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REPORT OF THE

Working Group on the Application of Genetics in Fisheries and Mariculture

La Tremblade, France
10–12 March 2003

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TABLE OF CONTENTS

Section **Page**

EXECUTIVE SUMMARY

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met in La Rochelle, France from 10–12 March, 2003. Seven persons representing six countries were present. Three terms of reference (ToR) were addressed, while one was deferred until the appropriate information was available for review.

WGAGFM first considered ToR a) *Review and report on the practical use of genome mapping in aquacultured organisms.* Genome maps are used extensively in the production of selected strains of agriculture plants and livestock. Studies on the genome of aquatic animals have been summarised in the WG report. Genome mapping projects in aquaculture exist for most major species and linkage maps are now becoming available. WGAGFM produced four recommendations:

1. Information networks, similar to those developed for model fish species, should be developed in order to provide a WWW community resource on aquaculture species genomes;
2. The co-development of domesticated, selected and inbred lines of aquaculture species is essential for future development of MAS in aquaculture species and should be initiated;
3. More links should be developed between model fish species and aquaculture and fisheries species genome projects;
4. Research on the development of physical maps for aquaculture and fisheries species should be encouraged.

Escapes of cultured salmonid fishes and their interaction with wild stocks have been a focus of attention for a number of years. Increasingly, marine species such as cod are being reared at a large-scale, and accidental escapes can be anticipated. The biological characterisitcs of marine species are different from salmonids and so the genetic impact of escapees will also differ. In ToR b) *Review and report on genetic issues related to escapes of farmed marine fish and shellfish*, WGAGFM considered the genetic interaction between cultured marine species and wild stocks (with cod as a case study) as well as the release of hatchery propogated marine species (with oysters as a case study).

1. Farming of marine fish species should be performed in a confined environment. Recommended measures of confinement include closed net-pens, recirculated inland systems, sterility (triploids) etc. This will reduce but not elliminate interactions due to accidental releases;
2. Unless proper confinement measures are taken, farming of marine species should be founded on local stocks. However, even taking this approach could be problematic due to rapid divergence between wild and farmed populations due to selection and low effective population size under aquaculture conditions.

For a number of years, WGAGFM has considered the management aspects of conserving genetic diversity. This year, a common ToR was held with the Working Group on Ecosystem Effects of Fishing Activities (WGECO) to develop advisory forms appropriate to the preservation of genetic diversity from detrimental impacts of fishing as requested by the EU. In ToR d) *Review and report on issues in relation to practical management options for the conservation of genetic diversity in marine fish and shellfish of economic importance,* WGAGFM advanced previous work by introducing categories of marine organisms with differing threats to genetic diversity. The content of this report was shared with WGECO, which met subsequently. Recommendations are identified in the WGECO report.

It was decided at the meeting to cancel ToR c) *Review and report on management recommendations for Atlantic salmon, developed by the SALGEN EU project*. This was due to the fact that the management recommendations and supporting documentation from the SALGEN EU project were not published at the time WGAGFM met, and pre-publication documents were not provided by the leader of the project who was unable to attend the meeting.

1 INTRODUCTION

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met at La Rochelle and at the IFREMER laboratory at La Tremblade, France, 10–12 March, 2003 to deal with its Terms of Reference (ToRs) for 2003 (Annex 1), with E. Kenchington (Canada) as the new Chair. The ToRs were decided in a Council Resolution (C.Res. 2002/2F01) adopted at the Statutory Meeting held in Copenhagen, Denmark, 2002. The meeting was opened at 9:00 hrs on Monday, 10 March, with the Chair welcoming the participants, particularly the new member Sylvie Lapègue, who has not previously attended WGAGFM meetings.

1.1 Attendance

Seven persons representing six countries attended the 2003 WGAGFM meeting (Annex 2). Apologies were accepted from F. Volckaert, P. Bossier (Belgium), G. Carvalho (UK), P. Prodohl, A. Ferguson (N. Ireland), M.-L. Koljonen (Finland), M. Møller Hansen (Denmark), A. Danielsdóttir (Iceland), J.-M. Sévigny (Canada), J. A. Sanchez (Spain), S. Stiles (US), and T. Paaver (Estonia).

Attendance was lower than in previous years and the WG felt that this compromised its ability to fully address the ToRs and to suggest ToRs for the 2004 meeting. Prior to the meeting, the Chair contacted all members regarding the timing of the meeting, as some felt that this might be the reason for the low attendance. However, only two members responded to that message and the timing was only a problem for one. Another factor is the large proportion of members who have been inactive for a number of years (in some cases more than 5 years). Others have been participating but are not official members. For some participants, travel funds are difficult to obtain if their status is not official. In response to this concern, ICES has initiated a survey of all members of all WGs to determine whether they wish to remain as members of their respective WGs. When this list is finalised it may be possible for WGAGFM to encourage new members to become official delegates.

1.2 Venue

The IFREMER laboratory at La Tremblade (represented by our hosts Pierre Boudry and Sylvie Lapègue) did an excellent job of arranging logistics and facilities for the meeting, including sightseeing and transport to La Tremblade on the last day. The meeting in La Rochelle was held at the Aquarium meeting room, which provided a very scenic backdrop to our discussions. The Working Group wants to thank both Pierre and Sylvie for all the work undertaken to arrange this meeting and for their very kind hospitality.

1.3 Meeting format

WGAGFM has an established framework for completing its ToRs. Prior to the meeting, small *ad hoc* working groups, under the leadership of one person, are established to prepare position papers related to specific issues in the Terms of Reference. The leader of the 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 is responsible for preparing the final report text from their sessions.

Unfortunately, not all ToR leaders followed this process this year and only one position paper was ready for presentation at the start of the meeting. To compensate, the WG worked interactively to prepare the report during and after the meeting. This was very stressful for the participants and it was decided that the working form, agreed upon in the 1999 meeting of the working group, must be adhered to. It was agreed that the Chair would be very clear to members that in proposing ToR for 2004, leaders must declare their intent to follow the WG framework and to attend the 2004 meeting.

The 2003 WGAGFM meeting proceeded under the following direction:

- E. Kenchington chaired business and general scientific sessions;
- P. Boudry chaired ToR a: *Review and report on the practical use of genome mapping in aquacultured organisms;*
- G. Dahle chaired ToR b: *Review and report on genetic issues related to escapes of farmed marine fish and shellfish;*
- E. Eg Nielsen and E. Kenchington chaired ToR d: *Review and report on issues in relation to practical management options for the conservation of genetic diversity in marine fish and shellfish of economic importance.*

It was decided at the meeting to cancel ToR c): *Review and report on management recommendations for Atlantic salmon, developed by the SALGEN EU project*. This was due to the fact that the management recommendations and supporting documentation from the SALGEN EU project were not published at the time the working group met, and pre-publication documents were not provided by the leader of the project who was unable to attend the meeting.

2 TERMS OF REFERENCE FOR 2003

2.1 Review and report on the practical use of genome mapping in aquacultured organisms (ToR a)

This text was based on a position paper prepared by P. Boudry, S. Lapègue and R. Guyomard and adopted by WGAGFM in La Rochelle/La Tremblade in 2003.

2.1.1 Introduction

In the last decades, genome maps have become available in various agricultural plants and terrestrial livestock. Comparatively, studies on the genome of aquatic animals, and especially aquaculture species, are relatively recent. Indeed the first Aquaculture Species Genome Mapping workshop was held in 1997 (Alcivar-Warren *et al.*, 1997) and annual progress is reported during the Plant and Animal Genome Conference in San Diego, California, USA (http://www.intl-pag.org). However, much progress has been made in some fish model species, such as the zebrafish, *Danio rerio*, for various biological and/or biomedical research applications. In aquaculture species, the rationale of genome mapping is to increase the efficiency of selective breeding. Selective breeding is more recent, and consequently relatively less developed, in aquaculture species compared with most agricultural plants and terrestrial livestock.

The development of a large number of polymorphic markers is first required to establish a linkage (*i.e.* meiotic) map, based on recombination between segregating markers. For physical maps, polymorphism is not necessary because these maps are based on the "partitioning" of chromosomes and the subsequent "grouping" of markers. Advances in molecular biology and genomic technology have made easier the development of markers. Consequently, one challenging question is to know how the biological and economical characteristics of aquaculture species will favour, or conversely disfavour, the application of genome mapping and the development of marker-assisted-selection (MAS) programmes.

On one hand, phenotypic and genetic diversity available in aquaculture species is likely to be larger than in most agricultural species (for which wild populations are almost extinct and/or introgressed by domesticated stocks genetically impoverished by selective breeding). This means that the identification and characterization of quantitative trait loci (QTLs) is likely to be made easier by the availability of extreme genotypes. Similarly, polymorphism at candidate genes is likely to be higher. Additionally, the high fecundity of most aquaculture species relative to terrestrial livestock is a clear advantage for mapping studies. Furthermore, viable double haploids can be produced in some species providing useful reference material for mapping and QTLs analysis.

On the other hand, in some species such as most fisheries species, controlled bi-parental crosses are technically difficult or even impossible to perform. Good individual phenotypic characterization of extreme parental phenotypes and of their segregating progeny is often difficult because of the high level of phenotypic plasticity and the lack of precise environmental control (and/or inter-individual competition) in many species (especially marine ones). Additionally, in many "new" aquaculture species, investment in genetic research is still low and, consequently, limited efforts/funds are devoted to selective breeding, development of markers and, finally, MAS.

2.1.2 Genome mapping in fish model species

Genome mapping in fish models was recently reviewed by Tong and Chu (2002).

Zebrafish *(Danio rerio)*

Zebrafish is used as a model for studying early development in vertebrates. The first zebrafish map was developed by Postlethwait *et al.* (1994) using RAPD markers, with a map distance of 2317 centimorgans (cM) and an average marker interval of 5.8 cM. Since then, SSCP, EST and microsatellite (> 2000 locus, Shimoda *et al.*, 1999) markers have been used, improving the average marker interval to 0.74 cM. The number of microsatellites mapped in zebrafish is only surpassed by those mapped in human, rat and mouse. Comparative studies with mammalian genomes lead to the identification of hundreds of conserved syntenies between zebrafish and humans (Barbazuk *et al.*, 2000).

A physical map of the zebrafish genome was developed using radiation hybrid cells, artificial yeast chromosome (YAC) and bacterial artificial chromosome (BAC) libraries. Sequencing of the genome of zebrafish (1700 Mb) was expected to be completed by the end of 2002. Updated information can be obtained from http://www.sanger.ac.uk/Projects/D_rerio and from http://zfin.org, http://www.tuebingen.mpg.de.

Pufferfish *(Fugu rubripes)*

The *Fugu* genome is the smallest among vertebrates (400 Mb), showing less than 10 % of intergenoic and intronic sequences. This makes this fish useful as a model as its genome contains a similar repertoire of genes to that of other vertebrates. So most published papers on mapping report comparative genomics studies between *Fugu* and humans. Updated data can be obtained from http://www.fugu-sg.org, http://fugu.hgmp.mrc.ac.uk, http://www.ensembl.org/Fugu_rubripes.

A sequencing project is also in progress on *Tetraodon nigroviridis*, a fish of the same family as *Fugu rubripes*. Approximately 70 % of the *Tetraodon* genome has been sequenced and assembled so far. Updated data can be obtained at http://www.genoscope.cns.fr/externe/tetraodon/.

Medaka *(Oryzias lapites)*

The availability of numerous mutant strains of medaka make it a model fish for mapping the genes responsible for these mutations. Following an initial RAPD-based map (Ohtsuka *et al.*, 1999), an AFLP-based map based on 633 markers is now available (Naruse *et al.*, 2001). Information can be obtained from http://biol1.nagoya-u.ac.jp.

2.1.3 Genome mapping in aquaculture species

Aquaculture species are very diverse in terms of systematic groups (fish, crustaceans, molluscs) and, more importantly, in terms of their level of domestication related to breeding and biological constraints. The development of meiotic maps requires proper control of pair mating or, alternatively, gynogenesis. This is still problematic in many aquaculture species such as brooding oyster species and some fish species. Indeed this is the case for most fishery species for which controlled reproduction is not handled. Genome mapping in aquaculture species, including salmonids, tilapia, catfish, shrimps and oysters, was recently reviewed by Tong and Chu (2002). The present status of mapping programmes in aquaculture fish and shellfish species is reported in Table 2.1.

Salmonids

Salmonids are the most important fish group for aquaculture. Domestication and large-scale selective breeding, mostly based on family designs, have been initiated for several decades. The first maps were developed more than 10 years ago, based on allozymes. Difficulties arose from the tetraploid ancestry of salmonids, as clearly demonstrated by the occurrence of duplicated chromosome regions. However, the development of gynogenetic (Lie *et al.*, 1994) and androgenetic progenies facilitated the obtention of meiotic maps. To date, the two most advanced species are rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) (see Table 2.1). Some QTLs have already been identified.

Tilapia *(Oreochromis* spp.*)*

Tilapia species are important fresh water fish species for tropical and sub-tropical aquaculture. Selective breeding aims to improve growth, cold tolerance and quality. Similarly to salmonids, gynogenesis was used to develop linkage maps (Kocher *et al.* 1998).

Catfish *(Ictalurus* spp.*)*

An important gene mapping programme is also currently undertaken on the channel catfish, *Ictalurus punctatus*, an important species used in aquaculture in the southern United States (Waldbieser *et al.,* 2001).

2.1.4 Discussion

Numerous mapping projects in aquaculture exist for most major species and linkage maps (at least low-density maps) are now available. However, up to now, few cases have demonstrated that MAS can be used efficiently when applied to aquaculture species. It should be noted that this is also the case in many agricultural species. Concerning traits exhibiting high heritabilities, MAS is unlikely to show effective benefits when compared to "classic" breeding

programmes. In many cases, selective breeding programmes in aquaculture species concern traits such as growth which are likely to be determined by a large number of genes. Additionally, QTL mapping requires accurate quantitative individual phenotyping, which can be difficult in aquaculture species. MAS is of special interest for traits which require sacrifice of individuals to be recorded and/or disease-resistance traits, which are clearly problematic unless *in vitro* assays are available. The latest progress in gene mapping of aquaculture species presented at the Plant and Animal Genome Conference in San Diego, California, USA can be obtained on http://www.intl-pag.org/11/abstracts/.

It should be noted that, up to now, few links have been established between model fish species and aquaculture of fisheries species. The large development of genome research on model fish species should lead to further identification of candidate genes in aquaculture of fisheries species. Comparative mapping based on new generations of gene maps will provide a very helpful approach in this respect. Such developments would be of special interest in fisheries species, for which experimental approaches are not feasible. For example, one objective could be to identify genes involved in age-at-maturity and subsequently monitor these genes in species for which selection due to fisheries might have led to a rapid evolution of this trait.

Few physical maps, based on Radiation Hybrid (RH) panels or other methods are yet available, and they are limited to model species (see Table 2.1). Alternative methods, such as Fingerprint Analysis (Marra *et al.*, 1997) or "Happy mapping" (http://www.mrc-lmb.cam.ac.uk/happy/happy-home-page.html), should be used in the future.

2.1.5 Recommendations

1. Information networks, similar to those developed for model fish species, should be developed in order to provide a WWW community resource on aquaculture species genomes;
2. The co-development of domesticated, selected and inbred lines of aquaculture species is essential for future development of MAS in aquaculture species and should be initiated;
3. More links should be developed between model fish species and aquaculture and fisheries species genome projects;
4. Research on the development of physical maps for aquaculture and fisheries species should be encouraged.

2.1.6 References

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Table 2.1. Summary of the present status of mapping programmes in aquaculture species.

Species	Number of Chromosomes / LGs	Minimum Genome Size (CM)	Meiotic Map	Radiation Hybrid Panel	BAC library	Physical map / Fingerprinting	ESTs	Most Relevant Web Sites/ References
Rainbow trout	30	2627.5	350 µsats 1000 AFLPs	in progress	Several USDA: 10×	no	90 000 USDA/ INRA	
Atlantic salmon	27–28		500 µsats	no	Three: 19× (in total)	200 000 BAC clones fingerprinted	150 000	http://www.ri.bbsrc.ac.uk/salmon http://web.uvic.ca/cbr/grasp
Brown trout	40/35	922	250 µsat	no	no	no	no	http://www.inra.fr/theses/these-integrale/Theses/gharbi/
Tilapia	22/22	≈ 2000	500 µsats	failed	Four: 6× to 65×	yes	yes	http://www.ri.bbsrc.ac.uk/tilapia http://tilapia.unh.edu http://hcgs.unh.edu
Carp	50/50	5789						
Seabass			in progress	no	One: 7×		yes	www.bassMap.org
Sea bream			in progress	in progress	no		yes	www.bridgemap.tuc.gr
Catfish (*Ictalurus punctatus*)	29/32	1958	243 AFLPs 263 µsats 19 SSRs 1 ESTs		Three: 17×			
Shrimp (*P. monodon*)	44/28	1412	29 AFLPs 8 µsats					www.aims.gov.au/shrimpmap
Pacific oyster (*C. gigas*)	10/10	880	98 µsats	no	yes	no	700 IFREMER	Hedgecock *et al.,* 2002 www.ifremer;fr/Gigas/Base

2.2 Review and report on genetic issues related to escapes of farmed marine fish and shellfish (ToR b)

This section was based on a position paper prepared by G. Dahle, P. Boudry, S. Lapègue, and E. Eg Nielsen and was adopted by WGAGFM at La Rochelle/La Tremblade 2003.

Escapes of cultured salmonid fishes, particularly Atlantic salmon, and their possible genetic impact on wild populations, has been an issue of concern for several years. This problem has been subject to a considerable number of research projects. More recently, culture of some marine species, e.g., cod, *Gadus morhua*, has reached a level where accidental large-scale escapes may soon be anticipated. Similarly, genetic impact of the release of hatchery propagated strains of

selected oysters is questioned (e.g., *C. virginica* selected strains in Chesapeake Bay). It is important to discuss how such escapes or releases of marine species may affect wild conspecific populations. Experience may, to some extent, be drawn from research on salmonid species, but there are also some important biological differences between many marine species and salmonids that may result in different genetic consequences of large-scale escapes.

2.2.1 Background

In a socio-economic context, marine species like cod are of extreme importance to ICES Member Countries. Consequently, aquaculture of marine species is expected to grow rapidly. Mariculture comprises more than one-third of global seafood farming by weight, and cultivation of marine finfish and shellfish has been the fastest growing segment within aquaculture. In 2000 more than half of global aquaculture production originated from marine or brackish coastal waters (FAO, 2002), although molluscs (46.2 %) and aquatic plants (44 %) are dominant in comparison to finfish (8.7 %) and crustaceans (1 %) in the marine environment. Species like seabass and sea bream have been in aquaculture for several years, but the production is nowhere as large as for oysters and mussels. Halibut and cod have been produced, although in low numbers, in aquaculture for some years. However, due to the decreasing quotas on wild catches, the potential and interest for breeding, especially cod, is increasing. The potential risk of interaction between farmed and wild marine animals is of great concern.

Due to the nature of domestication, introducing wild individuals to an aquaculture environment, and producing offspring from a selected proportion of the existing natural variants, there is the large possibility that the produced offspring will deviate from its wild relatives. Whether this is a genetic or behavioural differentiation, the impact on the wild conspecific populations is a potential problem for the wild population.

In general, there are two ways that individuals reared in aquaculture can have a genetic impact on wild conspecifics: either directly by interbreeding, or indirectly by introducing competition, predation or introduction of diseases (Waples, 1991; Hindar *et al*., 1991). The indirect effects are rather straightforward. They all lead to a reduction in the wild population size, ultimately reducing the genetic variation. Therefore, only direct genetic effects are discussed below.

Direct genetic impact

Hybridization between wild and aquaculture individuals can have two direct effects on wild populations: 1) reduction of between-population genetic variance; and 2) outbreeding depression. To understand these potential impacts, it is necessary to realize that the genetic make-up of aquaculture and wild fish can be very different. Atlantic salmon has been one of the model organisms estimating the potential genetic impact of aquaculture fish on wild populations, so we have chosen to use this species as an example. It is known that wild Atlantic salmon consist of a high number of local, genetically differentiated populations, potentially locally adapted to specific environmental conditions of their natal habitat. In contrast, aquacultured Atlantic salmon is genetically very homogeneous (i.e., most of the world's production of salmon originates from a very limited wild source). At the same time, many traits (such as growth rate and age at maturity) of salmon in aquaculture have been intentionally altered by selection of broodstock. But also domestication selection or adaptation to the aquaculture environment *per se* takes place. Finally, a high number of offspring is often produced from a limited number of parents, leading to a loss of genetic variation. In short, hatchery salmon originates from few wild sources, contains little genetic variation, and has been selectively changed both intentionally and unintentionally (domestication). So the direct genetic consequence of large-scale escapes or releases are that locally adapted and genetically variable wild salmon hybridize with genetically homogeneous salmon, adapted to and selected for a life in aquaculture. This results in a homogenization effect making the genetic composition of populations more similar, i.e., it reduces the interpopulation variance. At the same time, hybrids are likely to have reduced fitness leading to outbreeding depression.

Until recently, the general belief was that population structure in marine fishes was lacking or very limited. However, recently this "conventional wisdom" has been challenged by a number of publications (see Nielsen and Kenchington, 2001). The number of populations and proportion of genetic variance distributed among populations are generally less than in salmon, but population structure has been demonstrated in many marine fish species. This means that local adaptations can be common in marine fishes. So the potential direct genetic effects are similar for these species.

Present data indicate that there is genetic differentiation between cod from different regions along the Norwegian - Russian coast, and between the Norwegian coast and the Barents Sea (Jørstad and Nævdal, 1989; Dahle, 1991; Fevolden and Pogson, 1997; Pogson and Fevolden, 2003). In addition, studies of the migration pattern of released cod along the coast and in fjord areas indicate that cod is a stationary species, staying within the release area, and seldom moving more than 10 km from the release sites (Svåsand, 1990; Svåsand and Kristiansen, 1990). This stationary behaviour and possible genetic differentiation calls for a cautionary approach when doing aquaculture, as any released or accidentally escaped individuals, presumably will stay within a small distance of the aquaculture site. This will facilitate direct genetic and ecological interactions with local stocks.

Although, on a global scale, the number of individuals of most economically important marine species is enormous, and no evidence exists of any marine species becoming extinct due to overfishing, there are indications that overfishing in certain areas has removed a species temporarily from an area (east coast of Canada and the US; Kenchington, 2003). Keeping this in mind, one should pay attention to any possible implications on local populations due to genetic and/or ecological interaction from domesticated individuals to avoid local extirpation.

Population genetics studies on cod are still carried out in different areas, utilizing most available methods ranging from otolith, hemoglobin and allozymes analysis, to microsatellite analysis. Detailed knowledge about the population structure is vital to be able to evaluate the potential impact of interbreeding of escapes in any local population. In addition, several potential cod stocks are being evaluated for aquaculture in Norway. A survey of the heritable variation within and between populations is being carried out.

2.2.2 Case study: cod

The number of potential escapes compared to the numbers in the local population is an important factor in this context. Since the cod is a stationary species, the primary impact would be on the local population. Studies have shown that a fjord system seems to have a carrying capacity of a certain number of individuals, and releases of additional cod in the fjord system will not increase the number of cod in that area. This implies that the escaped cod will compete with the wild cod for food, and ultimately spawning area and mates (i.e., indirect genetic effects). A large number of escapees has the potential to displace indigenous individuals, thereby reducing the effective population size.

Today cod and other marine species in aquaculture are kept in net-pens or similar compartments in an area inhabited by their wild conspecifics. Unlike salmon, which must enter fresh water in a river to spawn, the farmed marine species are kept in an environment where they are able to spawn naturally at time of maturation. This will create large numbers of fertilized eggs floating out from the net-pen and swamping the area where the aquaculture farm is located. If this spawning in the net-pen occurs before the spawning of the wild individuals, and given that the plankton situation is such that the newly hatched larvae will be able to survive, these individuals would be a potential threat to the local egg and larvae production due to cannibalism. Even if the spawning in the wild and in the net-pen occurs at the same time, the newly hatched larvae will compete for the food available in the area. Further, cod milt and eggs are known to be able to survive for a long time after release, and thus could be fertilized in seawater even after 60 minutes. Wild cod outside the net-pen could therefore, potentially, interbreed with its farmed counterpart inside the net-pen, producing viable offspring. These scenarios could have unknown impact on the genetic structure of the local population, and if the local population has evolved some type of adaptation to the local environment, this could in the end be detrimental to the local stock.

Presently farmed cod will reach age at maturity very fast, often in less than two years, creating a large pool of genetic material that could be spread into the local area in a very short time. Due to its natural behaviour as a demersal fish, cod is known to be able to escape the net-pens more easily and more frequently than salmon. This increases the possibility of escaped cod in the area around the net-pens interacting or even interbreeding with the wild cod. Even without interbreeding these escapees will increase the biomass, thus putting a stress on the carrying capacity of the area, and therefore increasing the degree of "ecological" competition. This competition could have an impact on the genetic makeup of the stock/population over time.

2.2.3 Case study: oyster

During the last 20 years, oyster culture in Europe has principally been based upon natural spat, but also a smaller proportion has been developed through hatchery-propagated spat. Nowadays, this hatchery production reaches about 15 % of the spat production in France and is tending to increase. This is especially true for the cupped oyster, *Crassostrea gigas*. However, the European flat oyster, *Ostrea edulis*, may also be concerned as some resistant strains to Bonamiosis have been developed and will be proposed to producers. The use of these strains for replenishment of the flat oyster stocks has to be questioned because of underlying genetic concerns. Hence, what will be the genetic impact of a massive introduction of hatchery-improved spat in the wild?

To quantify the risk of "genetic pollution" by these stocks, an estimate of the numbers of wild parents contributing to the overall oyster population is needed, in both the hatchery and in the wild. At the individual level, females can be fertilized by a limited (down to one) number of males. Comparisons between data obtained using nuclear and mitochondrial markers clearly suggest that female effective population sizes are smaller than male ones. This seems to be particularly the case on the Atlantic coast. At the within-population level, data on spat genetic variability suggest that the dynamics of recruitment might also vary between the Atlantic Ocean and the Mediterranean Sea. At the species level, markers consistently show a clear pattern of isolation by distance. In any case, significant F_{st} values are found,

even at a rather small scale. At the same scale, gene diversities were also quite variable, showing that populations with different diversities may coexist in close proximity (for review see Boudry *et al*., 2002).

These results point toward the fact that, despite the possibility of larval dispersal, local stocks may be quite independent dynamically and harbour varied instantaneous effective sizes likely to shape the gene diversity they contain. We think that human activities (overfishing, stock transfer, etc.) are unlikely to have had a significant impact on genetic variability and population differentiation in this species. However, this may change if the production of hatchery-propagated spat in this species develops in the future. This is likely to efficiently contribute to the sustainability of the aquaculture of this species, but it should be managed in such a way that it will not have a negative effect on the local genetic variability. This will be especially true if disease-resistant strains are released, and the populations are subsequently challenged.

The same kinds of questions are asked for the American oyster, *Crassostrea virginica*, when dealing with the restoration of oyster reefs by hatchery-propagated stocks (Allen and Hilbish, Workshop "Genetic considerations for hatchery-based restoration of oyster reefs", 21–22 September 2000, Virginia Institute of Marine Science, USA). One main conclusion of this workshop was that the effective population size of wild populations was an essential parameter to predict genetic effects, before any restoration programme.

2.2.4 Possible solutions

2.2.4.1 Confinement

Today net-pens are the dominant system for storing the produced fish until slaughter. Milt, egg and fertilized eggs will escape from these net-pens. Using "plastic bags", a system that is available, will keep everything inside the rearing facility as long as it is unbroken. This will be a possible method to avoid unwanted genetic mixing of farmed and wild individuals.

2.2.4.2 Local stocks

If the fish farmers use individuals caught locally as their broodstock, the possible consequences of an interbreeding between the individuals inside the net-pen and individuals outside the net-pen, would most probably be insignificant. Escapees would most probably create fewer disturbances to the system than individuals from regions far away. On the other hand the benefits of selective breeding would be very difficult to realise using many different broodstocks in many different aquaculture facilities and highly productive strains could not be developed.

2.2.4.3 Triploids

The production of triploid fish and shellfish has been proposed as one of the most efficient ways to prevent genetic impact of aquaculture stock on wild populations. In some species (e.g., oyster), triploids are not fully sterile and/or stable (reversion from the triploidy to diploidy). However, triploidy strongly reduces the risk of gene flow from cultivated to wild con-specific stock (see for review Boudry and Chatain, 1999), although there may be remaining ecological concerns (indirect genetic effects).

2.2.5 Recommendations

1) Farming of marine fish species should be performed in a confined environment. Recommended measures of confinement include closed net-pens, recirculated inland systems, sterility (triploids), etc. This will reduce, but not eliminate, interactions due to accidental releases;
2) Unless proper confinement measures are taken, farming of marine species should be founded on local stocks. However, even taking this approach could be problematic due to rapid divergence between wild and farmed populations due to selection and low effective population size under aquaculture conditions.

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2.3 Review and report on issues in relation to practical management options for the conservation of genetic diversity in marine fish and shellfish of economic importance (ToR d).

This section is based on a position paper prepared by E. Kenchington and E. Eg Nielsen and adopted by WGAGFM in La Rochelle/La Tremblade in 2003. It forms part of a joint ToR with the Working Group on Ecosystem Effects of Fishing Activities (WGECO) and is cross-referenced in their 2003 report.

2.3.1 Background to the Terms of Reference

The Working Group on Ecosystem Effects of Fishing Activities (WGECO) was tasked in 2002 with developing advisory forms appropriate to the preservation of genetic diversity from detrimental impacts of fishing (ICES, 2002a). WGECO proposed a three-phase approach to the development of this advice: 1) identification of management objectives; 2) definition of acceptable risk and/or identification of appropriate reference points (when possible); and 3) development of a monitoring programme (ICES, 2002a). They further identified three considerations for defining management objectives for maintaining intra-specific genetic diversity. These were the genetic diversity within and between populations and the population structure, that is the preservation of the paths of geneflow (cf. Smedbol *et al.*, 2002). For each of these considerations, example management objectives were provided (Table 2.3.1.1).

Table 2.3.1.1. Examples of management objectives to address generic concerns related to the loss of genetic diversity in marine species (ICES, 2002a).

Consideration	Example management objective
1. Genetic diversity among populations	1. Maintain number of populations
2. Population structure and relative abundance	2. Maintain relative size of populations
3. Within-population genetic diversity	3.1. Maintain large abundance of individual populations
	3.2. Minimize fisheries-induced selection

However, WGECO was unable to establish reference points for these objectives and referred the matter to the WGAGFM for consideration in 2003.

The ICES Advisory Committee on Ecosystems (ACE) in its 2002 report (ICES, 2002b) reviewed the scientific advice for impacts of human activities, including fishing, on genetic diversity produced by WGAGFM between 1995 and 2000, and in the ICES (2002a) WGECO report, to address a request from the European Commision, Directorate General of Fisheries. ACE suggested four general measures to mitigate against the loss of genetic diversity:

1) Fishing mortality should be kept sufficiently low to maintain large populations;
2) The harvest should be widely distributed geographically and among all of the recruited populations to avoid local depletions and fragmentation;
3) Reduction of fishing effort rather than alternative management approaches that result in fisheries becoming even more selective;
4) Case-by-case evaluation of risks associated with loss of genetic diversity vs. benefits of imposed action (ICES, 2002b).

These were suggested as "common sense" approaches for managers to follow until the scientific community could recommend a more rigorous framework. The WGAGFM has considered these common sense approaches and deliberated further on the establishment of management objectives and reference points within the ICES context.

Why preserve genetic diversity?

Genetic diversity is the product of thousands of years of evolution, yet irreplaceable losses can occur very quickly (cf. Nielsen and Kenchington, 2001; Kenchington, 2003). This diversity is important for the long-term ability of a species to adapt to climate change, and loss of populations (extirpation) most likely equates to a loss of adaptive variation. Yet management units are often discordant with population structure. For example, in the blue whiting, *Micromesistius poutassou*, the main oceanic distribution is considered to represent a single stock and is managed accordingly. Population genetic studies have, however, indicated that partially separated stocks exist in the Mediterranean and in the eastern Barents Sea (Giæver and Mork, 1995; Giæver and Stien, 1998). If there are some relatively local stocks, the overall catch depletions could conceal community extirpation of a valuable prey resource to higher predators. Similarly, Ruzzante *et al.* (2001) report on the decadal stability of the genetic differentiation of five cod, *Gadus morhua*, spawning banks off Newfoundland and Labrador, Canada. This genetic structure persisted through the recent population collapse, with only some suggestion of post-collapse mixing between two of the spawning banks. This information is critical to recovery management as it indicates that population re-growth will be the mechanism for rebuilding the stocks, as opposed to migration from other areas. Pragmatically, genetic diversity is also very important for aquaculture, providing the raw material for selective breeding programmes and revitalization of inbreed broodstock.

2.3.2 Review of Management Objectives

WGAGFM reviewed the example management objectives suggested by WGECO (ICES, 2002a) and listed in Table 2.3.1.1 above. There was consensus that there would be many different management objectives for the different threats to genetic diversity and species affected. Further, WGAGFM endorsed the "common sense" objectives put forward by ACE (ICES, 2002b) with some reservations over recommendation 4. The example list (ICES, 2002a) was extended modestly to include an objective for the preservation of genetic diversity among populations: *Maximize the amount of*

diversity maintained when prioritizing populations. This objective was a reaction to the "common sense" ACE (ICES, 2002b) recommendation 4) paraphrased above. Managers are directed to the prioritization scheme proposed by Nielsen and Kenchington (2001) to assist with the decisions on which populations to protect. WGAGFM felt that it would be in a position to give species-specific recommendations for objectives if such requests were forthcoming.

2.3.2.1 The application of management objectives to different types of organisms

Marine organisms have a wide range of intraspecific genetic complexity and biological and ecological characteristics. Nielsen and Kenchington (2001) grouped marine organisms into five categories based on life-history characteristics and population dynamics (Table 2.3.2.1.1). These categories are not mutually exclusive, however most organisms reviewed fall into only one classification. There is some degree of overlap between classic marine organisms and benthic/sessile invertebrates. The latter classification is distinguished from the former because of their sedentary nature. They are not able to relocate in response to habitat disturbance or degradation. For broadcast dioecious spawners who are also sedentary, small-scale spatial structure (nearest neighbour distances) becomes critical to spawning success (e.g., scallops).

Nielsen and Kenchington (2001) provide a detailed description of these classifications and also put the threats to genetic diversity into context for each group. One threat which is not intuitive is the threat to among-population genetic diversity in classic marine organisms. Species within this group have generally been regarded as "safe" in a classical conservation genetic context. Beverton (1990) reviewed the well-documented population crashes for ten species of small pelagic marine fish. He demonstrated that even in the case of Icelandic spring-spawning herring, which had the worst population crash, the lowest census size in the time series was estimated at more than one million individuals. However, the use of new genetic markers has challenged this conventional wisdom in some cases by identifying substantive population structuring (cf. Ruzzante *et al.,* 1999), although the within-population genetic variance remains high.

Table 2.3.2.1.1. Categories of marine organisms which have differing threats to genetic diversity (drawn from Nielsen and Kenchington, 2001).

Classification	Defining characteristics	Examples
Classic marine species	Large population size; high fecundity; pelagic larvae; wide distributions	mackerel, herring, cod
Benthic/sessile invertebrates	Limited adult mobility; broadcast spawning in some	scallops, mussels, coral
Apex species	Slow growth; long-lived: low reproductive potential; large size and/or late age at maturity; restricted dispersal	sharks, rays, marlin, whales
Localized species	Restricted range; island habitats; or broad range with limited dispersal	coral, whelks
Hermaphrodite species	Sex change (protoandrous or protogynous)	groupers, snappers, shrimp

2.3.2.2 The primary genetic concerns for different types of marine organisms

The life histories and ecology of different types of marine organisms result in different population structures. In turn, the threats to genetic diversity are different for each group. Of course, these are generalizations and WGAGFM emphasizes that case-specific evaluations must be made and endorses the prioritization scheme put forward by Nielsen and Kenchington (2001) to assist in decision making. Clearly, factors such as overall species abundance are critical in determining the relevant genetic concerns and options. However, Table 2.3.2.2.1 identifies the generic concerns which are likely to be the most important to the management of genetic diversity.

Table 2.3.2.2.1. Generic *prima fascie* genetic concerns for divergent groups of marine organisms based on a review paper by Nielsen and Kenchington (2001). Bullets are in order of priority.

Classic marine species	Benthic/sessile iInvertebrates	Apex species	Localized species	Hermaphrodite species
• Among-population variation • Directional selection within populations	• Among-population variation • Preservation of small-scale spatial structure	• Population size • Among-population variation	• Population size • Among-population variation	• Population size • Among-population variation

2.3.3 Reference points

The WGECO report (ICES, 2002a) was only able to establish a limit reference point for individual population size. This was based on theoretical calculations of successful breeding population sizes required for long-term population viability (Lynch and Lande, 1998). WGAGFM was able to suggest an additional limit reference point for the management objective: *Maintain number of populations*. Here, the target would be to *Maintain all populations*, but a limit reference point could be to *Maintain all Evolutionary Significant Units* (ESUs *sensu* Waples, 1985). The concept of ESUs is drawn from the conservation biology literature and is a means of preserving evolutionary units above the level of population.

2.3.4 References

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3 WORKING GROUP BUSINESS

3.1 Suggestions for WG ToR and meeting place in 2004

During discussions on a meeting place in the year 2004, WGAGFM responded positively to a generous invitation from Dr. Jochen Trautner, Bundesforschungsanstalt für Fischerei, Hamburg, Germany, to host the 2004 WGAGFM meeting on 3–5 May 2004. The 2005 meeting is provisionally planned for Silkeborg, Denmark at the Danish Institute for Fisheries Research.

ANNEX 1: TERMS OF REFERENCE FOR 2003

2FFM The **Working Group on the Application of Genetics in Fisheries and Mariculture** [WGAGFM] (Chair: E. Kenchington, Canada) will meet in La Tremblade, France, from 10–12 March 2003 to:

a) review and report on the practical use of genome mapping in aquacultured organisms;

b) review and report on genetic issues related to escapes of farmed marine fish and shellfish;

c) review and report on management recommendations for Atlantic salmon, developed by the SALGEN EU project;

d) review and report on issues in relation to practical management options for the conservation of genetic diversity in marine fish and shellfish of economic importance.

WGAGFM will report by 28 March 2003 for the attention of the Mariculture Committee, and ACE.

Supporting Information

Priority:	WGAGFM is of fundamental importance to the ICES science process and contributes to the advisory process
Scientific Justification:	a) During the past few years, several genome mapping projects of species of importance for aquaculture have been initiated (e.g., salmonids, oysters). Such maps are essential for a better knowledge of the genome of these species. Recent developments in DNA technology have greatly eased the development of such maps. However, the practical application of such maps as tools in selective breeding programmes, such as the identification and use of QTL, remains to be demonstrated in aquaculture. The ToR will review the present state of development of mapping projects of aquacultural species and, further, identify the specific constraints that might slow down their development and potentially limit their use in selective breeding programmes. b) Escapes of cultured salmonid fishes, particularly Atlantic salmon, and their possible genetic impact on wild populations, has been an issue of concern for several years. This problem has been subject to a considerable number of research projects. More recently, culture of some marine species, particularly cod, has reached a level where accidental large-scale escapes may soon be anticipated. It is important to discuss how such escapes may affect wild conspecific populations. Experiences may to some extent be drawn from the research on salmonid species, but there are also some important biological differences between many marine species and salmonids that may result in different genetic consequences of large-scale escapes. c) SALGEN (www.salgen.marlab.ac.uk) is a project set up to review genetic studies on Atlantic salmon and develop management recommendations for the species. WGAGFM has been asked to review and discuss the recommendations resulting from this project. d) This is a request a request from WGECO concerning collaboration on developing practical management options for the conservation of genetic diversity in marine fish and shellfish of economic importance. The first task of WGAGFM will be to identify the specific genetic problems of relevance to marine organisms, which will then be addressed in more detail at forthcoming meetings.
Relation to Strategic Plan:	Responds to Objectives 1 (d), 2 (a, d) and 4 (a).
Resource Requirements:	None required other than those provided by the host institute.

Participants:	WGAGFM members
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	ACME, ACFM, ACE
Linkages to other Committees or Groups:	SIMWG (Delegates drew specific attention to the need to develop this link – the Chairs of these two Working Groups should correspond together to ensure that there is no unnecessary overlap in their work.)
Linkages to other Organisations:	OSPAR, HELCOM, EC, NASCO
Cost Share	ICES 100%

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ANNEX 4: RECOMMENDATIONS FOR 2004

The Working Group on the Application of Genetics in Fisheries and Mariculture (Chair: Dr E. Kenchington, Canada) proposes to meet in Hamburg, Germany, 3–5 May 2004 to:

a) review methods and applications for the estimation of effective population size in wild populations of marine fish and shellfish (lead E. Eg Nielsen (Denmark));
b) discuss and report on management recommendations for Atlantic salmon, developed by the SALGEN EU project (lead E. Verspoor (UK, Scotland) or intersessional group);
c) review the conservation genetics of eels (lead J. Trautner (Germany));
d) evaluate the use of reaction norms within a selective fishing framework (lead P. Boudry (France) with B. Ermande and U. Dieckmann (Austria))

Priority:	WGAGFM is of fundamental importance to the ICES advisory process.
Scientific Justification:	a) Population size is the single most important factor in sustaining a high level of genetic variation within a population of a species. Population size here refers to the genetically effective population size (Ne), and not the number of individuals in a population (N). *Ne* is considered to be the most appropriate variable for assessing population viability but there is a need to review the methods and applications for inferring Ne and to point out their limitations. b) SALGEN (www.salgen.marlab.ac.uk) is a project set up to review genetic studies on Atlantic salmon and develop management recommendations for the species. WGAGFM has been asked to review and discuss the recommendations resulting from this project. c) The return rate of glass eels from the spawning ground of the European Eel (*Anguilla anguilla*) in the Sargasso Sea to the coasts of Europe and North Africa has declined dramatically. Several factors are suspected to have caused this decline. A review on the currently available knowledge on the genetic structure of the European Eel should point out the potential dangers of losing genetic diversity and lead to management recommendations. The status of the European Eel as a catadromus species has caused confusion in scientific responsibility between ICES and EIFAC (European Inland Fisheries Advisory Commission) in the past. Therefore this review is also meant to target the levels of actions to conserve the stocks in the marine and/or freshwater phase. d) As presented in Theme Session Y of the 2002 ICES Annual Science Conference in Copenhagen, recent developments of "Adaptive Dynamics Theory" have shown how the evolution of reaction norms can be modelled to evaluate the genetic impact of selective fishing. Relatively little information is available on genetic variation of reaction norms in marine organisms. However, quantitative genetics experiments, using model and/or aquaculture species, can be performed and are likely to provide valuable data for fisheries species. We will review how these two complementary approaches can be used to study the selective effect of fisheries and related issues.
Relation to Strategic Plan:	
Resource Requirements:	None required other than those provided by the host institute.
Participants:	WGAGFM members
Secretariat Facilities:	None required

Financial:	None required
Linkages to Advisory Committees:	ACME, ACE
Linkages to other Committees or Groups:	SIMWG (Delegates drew specific attention to the need to develop this link – the Chairs of these two Working Groups should correspond together to ensure that there is no unnecessary overlap in their work.)
Linkages to other Organisations:	HELCOM, EC