with marine fisheries management

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3.1 Content

1. Introduction

While management of commercially exploited marine living resources aims at maximizing yield, profit and employment opportunities, these goals have to be reconciled with long-term sustainability as well as the maintenance of coastal and marine ecosystem health. Such thinking underpins many fisheries management and policy frameworks worldwide. The recently reformed Common Fisheries Policy (CFP; REGULATION (EU) No 1380/2013) stipulates that until 2015 the exploitation of marine living resources should be adapted such that populations of harvested stocks are maintained above levels that can produce the maximum sustainable yield (MSY). The legislation also puts much emphasis on the need to introduce the ecosystem approach to fisheries management (EAFM) and introduces a discard ban, the so called "landing obligation". Identical and similar provisions are embedded in fisheries legislation of other countries such as the US (Magnuson–Stevens Fishery Conservation and Management Act), Canada (Fisheries Act R.S.C., 1985, c. F-14 and Coastal Fisheries Protection Act), Norway (The marine resources act - Act of 6 June 2008 no. 37[1]), and Russia (**Federal Law No. 166-FZ on Fisheries and Conservation of Aquatic Biological Resources and Federal Law No. 7-FZ on Environmental Protection**). For the EU, additional challenges arise through the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC), which is linked to the Common Fisheries Policy (CFP), whereby good environmental status (GEnS) of the EU's marine waters must be reached by 2020. In order to achieve GEnS by 2020, each Member State is required to develop a strategy for its marine waters based on 11 descriptors. Descriptor 3 aims at the protection of commercially exploited fish and shellfish[2].

As with all biota, marine fish species are fragmented to varying degrees, into a series of locally interbreeding populations. The extent to which such populations differ biologically ("population diversity") and their distribution in time and space are among the most important drivers of species' survival and persistence in response to environmental change and also anthropogenic pressure (Schindler *et al.*, 2010). Fisheries biologists and managers have acknowledged and endorsed such thinking since the early

[1] http://www.fiskeridir.no/english/fisheries/regulations/acts/the-marine-resources-act

[2] MSFD descriptor 3: "Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock."

20th century, though management remains mostly based at the scale of large sea basins with fixed administrative boundaries and rectangular management areas. The latter geographically defined framework typically fails to match the biological structure of populations (e.g. Reiss *et al.*, 2009). It follows that in order to move towards sustainable fisheries, a central challenge is to incorporate spatial biological diversity into contemporary management schemes Moreover, population connectivity and dynamics must be monitored to support management strategy implementation. Genetic methods provide one important tool to achieve this goal.

As discussed previously (Martinsohn *et al.*, 2011; ICES, 2013a; Ovenden *et al.*, 2013a) fisheries genetics has clearly come of age. State-of-the-art genetic and genomic approaches are suited to address a plethora of fishery management relevant questions from basic species identification (e.g. for Ichthyoplankton analysis carried out for stock assessment) and stock (population) structure analysis, to more complex themes such as mixed-stock analysis (e.g. Bekkevold *et al.*, 2011) and ecosystem monitoring (ICES, 2013a). The general acknowledgement that genetics/genomics are valuable to fisheries management is also manifest by their increasingly frequent occurrence in fishery/aquaculture advisory documents and even legislation[3]. In its most recent review of scientific advice the Scientific, Technical and Economic Committee for Fisheries (STECF, 2014) stipulates that "the term 'stock' in some cases, may not reflect a likely biological unit, but rather a convenient management unit". Interestingly, in the same report, genetic analysis in the context of stock characterization and revision is referred to in many instances (see Table 1), and the report specifically states that "STECF suggests that, in order to provide rational fisheries based advice, there is a need to define groupings, which have a spatial coherence that facilitates management. STECF further suggests that continued efforts should be made to define biological units based on, for example, genetic studies."

Perhaps the biggest challenge is to find ways to truly integrate genetic data into current stock assessment and modelling frameworks, including Management Strategy Evaluation (MSE). First attempts have been made, including members of the ICES WGAGFM, during a recent interdisciplinary meeting on the JRC Assessment for All (a4a) initiative (see a4a kick-off report 2012, available at https://fishreg.jrc.ec.europa.eu/web/a4a), which was also presented at the World Conference on Stock Assessment Methods for Sustainable Fisheries (WCSAM, Boston, 2013), co–organized by ICES. However, further international effort is still needed to reduce prevailing barriers between fisheries genetics and other spheres of fishery science (Waples *et al.*, 2008). ICES is ideally placed to catalyse linkages between these traditionally rather separated domains. The current ToR provides a general synthesis of data that can be delivered by genetics and genomics in the context of current fisheries management schemes. Evidence is presented from salient examples incorporating such approaches that genetic data can be integrated readily with other relevant data in diverse fisheries management scenarios. Consequently, genetics and evolutionary thinking can add valuable information to the successful implementation of strategies to promote profitable and sustainable fisheries within an ecosystem context.

[3] An example is Council Regulation (EC) No 1224/2009 establishing a Community control system for ensuring compliance with the rules of the Common Fisheries Policy. In Article 13 is an explicit reference to genetic technology.

2. Data needed for stock assessment

A central early step in the management process is the identification of units used in the assessment models. For most stocks with relatively long assessment history, the assessment units have usually been defined decades ago, at a time when relevant genetic information was scarce. At designated benchmark meetings, new biological information of relevance to stock identity is often reviewed. While the statistical areas used for data collection are rarely changed, the meetings may result in the identification of new assessment and management units. This was recently the case for plaice (*Pleuronectes platessa*) in the Kattegat/Skagerrak, where the benchmark report recommended that Skagerrak should be included with the North Sea for assessment (ICES, 2012a). However, changes in established management units are generally problematic from a practical perspective, due to administrative issues such as historical regulation systems and quota distribution keys between countries, where temporal stability in management units is preferred.

For re-defined stocks where assessment and management systems are less well established, the most up to date genetic information plays an important role in defining stock areas (e.g. flounder in the Baltic Sea; ICES, 2014a). In the simplest cases, biological populations match management areas. However, often the cases are more complex and may include mixing of biological populations within management areas (Reiss *et al.*, 2009; Eero *et al.*, 2014). Here, assessment will need to account for complexity across assessment borders.

Stock assessments are typically conducted through fitting population dynamics models to estimate population size at age in a given management unit. The core information used in analytical stock assessment models include fisheries catch numbers by age and relative abundance indices from research surveys, supplemented by biological information on mean weight and maturity stage of individuals by age. To provide management advice, such quantitative assessments are often combined with forecasts of yield under different exploitation scenarios. Most assessment models aim at considering data within a single assessment/management unit (or combine data from several units). Thus, although biased assessments may arise from failure to incorporate spatial complexity (Berger *et al.*, 2012), few models have actually been developed to handle several assessment units simultaneously, or complex mixture scenarios by including information from more than one biological unit (e.g. Goethel *et al.*, 2014).

One option in mixture scenarios is to split the biomass into separate units, which are then allocated to units, and assessed separately. This is currently the case for Atlantic herring in the Kattegat/Skagerrak area, where otolith shape and - microstructure of individual herring is used to allocate catches to either the North Sea Autumn Spawning stock or the Western Baltic Spring-spawning stock (ICES, 2013b). Here, catches can be allocated to different units (assessments) because the mixture area is a feeding ground, and individuals are therefore expected to contribute to recruitment in their native spawning populations in the western Baltic Sea and the Atlantic/North Sea, respectively (see also "Case studies" below).

Thus, management options range in complexity, from simple scenarios, where biological information (including genetics) is used to identity units used for assessment, to more complex cases of mixture, where data requirements are more extensive and continuous monitoring is needed.

3. Data delivered by genetics/genomics

3.1 Identifying management units and tracking individuals

There is a long and well-documented history of the application of genetic principles and techniques to fisheries management and conservation (Ryman and Utter, 1987; Hauser and Carvalho, 2008; Ovenden *et al.*, 2013a). While there are a plethora of applications and questions tackled, including species ID, stock structure analysis, mixed-stock fisheries analysis, assessing the impact of fishing, establishing linkages between genetic diversity, population abundance and resilience and stock enhancement strategies, they can be grouped into two main approaches: 1) practical tools for *monitoring stocks and species*, 2) *elucidating the ecological and evolutionary forces* influencing distribution and abundance. Both aspects can contribute to the overall aim of securing sustainability in harvested fish stocks while avoiding stock depletion.

The ability to assess patterns in the distribution of adaptive diversity (Nielsen *et al.*, 2009; Limborg *et al.*, 2012; Milano *et al.*, 2014) is also transforming our notion of the nature and extent of local adaptation in wild and captive fish populations. Most notably is the discovery that even highly mobile fish populations of large effective population size, often display extensive divergence in genes under selection across small geographical scales (Nielsen *et al.*, 2009; Hemmer-Hansen *et al.*, 2013). Such data on local adaptation in the wild provides a baseline for assessing population resilience (Schindler *et al.*, 2010) and potential for stock recovery (Pinski and Palumbi, 2014). These insights have also resulted in a significant shift to the usage of molecular markers influenced by selection; so-called adaptive or gene-associated markers (Nielsen *et al.*, 2012). Markers under selection typically display elevated levels of population differentiation, which may therefore make them especially effective in discriminating marine fish populations exhibiting low genetic differentiation (Nielsen *et al.*,. 2009; Hemmer-Hansen *et al.*, 2014), population assignment and population traceability (Martinsohn *et al.*, 2011; Zelenina *et al.*, 2012), and yielding powerful tools for tacking illegal fishing and false eco-certification (Nielsen *et al.*, 2012).

3.2 Additional information

Recent reviews have stressed the importance of genetics and genomics for a range of other applications of relevance to fisheries management. For example, it is now possible to extend the application of molecular tools to using DNA as a biomarker for age, detection of pathogens and invasive species, ecosystem monitoring and analysis of the microbiome of captive and wild fish in relation to disease resistance (Ovenden *et al.*, 2013a; Llewellyn *et al.*, 2014). Moreover, the ability to identify putative and highly diagnostic domesticated genes in farmed fish allows the tracing of escapees back to source farm, together with investigation of the impact of stocking and escapees on native fish populations (Glover *et al.*, 2012).

Genetic data can also be used to estimate effective population size (Ne), which is a crucial measure in relation to predicting population ability to adapt to future changes and the effects of overexploitation. There are several examples of fisheries related genetically based Ne estimations. A 10-year study on Atlantic cod (*Gadus morhua*) revealed two separate biological populations inside and outside a Norwegian fjord on the Skagerrak coast (Knutsen *et al.*, 2011). When compared to the larger population that inhabits the skerries outside the fjord (that genetically resembles and may possibly represent a segment of the North Sea cod population), the cod population inhabiting the inner fjord presents a limited number of spawners and a limited genetically effective population size and may thus be vulnerable to local overexploitation. A 2-year

study on tiger prawns (*Penaeus esculentus*) showed high values for the genetic estimates of Ne, revealing that is unlikely to be affected by inbreeding, a useful result for the management of this exploited population (Ovenden *et al.*, 2007). The close coupling of Ne and Nc (census size) reported in a study with 5-cohorts of sandbar shark (*Carcharhinus plumbeus*; Portnoy *et al.*, 2009), revealed that despite heavy exploitation the species' evolutionary potential may not be compromised, as long as Nc is maintained well above 10,000.

3.3 Limitations

It is important to accommodate the limitations of molecular markers, in common with alternative approaches, as well as exploring opportunities for their integration with independent data sources. One such case derives from the challenges of detecting genetic differentiation among highly mobile species of high effective population size (Waples, 1998). The notion of "crinkled connectivity" (Ovenden, 2013b) describes a scenario when migration is above the threshold required to link populations genetically, but below the threshold for demographic links: that is, where population samples that exhibit genetic homogeneity respond independently to harvesting or other environmental perturbations. In such circumstances, there is particular merit in combining genetic estimates of connectivity with other data, such as estimates of population size and tagging and tracking data, to quantify demographic connectedness between these types of populations (Ovenden 2013b). Likewise, genetic data cannot reveal the detailed history of migratory behavior over the life time of individual fish. Here, combinations of genetics and tagging (e.g. Pampoulie *et al.*, 2008) or otolith microchemistry data could provide useful information.

3.4 Costs and time frames

As a result of recent technological developments, the time and cost of developing genetic markers to allow stock identification is decreasing, and the number of species for which markers and baselines are already available is increasing. For example, a recent FP7 funded project (FishPopTrace; https://fishpoptrace.jrc.ec.europa.eu), among other outputs, developed and validated genomic resources allowing stock discrimination of Atlantic cod, Atlantic herring, European hake and common sole within 3 years (Nielsen *et al.*, 2012). Outputs (Martinsohn and Ogden, 2009) have developed a range of cost-effective and reliable tools for identifying, monitoring and tracing marine fish populations. Such tools can promote fisheries governance by ensuring that the most effective tools can be applied to forensic standards, and thereby be legally supportive for prosecution and enforcement. In the case of FishPopTrace, tools are the focus of ongoing work with government and certifying bodies to promote the transfer of the technology to support enforcement and conservation policies of the EU CFP. In Atlantic salmon there is also a large component of baseline data covering rivers representing ~85% of the non-Baltic European salmon production, and fish can now be assigned to their river of origin with a high degree of accuracy (Verspoor *et al.*, 2012). These genetic data can be used not only to monitor wild populations, but also for monitoring escaped farmed salmon, and their hybridization with wild populations (Glover *et al.*, 2008, 2009).

A recent study assessed the costs and benefits arising from the use of DNA technology in support of fisheries enforcement and traceability, and included data from 32 countries (Guillen and Martinsohn, manuscript in preparation). Based on data from the control and enforcement authorities in the countries that currently use DNA testing on fish and fish products, it was shown that in all cases examined the benefits outweighed the

costs. It was also concluded that enhanced capacity building enabling the routine application of DNA-testing would significantly lower the costs. Additionally, several experts independently pointed out that (forensic) DNA-testing in the frame of criminal investigations has a highly deterrent effect further increasing the cost-effectiveness. The study concluded that routine application of DNA-testing could greatly support fishery control and enforcement as well as traceability and certification schemes in a cost-efficient manner, particularly if a coordinated effort by all stakeholders leads to a substantial building of capacity.

3.5 Routine collection of genetic data

As corroborated by the cases reviewed below, fisheries genetics has shown in a number of cases to provide added value to management strategies. However, an evident disadvantage and major challenge as compared to other fisheries data are the currently rather dispersed nature of genetic data and information. Such fragmentation compromises the integration of genetic information with data emerging from other sources. Ideally, genetic fisheries data should be centrally stored and collected under the remit of existing data collection schemes such as the EU Data Collection Framework (DCF; Council Regulation (EC) 199/2008), implemented through Commission Regulation (EC) No. 665/2008. In May 2014 an improved new Commission Regulation for data collection will be launched, though currently no such genetic data are collected within the DCF.

For a regular monitoring under the DCF Framework those cases should be taken into account where shifts in the genetic structure of populations can be expected, either their spatial and temporal distribution or levels of genetic diversity within populations. Such shifts can be caused by climate change, predation, food availability and other environmental facts but also by overfishing or selective fisheries. In these cases standard procedures for collection, marker systems and data analysis have to be agreed on to provide consistent data over several years. The existing mechanisms for the collection of biological information already being undertaken by EU member states in the frame of the DCF can provide such a cost-effective platform (ICES, 2012b).

4. Case studies

4.1 Cases where genetics has already been applied

4.1.1 Species identification of fish eggs

Biomass based models of fish population dynamics describe the overall change in population level from one year to the next without making any specific assumption on recruitment, growth, or natural mortality. The daily egg production method (DEPM) is a direct assessment method to evaluate the reproductive biomass of fish species. It consists of estimating the spawning-stock biomass as the ratio between the total daily egg production (P) and the daily fecundity estimates (DF). In consequence, this method requires survey to collect eggs for estimating the P and adults for estimating the DF. Currently the DEPM is used for anchovy (AZTI-Technalia, Spain) and sardine (Instituto Español de Oceanografía, IEO, Spain), and is being evaluated to be applied to mackerel and horse mackerel. Application of DEPM requires the individual identification of each egg, which is a tedious and time consuming task. Genetics could be used as an alternative to species identification, either applied to individual eggs (barcoding; Costa and Carvalho, 2007) or to a bulk zooplankton sample (metabarcoding). The former method requires manipulating eggs individually and does not represent a huge decrease in cost compared to traditional methods. However, it has potential for species

where eggs are not easily identified by eye (e.g. Bluefin tuna and gadoids, see also below). The latter method is most promising as it would allow the analysis of bulk plankton samples; yet, this approach requires some development to allow quantification or biomass estimation of eggs.

In recent surveys in the Irish and North Seas, a genetic identification technique (Taylor *et al.*, 2002) has been used to allocate gadoid eggs to species (Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and blue whiting (*Merlangius merlangus*)) in the large-scale egg surveys carried out by the Centre for Environment, Fisheries & Aquaculture Science (Cefas) in the North and Irish Seas (Fox *et al.*, 2005), and has also been successfully applied to samples fixed in formalin (Goodsir *et al.*, 2008). The rt-PCR test takes only 1–2 hours to screen samples for all three species, and the application of the genetic test in combination with the traditional plankton survey has yielded new insights into the distribution of species' spawning stocks.

4.1.2 Atlantic cod off Norway – real-time monitoring and closure of fishing

In spring the Atlantic cod in Norwegian waters is a mixed-stock fishery for northeast arctic cod (NEAC) and coastal cod (NCC). While NEAC is all time high, the NCC is below safe limits and needs protection. As the NCC is below safe limits and needs protection. Until the mid-1970s NCC was managed as part of the NEAC stock. From the mid-1970s to 2003 an expected catch of 40 000 tonnes NCC was added annually to the quota for NEAC. In 2004 and later years the additional catch expected from NCC was set near 20 000 tonnes. Not managing the NCC as a separate stock unit might be the reason for the apparent collapse in the NCC fishery. Due to continued decline in survey results, ICES advised zero catch for the years following the 2006 season (Anon, 2008, 2012), and at the same time recommended establishing a recovery plan to rebuild the NCC stocks. As a part of the rebuilding plan of the NCC, the Norwegian Directorate of Fisheries, in cooperation with the Institute of Marine Research, has been monitoring the cod fisheries for NCC around two closed grounds in the two main spawning areas off Norway over the last 8 years. The monitoring is protecting NCC when there is only NCC in the area and opening the ground for fisheries whenever the level of NEAC is high. This decision is based on the results from genetic analysis (PanI) of finclips from landings, mainly from the areas outside the closed grounds, and the analyses disclose the fraction of the NEAC component within 24 hours. Based on this information the Directorate can react to any changes in the composition of the catches and decided whether or not to open the closed area. For transparency, the Directorate has set a 70% limit; unless the fraction of NEAC outside the closure exceeds 70% over a short period it will remain closed for all fishing gears except handlines. The Directorate of Fisheries now opens and closes the areas to fishing based solely on the genetic analysis.

4.1.3 Atlantic cod in Greenland – definition of management units

Recent genetic studies have documented that the ocean around Greenland is inhabited by at least three genetically distinct populations of Atlantic cod: West Greenland inshore, West Greenland offshore and East Greenland offshore (Therkildsen *et al.*, 2013). These results are consistent with previous genetic studies, egg distribution survey and tagging experiments (Buch *et al.*, 1994; Storr-Paulsen *et al.*, 2004; Pampoulie *et al.*, 2011). Although the different groups show independent dynamics and considerable geographic separation during the spawning season, there are also areas of overlap and mixture (e.g. Southwest Greenland). Accordingly, spatially differentiated management plans could optimize the exploitation of each population to increase overall sustainable

yields while protecting the most vulnerable populations. Based on the genetic and stock dynamic differences, the ICES North Western Working Group (NWWG) has recently assessed the West Greenlandic inshore cod component separately from the offshore component (ICES, 2013c). Following these analyses, the working group recommended a 2013 inshore quota of 8,000 tonnes, a quota which the Parliament of Greenland has since increased to 15,000 tonnes. Further genetic investigations are currently being undertaken to clarify the origin of cod harvested in different management areas (e.g. NAFO 1A – 1F) during the historical mixed-stock fishery in West Greenland.

4.1.4 Redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters – definition of management units

The fishery for *S. mentella* traditionally targets fish on the continental slopes of Iceland, Greenland, Norway, Canada and the Faroe Islands, and a pelagic fishery developed in the Irminger Sea in the early 1980s (Sigurðsson *et al*., 2006). In 2009, the International Council for the Exploration of the Sea (ICES) organized an interdisciplinary workshop to reconcile all available data on stock structure in *S. mentella*. The aim was to recommend practical management units and provide clear advice for fishery science and management in the Irminger Sea and adjacent waters (ICES, 2009a).

Based on current data, four genetic stocks of *S. mentella* were suggested in the Irminger Sea and adjacent waters (Figure 1). However, as these stocks were partially defined by depth, it was recognized that the definition of management units by depth and the associated fishery monitoring by depth would be impractical (Cadrin *et al*., 2010). Based on genetic advice, ICES has revised its recommendations for the management of *S. mentella* fisheries, with three management units based on geographic proxies for biological stocks that minimize mixed-stock catches in the Irminger Sea (ICES, 2009a). ICES also revised its advice to account for genetic differences between the deep-pelagic and shallow-pelagic stocks (ICES, 2009b). Advice for the shallow-pelagic stock was "given the very low state of the stock, the directed fishery should be closed", and for the deep-pelagic stock "given the reduced abundance of this stock in recent years, a total catch limit of no greater than 20 000 tonnes should be implemented in 2010". The difference in advice for the two stocks illustrates the importance of stock identification for fishery management.

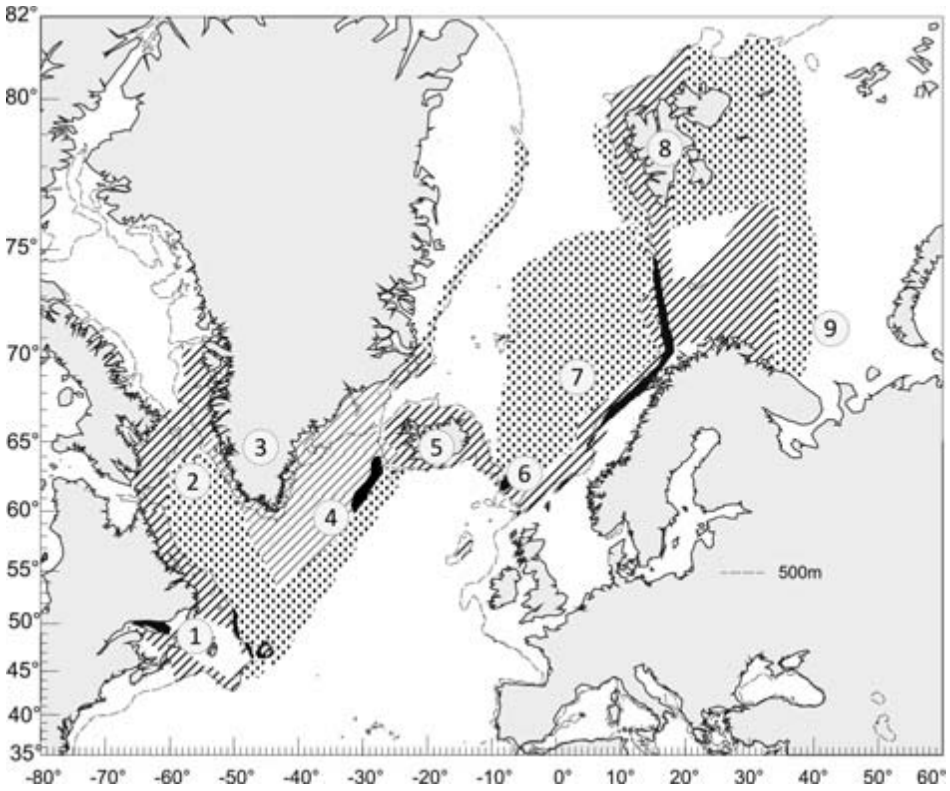

Figure 1. Geographic range of *Sebastes mentella*. The hatched area shows the centre of abundance. The dotted area is the outer sector of the distribution range, the black area along the slope shows the main area of larva release, and the dashed line indicates the 500-m depth contour. Numbered locations are 1, Newfoundland; 2, Davis Strait; 3, Greenland; 4, Irminger Sea; 5,Iceland; 6, Faroe Islands; 7, Norwegian Sea; 8, Svalbard; 9, Barents Sea. The four genetic units identified in the cross-disciplinary workshop were 1: A Western stock extending south of Newfoundland (1) and west to the Gulf of St Lawrence, 2: A shallow-pelagic stock extending from the Grand Bank to the Faroe Islands (6), perhaps farther east, 3: A deep-pelagic stock also primarily consisting of *S. mentella* in pelagic habitats, but including demersal habitats west of the Faroe Islands. Note that this genetic stock does not necessarily equate to the deep-sea phenotype, 4: An Iceland slope stock inhabiting demersal habitats of the continental slope; the northwest Faroese slope may be part of this stock. Figure copied from Cadrin *et al.*, 2010.

4.1.5 Blue threadfin (*Eleutheronema tetradactylum*) in Western Australia – definition of management units

The blue threadfin (*Eleutheronema tetradactylum*) is a widespread shore fish with a distribution ranging from the Persian Gulf to Northern Australia and is among the most important fisheries species in many Asian countries (Motomura, 2004). In Australia, this species has heretofore been managed as a single-stock from Queensland to Western Australia. However, a genetic survey of this species in Australian waters indicated a lack of migrant exchange between adjacent areas at an unusually small geographic scale for a marine fish (Horne *et al.*, 2011; Horne *et al.*, 2013a), suggesting that this species should be managed at a local scale. On Western Australia's Kimberly Coast, where genetic diversity was particularly low, the looming possibility of local depletion in specific areas prompted the government of Western Australia to terminate commercial gillnet fisheries in Roebuck Bay, near the city of Broome, to reduce fishing pressure (www.fish.wa.gov.au). The local community responded favourably to the decision as a move that will lead to healthier fish stocks and improve fishing tourism to the area.

4.1.6 Pacific salmon – population-based management

Management of Pacific salmon fisheries is a classic example of genetics assisted real-time monitoring and management of a fishery. The population structure of sockeye salmon has been investigated using different types of genetic markers for many years; however the best level of resolution was achieved using SNPs.

The comparative analysis of numerous sockeye salmon populations of the Bristol Bay drainage and sea samples from the Port Moller test fishery located on the migration routes of sockeye salmon to the spawning sites in the Bristol Bay presents a good example of population-based management (Dann *et al.*, 2013; Figure 2).

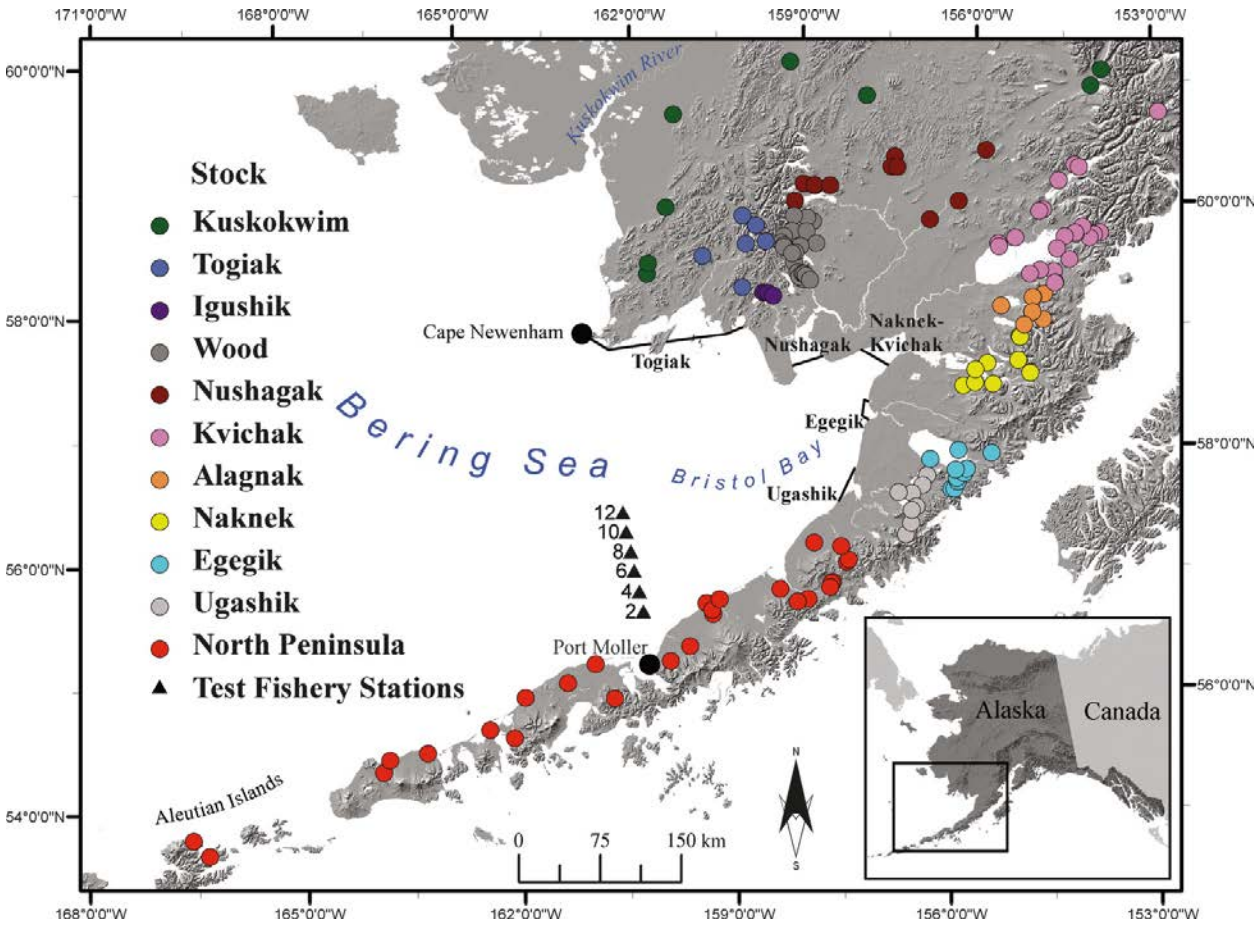

Figure 2. Southeastern Bering Sea showing locations of 96 populations from 11 stocks of sockeye salmon used for baseline data, Bristol Bay fishing districts, and stations sampled during Port Moller test fishery. Populations are color-coded by stock. Districts where the commercial fishery occurs are marked in bold. Sampling stations were spaced every 18.52 km (10 nautical miles) along a transect between Port Moller and Cape Newenham that is perpendicular to the migration of sockeye salmon returning to Bristol Bay (from Dann *et al.*, 2013).

Genotyping by 38 SNP loci of sockeye salmon in Bristol Bay during spawning migration, conducted in 2006–2010, allowed an assessment of the relative abundance of each stock in the mixed catch and spatio-temporal distribution of fish originated from different fishing districts. These data lead to the development of fundamentally new principles for salmon fishery management. Traditionally, distribution and timing of fishing efforts is based mainly on preseason forecasts of salmon abundance returning to each of the major rivers followed by adjustments based on fish counts in rivers during the

spawning run. The real-time genetic analysis of sockeye salmon samples caught in the test fishery allowed an estimation of the number of fish in each fishing district 2–3 days prior to their arrival to the final destination. Fishing effort was shifted according to the relative stock-abundance data in the test fishery which deviated from estimates based on preseason forecasts (Dann *et al.*, 2013).

4.1.7 Spanish mackerel (*Scomberomorus commerson*) in Australia – genetic tagging and estimation of harvest rates

Adult Spanish Mackerels in northern Australia are thought to form highly localized subpopulations that have low levels of mixing. Consequently, obtaining information on harvest rates for stock assessment is difficult using traditional monitoring methods, such as abundance surveys and age structure analyses. In addition, these large pelagic predators are highly vulnerable to conventional tagging procedures as they may suffer high mortality rates when they are line-caught and brought aboard vessels.

A team of Australian scientists have developed an innovative method to 'Genetag' fish by taking a small tissue sample with a special pliable hook. Directly after sampling of the tissue via the special hooks each "gene tagged" fish is automatically let go by the hook, leaving only the tissue for genotyping behind without further emphasizing the fish. Harvest rates can subsequently be estimated through sampling a proportion of commercially landed fish and comparing these genotypes to the database with the "gene tagged" fish. Within the Genetag project around 10,000 samples from Spanish mackerel have been collected and the method is now being used in sharks, which are also commercially fished (Buckworth *et al.*, 2012).

4.1.8 Design of marine protected areas (MPAs)

There is now overwhelming evidence to suggest that spatially explicit management programs, such as marine protected areas (MPAs) can be effective tools for conservation and yield significant benefits for fisheries. Australia's Great Barrier Reef Marine Park comprises a large MPA network and it has been shown that MPAs on the barrier reef have resulted in significant and rapid benefits for fishery species and sharks, as well as reduced outbreaks of coral-eating crown-of-thorns starfish (McCook *et al.*, 2010). Worldwide, the fruits of MPAs are recognized; the challenge that researchers and policy-makers now face is how to best design marine preserves so that they produce the maximum benefits, to the maximum number of species, at the lowest cost to commercial and recreational fisheries (Gaines *et al.*, 2010).

In order to be viable into the long-term future, populations in MPAs need to be able to exchange migrants with other protected populations (Carr *et al.*, 2010). Managers with stewardship over marine resources regularly face difficult decisions about the amount of marine real estate that should be allocated to reserves. If MPAs are spaced to thin they will fail to exchange migrants. On the other hand, if too much space is allocated for MPAs, negative impacts to commercial and recreational fisheries will be too great.

For many marine species, which migrate only during the early life-history stages (eggs and larvae), using DNA markers is the only feasible way to investigate dispersal distances and barriers to marine dispersal (Hellberg, 2007). For example, genetic data have revealed Point Conception, on the California coast, to be a major transition zone for many marine species (Pelc *et al.*, 2009). This and other genetic data were important sources of information used to design California's current network of MPAs (Carr *et al.*, 2010), illustrating the important role for genetic data for defining networks of protected areas.

In addition to genetically surveying populations to assess genetic connectivity, genetically based parentage analysis (genetically identifying parent offspring pairs) can be used to assess whether protected populations are successfully exporting migrants to unprotected areas (Planes *et al.*, 2009; Christie *et al.*, 2010). Kinship analysis (genetically identifying sibling pairs) has also been used to assess demographic exchange, sometimes across large distances (Horne *et al.*, 2013b). Therefore, genetic methods can be used to detect long-term stock structure, but also short-term patterns of migrant exchange needed to improve the design of spatially explicit management programs, such as MPAs.

4.2 Cases where genetics could be readily adopted

4.2.1 Atlantic herring in the Skagerrak/Kattegat – mixed-stock fisheries

Atlantic herring in the Eastern North Sea, Skagerrak and Kattegat (ICES areas IVaE and IIIa) is currently managed as a mixed-stock composed of Western Baltic Spring-spawning (WBSS) herring and North Sea Autumn Spawning (NSAS) herring. These groups mix at feeding grounds in the area during summer feeding migration but return to their respective spawning grounds, thus contributing to recruitment in the western Baltic and North Sea, respectively. ICES advises on catch options by fleet for the entire distribution of WBSS and NSAS herring stocks separately, however, the fisheries are managed by areas covering the geographical distribution of the stocks. To perform separate stock assessments, splitting keys are constructed on an annual basis and applied to catches to separate NSAS herring from WBSS herring. The methodology for constructing these splitting keys has developed from use of sample-based mean vertebral counts (1991–1996) to include otolith microstructure (1996–2009; Clausen *et al.*, 2007). From 2009, otolith shape analysis has been used as a supplementary method to increase sample size for estimating stock proportions of NSAS and WBSS in the mixing areas of Division IIIa. For each assessment year individual population identity has been established by otolith microstructure visual inspection and used as a baseline for assignment of shape characteristics to the involved stock components. A baseline of about 800–1200 otoliths with known hatch type has then been used as calibration in an age-structured discriminant analysis where additionally 3000–4000 otolith shapes have been assigned to one of the two stocks using a combination of shape Elliptic Fourier Coefficients, otolith metrics, fish metrics, length, weight and maturity as well as longitude–latitude and seasonal parameters (ICES, 2013b). Recent studies have shown that additional complexity may be present within the area (Ruzzante *et al.*, 2005; Bekkevold *et al.*, 2005, 2011), and genetic markers have high statistical power for identifying individual population components in the mixed feeding area (Bekkevold *et al.*, 2011). The mapping of genetically based differences among herring populations in the region has led to novel procedures for identification and estimation of stock contributions to the mixed-stock fisheries as well as providing baselines for less expensive routine measures of stock identity. The method of choice for future stock identification analysis should depend on the purpose of the analysis. If it is for identification of population diversity in the area, only genetic markers can give the desired resolution at present. However, if the aim is tracing the management stock of herring in a random sample, otolith analyses has the potential for giving the answer if the assignment of the local autumn- and winter spawners can be assigned to the stock in the transition area and not to the North Sea (as is done currently).

4.2.2 European hake in the Mediterranean – definition of management units

In the Mediterranean stock assessment is normally directed by subareas set by the General Fisheries Commission of the Mediterranean (GFCM). Due to the large number of countries with access to its waters, a large proportion of small-scale artisanal fisheries and other reasons, the management of Mediterranean fisheries is notoriously difficult. An evaluation of the status of Mediterranean and Black Sea resources in European Waters in 2013 showed that the majority of stocks remain unassessed, and that between 94% and 95% of the stocks are overexploited compared to FMSY (Osio and Cardinale, 2013).

Such assessment also applies to European hake (*Merluccius merluccius*), where all assessed stocks are fished mostly far above FMSY (STECF, 2013). Recent genetic work has, however, demonstrated the value that genetics could add to the management in such a complex scenario. The genetic relatedness among the samples is shown in Figure 3 in the context of the stock assessment subareas, highlights the potential value of genetic data for an enhancement and rationalization of sampling and assessment efforts. An example is the Adriatic Sea encompassing areas 17 and 18, where apparently one hake population exists. This indicates that one instead of two stocks is targeted for fisheries in this region and demonstrates that genetic analysis can rationalize fisheries management not only by identifying populations but also by determining their extent. Moreover, genetic analysis can be used to determine the origin of landings of mixed composition, for instance from regions 15, 16 and 19.

This example shows how genetic analysis, provided robust genetic reference baselines are available, can be applied routinely to guide stock assessment and at the same time to provide additional valuable information. In the Mediterranean, roughly 100 commercially valuable species are exploited. Several hundred stocks would have to be assessed to reach full spatial coverage, showing the need to rationalize assessment effort to foster a move towards more sustainable fisheries in this highly challenging region.

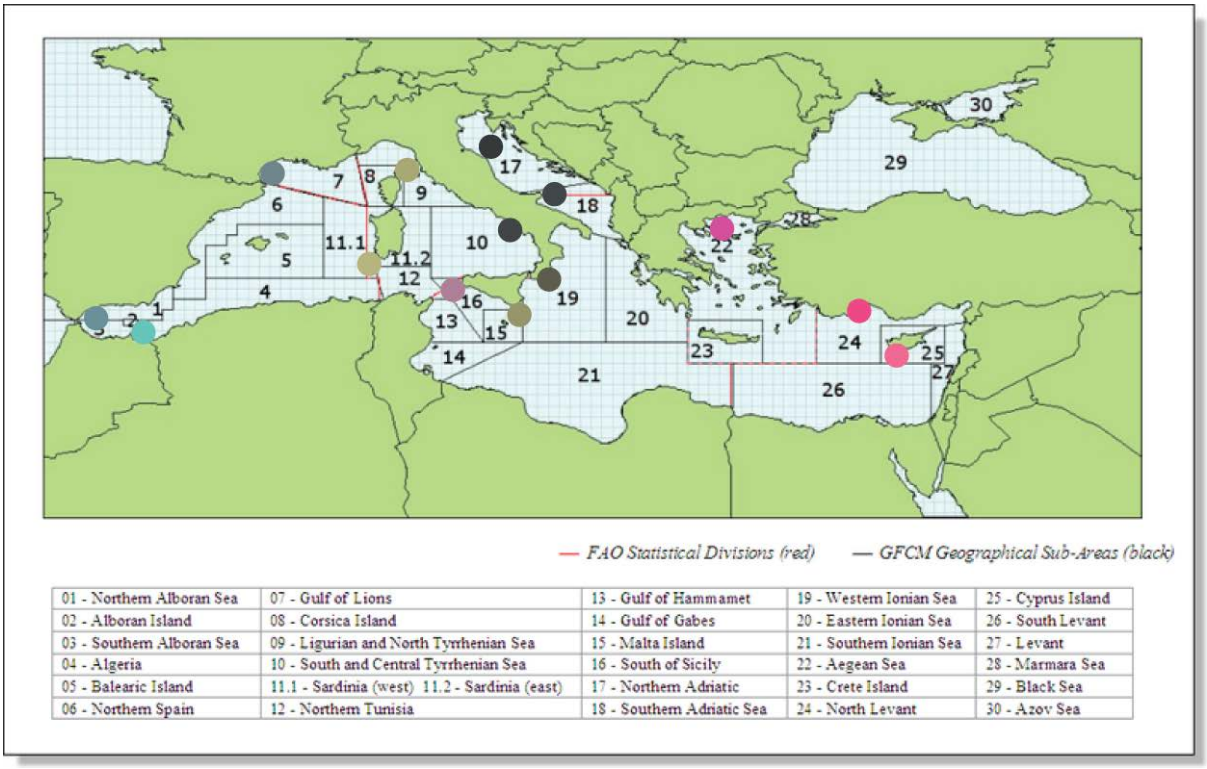

Figure 3. Mediterranean genetic population map of European hake (*Merluccius merluccius*) overlaid on GFCM geographical subareas (http://www.gfcm.org/gfcm/topic/16162/en). Couloured dots illustrate genetic relatedness revealed by discriminant analysis of principal components (DAPC) based on Single Nucleotide outlier loci. For details see https://fishpoptrace.jrc.ec.europa.eu/map/genetics_geobrowser and Milano *et al.* (2014).

4.2.3 Red king crab (*Paralithodes camtschaticus*) in the North Pacific – definition of management units

The red king crab has been harvested in the North Pacific since 1920's, mostly by Japanese, Russian and US fleets. It had its peak in the early 80's with 91 tons of catches, having a decline up to 90% afterwards in some places (http://www.afsc.noaa.gov/Education/factsheets/10_rkc_fs.pdf). Most of today's red king crab harvest comes from Bristol Bay and represents one of the most valuable fisheries in the United States valued at more than $90 million in 2012 (http://www.fishwatch.gov/seafood_profiles/species/crab/species_pages/red_king_crab.htm).

In Alaska, fishing stocks of the red king crab **are managed by National Marine Fisheries Service (NMFS) and the State of Alaska through the** North Pacific Fishery Management Council (NPFMC), NOAA Fisheries, and the Alaska Department of Fish and Game. Currently, large fisheries in the southeastern Bering Sea and Gulf of Alaska were reduced or closed (Bechtol and Kruse, 2009) based on population declines probably derived from climate fluctuations (Mantua and Hare, 2002; Kruse, 2007) and overexploitation (Bechtol and Kruse, 2009). Some stocks of the red king crab have failed to recover after 25 years of fishery closures, such as off Kodiak Island (Bechtol and Kruse, 2009), but Bristol's Bay stock in the southeastern Bering Sea have recovered and have exceeded the rebuilding target levels since 2003 (Vining and Zheng, 2004). Nine registration areas were defined (Figure 4).

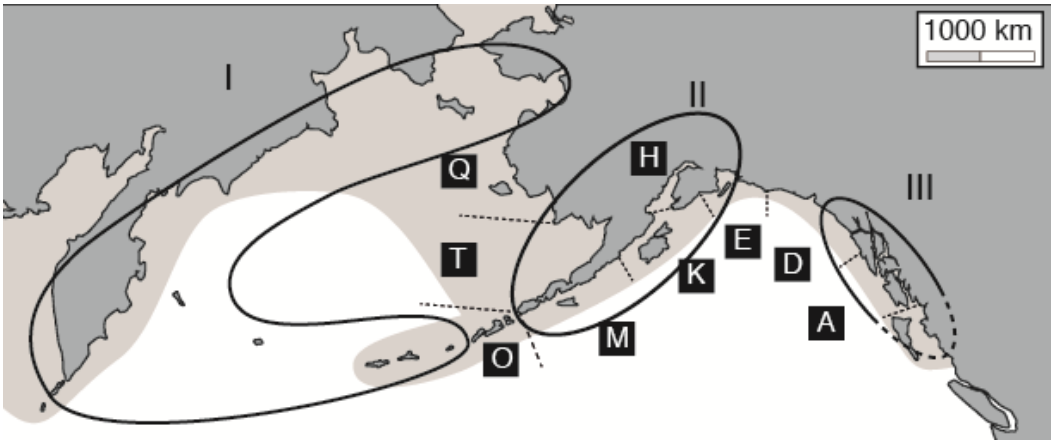

Figure 4. Geographic distribution of red king crab (light grey), letters in squares represent State of Alaska harvest management areas (registration areas: A, D, E, H, K, M, O, T), dashed lines indicate management area boundaries and full lines represent genetic units (I, II, and III). Figure adapted from Grant and Cheng (2012).

In the North Pacific, three main genetic populations were found (Figure 1). Processes influencing population structure within the groups appear to differ, because of differences among regions in oceanic and shoreline barriers to dispersal (Grant and Cheng, 2012). Genetic divergence, together with independent demographic responses in each area in the last few decades (Bechtol and Kruse, 2009), indicates that southeastern Bering Sea populations warrant continued management as a separate unit as it represents a single evolutionary unit indicating demographic independence from the Bering Sea and Western Gulf of Alaska pools. These data do not conflict with current management practices in the species. However, within the southeast Alaska population (population III in Figure 4), partially isolated populations in semi-enclosed fjords may have dispersal limitations that promote some degree of isolation of localized populations. This group also shows low levels of genetic diversity, which may impede adaptive responses to environmental changes and may predispose these populations to extinctions from climate shifts. Therefore, based on genetic data, southeast Alaska king crab populations would be best managed on a finer geographic scale (Grant and Cheng, 2012; Figure 4).

4.2.4 Atlantic cod in the North Sea – definition of management units

Atlantic cod within the North Sea is currently assessed and managed as one unit (ICES, 2013d). However, a recent example serves to illustrate the potential of using genetic data to identify discrete stocks, and then to incorporate spatially resolved data to simulate the impacts of alternative harvesting regimes within the North Sea. Using new genetic data showing the existence of two discrete populations of cod, *Gadus morhua*, within the North Sea (one northern and one southern), Heath *et al.*, (2014) found that the current strategy of collapsing such sub-specific structuring into single-stock models with contemporary harvesting rates is likely to lead to local extinction of the weaker stock component due to competition between the early life history pelagic stages. The model provides a method to quantify adjustments to regional fishing mortality rates to strike a balance between maximizing sustainable yield and conserving vulnerable populations. This and other recent work on life-history and genetic variation within the North Sea was recently reviewed by the ICES North Sea assessment working group (WGNSSK), which also highlighted the need to further explore sub-structuring within the North Sea for this species (ICES, 2013d).

4.2.5 Atlantic cod in the Baltic Sea – population mixing/mixed-stock assessment

Atlantic cod in the Baltic Sea is currently assessed and managed as two different units, eastern and western Baltic cod, separated at the transition from SD24 to SD25 around the island of Bornholm. However, the two populations likely mix in the eastern part of the western Baltic management area (in SD 24; see Eero *et al.*, 2014 and Figure 5). The migration patterns over a lifespan of cod and contribution of the cod found in the mixing zone to recruitment in either eastern or western Baltic management area are not well understood at present. Possible interannual and seasonal variability of mixing rates creates uncertainty in stock assessment that assumes no migration in or out of the assessment area. Further, the developments in local biological populations in the western Baltic Sea may be masked by occasionally abundant immigrants from the eastern stock.

The transition zone between the North Sea and Baltic Sea has been subject to several studies applying genetic markers to identify biological units (e.g. Nielsen *et al.*, 2001, 2003, 2005, 2009; Hemmer-Hansen *et al.*, 2013; Hemmer-Hansen *et al.*, 2014). As in many other species, large genetic differences are generally found between sampling sites in this geographical region, suggesting the presence of independent genetic units (Nielsen *et al.*, 2003). Genomic data have shown that a number of genetic markers are highly divergent between the North Sea and Baltic Sea populations (Nielsen *et al.*, 2009; Hemmer-Hansen *et al.*, 2013), and some of these have successfully been applied to differentiate between western and eastern Baltic cod individuals with high statistical power (Eero *et al.*, 2014). Thus, the genetic tool for assigning individual fish to biological population is already available and operational on larger scales (Nielsen *et al.*, 2012), and is currently applied to screen further samples to provide some of the missing information needed for successful management of this resource.

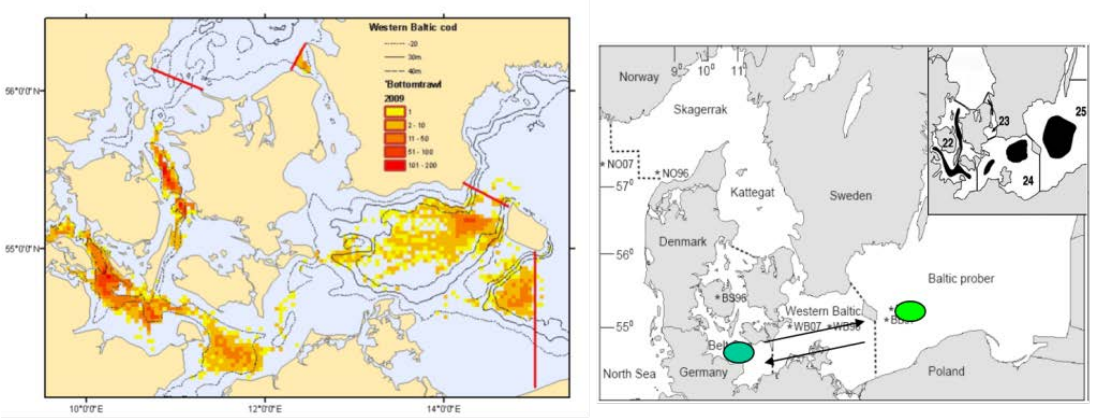

Figure 5. Left: Fishing pattern in the Western Baltic, showing high intensity towards the Eastern boundary of the management area for the Western stock. Right: With only 20 genetic markers (SNPs), individuals can be assigned unambiguously to sea area of origin.

5. Discussion

The application of genomic approaches for natural renewable resource management is developing quickly and is now also available for many marine fish species. The ability to screen genomes for variation associated with adaptation to local environmental conditions promises to be particularly useful for fisheries management, as it can facilitate integration of evolutionary and ecological population concepts (Waples and Gaggiotti,

2006), which are normally the units targeted by fisheries management and population genetics, respectively.

We have identified a number of cases, where genetics has been used as a tool for fisheries management, and have also reviewed cases where it could be readily adopted. In most examples, genetics has been used to identify management units. However, genetic tools also show great potential in mixed-stock scenarios because of the ability to identify origin of single fish with high statistical certainty. Yet, important communication gaps between population geneticists and fisheries biologists and assessment scientists may still challenge the routine incorporation of genetic information in fisheries management. Thus, geneticists should be encouraged to engage more directly with the assessment work, for example via contributions at benchmark meetings, where assessment units are defined based on available knowledge.

If mixed-stock fishing is exploiting feeding assemblages (as in Atlantic herring in the Kattegat/Skagerrak), it is relatively straightforward to allocate proportions to different assessment units. However, if mixing also involves reproduction of several populations within management units, most current assessment models appear to be challenged, as only few can handle multiple assessment units simultaneously. Since genetics is likely to address such complexity effectively, such scenarios necessitate the further development of assessment models able to handle spatial and temporal heterogeneity.

It is also clear that while genetics can be useful for addressing questions of relevance to fisheries management, there is a need for cross-disciplinary work where genetic information is combined with other types of data. The integrative approach used by Heath *et al.* (2014) for cod in the North Sea reviewed in this ToR shows the power of combining genetics with spatial modelling. In addition, combinations of tagging (e.g. data storage tags, DST) and otolith microchemistry, to track individual migration patterns, with genetics, to identify population of origin, could be powerful approaches to identify population dynamics on local geographical scales.

There is little doubt that genetics will also play a significant role under future management schemes, such as the ecosystem based approach to fisheries management and the EU landing obligation. However, such future management schemes are challenging even from a management and policy perspective. Thus, incorporating genetics more efficiently into current management schemes will be a first step to harvest marine fish genomic data for sustainable fisheries management.

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Table 1. Genetic analysis mentioned in the context of stock identification and assessment in the latest "Review of scientific advice form the Scientific, Technical and Economic Committee for Fisheries" (STECF, 2014).

Species	Area	STECF Comment
Anchovy (*Engraulis encrasicolus*)	Sub-area IX	Recent studies on genetics indicate that the stock inhabiting Division IXa South (Algarve and Cadiz) is different genetically from the one inhabiting the remaining parts of Division IXa (Zarraonaindia *et al.*, 2012). Given the differences in genetics and stock dynamics between the northern and southern parts of the area, this might imply separate management in these two regions of Division IXa.
Greenland halibut (*Reinhartius hippoglossoides*)	Sub-areas V, VI, XII, XIV	Available biological information such as tagging and genetic studies and the distribution of the fisheries suggest that Greenland halibut in Subareas XIV and V belong to the same stock entity and that a common management is therefore required.
Cod (*Gadus morhua*; Norwegian coastal cod)	area I and II	Genetic studies indicate that the cod in some fjords may be separate stocks. An assessment of the combined stocks is not likely to detect fluctuations of the smaller components, and thereby the current assessment approach involves some risk to local stocks. The stock complex is still not fully mapped, but the existence of local stocks also calls for special attention to protect genetic diversity and smaller components.
Spurdog (*Squalus acanthias*)	Northeast Atlantic	Analyses of microsatellite data conducted by Verisimmo *et al.* (2010, a WD submitted to WGEF) found genetic homogeneity between east and west Atlantic spurdog, but the authors suggested this could be accomplished by transatlantic migrations of a very limited number of individuals.
Basking shark (*Cetorhinus maximus*)	Northeast Atlantic	According to WGEF, a single-stock of basking sharks Cetorhinus maximus exists in the ICES area. The stock structure is unknown. In the absence of such information, the basking shark population in the Northeast Atlantic is presumed to be a single-stock. There are indications that this stock has connectivity with the western and southern Atlantic..A genetics study underway in the UK aims to differentiate distinct stocks globally. They are known to congregate in areas with a high zooplankton biomass (e.g. fronts) and, therefore, may be locally important, but the locations of these areas are variable.

Species	Area	STECF Comment
Tope (*Galleorhinus galeus*)	Northeast Atlantic	A genetic study (Chabot and Allen, 2009) on the eastern Pacific population including comparisons with samples from Australia, South and North America and UK, shows that there is little to no gene flow between these populations, meaning an apparent lack of migration.
Porbeagle (*Lamna nasus*)	Northeast and Northwest Atlantic	A recent genetic study suggests that the stock is genetically robust, although further confirmation is required.
Tusk (*Brosme brosme*)	The majority of landings are from ICES Subareas IIa, IIIa, from along the Norwegian coast of IVa, Va (around Iceland), and Vb (around Faroe Islands).	The new perception of the stock structure is based on considerations of new genetic information in 2009 (Knutsen *et al.*, 2009). Studies using recently developed microsatellite primers detected highly significant genetic differentiation in tusk within its North Atlantic range. In particular, tusk around Rockall, the Mid-Atlantic Ridge, and off Canada, most likely represent different biological populations that clearly warrant separate management considerations. As in 2011, ICES provided advice on separate stocks of tusk on the basis of new genetic evidence and advice is presented for the following revised management units: I and II (Arctic) Division Va and Subarea XIV The Mid-Atlantic Ridge (Division XII excluding XIIb) Subarea VIb (Rockall) IIIa, IV, Vb,VIa, VII, VIII, IX, XIIb, . (This latter grouping is a combination of isolated fishing grounds and these areas are grouped due to their mutual lack of data.)
Red (blackspot) sea bream (*Pagellus bogaraveo*)	Subareas VI, VII, VIII, IX and X (Azores)	The stock structure is uncertain. This section deals with a species distributed over a wide area, which may be composed of several populations. Three units are considered: Subareas VI, VII, and VIII; Subarea IX; Subarea X. This management units division are supported by information on genetics and tagging.
Shrimp (*Pandalus borealis*)	NAFO Division 3LNO	Recent genetic analysis shows that this stock is part of a wider population spanning NAFO Subarea 2 and at least Div. 3KL. Migrations of shrimps across the management-area boundaries are not accounted for in the assessment and therefore introduce additional uncertainty. Scientific Council recommends exploration of alternative approaches that take into account the entire stock area.

Species	Area	STECF Comment
Porbeagle (*Lamna nasus*)	Northwest Atlantic	A recent genetic study suggests that the stock is genetically robust, although further confirmation is required.
Yellowfin (*Thunnus albacares*)	Eastern, Western and Central Pacific	While it is likely that there is a continuous stock throughout the Pacific Ocean (with exchange of individuals at a local level, although there is some genetic evidence of local isolation) the movements of tagged yellowfin are generally over hundreds, rather than thousands, of kilometers, and exchange between the eastern and western Pacific Ocean appears to be limited.
Swordfish (*Xiphias gladius*)	Eastern Pacific	The best available scientific information (genetic and fishery data) indicate that the swordfish of the Northeastern Pacific Ocean and the Southeastern Pacific Ocean (south of 5°S) constitute two distinct stocks. Also, there may be movement of a northwestern Pacific stock of swordfish into the EPO at various times.
Striped Marlin (*Kajikia audax*)	Pacific	Genetic studies provide a more detailed picture of stock structure. McDowell and Graves (2008) suggest that there are separate stocks in the northern, northeastern, and southeastern, and southwestern Pacific.
Pacific jack mackerel (*Trachurus symmetricus*)	Pacific	From genetic studies it has been identified as a distinct species and supports one of the largest single-species fisheries in the world, with annual landings approaching 2.5 million tonnes (FAO, 2004).
Patagonian tootfish (*Dissostichus eleginoides*)	Subarea 48.3, South Georgia and Subarea 58.5.2., Heard and McDonald Islands	There is genetic separation between Subarea 48.3 and the Patagonian Shelf (FAO Area 41). The SGSR stock, occurring within management areas A, B and C is genetically separate from fish taken in the extreme north and west of Subarea 48.3. All assessments consider only the SGSR stock. The stock in Subarea 48.3 is considered fully exploited. / Genetic studies have demonstrated that the population at Heard Island and McDonald Islands is distinct from those at distant locations such as South Georgia and Macquarie Island, but that within the Indian Ocean sector there appears to be no distinction between fish at Heard, Kerguelen, Crozet or Marion/Prince Edward Islands.

4 Term of Reference c): Quantifying the presence and impact of domesticated Atlantic salmon in the wild: approaches and strategies for studying introgression

4.1 Summary

Domesticated strains of a species differ genetically from their wild counterparts due to generations of selective breeding and husbandry practices. As a result of this divergence, studies on Atlantic salmon (one of the major species in world aquaculture) suggest that hybridization and introgression of escaped farm fish with wild conspecifics may incur a fitness cost to wild populations. On a global scale, there is increasing awareness and concern regarding the conservation of native fish gene pools and preservation of wild populations, in the face of escaped domesticated individuals. Consequently, there is a need for the identification and development of molecular and statistical tools to assess and quantify the degree of hybridization and subsequent introgression of escaped farm and wild fish. Given the dynamic nature and inherent complexity both within and among aquaculture strains and wild populations, quantifying hybridization and subsequent introgression using standard population genetic methods is often difficult, and represents an ongoing challenge. A review of the literature indicated that most studies have focused on the identification of farm escaped salmon or domesticated strains, and have utilized microsatellite loci or earlier molecular methods in conjunction with assignment tests or Bayesian clustering. These studies generally report that accurate identification is possible, though differentiation of individual cage sites seems characterized by reductions in accuracy. In contrast, studies attempting to quantify the frequency of F_1 hybridization and subsequent introgression are much rarer, each represented by only a handful of studies. In the case of quantifying rates of hybridization (F_1) and recent interbreeding, hybrid identification usually involves the use of Bayesian genetic models, and highly selected SNPs which together provide ample power for robust F_1 identification. Genetic and genomic based methods to study introgression have largely been based on either estimating effects of farm escapees on the distribution of genetic variation within and between populations, often using F_{ST} or similar estimators or estimating the degree of individual or population-wide admixture present, again using Bayesian based methods. In both cases, recent studies suggest significant promise in using highly selected panels of SNPs to identify hybrid individuals or the presence of introgression. Given recent access to genome wide markers and a published genome, the potential exists to explore changes in genomic architecture (i.e. LD, F_{ST}) associated with introgression and further refine our ability to detect subtle impacts. Nonetheless, careful consideration regarding the influence of increased diversity among possible domesticated sources is required as this inter-source diversity has been shown to significantly reduce statistical power to detect introgression. Overall, genetic and genomic quantification of the impact of farmed escaped salmon on wild populations remains challenging, though recent advances in genomic and statistical resources have made it increasingly feasible in many situations.

4.2 Introduction

The monetary value of aquaculture production has now surpassed the total value of wild fisheries (FAO 2012; ICES 2013). Balancing the rapid industry expansion with environmental sustainability remains a challenge, with impacts both for wild conspecific populations and industry production levels. Atlantic salmon (*Salmo salar* L.) farming, largely in freshwater hatcheries and sea cages, has increased exponentially in recent

years (Thorstad *et al*. 2008), developing into a global industry with over 95% of adult salmon now in existence being of domesticated farm origin (Naylor *et al*. 2005). Global production is currently estimated at around 2 million tonnes per annum, a 30% increase on the previous 5-year average (ICES 2013), and the worldwide production of farmed Atlantic salmon is more than 1300 times the reported catch of wild fish from the North Atlantic (ICES 2013). This rapid expansion globally ultimately involves the use of highly domesticated and selectively bred strains, developed by breeding wild North American and European salmon populations. Because of the enormous scale of production, although a small proportion of those being farmed escape, large numbers of reared salmon escape aquaculture facilities at all life stages (Naylor *et al*. 2005; Thorstad *et al*. 2008). Total number of escapes into the North Atlantic has been estimated at 2 million individuals annually (McGinnity *et al*. 2003), and although the proportion of escapes has decreased (Jensen *et al*. 2010; Thorstad *et al*. 2008), the absolute numbers remain high and are likely growing due to the continued expansion of the industry.

The ultimate impact of these escapes on wild salmonid populations is a growing concern across the natural and introduced range (Ford and Myers 2008). Wild natural populations of Atlantic salmon are in major decline (ICES 2013) with many populations threatened or endangered, particularly in the south of their range on either side of the North Atlantic (COSEWIC 2011). Aquaculture escapes represent a continued threat to wild populations through genetic, pathological and ecological interactions (Fleming *et al*. 2000; Glover *et al*. 2013; Hindar *et al*. 2006; McGinnity *et al*. 2003; Naylor *et al*. 2005; Thorstad *et al*. 2008). Farm escapes have been commonly documented to interbreed with wild fish, resulting in population-level changes, including an erosion of local adaptation due to genetic introgression (Glover *et al*. 2013; Skaala *et al*. 2006). The magnitude of changes and subsequent risk to wild populations will be dependent on the relationship between wild populations and farm strains resulting both from the ancestral relationship (Baskett *et al*. 2013), and the degree of selective change associated with the domestication process and subsequent deliberate selection for traits such as enhanced growth and delayed sexual maturity (Gjedrem *et al*. 1991). As a result of both ancestry and domestication selection, farm escapes and their offspring (including F1 and subsequent hybrids and backcrosses between farm escapes and wild) are generally poorly adapted to wild conditions and suffer reductions in fitness (Fleming *et al*. 2000; McGinnity *et al*. 2003; Skaala *et al*. 2012). (Hereafter we use the term "hybrid" to refer to F_1 farm X wild offspring and "introgressed individuals" to refer to F_2 and subsequent hybrids and backcrosses respectively categories B and C in Table 1.).

Accordingly, there is a need to understand the risks and potential impact of reproduction between various farm strains and wild Atlantic salmon, in order to assess the impact of interbreeding and introgression. The ultimate ecological and evolutionary impacts of aquaculture escapes on wild populations will be a function of (1) the presence and abundance of escapes in natural rivers; (2) the subsequent interbreeding (frequency and magnitude) producing hybrid individuals; (3) the survival of hybrid offspring and subsequent introgression of farmed alleles into wild populations. Ultimately, quantifying these impacts necessitates accurate identification of direct farm escapes, F_1 hybrids and progeny at subsequent levels of introgression. Thus the aim of the present study is to review work on the identification of farm escape salmon, F_1 hybrids, and subsequent introgression, and to highlight limitations and successes. Specific objectives include: (1) to review studies identifying escape farm salmon in the wild and to summarize existing tools, and the assumptions involved; (2) review studies

identifying recent F_1 hybridization in the wild, (3) evaluate studies quantifying subsequent introgression; and (4) to provide recommendations on usage of analysis tools and their limitations.

4.3 Literature review

Studies which evaluate the potential impact of escaped farm salmon vary in approaches used and the temporal and spatial scale examined. Specific methodologies commonly applied (i.e. individual assignment, Bayesian clustering) have been reviewed elsewhere (Kalinowski 2004; Koljonen *et al*. 2007; Manel *et al*. 2005) but rarely in the context of farmed escape identification. Admittedly, given the breadth of literature on interactions of wild and domesticated organisms in general, our analysis is not inclusive. Nonetheless, the restricted focus on domesticated Atlantic salmon seems appropriate given rapid growth of the industry and status of wild populations. Also, as the goal here is to review studies focusing on impact detection and quantification, we have also chosen not to include quantitative trait based studies which document significant evolutionary and demographic changes associated with introgression of farmed and wild salmon (e.g. Fleming *et al*. 2000; McGinnity *et al*. 2003; Milot *et al*. 2013). Studies were only included here if genetic or genomic approaches were utilized to identify farm escapes or hybrids in the wild or quantify the degree of subsequent introgression. For each study, the marker used, number of loci, and statistical approach were noted. When possible, estimates of assignment power examined using simulated mixtures or leave one-out cross validation (e.g. Anderson *et al*. 2008; Kalinowski *et al*. 2007) were also included. To assist in comparing and contrasting approaches, studies were organized into the three categories described above: (A) direct farm escape identification, (B) F_1 hybrid identification, (C) quantifying subsequent introgression. Details and sources of each estimate are contained in Table 1.

4.4 Distribution of studies

Our review of the literature identified 29 studies which broadly addressed the issue of Atlantic salmon farmed escape impacts using genetic and genomic approaches (Table 1). Of these, the majority of studies focused on escape identification (~70%), with fewer attempting to identify farm-wild hybrids (12%) or to quantify introgression (18%; Figure 1). Study attributes, success and limitations are discussed below for each of these groups separately.

Identification of farmed escape salmon in the wild. Escape farm salmon are increasingly captured both in freshwater and at sea (e.g. Jensen *et al*. 2013). Based on the reviewed 19 studies, the identification of farm escaped salmon using genetic and genomic tools has been more commonly (> 2 times) applied to assigning individuals to specific cage sites or aquaculture facilities (e.g. Glover *et al*. 2011) than the distinction of wild and farmed origin, though both exist in the literature. Most studies to date have employed microsatellite loci (n = 12), followed by SNPs (n=4), and allozymes (n=3), although a few studies evaluated multiple marker types (e.g. Glover *et al*. 2010). Not surprisingly, we observed a temporal trend in approaches utilized and markers, transitioning from allozymes in the 1990s to microsatellites, and more recently the use of SNPs (e.g. Glover *et al*. 2013). With the increasing use of single nucleotide polymorphisms, SNPs, for individual assignment in recent studies, there is also a shift from randomly distributed loci, to targeting genomic regions that display elevated divergence associated with domestication (e.g. Karlsson *et al*. 2011). This trend seems likely to continue and will improve assignment power in future. For example, Karlsson *et al*. (2011) identified 60 diagnostic SNPs that have seem to provide high accuracy (~100%)

for the identification of Norwegian farmed Atlantic salmon (Table 1). These loci were chosen from a panel of 7K loci to maximize assignment power. This panel is already getting widespread use, in the eastern Atlantic (e.g. Coulson 2013; Glover *et al*. 2013; Jensen *et al*. 2013), though its utility in resolving non-Norwegian domesticated salmon may be limited (Coulson 2013).

The most common statistical approach used for identification of farm escaped individuals was individual assignment tests primarily implemented in GENECLASS (Piry *et al*. 2004) or STRUCTURE (Pritchard *et al*. 2000). Other and often older approaches involved allele frequency comparison, or the presence and absence of rare alleles. The accuracy of individual assignment reported by various authors varied significantly with the nature of the groups being evaluated. The accuracy of the identification of farm escaped salmon from wild salmon was significantly ($p < 0.001$) higher on average (92.2% ± 6.1) compared with the identification of specific farm salmon strains or cage locations (67.2% ± 13.1). In several studies this seemingly low accuracy could be explained by the use of individual cages in a baseline with very low (28%) self-assignment. Several recent studies (e.g. Glover *et al*. 2009) seem to indicate that Bayesian clustering (i.e. STRUCTURE) may outperform other methods as noted elsewhere (e.g. Ensing *et al*. 2011; Griffiths *et al*. 2010). Overall, it seems that recent advances allowing genome-wide SNP surveys offer the greatest potential for developing highly accurate SNP panels for farm escape salmon identification. The broad scale applicability of these panels across aquaculture strains, and regions though remains to be evaluated (but see Vasemagi *et al*. 2012).

Identification of wild × farm F_1 hybrids. Surprisingly few studies have attempted to identify recent hybrids among wild populations and farm escaped Atlantic salmon. Only four such studies (See Table 1) were identified which specifically addressed this issue. Of these, one (Crozier 1993) used allozymes, one (McGinnity *et al*. 2003) used microsatellites, and two (Coulson 2013; Karlsson *et al*. 2011) used SNPs. While we primarily restrict the term 'hybrid' to F_1s which represents the focus of most the studies here, McGinnity *et al*. (2003) further identified F_2 and backcrossed individuals via parentage analysis from both wild and farmed broodstock. Given the relative lack of studies in this category, identification of particular trends is challenging. Crozier (1993) used allelic variant frequencies to identify hybrid individuals from pure farmed or wild individuals as evidence of successful spawning between wild and farmed salmon. They found that in four of their seven loci, allele frequencies in the wild had shifted toward those of the farmed strain. McGinnity *et al*. (2003) used pedigree reconstruction to identify pure, F1, F2 and backcrossed individuals and were further able to assess the degree of fitness reduction (2–89%) of the various hybrid classes. With increasing sequencing and genomics capability, SNPs seem poised to become the marker of choice for the identification of hybrids in the field. Karlsson *et al*. (2011) reported on a subset of 60 SNPs that distinguished between their Norwegian wild and farmed baselines (see above). While this study was primarily focused on the identification of the marker subset, they did elude to the potential ability for hybrid identification, tested on simulated individuals. A subset of the SNPs identified by Karlsson *et al*. (2011) have been adopted and tested in Scotland (e.g. Coulson 2013), for both identifying Norwegian escapees, and for testing both Scottish west coast fish sampled in the wild and simulated F_1 individuals, for possible Norwegian ancestry. Several locations showed significant evidence of intermediate genotypes, with moderate success in the identification of F_1 hybrids. Notably however, a sample of Scottish origin farmed fish could not be distinguished from wild Scottish fish, suggesting that more and/or different sets of markers

are needed (Coulson 2013). This point underlies the potential challenges in the identification of common sets of markers or genomic regions across all domesticated strains. Overall given the scarcity of studies, it seems likely that statistical power has likely been lacking to accurately identify farm – wild hybrids using allozymes or microsatellites, unless approaches like pedigree analysis are being used (see (McGinnity *et al*. 2003). This limitation seems to have been recently alleviated using targeted panels of SNPs but further examination is required to delineate situations and scenarios where this approach can be successful. Simulation studies using wild and domesticated salmon with large numbers of genome-wide SNPs are needed to explore ultimate power and limitations of this approach.

Studies evaluating introgression. Several genetic marker studies have demonstrated altered genetic signatures in wild salmon populations following exposure to farm escapees (Bourret *et al*. 2011; Clifford *et al*. 1998a; b; Crozier 2000; Glover *et al*. 2012; Skaala *et al*. 2004) as well as in response to active stocking with hatchery fish, e.g. in attempts to 'rehabilitate' dwindling local populations (e.g. Perrier *et al*. 2011; Tessier *et al*. 1997; Vasemägi *et al*. 2005). Whereas a qualitative demonstration of 'genetic change' can be done fairly easily by comparing haplotype- or allele frequencies in farm strains, unaffected and affected wild populations (Clifford *et al*. 1998a; b; Mork 1991) or in samples collected from the same population before and after farm exposure (Crozier 2000; Glover *et al*. 2013; Skaala *et al*. 2006), also see Hansen (2002) and references therein for examples for brown trout *S. trutta*), quantitative estimates of long-term admixture and introgression remain rare in salmon (Glover *et al*. 2013). Compared with the relatively simpler identification of farm escapes and hybrid individuals in samples of wild-caught fish (see above), quantitative estimates of levels of genetic introgression commonly require more detailed analyses and more extensive information about the gene pools of hatchery strains, pure wild populations, as well as fish at various generation levels of hybridization. However, to fully understand the extent of impacts of escaped farm fish on wild populations, quantification of the long-term dynamics of hybridization processes are needed. Genetic marker based methods have largely been based on one of two approaches depending on available data: 1) Estimating effects of farm escapees on the distribution of genetic variation within and between populations, often using F_{ST} or similar estimators (see Glover *et al*. 2013; Glover *et al*. 2012); 2) Estimating the degree of individual or population-wide admixture.

Under the first approach F_{ST} based estimates of the distribution of genetic variation within and among samples from populations (rivers) pre- and post-farm escapes have shown that genetic profiles become more similar among river populations over time (Bourret *et al*. 2011; Glover *et al*. 2012, Perrier *et al*. 2011), suggesting that local gene-complex signatures may erode under the impact of farm introgression. In the study by Glover *et al*. (2012), there was a strong positive relationship between river-specific estimated numbers of escapees and the relative change in the genetic profile of wild populations. However, their study also showed that genetic changes were not always predicted by rates of escapes. In some rivers subjected to many escapes, there was little genetic change, signifying that local populations may respond very differently to the presence of non-native fish. That feral domesticated fish are often not able to make a genetic contribution comparable to that of wild fish is a general observation, as for example is also shown in brown trout *S. trutta* populations stocked excessively with a hatchery strain that in some cases outnumbered local fish, but nonetheless only had minor genetic contribution (Hansen 2002).

Under the second approach, admixture analysis aims to determine in wild-caught fish the proportional contribution of each parental strain or population (in this case wild

vs. one or more farm strains) in a hybridizing population. Following the next-generation-sequencing driven explosion in genome-wide mapping studies in humans, recent years have witnessed a marked development of analytical methods to determine population structure and individual ancestry, and also admixture (e.g. Alexander *et al*. 2009; Chikhi *et al*. 2001; Dupanloup and Bertorelle 2001; Falush *et al*. 2003; Loh *et al*. 2013; Pritchard *et al*. 2000; Skotte *et al*. 2013). An important prerequisite in admixture analysis is the correct determination of contributing parental populations. Ultimately, reliability of admixture estimates depends on the degree of differentiation of the parental populations, and whether allele frequencies can be estimated reliably (Bertorelle and Excoffier 1998). Correspondingly, estimating admixture may be complicated by introgression stage. It will, for instance, often be statistically more challenging to evaluate contemporary levels of admixture in wild populations that have been subjected to introgression by farm fish for several generations or from multiple sources. This is because the accumulated genetic change in the wild gene pool towards becoming more 'hatchery strain-like' means decreased genetic resolution for quantifying contemporary levels of admixture. Estimation can be especially challenging if information about genetic profiles in pre-introgression stages is lacking. However, new statistical developments emanating from human genome research (e.g. Skotte *et al*. 2013) are likely to yield much increased power to estimate admixture in such cases, although analyses may in some cases require much increased genomic marker coverage. Nonetheless, significant genetic changes have been demonstrated, even with more limited genomic marker coverage. Based on 72 SNP markers in samples from farm strains and temporal samples from the wild, Glover *et al*. (2013) recently reported introgression, with admixture estimates (i.e. the proportion of gene pool originating in aquaculture fish) ranging between 2–47%, and marked changes towards more 'farm-like' gene pools in several wild Norwegian populations. Likewise, Bourret *et al*. (2011) used linkage disequilibrium as an estimator of admixture and found that wild Canadian salmon populations showed significant changes in candidate gene variation and possible loss of local adaptation following farm introgression.

4.5 Discussion and Future Directions

In light of rapid increases in domesticated salmon production in recent years and the current depressed status of many wild populations, there is a growing need to delineate the risks and potential impact of reproduction between various farm strains and wild Atlantic salmon. Here we reviewed the literature focusing on studies which have attempted to identify farm escapes in the wild, recent hybridization, and to quantify introgression among farm strains and wild populations. The majority of studies reviewed clearly demonstrate that highly accurate identification of farm escapes is possible using assignment tests or Bayesian clustering in conjunction with microsatellites or SNPs. In contrast, studies which attempted to identify hybrid individuals (F_1) were rare suggesting this remains challenging. Hybrid identification seems to only recently have become possible, outside of experimental systems, through the availability of large numbers of genome wide loci. Several studies have reported accurate F_1 identification. Although only one investigation has attempted this through a broad survey of wild salmon (Coulson 2013) the number of such studies is likely to increase in the coming years. Genetic and genomic based methods for studying introgression have largely been based on either estimating effects of farm escapees on the distribution of genetic variation within and between populations, often using F_{ST} or similar estimators, or on estimating the degree of individual or population-wide admixture present, again using Bayesian based methods. In both cases, recent studies suggest significant promise in using highly selected panels of SNPs to identify F_1 hybrid individuals or the presence

of introgression. Overall, genetic and genomic quantifying the impact of farmed escaped salmon on wild populations remains challenging though recent advances in genomic and statistical resources have made it possible in many situations.

Perhaps surprisingly, few studies actually attempted to distinguish farm escapes from wild populations. More commonly, studies have attempted to distinguish among cage sites (e.g. Glover 2010; Glover *et al*. 2011) although reported accuracy has been less than perfect. Comparisons of marker types and assignment approaches have been limited but seem to suggest that the use of SNPs and Bayesian clustering (i.e. STRUCTURE) is associated with elevated assignment power (e.g. Glover *et al*. 2010; Glover *et al*. 2009; Rengmark *et al*. 2006). With the recent shift to using SNPs selected specifically for the identification of farmed escapes, comes the issue of how transferable these panels of makers are across farmed and wild strains. On some level, selection associated with domestication of different strains selects for a common phenotype, however the genetic basis of this convergence may differ among strains. Accordingly, Coulson (2013) reported that the panel of SNPs developed for Norwegian escapee identification, was unable to distinguish farmed Scottish salmon from wild Scottish salmon. Certainly when the genomic regions associated with divergence among wild and domesticated strains of salmon have been compared, little overlap seems to exist in SNPs displaying elevated divergence, suggesting that the domestication process may target different genes in different instances (e.g. Vasemagi *et al*. 2012). This ultimately may mean that the identification of broadly applicable diagnostic SNPs for domestication in salmon (i.e. domesication genes; e.g. Flink *et al*. 2014) may be unrealistic and these panels may need to be developed independently for each instance of domestication or at least in each region. Admittedly, as the number of loci involved in screening for diagnostic SNPs increases from thousands (e.g. Bourret *et al*. 2013) to hundreds of thousands (e.g. Houston *et al*. 2014), the chances of resolving common pathways and genes associated with domestication should increase.

Compared with farm escape identification, the successful identification of F_1s using genetic and genomic approaches has been rarely reported in the literature (but see Coulson 2013; Karlsson *et al*. 2011). In fact, only one assignment-based attempt to identify hybrids in the field could be found (Coulson 2013), and suggested extensive hybridization was occurring. A variety of statistical tools exist for the identification of hybrid individuals are available (e.g. Anderson and Thompson 2002; Pritchard *et al*. 2000; Wilson and Rannala 2003), but their application to wild × farmed escape hybrids has been limited. Both the lack of examples and tool application seems likely to be due to the lack of statistical power associated with the markers used (i.e. microsatellite loci). In fact, in a simulation study evaluating power of Bayesian approaches (i.e. STRUCTURE and NEWHYBRIDS) to detect hybrids, Vaha and Primmer (2006) report upwards of 24 microsatellite loci are required at an F_{ST} of 0.12 to accurately detect F_1 individuals. As such, it seems likely that in many situations hybrid identification has not been possible with microsatellite loci. Nonetheless, with recent access to large SNP panels, screening for loci which display elevated divergence now means that accurate F_1 identification may be possible with< 100 deliberately chosen loci (Coulson 2013; Karlsson *et al*. 2011). Further simulation studies are needed to evaluate the potential for SNP panels, such as these, to provide accurate F_1 identification and possibly other hybrid classes under a variety of demographic situations.

Levels of introgression among wild and domesticated strains may be higher in many domesticated animals than previously thought, with recent analysis suggesting the history of domestication is characterized by frequent gene flow between wild and captive

populations (Marshall *et al*. 2014). In Atlantic salmon, levels of introgression with aquaculture escapes have rarely been examined, but the few studies which exist suggest significant variation among populations and that the extent of introgression is difficult to predict in advance (Glover *et al*. 2012). Qualitatively, several studies have documented genetic change over time attributed to introgression with farmed escapes (e.g. Clifford *et al*. 1998a; Clifford *et al*. 1998b; Crozier 2000; Skaala *et al*. 2006) but to date only a single study has estimated the degree of introgression at the population level (e.g. Glover *et al*. 2013). The ability to resolve introgression seems to be complicated by the degree of diversity within the aquaculture strains involved, and modelling work suggests that with increases in the number or diversity among the strains used, the ability to detect introgression is dramatically reduced (Besnier and Glover 2011). The use of highly selected SNP panels seems to alleviate this issue somewhat, presumably through screening outlarge numbers of loci for SNPs and alleles common to all strains (Glover *et al*. 2013). As noted above, it seems likely that SNP panels will have to be situation specific to maximize power to identify and quantify introgression. In addition to highly selected panels of SNPs, the potential for using genome wide distributed loci to explore changes in genomic architecture associated with introgression exists and has barely been explored. For instance, we might expect changes in distribution of linkage disequilibrium and differentiation (Bourret *et al*. 2011) across the genome as introgression progresses, and this is expected to be more pronounced in some genes or gene regions than others. It thus seems likely that genome resequencing and high density genome scans will dramatically improve our ability to resolve and quantify introgression among wild and farmed escaped salmon.

In summary, our review of the literature indicates significant progress in the resolution of genetic impacts from farmed escape Atlantic salmon on wild populations. At present, the largest challenge seems to remain the identification of recent hybrids (F_1) and recent introgression (F_2, Backcrosses), although long-term introgression under complex scenarios of multiple aquaculture strains remains a challenge as well. The recent use of both highly selective SNP panels and genome wide analysis has significantly advanced our understanding in recent years and is poised to continue this trend. It is critical that during the implementation of these new methods and approaches, simulation studies continue to be used to provide robust evaluations of accuracy, and identify limitations.

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Table 1. List of studies which identified Atlantic salmon direct farm escapes (A), F_1 wild X farm hybrids (B), or the presence of introgression in the wild (C), using genetic or genomic approaches. See methods for details regarding review criteria.

Reference	Study Goal	Genetic marker (s)	# Loci	Statistical approach	Evaluation of Accuracy	Comparison	Notes
A) Studies Identifying Farm Escapes							
Clifford et al. (1998c)	A	mtDNA, minisatellites	2, 3	Allele frequency comparisons	-	F-W	Evidence of escaped farm salmon completing their life-cycle, returning to breed with native fish
Glover et al. (2008)	A	microsatellites	13	Individual Assignment (GC) exclusion 0.01 and 0.05)	LOO - 62.5%	F-F	21/29 escapees assigned to a single farm
Glover et al. (2009)	A	microsatellites	17	Bayesian Clustering (STR), Individual Assignment (GC exclusion 0.01 and 0.05)	LOO - 44%, ST Self Assignment - 99%	F-F	Comparison of self assignment in GC and STRUCTURE
Glover et al. (2010)	A	microsatellites, SNPs	15, 300	Bayesian Clustering (STR), and Individual Assignment (GC)	Hold out individuals (GC-65% MS, 80% SNP; ST - 73% MS, 88% SNP)	F-F	Comparison of power of SNPS and Microsats for assignment
Glover (2010)	A	microsatellites		Individual Assignment (GC)	LOO - 62.5%	F-F	Single cage and farm identified.
Glover (2010)	A	microsatellites		Individual Assignment (GC)	LOO - 67.2%	F-F	Multiple cages, farms
Glover (2010)	A	microsatellites		Individual Assignment (GC)	LOO - 64.8%	F-F	Single cage and farm identified
Glover (2010)	A	microsatellites		Individual Assignment (GC)	LOO -63.5%	F-F	Single cage and farm identified
Glover et al. (2011)	A	microsatellites	18	Bayesian Clustering (STR), and Individual Assignment (GC)	LOO - 64%	F-F	Cage specific assignment possible
Jensen et al. (2013)	A	SNPs	76	STR & GC (GC, exclusion 0.01)	No	F-W	Farmed fish caught at sea migrating with wild
Milot et al. (2013)	A	microsatellites	8	parentage reconstruction	generation of 'pseudo-offspring' for parentage assignments	W-F	11-41% of returning spawners were hatchery born; RRS was 0.55 of wild-born; parentage analysis
Mork (1991)	A	allozymes	3	Temporal changes in GST	-	F-W	Substantial reduction (50-70%) in differentiation (GST) with reported farm escapees (~30%)
O'Reilly et al. (2006)	A	microsatsatellites, mtDNA	3, 1	Presence/absence of alleles/haplotypes	No	F-W	European origin screened for
Rengmark et al. (2006)	A	microsatellites, SNPs	16, 26	Individual Assignment (GC, exclusion 0.05)	LOO -82.4, 95.4%	F-W	Comparison of SNP and Microsatellite performance
Sægrov et al. (1997)	A	allozymes	2	identification of f-w status of breeding individuals using markers + enzyme	-	F-W	45% of spawning females confirmed to be of farmed origin
Skaala et al. (2004)	A	microsatellites	12	Individual assignment (GC)	LOO - 96-97%	F-W	High discriminatory ability between f-w strains
Withler et al. (1994)	A	Southern Blot	1	Discriminant Function	simulated mixture (83-97%)	F-W	Chinook domestic and wild comparison
Withler et al. (2007)	A	microsatellites	13	Individual Assignment (CB)	simulated mixture (>90%)	F-W	Chinook domestic and wild comparison
Zhang et al. (2013)	A	microsatellites		STR & GC (exclusion 0.001)	LOO self-assignment (59%)	F-F	The majority of the escapees originated from a single farm, escapees captured in later period from multiple farms.
B) Studies Identifying Hybrids							
Crozier (1993)	A, B	Allozymes	7	Allele frequency comparisons	No	F-W	Two variant alleles unique for farmed s. Folow up on F1s (0+ fry). Shifted allele-freq in juveniles.
Karlsson et al. (2011)	A, B	SNPs	60	Bayesian Clustering (STR), and Individual Assignment (GC, exclusion 0.001)	100%	F-W	SNPs screened for loci to ID escape, simulated F1s evaluated as well.
MIAP RAFTS report Coulson (2013)	A, B	SNPs	35	Bayesian clustering (STR)	self-assignment using clustering of baseline & simulated F1 hybrids	F-W	High accuracy for direct escapees and moderate-high for F1
McGinnity et al. (2003)	B	microsatellites	6	parentage reconstruction	-	F-W	Stocked farm and wild hybrids evaluated for fitness reductions over two generations
C) Studies Evaluating Introgression							
Bourret et al. (2011)	C	microsatellites, SNPs	8, 112	Temporal change in allele freq, change in LD, number of outliers	No	F-W	Farm strain based on local population 20 years back. Temporal samples show reduction in the number of observed markers potentially under the effect of divergent selection
Clifford et al. (1998c)	C	mtDNA, minisatellites	1, 1	Allele frequency differences	No	F-W	Temporally replicated samples
Crozier (2000)	C	Allozymes	8	Allele frequency comparisons	No	F-W	Follow up on "nr 12". Wild population remains significantly different from the pre-escape population. Not quantifying introgression, but claim to see deminishing impact.
Glover et al. (2012)	C	microsatellites	22	Bayesian Clustering (STR), and Individual Assignment (GC, exclusion 0.001)	-	W-W	Historical population genetic structure throughout Norway still appears to be retained
Skaala et al. (2006)	C	microsatellites	8	Assignment (BAYES & GC); FST	-	W-W	significant changes in some rivers but not others; reduction in differentiation over time
Glover et al. (2013)	C, A	SNPs	72	Temporal change using Bayesian Clustering (STR), Individual Assignment (GC), ABC	-	W-W, W-F	First study to estimate introgression, estimated introgression of farmed fish ranged from 2-47%

Key: A-farm escape ID attempted, B- hybrid ID attempted, C-level of introgression evaluated; STR – STRUCTURE, GC – GENECLASS; LOO- Leave One Out Validation; W – wild, F – Farm

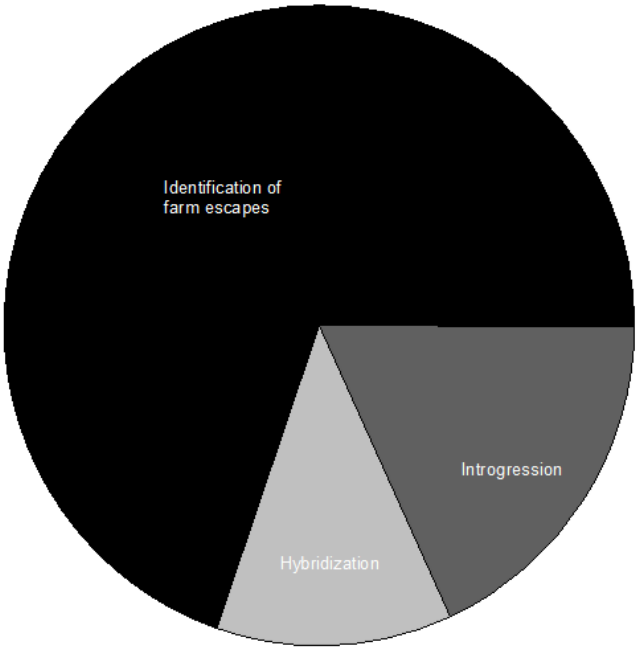

Figure 1. Proportion of papers addressing escape identification, F_1 hybridization, and introgression of farmed salmon in the wild. See Methods for details regarding literature review and Table 1 for list of studies included.

5 Term of Reference d): Produce an update on SNP-technology assessment

Geir Dahle, and Naiara Rodríguez-Ezpeleta

In 2012 the WGAGFM presented an in-depth discussion on the assessment of Single Nucleotide Polymorphism "SNP" technology. There, we focused on RAD sequencing as one of the most promising approaches for "Reduced Representation Library" sequencing able to identify and genotype thousands of SNPs in non-model species. The report also raised the issue of ascertainment bias as well as the value of validating the SNPs. Different platforms and software were discussed as well as infrastructure and cost. Two years later in 2014 the development of SNP usage in genetic studies is still in a positive development with new methods for Reduced Representation Library sequencing (e.g.GBS, ddRAD, 2bRAD) arising and new platforms for analysis (e.g. Stacks, pyRAD, TASSEL) being developed. Thus, there is an ongoing process of identifying suitable laboratory and analysis protocols to identify the "useful" SNPs for different studies, including those of relevance for the application of genetics in mariculture and fisheries. Moreover, sequencing technologies are rapidly evolving, moving from second generation sequencing producing short reads and requiring a previous amplification step (e.g. Illumina, 454) to third generation technologies that do not require PCR (e.g. Oxford Nanopore) or are able to produce longer reads (e.g. PacBio). This opens up a whole new area of population genomic tools that will become increasingly available also in non-model species, such as most exploited fish and shellfish.

6 Term of Reference e): Request from OSPAR: "genetic impacts on marine environment and on wild fish stocks, specifically in connection with introgression of foreign genes, from both hatchery-reared fish and genetically modified fish and invertebrates, in wild populations"

Dorte Bekkevold, Ian Bradbury, Mark Coulson and Tom Cross

The Working Group for the Application of Genetics in Fisheries and Mariculture (WGAGFM) has a long history of providing advice to ICES, including reviews of genetic aspects of aquaculture, both with regards to use of molecular approaches to set up and optimize breeding designs, as well as on assessment of potential effects of aquaculture (including escapees) on ecosystems, and fish and shellfish resources. Several of the group's members are involved in or responsible for giving advice to national governmental bodies on genetic and ecological effects of aquaculture escapes, and several members engage in internationally leading research on the same issues. The following text provides comments on the three points:

1) Updating the available knowledge of genetic impacts on marine environment and on wild fish stocks,
2) Concrete examples of management solutions to mitigate genetic pressures on the marine environment, and
3) Advice on which pressures have sufficient documentation regarding their impacts, so as to implement relevant monitoring and suggest a way forward to manage these pressures; as requested by the OSPAR commission.

6.1 Update on the available knowledge of genetic impacts

One of the pervasive findings in genetic studies of fish and shellfish is that wild populations generally are spatially structured and this is evident both in neutral and functional genetic variation (review in Hauser and Carvalho 2008). A fish or shellfish individual from one area can thus be distinguished from individuals from other areas based on their genetic composition. These unique genetic population signatures are the results of local demographic and evolutionary processes (i.e. colonization, genetic drift, natural selection). Likewise, the vast majority of fish produced in aquaculture constitute selectively bred and highly domesticated strains, which in some cases have been developed from targeted breeding programmes over multiple generations (Gjedrem 2010). The implication of domestication divergence being that introduction of farm escapes can significantly alter the composition of local gene pools of wild fish. This, in the popular press, 'genetic contamination' more correctly and formally known as 'genetic introgression' is the result of reproduction between 'pure' farm and 'pure' wild fish (hybridization) and their offspring's interbreeding and backcrossing in subsequent generations. Introgression is of concern as it leads to an erosion of locally adapted gene complexes, and thus to maladaptive changes in functional traits that govern the productivity and ultimately the survival of local populations (Hindar *et al*. 1991; Lynch and O'Hely 2001; Ford 2002; Naylor *et al*. 2005; Araki *et al*. 2007; Satake and Araki 2012). Perhaps counter intuitively, farm escapes can also lead to an overall lowering of the total amount of genetic variation in introgressed populations (Box 1), potentially leading to loss of adaptive potential and the risk of inbreeding depression. Collectively, these concerns have fuelled a multitude of both theoretical and experimental studies. There has been strong experimental focus on salmonids fish, which can be bred experimentally relatively easily, and where genomic resources are ample, facilitating genetic assessment.

Box 1. *The Ryman-Laikre effect*

Escaped farm (or actively stocked) fish of hatchery origin are often based on strains established from a relatively small number of broodstock individuals. This means that levels of genetic variation maintained in the hatchery strain commonly are much lower than levels maintained in the wild. When large numbers of individuals representing a small number of families enter wild populations and compete for mating with wild fish, the effect is expected to be an overall lowering of the total genetic variation in that population compared with an un-manipulated scenario (Ryman and Laikre 1991).

This so-called Ryman-Laikre effect was demonstrated in steelhead trout, *Oncorhynchus mykiss*, where Christie *et al*. (2012) used genetic marker analysis in all spawners returning over a 12 year period to an Oregon river which was supplemented with a hatchery strain. They found that despite released hatchery fish leading to a near doubling of the census population size, they also led to a reduction in the genetically effective number of breeders, relative to a situation where no hatchery fish had been released. They moreover identified a strong relationship between the numbers of hatchery origin fish recorded on spawning sites in a specific year and the reduction in total genetic diversity relative to no fish being released, showing that the relative proportion of wild vs. farm fish competing for reproduction has a strong effect on the population's total genetic variation.

Evidence of genetic effects of farm fish has generally been of either, 1) a quantitative genetic nature, where fitness traits such as survival, growth-rates, mating- and reproductive success have been compared in farm, wild and hybrid individuals under natural or experimental conditions (see Box 2 for examples); or 2) assessed using genetic marker studies commonly evaluating changes in the genetic signatures of impacted wild populations (see Box 3 for examples). Whereas the first type of approach is able to generate direct estimates of fitness effects of farm escapes at one or more life-stage in one or more scenario, marker based studies enable assessment and monitoring of the actual longer-term impact of escapees, including under different escape scenarios. The latter issue could, for example, be related to comparing effects under chronic 'leakage' where smaller numbers of fish escape regularly, vs. where escape events are rarer but massive, as when entire net pens rupture.

Box 2. *Fitness differences in farmed, wild and introgressed populations*

Several salmonids studies have demonstrated heritable differences in various behavioural and fitness traits among farm, wild and hybrid fish (e.g. Solberg *et al*. 2013 and references herein). A commonly applied approach is to experimentally produce large numbers of families of known genetic origin (pure wild, farm and hybrid) and then test their expression of one or more fitness traits for one or more life-stage in a controlled so called 'common garden' environment.

Fraser *et al*. (2010) used a common-garden experimental approach, implemented over an 8-year period, to compare embryonic development rates in wild, farm and hybrid strains of Atlantic salmon. They demonstrated that embryos of farmed salmon, first-generation hybrids between wild and farmed fish and their subsequent multigenerational hybrids had slower developmental rates than those of two wild populations, showing a clear mismatch in farm genetic make-up to wild conditions. However, hybrid developmental rates sometimes overlapped with wild fish and under prevailing environmental conditions hybrids and backcrosses would thus persist and admix into the wild population, despite their maladaptation.

In another Atlantic salmon study, McGinnity *et al*. (2003) produced wild, farm, hybrid and various back-crosses to examine a suite of fitness traits, including embryo survival, juvenile survival and migration phenology, as well as adult reproductive tactics. For most traits, they found a clear negative relationship between the proportion of a fish's genetic make-up that came from the farm strain and its survival. Taken across all life stages from embryo to spawner, the authors estimated farm fish to have a mere 2% of the survival of pure wild fish, and the different categories of hybrid crosses had intermediate survival rates. Thus, the more 'wild' a fish is the higher its lifetime fitness. Nonetheless, farmed fish and hybrids grew faster as juveniles and were able to behaviourally displace wild juveniles, showing that during some life stages farm fish may be superior to wild fish. When fish of farm or admixed origin first outcompete wild fish and then do relatively poorly in subsequent life stages, this can jeopardize the production of an entire population relative to its natural state.

Importantly, fitness effects can be difficult to predict from short-term studies. Thus, in hatchery origin rainbow trout, *Oncorhynchus mykiss*, that hybridized with wild cutthroat trout, O. clarkia, Muhlfeld *et al*. (2009) showed that whereas first generation hybrids had high fitness, fitness in subsequent generations declined by nearly 50%, following a 20% introgression into the wild population.

The magnitude of genetic changes or degree of introgression will depend on 1) escape numbers, 2) escape frequencies, 3) the life-history or reproductive stage, 4) levels of interbreeding and 5) the genetic make-up of escaped fish (generations of hatchery breeding and genetic distance from wild populations), which will ultimately affect the survival and reproductive success of hybrid and admixed fish in comparison with pure wild fish (Baskett *et al*. 2013). The fitness of farm escapes in the wild depends on their genetic make-up, which is the combined result of 1) which population(s) and how many individuals were used to produce the broodstock, 2) levels of directed selection for increased production traits such as growth-rate and parasite resistance, and 3) domestication selection. Domestication selection is the ultimate result of adaptation to life in a captive environment, selection for desired traits (i.e. often growth or size) and a relaxation of selection associated with predators, diseases or parasites. Both theoretical

and empirical studies have shown that even without targeted breeding selection, unintended domestication selection can lead to marked maladaptation if hatchery strains are released into the wild (Araki *et al*. 2007). Population response to introgression of maladapted genes or gene complexes will conversely also depend strongly on the genetic make-up and especially population size of the recipient population (Glover *et al*. 2012). Thus, larger and genetically more diverse populations are expected to be more resilient to introgression. This is due to the fact that natural selection is more efficient at 'weeding out' maladapted gene complexes in large populations compared to small populations, which are also more easily 'swamped' by gene pools from farmed fish. It can also be expected that species exhibiting extensive population and life-history diversity (biocomplexity) are likely to be overall more resilient to negative impacts of farm escapees on an ecosystem level.

Box 3. *Wild population genetic signatures following introgression*

Genetic markers have been widely used to analyse the genetic profiles of wild populations subjected to farm escapes or to stocking with non-native strains in a suite of salmonids fish (see, for example, Bradbury *et al*. 2014 for a recent review of studies in Atlantic salmon and Hansen *et al*. 2009 for an example from brown trout, *S. trutta*), and in a few fully marine species (e.g. Coscia and Mariani 2011).

Glover *et al*. (2013a) used diagnostic genetic markers to genotype historical and contemporary samples from 20 Atlantic salmon populations spanning all areas of Norway where salmon escapes occur, sometimes in massive numbers. They found that the genetic signatures were altered in several populations to becoming more 'farm-like', and there was a strong positive relationship between river-specific estimated numbers of escapes and the relative change in the genetic profile of wild populations. However genetic changes were not always predicted by rates of escapes, as, in some rivers subjected to many escapes, there was little genetic change, signifying that local populations may respond very differently to the presence of non-native fish.

In recent years, such analyses have been combined with genomic studies aiming to identify the genetic architecture of the traits under divergent selection in wild vs. farm environments (e.g. Vasemagi *et al*. 2012). Answering questions like whether trait differences are governed by few loci of large effect or many loci of small effect will aid in predicting the rate with which maladapted farm traits will be purged from introgressed populations.

Major knowledge gaps fall into two main categories. First, in contrast to the situation in an increasing number of salmonid species and populations, there is generally very limited information about genetic effects of the farm escapes on populations of fully marine fish (Bekkevold *et al*. 2006, see Glover *et al*. 2011 for a genetic marker based traceability study in Atlantic cod *Gadus morhua*). Relative to finfish, establishment of selective breeding of shellfish is even more recent and less developed, and the genetic impact of escapes is rarely evaluated (Gilbey *et al*. 2014). Discrimination between wild and farmed strains of marine fish is complicated by the fact that in most cases each farmed strain has its own history of selection and domestication, sometimes including repeated backcrosses to wild-caught broodstock. These breeding processes have typically not been documented and may mask the frequency and direction of interactions. Projects, such as the EU framework projects Genimpact ("Genetic impact of aquaculture activities on native populations", http://genimpact.imr.no/) and Aquatrace ("Development of tools for tracing and evaluating the genetic impact of fish from aquaculture" https://aquatrace.eu/) have recently, respectively, reviewed and started

developing genetic tools for addressing this knowledge gap. It is therefore expected that genetic tools for estimating genetic impacts of several fully marine fish and shellfish will become available within a couple of years. However, as fully marine species commonly exhibit large populations with more gene flow it is expected that conducting a comprehensive assessment of the genetic impact of farm escapees often will be even more challenging than in salmonids.

A second major knowledge gap is linking the degree of hybridization and introgression with actual effects on fitness and survival in subsequent generations. Although modelling can be applied to predict effects of introgression on quantitative traits (e.g. Satake and Araki 2011) it is very difficult to accurately predict fitness and survival across entire life cycles and over multiple generations. One of the reasons for this is that the response will depend on the relative balance between rates of maladapted genes entering the population (~ numbers of escapes) and the strength of natural selection acting to 'weed out' those genes. Clearly, there are large differences in these parameters, both among species and among populations within a species, making it very difficult to predict long-term responses beyond a case-by-case basis. Theoretical models predicting fitness effects of domesticated escapees should be applicable across taxa and environments. However, in fully marine populations there have yet been almost no attempts to estimate the effects of farm escapees on fitness and productivity. The reasons for this are multiple, and include the general lack of genomic resources available examine links between key fitness and life-history traits and specific functional genetic variation between wild and farmed fish, as well as the general difficulty in conducting extensive full life cycle experiments in marine fish and shellfish. Novel genomic analytical methods have found use not just for better targeting aquaculture breeding designs (McAndrews and Napier 2011), but have also further opened possibilities for direct assessment of genetic changes of specific genes known to be under divergent selection pressures in farm vs. wild environments, including in marine taxa. Such approaches are expected to be extremely useful for merging information about quantitative levels of genetic changes with qualitative assessment of direct fitness impacts.

In conclusion, genetic effects of escaped farm fish have been demonstrated in several natural populations, where hybridization and introgression is pervasive. Negative fitness effects of introgression have been clearly demonstrated in several species and populations, although natural selection is expected to act against propagation of maladapted traits. The relationships between a specific level of genetic introgression and the level of fitness decrease in wild populations are commonly difficult to predict. There is a strong study bias towards salmonids, which is mainly driven by the proliferation of the farming of Atlantic salmon and associated concerns about their effects on wild populations in combination with ample genomic resources facilitating genetic study. Similar tool developments and studies in farmed marine organisms are strongly warranted.

6.2 Concrete examples of management solutions to mitigate these pressures on the marine environment

1) Maximize containment of farm fish

If farmed individuals, their diseases and parasites never or rarely escape into the wild, this will always be the most efficient way to minimize adverse genetic impacts. A tool could be to define equipment standards for cage structures and moorings, in combination with reporting of losses.

2) Concentrate aquaculture to as few species as possible

In contrast to aquaculture, where several hundred species to date have been attempted used in production (Teletchea and Fontaine 2014), agriculture relies on a very limited number of highly domesticated species of animals and crops. One way to minimize genetic impact of farm escapees, at least in terms of the number of wild species under concern, would thus be to follow the same approach in aquaculture as in agriculture. A potential positive side effect would be if focus is on selectively breeding a few species to an extent where there become highly domesticated, their survival and reproduction under wild conditions might be minimized.

3) Use sterile farm fish

A conceptually easy and efficient safeguard against genetic pollution is to only use sterile fish in farms (e.g. using all-female triploidisation), although this will not alleviate direct interaction or parasite transfer, and problems associated with growth of triploids have been noted.

4) Conduct fully informed risk assessments

In connection with risk assessment, genetic impact should be included as a parameter (see Taranger *et al*. 2011; Palme *et al*. 2012 for examples). The evaluation could also include spatially explicit assessment of whether the use and escapes of specific farm strains is more likely to inflict genetic damage in some areas compared to others, e.g. in areas inhabited by vulnerable, genetically unique populations. The usefulness of 'indicator based management systems', as suggested for Norwegian Atlantic salmon, could be examined.

5) Establish genetic monitoring tools based on genetic databases

It should be an aim to develop genetic marker tool systems that are reliable, cost-effective and fully transferrable, that allow both robust assessment and monitoring of interactions between wild populations and aquaculture strains, as well as the traceability of individual farm strains. The efficiency of such systems relies on access to databases with genetic information for wild and farm strains. To facilitate genetic monitoring and enable maximum power for estimating potential impacts, samples from 'pure' wild gene pools, i.e. prior to establishing farms in pristine areas, should be collected or, where available, secured from archived collections. Applicability of such systems would be facilitated (but not dependent on), a situation where the use of farm strains were regulated, so that on-growing farms were responsible for accounting for the (genetic) identity of their farmed material.

6.3 Advice on which pressures have sufficient documentation regarding their impacts to implement relevant monitoring and suggest a way forward to manage these pressures

Genetic change due to escaped farm fish is a generally acknowledged threat and has been demonstrated in several species and populations (see review in Bradbury *et al*. (2014) for Atlantic salmon, EU framework projects GENIMPACT and AQUATRACE which also include farmed marine species, and Norwegian Knowledge platform "Quantifying genetic effects of escaped farmed salmon on wild salmon" (QUANTESCAPE). A recent risk assessment of Norwegian salmon farming concluded that genetic change resulting from interaction with escapesand salmon lice infections, were the two most important farm-related threats to wild conspecifics. Genetic monitoring methods are already in place in some areas and species (Norwegian salmon), and ongoing ge-

netic marker based developments in other species (sea bream, sea bass, turbot) are expected to facilitate implementation of monitoring to determine degrees and effects of farm-wild hybridization and introgression, ranging from local to global geographical scales. Moreover, genetic monitoring tools have proven highly advantageous in the implementation of regulation and enforcement (Glover *et al*. 2008; 2013b). It is highly recommended that risk assessments be conducted (see for example Taranger *et al*. 2011 for Norwegian salmon, and Palme *et al*. 2012 for Baltic salmon, also see DFO 2013 for Newfoundland salmon) incorporating genetic considerations, from which management plans can be developed.

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7 Special request: Interactions between wild and captive fish stocks (OSPAR 4/2014)

a) Recalling the conclusion of the QSR 2010 that mariculture is a growing activity in the OSPAR maritime area, EIHA 2012 considered the potential for increasing environmental pressure relating to the growth of this industry. As yet this is not an established work stream within EIHA, and Contracting Parties have requested that more information be brought forwards on this issue. This was reiterated by EIHA 2013.

b) Mariculture has a number of associated environmental pressures such as the introduction of non-indigenous species, which can have ecological and genetic impacts on marine environment and especially on wild fish stocks; in addition, pressures from mariculture might include:

   i) introduction of antibiotics and other pharmaceuticals;

   ii) transfer of disease and parasite interactions;

   iii) release of nutrients and organic matters;

   iv) introgression of foreign genes, from both hatchery-reared fish and genetically modified fish and invertebrates, in wild populations;

   v) effects on small cetaceans, such as the bottlenose dolphin, due to their interaction with aquaculture cages.

c) EIHA proposes that OSPAR requests ICES to provide:

   i) an update on the available knowledge of these issues;

   ii) concrete examples of management solutions to mitigate these pressures on the marine environment;

   iii) advise on which pressures have sufficient documentation regarding their impacts to implement relevant monitoring and suggest a way forward to manage these pressures.

d) It may be appropriate to explore cooperation with other competent authorities working in this field, such as the European Food Safety Authority with respect to disease transfer or parasites, or the North Atlantic Salmon Conservation Organisation (NASCO), in particular with respect to existing cooperation between NASCO and ICES on issues pertaining to pressures from mariculture.

WGAGFM provide comments to the OSPAR request on the following issues: 1) Updating the available knowledge of genetic impacts on marine environment and on wild fish stocks, 2) Concrete examples of management solutions to mitigate genetic pressures on the marine environment, and 3) Advice on which pressures have sufficient documentation regarding their impacts, so as to implement relevant monitoring and suggest a way forward to manage these pressures. All responses are listed in Term of Reference e) in Section 6 of this report.

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Annex 2: Agenda

Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) Faro (Olhão), Portugal 7–9 May 2014

The meeting venue address: *Hotel Real Marina, OLHÃO – PORTUGAL, Phone (+351) 289 091 300.* http://ccmar.ualg.pt/wgagfm

Wednesday 7th

9.00 Welcome by local host Dr. Rita Castilho

9.30 Welcome and updates from WG chair Dorte Bekkevold

9.45 - 12.30 Presentation and discussion of position papers for ToRs a–e

e) Produce a review of the identification and use of adaptive gene markers in shellfish aquaculture and for the genetic characterisation of wild populations

f) Review and consider methods for integrating genomic methods with marine fisheries management

g) Review and consider molecular methods for quantifying genetic introgression of farmed fish in native populations

h) Produce an update on SNP-technology assessment.

i) Request from OSPAR: "genetic impacts on marine environment and on wild fish stocks, specifically in connection with introgression of foreign genes, from both hatchery-reared fish and genetically modified fish and invertebrates, in wild populations"

i. Update on the available knowledge on these issues;

ii. Concrete examples of management solutions to mitigate these pressures on the marine environment;

iii. Advice on which pressures have sufficient documentation regarding their impacts to implement relevant monitoring and suggest a way forward to manage these pressures.

13.00 - 14.00 Lunch

14.00 - 16.00 Presentation and plenum discussion of position papers for ToRs a–d (continued)

16.30 – 17.00 Formation of ToR working groups

17.00 – 18.00 Scientific presentation Claudia Junge: Genetics in fisheries management: an Australian perspective.

19.30 Joint dinner

Thursday 8th

9.00 Morning assembly w. updates on activities and practical information

9.15 – 12.15 Parallel work sessions on ToRs a-d

13.00 - 14.00	Lunch
14.00 - 15.30	Work in groups on ToRs a-d (continued)
15.30-16.30	Final planning of response to OSPAR
16.30 – 17.30	Status of work in ToRs groups – each ToR lead gives an update
19.30	Joint dinner

Friday 9th

9.00	Morning assembly
9.15 – 12.15	Presentation of ToR reports/recommendations
12.15 - 13.30	Suggestions for new ToRs for 2015, new chair for 2015-2017, discussion of next meeting venue.
13.30	End of meeting

Annex 3: WGAGFM terms of reference for the next meeting

A **Working Group on Application of Genetics in Fisheries and Mariculture** (WGAGFM), chaired by Gary R Carvalho, United Kingdom, will meet in Ispra, Italy, 6–8 May 2015, to work on ToRs and generate deliverables as listed in the Table below.

WGAGFM will report on the activities of 2015 (the first year) by 31 May 2015 to SSGEPI.

ToR descriptors

ToR	Description	Background	Science Plan topics addressed	Duration	Expected Deliverables
	This should capture the objectives of the ToR	Provide very brief justification, e.g. advisory need, links to Science Plan and other WGs	Use codes	1, 2 or 3 years	Specify what is to be provided, when and to whom
a	Review and assess the utility of molecular techniques to evaluate disease and parasite spread from transferred seafood into wild populations	There is a science and advise need for knowledge of potential risks of disease and parasite spread from imported seafood, and this ToR reviews the scope for genetic marked based monitoring.	Topic 25-251+252 Topic 34-344	year 1	Review paper, May 2015, to SCICOM
b	Review and map decision channels for integrating WGAGFM advice into fisheries assessment and management	It is a scientific aim to integrate genetic monitoring and assessment methods into advise and management. This ToR will identify implementation processes and advise on how potential obstacles can be removed.	Topic 12-121 Topic 14-143+144+147 Topic 16-162 Topic 31-134 Topic 34-344+346	3 years	Report, May 2017, to ACOM
c	Review application of quantitative genetic techniques into non-mariculture marine species	There is a science and advise need for providing an integrated understanding of the functional variation within and among marine fish and shellfish populations. This ToR will review the application of quantitative genetic techniques to this end.	Topic 11-112 Topic 12-121+122 Topic 14-146 Topic 34-346	3 years	Review paper, May 2017, to SCICOM

Summary of the Work Plan

Year 1	
Year 2	
Year 3	

Supporting information

Priority	The current activities of this Group will lead ICES into issues related to the ecosystem effects of fisheries, especially with regard to the application of the Precautionary Approach. Consequently, these activities are considered to have a very high priority.
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants	The Group is normally attended by some 20–25 members and guests.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	There are no obvious direct linkages.
Linkages to other committees or groups	SIMWG, WGEVO, WGBIODIV, WGAQUA
Linkages to other organizations	Linkage with the EC Joint Research Centre at Ispra, Italy.

Annex 4: Recommendations

RECOMMENDATION	ADRESSED TO
1. Support the further development and use of diagnostic markers to identify shellfish taxa, particularly in those where cryptic species and/or introgressed hybrids exist. Promote the development and use genetic markers, particularly taking advantage of the potentially higher resolution which may be obtained with adaptive markers, to aid in assessing the genetic impact of transfers of adults and/or juveniles outside of their natal areas on both wild and cultured shellfish stocks. Encourage the use of neutral and particularly adaptive markers to assess interactions of hatchery propagated stocks with wild populations, particularly focusing on potential impacts on effective population size, local adaptation and genetic diversity.	WGAQUA
2. SCICOM and relevant working groups support the development of assessment models which are able to handle spatial and temporal genetic complexity.	ICES SCICOM
3. WGAGFM members participate in salient assessment working group benchmark meetings, to enable the incorporation of genetic data relevant to assessment methods and management scenarios.	ICES ACOM
4. That ICES establish a new training component to their portfolio of training courses and opportunities within the field of "Genetics in fisheries and aquaculture" to contribute to a multidisciplinary approach to fisheries management.	ICES TRAINING
5. Since genomic tools to discern levels of introgression and presence of hybridization among farmed and wild Atlantic salmon now exist, they should be applied, with careful consideration of diversity within and among baseline groups.	ICES ACOM
6. That ToR d be terminated as an annually recurrent ToR	ICES SCICOM

Annex 5: Technical Minutes from the Review Group Interaction between Wild and Captured Fish Stocks (RGFISH)

- RGFISH
- Review deadline 17 June 2014
- Peer Reviewers: Luc Comeau (Canada); Ellen Kenchington (Canada; RG Chair)
- Working Group: WGAGFM

WGAGFM Summary

WGAGFM received an advice request from OSPAR (4/2014) on "Interactions be-tween wild and captive fish stocks". WGAGFM contributed information on genetic effects and potential management solutions to mitigate adverse impact. Several studies have demonstrated that the gene pools of wild populations change when hatchery produced farm fish escape (or are released) at large scales. Several studies also report that introgression by escaped farm fish can incur a fitness cost to wild populations, causing increasing concern for the continuing health and viability of wild populations and awareness about conserving native fish gene pools. Knowledge is mainly based on salmonids fishes but should be transferrable to fully marine organisms, making aquaculture escapees a general concern. Molecular quantification has proved valuable for demonstrating introgression by farm fish. However, WGAGFM reviewed studies and found that in many cases, the introgression process is complex, e.g. with respect to escape rates and genetic makeup of escapees, and impacts can therefore be difficult to assess and predict. Members concluded that in order to develop and implement reliable management strategies and advice, locally and internationally, it is of importance to consider on a case-by-case basis the different options for the analysis of genetic data to quantify level of introgression.

Review of ToR e: WGAGFM response to OSPAR Request (04/2014): Interactions between wild and captive fish stocks

The WGAGFM report highlights the types of genetic interactions that may occur between wild and captive fish and shellfish stocks and so addresses pressure iv) introgression of foreign genes as outlined in paragraph b of the OSPAR Request (Section 1). The report is helpfully formatted with separate sections for each of the three items detailed in paragraph c of the Request (see Section 1 above).

The WGAGFM nicely summarizes the available knowledge on this topic and includes case studies where introgression between wild and domesticated stocks has been demonstrated. This is very useful as it demonstrates that this is a valid concern, despite the many obstacles for successful survivorship of adult or juvenile escapees in the wild and of survivorship of hybrid larvae through to adulthood as detailed in Section 4.1.2 Escaped Fish of the WGAQUA report.

Concrete examples of management solutions to mitigate these pressures on the marine environment are provided and the report appears to be technically correct, is well written with existing knowledge thoroughly summarized and referenced. Specifically WGAGFM provides five "*concrete examples of management solutions to mitigate" the* introgression of foreign genes. Measure 3 "Use sterile farm fish" is described as: "A conceptually easy and efficient safeguard against genetic pollution is to only use sterile fish in farms (e.g. using all-female triploidization), although this will not alleviate direct interaction or parasite transfer". RGFISH notes that the use of triploidization for biological containment has compelling strengths as well as major weaknesses

(NRC,2004; Kenchington, 2006). There are also special ecological risks to consider with such stock, some associated with gigantism. Because gonad maturation is reduced in triploid fish and shellfish, energy normally expended on reproduction can be allocated to somatic growth. For many species, triploids attain a larger size-at-age than conspecific diploids, although this may not be expressed until their productive season (Benfy, 1999). Yield increases maybe due to increased heterozygosity (Hawkins et al., 2000) to larger cell size (hypertrophy) or to an increased number of cells (hyperplasia). Triploidy will have associated higher metabolic demands which may translate into higher feeding rates. When physically contained in open-water systems this increased metabolic activity may have enhanced effects on the surrounding communities and in the case of filter feeding bivalves may significantly alter the carrying capacity of the environment (Kenchington, 2006).

Benfy, T. 1999. Physiology and behavior of triploid fishes. Rev. Fish. Sci., 7: 39–67.

Hawkins, A. J. S., Magoulas, A., Héral, M. *et al.* 2000. Separate effects of triploidy, parentage and genomic diversity upon feeding behaviour, metabolic efficiency and net energy balance in the Pacific oyster *Crassostrea gigas*. Genet. Res., Camb., 76: 273–284.

Kenchington, E. 2006 Triploidization as a means of biological containment for exotic species. Bulletin of the Aquaculture Association of Canada, 106: 6–12.

National Research Council of the National Academies. 2004. Bioconfinement of animals: Fish, shell fish and insects. In: Biological Confinement of Genetically Engineered Organisms. Chapter4, pp 130-158. Committee on Biological Confinement of Genetically Engineered Organisms. Board on Agriculture and Natural Resources. Board on Life Sciences. Division on Earth and Life Studies. The National Academies Press, Washington DC.