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REPORT OF THE

WORKING GROUP ON THE APPLICATION OF GENETICS IN FISHERIES AND MARICULTURE

Halifax, Canada
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1 INTRODUCTION

As decided in C.Res.2001/2F03 adopted at the 2001 Annual Science Conference in Oslo, Norway, the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) [Chair M.M. Hansen, Denmark] met at the Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, Canada, March 18–20, 2002 to deal with its Terms of Reference for 2002 (Annex 2).

1.1 Attendance and meeting place

Eleven persons representing eight countries attended the 2002 WGAGFM meeting in Dartmouth (Annex 3). As in previous years, the representation on the quantitative genetics was lower than on the qualitative genetics side, despite strong efforts to involve more quantitative geneticists.

The Bedford Institute of Oceanography, represented by our host, Ellen Kenchington, Benedikte Vercaemer and Patrick O'Reilly, offered excellent logistics and facilities for the meeting, including sightseeing and transport back and forth between the Bedford Institute of Oceanography and our accommodation. The Working Group would like to thank Ellen Kenchington and her colleagues for all the work undertaken to arrange this meeting and for their very kind hospitality.

1.2 Working form

Prior to the meeting, small *ad hoc* working groups, with one main responsible person, had been established to prepare position papers related to specific issues in the Terms of Reference, and to chair the respective sessions. During the meeting, the position papers were first presented and discussed in plenary. Thereafter, volunteers undertook the task of editing and updating position papers according to points raised in the plenary discussions:

- M.M. Hansen chaired business and general scientific sessions;
- M.M. Hansen chaired ToR a) *Update the provisions regarding GMOs in the ICES Code of Practice on Introductions and Transfers of Non-indigenous Organisms and transmit this material to WGITMO*;
- M.M. Hansen and E. Verspoor chaired ToR c) *Review and report on developments in the use of DNA from archived samples (scales, otoliths bones, etc.) for analysing fish populations;*
- J. Trautner chaired ToR d) *Review and report on the utility of molecular genetic methodologies for assessing the biological effects of contaminants on fish and shellfish;*
- R. Doyle chaired ToR e) *Review and summarize principles for minimizing diversity loss in the early generations of a captive broodstock;*
- J. Trautner chaired ToR f) *Prepare a position paper for the Working Group on Biological Effects of Contaminants on the possible use of gene array techniques in the detection and quantification of responses in fish to pollution.*

It was decided prior to the meeting to cancel ToR b) Assess and evaluate the utility of interspecific comparisons of population genetic parameters in understanding population structure in fish species. This was due to the fact that most of the persons involved in this ToR who were supposed to deliver data sets for the "plenary data analyses" were unable to attend the meeting, combined with the addition of the labour-intensive ToR a) to the agenda of the 2002 meeting.

The session Chairs were responsible for leading the plenary sessions and group work, and (in collaboration with their respective *ad hoc* working groups) for preparing the final report text from their sessions. A preliminary version of the report was made available on the (external) WGAGFM homepage for final comments by members before submission to the ICES Secretariat.

2 TERMS OF REFERENCE FOR 2002

2.1 Updated provisions regarding GMOs in the ICES Code of Practice on Introductions and Transfers of Non-indigenous Organisms (ToR a)

Position paper by M.M. Hansen and Pierre Boudry, adopted by WGAGFM in Dartmouth, 2002.

WGAGFM has been asked to update the provisions regarding GMOs in the "ICES Code of Practice on Introductions and Transfers of Non-indigenous Organisms", which dates back to 1994, and transmit this material to the Working Group on Introductions and Transfers of Marine Organisms (WGITMO). WGAGFM has previously addressed issues and provided updates on possible environmental effects of escaped/released GMOs in the 1995, 1996, 1997, and 1998

WGAGFM reports. Based on the recommendations given in these reports, and recent papers on the issue, WGAGFM has revised the section on GMOs in the "ICES Code of Practice on Introductions and Transfers of Non-indigenous Organisms". Below, we first list the recommendations from the 1994 Code of Practice. Next, we give our suggestion for a revised section on GMOs, and finally, we give some comments and justifications for our revised recommendations.

Recommendations regarding GMOs in the 1994 "Code of Practice"

In the 1994 "Code of Practice" the section regarding GMOs was as follows:

Recommended procedure for the consideration of the release of genetically modified organisms (GMOs)

Recognizing that little information exists on the genetic, ecological, and other effects of the release of genetically modified organisms into the natural environment (where such releases may result in the mixing of altered and wild populations of the same species, and in changes to the environment), the Council urges Member Countries to establish strong legal measures[1] to regulate such releases, including the mandatory licensing of physical or juridical persons engaged in genetically modifying, or in importing, using, or releasing any genetically modified organism.

Member Countries contemplating any release of genetically modified organisms into open marine and fresh water environments are requested at an early stage to notify the Council before such releases are made. This notification should include a risk assessment of the effects of this release on the environment and on natural populations.

It is recommended that, whenever feasible, initial releases of GMOs be reproductively sterile in order to minimize impacts on the genetic structure of natural populations.

Research should be undertaken to evaluate the ecological effects of the release of GMOs.

Furthermore, it included a definition of GMO:

Genetically modified organism (GMO): An organism in which the genetic material has been altered anthropogenically by means of gene or cell technologies.

Suggestion for new section on GMOs in the "Code of Practice"

WGAGFM suggests the following recommended procedure:

V General considerations regarding the release of genetically modified organisms (GMOs)

Recognizing that little information still exists on the genetic, ecological, and other effects of the release of genetically modified organisms into the natural environment (where such releases may result in the mixing of altered and wild populations of the same species, and in changes to the environment), the Council urges Member Countries to establish strong legal measures[2] to regulate such releases, including the mandatory licensing of physical or juridical persons engaged in genetically modifying, or in importing, using, or releasing any genetically modified organism.

VI Recommended procedure for all GMOs prior to reaching a decision regarding new releases

a) Member Countries contemplating any release of genetically modified organisms into open marine and fresh water environments are requested at an early stage to notify the Council about such releases. This notification should include a risk assessment of the effects of this release on the environment and on natural populations.
b) GMO risk assessment should particularly involve consideration of:

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[1]Such as the European Union "Council Directive of 23 April 1990 on the Deliberate Release into the Environment of Genetically Modified Organisms (90/220/EEC)", Official Journal of European Communities, No. L, 117: 15–27 (1990).

[2]Such as the European Economic Community "Council Directive of 12 March 2001 on the Deliberate Release into the Environment of Genetically Modified Organisms (2001/18/CE)", Official Journal of European Communities, No. L 106: 1–39 (2001).

i) the genetic and phenotypic characteristics of the modified organism, i.e., both the traits introduced or modified and other secondary phenotypic changes induced by the genetic modification, such as the construction and/or vector employed. The significance of the introduced or modified trait in relation to the biology of the parental organism should be evaluated;
ii) characteristics of the ecosystems that the GMO might access;
iii) possible interactions of the GMO with species of the ecosystems that might be accessed, in order to determine if the release of the GMO poses genetic and/or ecological hazards.

c) If possible, experiments in simulated natural environments are recommended. Such experiments should be conducted using secure systems to prevent escapes of GMOs from the experimental facilities at any life stage. The following points should be particularly assessed and reported:
i) phenotypic traits associated with the GMO in a simulated natural environment;
ii) the behaviour of transgenic marine organisms in a simulated natural environment;
iii) the competitive advantages/disadvantages of transgenic marine organisms;
iv) the degree to which transgenic marine organisms are capable of mating with a native population, including their reproductive performance in competition with wild conspecifics;
v) the success of that mating as defined by numbers of offspring;
vi) the relative fitness of juveniles of pure transgenic crosses, hybrids between native and transgenic crosses, and the pure native crosses.

VII If the decision is taken to proceed with the release, the following action is recommended:

a) It is recommended that initial releases of transgenic organisms be reproductively sterile in order to avoid transfer of the gene construct to wild organisms. However:
i) mass production of sterile progeny requires the maintenance of fertile transgenic parental stocks. The risk assessment of these stocks should also be addressed;
ii) it should be noted that many current sterilization techniques are not 100 % efficient and that most marine species have very high fecundity;
iii) mass releases of sterile organisms could still negatively impact the ecosystem and affect wild populations through competition.

b) Monitoring should be undertaken to ensure that GMOs, due to their nature, do not negatively affect wild populations and ecosystems after the release.

Definitions

Genetically modified organism (GMO)

An organism in which the genetic material has been altered anthropogenically by means of recombinant DNA technologies. This definition includes transgenic organisms, i.e., an organism bearing within its genome one or more copies of novel genetic constructs produced by recombinant DNA technology, but excludes chromosome manipulated organisms (i.e., polyploids), where the number of chromosomes has been changed through cell manipulation techniques.

Release

Voluntary or accidental dissemination of an organism, or its gametes, outside its controlled area of confinement.

Justification for recommendations

V a) It is important to keep this point. Despite some studies on the safety evaluation of transgenic fish (e.g., Duham *et al.*, 1999; Guillén *et al.,* 1999), there is clearly still insufficient knowledge about the possible impact of GMOs on wild populations and ecosystems, calling for use of the precautionary principle. Recent papers by Muir and Howard (1999, 2001) and Hedrick (2001) demonstrate the complexity of the problems. They found that if a transgene decreases the viability of an organism, but at the same time has a positive effect on some fitness components (for instance, transgenic males with an inserted growth hormone gene causing faster growth/larger size may be superior in spawning competition relative to unmanipulated wild conspecifics), then the transgene could spread in wild populations despite the lowered viability. This would lead to a decreased mean fitness of the wild populations and could eventually cause their extirpation (the "Trojan gene hypothesis"). Very fast growing transgenic Atlantic salmon have recently been developed, which stresses the actuality of the "Trojan gene hypothesis" (Reichhart, 2000).

VI a) We have kept this recommendation, but have added a sentence stressing that all ICES Member Countries should consider risk assessment protocols.

VI b) We list here the basic points that any risk assessment should address (as described in, e.g., Hallerman and Kapuscinski, 1995).

VI c) We give the recommendation that, if possible, field trials should be undertaken in order to assess a number of points that cannot be immediately assessed by considering the phenotype of the GMO, e.g., behaviour of GMOs relative to non-GMOs, the fitness of GMOs, wild conspecifics and their offspring at various life stages, etc. (this recommendation is taken from the 1997 WGAGFM report). The importance of this recommendation is further stressed by the recent publications by Muir and Howard (1999, 2001) and Hedrick (2001).

VII a) This is an important recommendation that we kept from the 1994 "Code of Practice". However, we also find it important to stress that the production of sterile individuals requires non-sterile parents, that many currently used sterilization techniques are not 100 % efficient, and that massive releases of sterile organisms may nevertheless have, at least, a short-term negative ecological impact on wild conspecifics and the ecosystem as a whole (also recommendations from the 1997 WGAGFM report).

VII b) We added this point to stress the importance of monitoring the effects of GMOs after they have been released, i.e., even despite a positive outcome of risk assessment, follow-up monitoring is required. This is due to the fact that negative effects may not be immediately apparent but could occur and accumulate over time (e.g., biological invasions after the GMOs have become established).

Definitions. We have found it important to clarify the definition of GMO used by ICES and make sure that the definition is equivalent to other definitions of GMO, for instance, as applied by the EU. We find it important to use a definition of "transgenic" that includes organisms manipulated by genes from their own genome, e.g., salmon, where a number of its growth hormone genes have been isolated and again inserted in its genome. Chromosome manipulated organisms are now clearly not included in this definition. Specific recommendations concerning polyploids were presented in the 1999 WGAGFM report. We also propose a definition of release to be added to the definition list of the ICES Code of Practice.

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2.2 Assess and evaluate the utility of interspecific comparisons of population genetic parameters in understanding population structure in fish species

This ToR was cancelled due to the fact that most of the persons involved in the ToR and supposed to deliver data sets for the "plenary data analyses" were unable to attend the meeting, combined with the addition of the labor-intensive ToR a) to the agenda of the 2002 meeting.

2.3 Developments in the use of DNA from archived samples (scales, otoliths, bones) for analysing fish populations (ToR c)

Position paper by E.E. Nielsen, adopted by WGAGFM in Dartmouth, 2002.

DNA from archived samples

The development of molecular biological methodology in general, and PCR techniques in particular, has led to studies of DNA from museum and archaeological samples or so-called "ancient DNA" (Pääbo, 1989; Ellegren, 1991; Janczewski *et al.,* 1992; DeSalle *et al.,* 1992). Initially, the main application was for phylogeny reconstruction, but subsequently population genetic aspects were included for various species (Taylor *et al.,* 1994; Hardy *et al.,* 1994; Zierdt *et al.,* 1996). Besides technical problems related to DNA quality, the main problem with using historical samples as a source of DNA for population studies has been that samples were typically not taken with a population genetic purpose. Therefore, the number of individuals sampled and knowledge regarding the population to which they belong was rather limited.

An exception to this general rule can be found for many species of fishes. Fish biologists have collected scale and otolith samples for more than a century, which have been used for age and growth pattern determination, on a population basis, to investigate spatial or temporal differences. In particular, for salmonid fishes and for commercially important marine fishes, such as cod and herring, samples are plentiful. This leaves population geneticists working with these species with an outstanding opportunity to conduct temporal studies of natural evolutionary processes, as well as anthropogenic influences in fish populations, without having to infer changes in levels and distribution of genetic variability from contemporary patterns alone.

In this paper we review the developments in application of archived samples for analysing fish populations and point to future directions for use of this unique source of temporal genetic data unmatched by any other group of organisms.

Extraction and amplification

The best DNA quality can be achieved from living cells. As soon as an organism dies or DNA is removed from the cell, degradation sets in. Depending on the external environment, DNA has a half-life varying from minutes to thousands of years. Bacteria, light and oxygen can degrade DNA and, therefore, the best environment for preservation of DNA is sterile, anoxic, dry and cold. Still, depending on the age of the samples, archived DNA material will be somewhat degraded, which has to be accounted for in the extraction method and the selection of genetic markers.

Extraction method

The two paramount problems when trying to extract DNA from historical material are the small amounts of DNA present and the poor DNA quality. For scales and otoliths, it is assumed that the DNA is found predominantly in dried cells on the outside and not in the collagen or calcareous matrix of the scales or otoliths, respectively. Therefore, it is sufficient to digest the overlying cells with proteinase K (see Nielsen *et al.*, 1999a; Hutchinson *et al.,* 1999). Several methods have been employed for the extraction of DNA from scales and otoliths, ranging from simple chelex procedures (Estoup *et al.,* 1996; Yue and Orban, 2001) to more expensive and time-consuming methods like the use of

microconcentrators described by Nielsen *et al.* (1999a). The only comparative study of extraction methods for scales (or otoliths) by Nielsen *et al.* (1999a) compared four different methods on salmon scale samples collected from 1913 to the present: 1) a phenol/chloroform method with ethanol precipitation as described by Taggart *et al.* (1992); 2) a modified version of this method employing microcon 50 microconcentrators (Amicon) instead of ethanol precipitation (Nielsen *et al.* 1997); 3) a Chelex protocol by Estoup *et al.* (1996); and 4) a guanidinethiocyanate method described by Gerloff *et al.* (1995). According to this study, the only method which gave consistently good results was method 2. Again, according to the authors, this is probably due to the specific qualities of the microconcentrators used. They keep the DNA in solution (which minimises loss of DNA during precipitation and reduces danger of contamination) which facilitates washing and concentration of the DNA. The results are in line with previous results on "ancient" DNA (Pääbo, 1989).

Fish bones are commonly found along with scales and otoliths in archaeological excavations. Whereas isolation of DNA from excavated samples of scales and otoliths is often impossible due to the position of the DNA on the outside, DNA is found within bones and has accordingly not been subject to degradation. A recent study (Consuegra *et al.,* in press) demonstrates that such samples, although with some difficulty, can be used as a source of DNA for population genetic studies. However, very stringent conditions have to be employed, i.e., protocols for extraction of "real" ancient DNA (Pääbo, 1989; Kwok and Higuchi,1989; Greenwood *et al.,* 1999).

Consuegra *et al.* (in press) reduced the risk of exogenous contamination by carrying out all manipulations prior to PCR in a forensic laboratory free of salmon DNA and physically separated from post-PCR procedures. Vertebrae were pre-treated with UV light before being pulverized. Three different methods of extraction were employed. However, only the Geneclean (Bio-101) and concentration with microconcentrators gave successful amplification and not for all samples. In conclusion, many methods for extraction of DNA from scales, otoliths and now also bones, have been described and applied with success. Before initiating studies of historical collections of scales and otoliths, careful evaluation of the age of samples, time available, and financial opportunities should be conducted. However, even though methods employing commercial kits such as microconcentrators are expensive and time consuming, they probably provide DNA of the highest concentration and quality and can be viewed as a "last resort".

Choice of markers

Since DNA from historical samples is more degraded than DNA from fresh tissue, the choice of genetic markers must be made accordingly. Repair of degraded DNA templates is possible and has been done for samples of ancient DNA, but is not recommended due to the uncertainty of what is incorporated during the repair process (Pääbo, 1989). Therefore, it is of paramount importance to use short DNA segments for PCR amplification, preferably segments not spanning much more than 250 base-pairs.

For mtDNA, primers spanning 2–3 kilobases are commonly employed for population genetic studies of fishes. To amplify such large segments of DNA from historical samples is probably unrealistic under most conditions. Instead, shorter segments should be amplified. Knox *et al.* (2002) and Consuegra *et al.* (in press) designed primers for amplification of smaller fragments of the ND1 mtDNA gene in Atlantic salmon known to encompass most of the previously known polymorphisms of that gene. Likewise, Adcock *et al.* (2000) used mtDNA primers spanning less than 200 bp. for detection of mtDNA polymorphisms in historical samples (50 year-old scales) of New Zealand snapper.

Microsatellites are very well suited for studies employing historical samples, since the amplified segments generally are short. Most studies using historical samples of fishes to date have employed microsatellites (Adcock *et al.,* 2000; Hansen, 2002; Heath *et al.,* 2002; Hutchinson *et al.,* 1999; Koskinen *et al.,* 2002; Martinez *et al.,* 2001; Miller and Kapuscinski, 1997; Nielsen *et al.,* 1997, 1999a, 1999b, 2001; Ruzzante *et al*., 2001; Tessier and Bernatchez, 1999). In general, the employed loci have been selected according to size, so that loci with small amplification products have been preferred (e.g., Nielsen *et al.,* 1999a; Adcock *et al.,* 2000). Further, it has been demonstrated that amplification success often is dependent on the age of the samples (e.g., Nielsen *et al.,* 1999a; Ruzzante *et al.,* 2001). Therefore, the selection of shorter loci becomes more important as the age of samples increases. Further, loci with large differences in allele sizes should be treated with care, since the number of templates of the longer allele will be less than that of the shorter allele and could result in the phenomenon of "large allele dropout". One major disadvantage of using microsatellites is the low copy number of nuclear compared to mtDNA genes. However, this is most likely to be of importance for "true" ancient DNA collected from archaeological excavations and not for historical collections.

PCR conditions

DNA concentration, and especially quality, varies considerably among individuals. This makes it difficult to make recommendations for the amount of template DNA to add to the PCR reaction, since the same amount of DNA rarely contains the same number of perfect templates. It is advisable not to be too fixed on a specific amount of DNA.

Recommendations from Nielsen (1999a; modified from Pääbo, 1989) should be followed. In short, if the amplification is not successful but primer-dimers can be observed, more template DNA should be added. If no primer-dimers can be observed, the amount of template DNA should be reduced until amplification or primer-dimers can be observed. If these procedures do not result in amplification of the desired target sequence, either the number of perfect templates in the extracted DNA is too low or the amount of inhibiting substances is too high for amplification. In both cases, a new extraction or purification of the extracted DNA should be tried. Another approach when amplification is limited or missing is to use more PCR cycles.

Authenticity

PCR is a very effective way of amplifying specific segments of interest when the DNA concentration is low. This makes PCR-based methods the obvious choice for analysis of DNA historical samples. On the other hand, this also allows amplification of contaminant DNA, which is a particularly serious problem in cases like this, working with a low number of templates. Therefore, it is necessary to set very high standards for laboratory conditions and procedures (Kwok and Higuchi, 1989; Greenwood *et al.,* 1999), such as to avoid extraction and PCR amplification of DNA from fresh tissue when working with old scales. There are several guidelines, which can be used to assure the authenticity of the amplified DNA (modified from Pääbo, 1989):

- Use several extractions from the same individual;
- Use control samples without template;
- Check if there is an inverse relationship between amplification length and effectivity;
- Unambiguous results (one sequence for mtDNA, maximum two alleles for nuclear DNA);
- Extract and amplify in a number of labs;
- Do the results make sense? (e.g., do you get trout sequences when you expect cod!)**Applications and case studies**

Temporal stability of population structure

Knowledge of temporal stability of the population structure is of paramount importance for our understanding of the evolution of fishes. It has a major influence on many population genetic parameters such as estimation of historical gene flow or genetic drift, which in turn determine the likelihood of adaptive differentiation among populations caused by natural or anthropogenic selection. Most studies of archived material have been conducted on a short time scale in an evolutionary context. However, in a recent study, Consuegra *et al.* (2002) investigated mtDNA variation in the late Pleistocene (16,000–40,000 years BP) from bones from the Iberian Peninsula to test if this region was a glacial refugium for salmon in other European regions. Their study determined the Iberian Peninsula as the most likely origin of the most common haplotype in Europe. Furthermore, significant changes of the Iberian refugial stock had taken place since the last ice age, demonstrating that caution of inferring evolution from current phylogeographic patterns should be exercised.

Most studies are based on archival material collected by fisheries biologists, i.e., scales and otoliths. Therefore, time intervals studied are generally between 50 and 100 years. Several studies focusing on temporal stability have been conducted on salmonid fishes (Nielsen *et al*., 1997, 1999b; Tessier and Bernatchez, 1999; Heath *et al*., 2002; Hansen, 2002). Even though many of these populations have suffered from anthropogenic disturbance, the general picture is a remarkable temporal stability of population structure (but see Heath *et al.,* 2002). If this is a general phenomenon, then population turnover in salmonids is low and therefore not equivalent to a metapopulation structure (Hansen *et al.,* submitted). Instead, the stability strengthens the argument for local adaptations in salmonid fishes (Taylor, 1991; Adkison, 1995), illustrating that estimates of genetic differentiation and therefore of migration and genetic drift based on contemporary samples are valid (see also section on estimating effective population size).

The only comparable study currently available from marine fish, shows a similar picture of temporal stability. Ruzzante *et al.* (2001) studied the long term (1964–1998) stability of the population structure of cod off the coast of Newfoundland. They found that despite population crashes, no component of variance in allele frequencies could be attributed to temporal changes.

These studies demonstrate that, even though most studies of temporal stability of population structure have not been conducted on an evolutionary time scale, they can still provide insight on the temporal variation of important population parameters.

Identification of native populations

The preservation of native populations is one of the main goals in conservation biology of freshwater fishes (Allendorf and Waples, 1996). The reasons for this are to secure large-scale genetic diversity and to preserve possible local adaptations (Taylor, 1991). However, in many areas, fish populations have experienced some kind of influence by escapes or "stocking". Consequently, it is of primary importance to identify populations with limited or no introgression from foreign conspecifics to ensure a high level of protection for these populations. For this purpose historical collections and, in particular, old scales have proven to be a very effective tool and have gained wide application. Most of these studies have been conducted on Atlantic salmon. Nielsen *et al.* (1997) studied scale collections from the severely decimated Skjern River population in Denmark, and found that the present population was most likely decendants of the original population represented by samples from the 1930s. Tessier and Bernatchez (1999) studied three populations of landlocked Atlantic salmon and found that despite population declines and stocking during this period, no statistically significant changes in intrapopulation genetic diversity were apparent. Martinez *et al.* (2001) studied a French population of Atlantic salmon from the Nivelle River and found some, but limited, introgression of foreign genes into the native gene pool. Similarly, Nielsen *et al.* (2001) found that despite several years of intensive stocking of Danish rivers (where the indigenous populations presumably had died out) with salmon from foreign populations, remains of the indigenous populations could still be identified in some rivers. Also for brown trout, there is evidence of resilience towards introgression. Hansen (2002) found that, despite decades of intense stocking with domesticated trout in one population, there was little evidence of any long-term reproductive success of the hatchery-reared fish. Instead, the present population was descendants of the indigenous population. However, in a second population stocked with domesticated trout from the same strain strong introgression had occurred, stressing the difficulties in making general predictions about the outcome of spawning intrusion of non-native stocked fish into wild populations.

In summary, these studies, although limited in number, point to a remarkable resilience of indigenous populations to withstand introgression from foreign or hatchery conspecifics. The most likely explanation is local adaptation of the native population. This should, however, not be interpreted as a "carte blanche" for releases of non-indigenous fish. Even though they have limited success in the long run, they can still impose negative effects on a short time scale by hybridisation and ecological interactions reducing the native population (Hindar *et al*., 1991; Waples, 1991) and they impose a genetic load on the wild populations that may result in reduced fitness for many generations (Lynch and O'Hely, 2001).

Estimation of effective population size

When population size is small, allele frequencies are expected to vary due to the sampling variance of gametes from a limited number of parents, i.e., the process of genetic drift. By estimating this variance, it is possible to get an estimate of the effective population size (N_e). Several approaches for estimating the temporal drift variance in fish populations can be found (see Waples 1989, 1990; Tajima, 1992; Jorde and Ryman, 1995, 1996; Berthier *et al.,* 2002). Microsatellite data from old scales collected at intervals should, accordingly, be well suited for estimating historical effective population sizes.

Miller and Kapuscinski (1997) used this approach to estimate effective population size in the northern pike (*Esox lucius*). They compared samples collected from a northern pike population in Lake Escanabe, Wisconsin in 1961 and estimated N_e based on variance in allele frequencies at seven microsatellite loci. Even though the variance of the estimate was substantial, they were still able to conclude that the effective size of the population in general had been low during the whole period. Additionally, the census population size was much higher than the effective size, with a low N_e/N relationship. Heath *et al.* (2002) also used the temporal method to estimate the effective number of breeders (N_b) in three large steelhead populations. The estimate was very low compared to the census size. This could, according to the authors, be caused by gene flow between populations. These studies provide examples of the opportunities for using old scales for estimating historical effective population sizes. However, they also illustrate that there are several potential pitfalls, which have to be evaluated before employing these methods. First of all, the method described is very sensitive to sampling variance. This means that it is most efficient when effective population size is small (large drift variance) and sample size is large (low sampling variance). Obviously, examples of populations with a small effective population size and the possibility of getting a large sample are rare. Additionally, variance in allele frequencies can have other causes than genetic drift, such as migration as suggested by Heath *et al.* (2002). Furthermore, non-random sampling of individuals with respect to life-stage (adults/juveniles, see Allendorf and Phelps, 1981; Hansen *et al.,* 1997), location (subpopulation structure within watershed, see Ryman, 1983) and time (different "runs", Phelps *et al.,* 1994) are also potential causes of variance. All of the mentioned causes of bias are particularly relevant in relation to studies using old scales, since the effects are expected to accumulate over time and exact sampling schemes cannot be repeated. This will lead to an underestimation of the effective population size. So, in conclusion, the temporal method should be applied with caution when the knowledge of when and where the samples have been taken is limited, if migration is possible and sample sizes are small.

Determination of levels of genetic variation

Loss of genetic variation can, on a short time scale, lead to inbreeding and associated inbreeding depression. On a long time scale, it is expected that the loss of genetic variation will impair the adaptability of the population. Many fish populations have suffered a substantial reduction in number during the last century, due to anthropogenic disturbance such as habitat degradation and overexploitation. However, very little empirical evidence on changes in levels of genetic variation can be found for fishes. For this reason, historical samples predating the time of disturbance are an ideal source of information. Genetic variability can be evaluated both as changes in heterozygosity and/or number of alleles, though significant changes in levels of heterozygosity are not expected, except if the genetic bottleneck is very severe and/or long lasting. More likely, the bottleneck will reduce the number of rare alleles in the population (Frankel and Soulé, 1981).

Nielsen *et al.* (1999b) observed a consistently lower number of microsatellite alleles in a recent (1998) sample of Atlantic salmon from the Skjern River than from historical samples (1930s) even though the population was still quite variable. Heath *et al.* (2002) were not able to detect any differences in levels of variability for microsatellites over 40 years in three populations of steelhead (*Oncorhynchus mykiss*) even though population structure was changed.

By employing historical collections for evaluation of changes in levels of variability, it is possible to evaluate changes using intrapopulation changes instead of making inferences by comparing to other contemporary populations with a (suspected) similar history.

Perspectives (copied from Nielsen *et al.,* 1999a)

In recent years, genome mapping projects have been initiated for many organisms including fish (see Slettan *et al.,* 1997; Young *et al.,* 1998; Kocher *et al.,* 1998). Potential “spin-offs” of such projects for population geneticists are numerous, but among the most important ones are the increased knowledge of the location and DNA sequence of selected loci. This will allow us to take population genetics “one step further”, to genetically based studies of the frequency of occurrence and the spatial scale of local adaptations in natural populations. In combination with temporal samples of DNA from old scales and neutral markers such as microsatellites, it is possible to study the fate of such adaptations in time, including changes induced by anthropogenic disturbance.

Obvious opportunities lie in the study of enzyme loci, of which many have well-documented kinetic differences between allelic variants (see Kirpichnikov, 1992 and references therein for examples). Additionally, natural selection on isozymes has often been suggested in wild populations (see Taylor, 1991; Pogson *et al*., 1995, for examples). Identification of sequence divergence between alleles with known kinetic and possibly adaptive differences in combination with DNA from old scales allows us to look at temporal changes in allele frequencies in relation to natural or man-induced changes of the environment. For instance, how has the building of a dam, which is known to have a significant influence on water temperature, affected the distribution of alleles with known different temperature optima?

For several years, population geneticists have been attracted by the study of genetic variation at MHC (Major Histocompatability Complex) genes. The main reason for this interest is the extreme level of variability apparently caused by overdominant selection in relation to disease resistance and dissortative mating (Hughes and Nei, 1988; Potts *et al.,* 1991). MHC loci have been identified and characterised for salmonid species (Grimholt *et al.*, 1993; Miller *et al.,* 1997), and evidence of the adaptive value of different MHC genotypes (Grimholt *et al.,* 1994; Miller and Withler, 1996) has been demonstrated. Consequently, these loci offer an opportunity to study potential local adaptations caused by natural selection. In relation to old scales, the study of MHC variation in time will allow us to get an idea of the dynamics of such loci in natural populations by correlating changes in allele distributions with environmental variables such as known historical outbreaks of diseases.

A common denominator of the above-mentioned loci is that they are single genes with an expected major effect. However, many traits of adaptive importance are believed to be under the control of many genes, i.e., they are inherited quantitatively. Such loci can be identified by constructing a physical map of the location of a large number of polymorphic markers on the chromosomes and thereby locate so-called quantitative trait loci (QTL). Due to the high variability, microsatellites have often been suggested as the best markers for this purpose (Ferguson and Danzmann, 1998). Although this application is still in its infancy (see Ferguson and Danzman, 1998 and references therein), its use is expected to grow tremendously in the near future, due to large economic value of selecting for traits such as growth, spawning time, age at maturity, etc., in aquaculture. Since selection for such traits has been going on for a very long time in hatcheries, a very interesting application of old scales and QTL would be to compare hatchery populations with their wild source populations and to investigate the speed and magnitude of domestication.

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Recommendations

- Appropriate extraction methods, genetic markers, PCR conditions, and authenticity guidelines should be selected and followed as discussed here to maximize efficiency and increase the likelihood of achieving success;
- Historical collections represent unique and irreplacable information on the genetic composition of populations. Therefore, processing protocols for hard parts (otoliths, scales, bones) should be adjusted with consideration of the potential for DNA analyses, i.e., by avoiding treatments that later make it impossible to extract DNA or, alternatively, by keeping unprocessed material that could later be used as a source of DNA. Likewise, current genetic analysis of historical collections should be undertaken with consideration of saving parts of the material for future studies;
- Information derived from historical or archived samples for DNA analysis could be compared with other data and records on the biology, ecology and life history of various species and populations to aid in interpretation of results. In other words, if populations differ in some biological trait over time, DNA analysis of historical material could be used for verifying that it is in fact samples from the same population that are being compared.

2.4 Molecular genetic methodologies for assessing the biological effects of contaminants (ToR d)

Based on a position paper by J. Trautner, adopted by WGAGFM in Dartmouth, 2002.

Introduction

The interactions between contaminant exposure and the genetics of individuals and populations have been the subject of a number of investigations. In the past, several different methods have been applied, ranging from classical approaches of counting abnormal developments of embryos and larvae, to transgenic reporter genes. Here some important molecular genetic methods for assessing the biological effects of contaminants are described and evaluated. The subject matter of this report draws extensively from recent reviews in the literature on this subject (Hebert and Luiker, 1996; Bickham *et al.,* 2000; Dixon and Wilson, 2000; Belfiore and Anderson, 2001).

Molecular genetic methods assessing the biological effects of contaminants can be split into two sections: those methods assessing the effect on an individual basis and those assessing the effects on populations. The methods reviewed are specifically evaluated for their use in field experiments, as for laboratory experiments most of them are already known to be efficient.

Methods for assessing the biological effects of contaminants within individuals

Methods for detecting DNA adducts

Some environmental contaminants are known to directly interact with the DNA strand through covalent binding, making them genotoxic by inhibiting transcription, replication, or by initiation of mutations. Some methods have been developed to detect and quantify the formation of DNA-contaminant formations.

^{32}P-postlabelling

By ^{32}P-postlabelling, specific DNA adducts were found and shown to survive for several months after exposure to benzo[*a*]pyrene (Stein *et al.,* 1993; Holbrook *et al.,* 1992). This method has also been used for fish populations where there was evidence for association between contaminant exposure and the abundance of adducts (Dunn *et al.,* 1987; Varanasi *et al.*, 1989). However, other studies have shown that those DNA adducts also exist in unexposed populations with a seasonal variation peaking prior to reproduction (Garg *et al.,* 1992), which makes it difficult to estimate the effects of contaminants against this background.

Chromatography and spectrometry

Combined gas chromatography-mass spectrometry and infrared spectrometry allow for recognition of OH- adducts and ring openings and show a clear association between DNA damage and contaminant exposure (Malins and Haimanot, 1991; Malins and Gunselman, 1994). The impact of DNA adducts on DNA replication and function is still uncertain, so measuring them might not give an answer to the question of how toxic contaminants are.

Methods to detect DNA strand breakage/damage

DNA is continuously maintained in the living cell. This involves partial unwinding and opening of the double strand to repair damaged parts, but also to amplify for replication leading to single strand breaks in the DNA. Some chemicals are suspected to either directly produce those strand breakages, or form the excision of adducts induced by contaminants (Shugart and Theodorakis, 1994). So, quantification of strand breakage could give an estimate on genotoxicity of contaminants.

Alkaline unwinding

Alkaline unwinding is a method which allows quantifying of DNA fragments due to the fact that at high pH the DNA denaturation initiates preferably at single strand breaks (Shugart, 1988). Consequently, it is possible to infer the incidence of strand breakage by ascertaining the rate of strand degradation.

Agarose gel electrophoresis

Another method is agarose gel electrophoresis, which can be used to directly estimate the size of DNA fragments, thus estimating the incidence of strand breakage and even distinguish between single or double strand breaks by using denaturing and non-denaturing gels (Theodorakis *et al.,* 1994). These assays have already been used in fish (Di Giulio *et al.,* 1993) and have shown that contaminant exposure elevates the strand breaks.

Comet Assay

The Comet Assay (Singh *et al.,* 1988) is based on the isolation of nuclei from cells. Nuclei isolated from cells exposed to specific chemicals are placed in an electric field, and subsequently the DNA is stained. After this treatment, DNA fragments can be seen as a “tail” of a comet (=nucleus) under a fluorescent microscope. The more breaks occurring, the larger and brighter the tail becomes. It has been shown that nuclei from fish leukocytes and cultured epithelial cells (common carp) show a clear signal when exposed to certain contaminants (Kammann *et al.,* 2001). However, this method is extremely dependent on an accurate and standardised screening procedure, as usually the nuclei are subdivided into certain classes depending on the magnitude of tail formation. The abundance of class-specific nuclei within one sample is then counted and compared to other samples.

TUNEL assay

The TUNEL assay, which detects DNA strand breaks in tissue sections, allows quantification of apoptotic cells by fluorescence and light microscopy. Common experience seems to be that the TUNEL assay is prone to false positive or negative findings. This has been explained by the dependence of the staining kinetics on the reagent concentration, fixation of the tissue and the extent of proteolysis. For the TUNEL assay, snap-frozen tissue is sectioned using a microtome, sections are fixed in formalin and transferred to slides. To stain for apoptosis, TdT directed FITC-dUTP nick-end labelling is performed (Boehringer-Mannheim). For nuclear staining, a solution of propidium iodide is used. The significance of apoptosis has mostly been studied with this method (Lacorn *et al.,* 2001).

Radiolabelling and autoradiography or scintillation counting

When damaging of DNA occurs, the cellular repair mechanism tries to fix it mostly by synthesis of new DNA. Autoradiography or scintillation counting are methods used to report those unscheduled DNA syntheses in fish (Ali *et al.,* 1993) through incorporation of radiolabelled nucleotides during DNA synthesis. The test requires the use of tissue-cultured cells which are exposed to certain contaminants; thereafter, radiolabelled nucleotides are applied and incorporated. Efforts have been made to develop combined *in vitro*/*in situ* assays (Madle *et al.,* 1994). These methods have the disadvantage that not only unscheduled DNA synthesis is measured but also normal DNA replication, which is a particularly serious problem if the rate of mitotic cell division is high. Another disadvantage of using unscheduled DNA repair as a marker system is that it reports DNA repair rather than damaging.

Methods detecting cytogenetic effects of contaminants

Damages of the DNA strand induced by contaminants can lead to changes at the chromosomal level. The extent of damage can be quantified by several cytogenetic methods.

Micronuclei staining with DNA-specific dye or centromeric probes

Micronuclei are masses of DNA found in the cytoplasm and typically formed as a result of chromosomal breakage or dysfunction of the spindle mechanism (ASTM, 1994). The influence of contaminants on the formation of micronuclei can be assessed by staining exposed tissue with DNA-specific dye and subsequent counting of micronuclei under a microscope. Another way is staining with centromeric probes (Natarajan, 1994).

The incidence of micronuclei has been investigated in fish (e.g., Al-Sabti, 1992) and bivalves (Wrisberg *et al.,* 1992). The existence and number of micronuclei were shown to vary seasonally (Fernandez *et al.,* 1993) and among populations (Wrisberg *et al.,* 1992). As this is the case, the main difficulty with these tests is to distinguish between naturally occurring micronuclei and micronuclei formed due to an exposure to contaminants. Hence, Carrasco *et al.* (1990) did not find any linkage between contaminant exposure and micronuclei in fish populations. Nevertheless, other studies have reported up to a 3-fold increase in micronuclei frequency, but they mostly failed to exercise sufficient control over other possible factors (disease, nutritional status, age and gender of fish) (Hebert and Luiker, 1996).

5-bromodeoxyuridine labelling

The event of sister chromatid exchange can be shown by differential labelling of chromatids with 5-bromodeoxyuridine. This thymidine analogue is incorporated during replication and quenches the fluorescence of some DNA-specific stains. This allows us to distinguish between sister chromatids and to track exchange events. Some contaminants are believed to block the movement of the DNA replication fork, leading to a high level of sister chromatid exchange (Tucker *et al.,* 1993). This method has been used in fish, too (Hooftman and Vink, 1981; Vigfusson *et al.,* 1983). However, as 5-bromodeoxyuridine itself increases the rate of sister chromatid exchange and creates a serious background, and as the reciprocal exchange of sister chromatids does not have a direct effect because they are genetically identical, this method may be of limited use for future investigations on the effect of contaminants.

Staining of chromosomes

Chromosomal investigations on cells stained by G/C banding or “chromosome painting” will show shifts in ploidy and chromosomal aberrations. Most studies on ploidy shifts have concentrated on the occurrence of aneuploidy – the gain or loss of chromosomes. However, as it has been shown in human populations that aneuploidy naturally occurs (Ohtaki *et al.* 1994) and shifts caused by chemicals (Mailhes and Marchetti, 1994) vary between gender, data interpretation is difficult. Chromosomal aberrations such as deletion, duplication, and rearrangement take place during DNA synthesis. *In vivo* studies have been carried out in fish (Hooftman, 1981; Hooftman and Vink, 1981) showing linkage between contaminant exposure and aberrations (Sofuni *et al.,* 1985; Wilcox and Williamson, 1986). Also, a study on American oysters (Stiles, 1990) and a combined laboratory and field study on hard clams (Stiles *et al.,* 1991) were performed using cytogenetic methods to evaluate the effect of contaminants. Only a few studies have examined the effects of contaminants on chromosomal aberrations in nature due to very stringent cytogenetic requirements (McBee *et al.,* 1987), so that this method might be difficult to apply in field experiments.

Methods monitoring mutagenesis in the genome

The mutagenic activity of several contaminants is already known from laboratory experiments, mainly carried out on tissue cultures. For mitochondrial DNA 50–600-fold higher binding levels for polycyclic aromatic hydrocarbons in relation to nuclear DNA have been reported (Allen and Coombs, 1980; Backer and Weinstein, 1980; Niranjan *et al.,* 1982). In contrast, it was confirmed that exposure to mutagenic agents induces only a low number of sequence changes in mtDNA (Mita *et al.,* 1988). Approaches for determining the magnitude of mutations were greatly improved by the invention of the polymerase chain reaction (PCR) technique.

Natural reporter genes

Mutants can sometimes be recognised by simple screening of phenotypes. The genes responsible for a particular trait that can be scored are called reporter genes. Studies performed on test systems using the APRT (adenine phosphoribosyl transferase) and HPRT (hypoxanthine guanine phosphoribosyl transferase) loci as reporter genes in mammalian tissue culture systems have shown a significant increase in mutation rates in response to contaminant exposure (Bridges *et al.,* 1991; Tates *et al.,* 1991; Perera *et al.,* 1993; Zimmer *et al.,* 1991). Unfortunately, until now no reporter genes are known in fish but the extended research on zebrafish gives reason to expect the discovery of such

genes in the future. Another remaining problem is the high number of individuals necessary for detection of shifts in mutation ratio by contaminant exposure, and the fact that these tests will be restricted to laboratory experiments.

Transgenic reporter genes

In mice and rat tissue, foreign DNA has been inserted containing a portion of the *lac* operon from *E. coli* and lambda phage shuttle vector to ease the recovery of the transgene (Gossen *et al.,* 1989; Kohler *et al.,* 1990). After exposure to contaminants, total DNA is extracted and transgenic DNA is recovered by exposing to lambda phage protein extracts, which excite the shuttle factor and encapsulate it. These bacteriophages can then be used to infect *E. coli* lacking a complete *lac* operon. Plating on media containing β-galactoside selects for positive clones and allows discrimination between wild type (blue) and mutants (white; *lac* operon not functioning). This method could also be applied to fish, but would require a larger amount of basic work and a long time scale of the experiments due to the longer generation cycle. They would also be restricted to laboratory experiments.

Monitoring mutagenesis within native genes with denaturing/reannealing techniques

As the spontaneous mutation ratio is less than one change in 100,000 base pairs, even a 10-fold increase would require around 200,000 bp to be sequenced (Hebert *et al.,* 1996). So direct sequencing of target genes is not feasible even with automated sequencers. But, as even single base pair mutations change the melting properties of DNA fragments, methods have been developed that use this property. Denaturing gradient gel electrophoresis (DGGE) denotes electrophoresis coupled with either a temperature gradient (Wartell *et al.,* 1990) or a denaturant gradient (Fischer and Lerman, 1983). Heteroduplex analyses (HA) are based on the fact that a mixture of native gene and mutant single-stranded DNA forms three different complexes after reannealing, which can then be separated using polyacrylamide gel electrophoresis (PAGE): one complex double strand native gene, one involving only the mutant gene, and finally, a hybrid between the native and mutant gene moving slightly differently in an electric field due to the mismatch at the mutation site. These kinds of analyses have already been shown to be very useful in detecting mutations caused by contaminants (Cariello *et al.,* 1991). Single nucleotide polymorphisms can also be detected by SSCPs (single strand conformation polymorphism) where the different DNA fragments are denatured and run on a PAGE. Due to the different weights and conformations of native and mutant DNA single strands, fragments up to 300 bp can be distinguished.

BESS base excision sequence scanning

Base excision sequence scanning (BESS; Hawkins and Hoffman, 1997, 1999) is a PCR-based mutation scanning method that locates and identifies all DNA mutations. The BESS method consists of two procedures that generate “T” and “G” ladders analogous to T and G ladders of dideoxy sequencing. The samples are analysed on standard sequencing gels or on automated DNA sequencers. The BESS method is versatile, having applications not only for mutation detection, but also single nucleotide polymorphism (SNP) discovery and analysis, DNA fingerprinting (including viral and bacterial typing), and clone identification.

DT-PCR (directed termination-PCR)

Chen and Hebert (1998) developed a method called directed termination-PCR (DT-PCR) to detect mutations. It is based on a PCR assay with site-specific primers, where one of the four nucleotides is limited in concentration five to ten times relative to the other three nucleotides. During PCR the lower concentration nucleotide causes the reaction to terminate prior to the full-length amplification as it becomes the limiting factor in DNA synthesis, increasingly with an increasing number of cycles. A specific banding pattern for a defined sequence is the result of this modified PCR reaction that can be compared between different template DNA samples.

CA chemical mismatch cleavage

This methodology (Cotton *et al.,* 1988) allows detection of point mutations, small insertions, and deletions and was extended by the development of a non-isotopic cleavage product detection system using silver staining after gel electrophoresis (Saleeba *et al.,* 1992). The complete mutation detection is achieved by use of mutant and wild-type DNAs in equimolar quantities in duplex formation, thus any mismatches that are resistant to chemical cleavage (e.g., some T.G mismatches) are easily detected by cleavage of the complementary heteroduplex (e.g., A.C mismatch). With such a strategy, mutant DNAs can be screened for mutations and polymorphisms.

Molecular genetic methods to evaluate the effects of contaminants at the population level

There have been some studies on the effect of contaminants on genetic patterns in populations. Most of them are referred to in an excellent recent review by Belfiore and Anderson (2001).

For studies investigating the effects of contaminants on gene pools at the population or species level in the field, it is important that different exposure levels can be clearly assigned for populations and that the exchange of individuals between populations is low enough for a few generations, so that an effect can be established within the population. "Before/after" data would be particularly useful, but are probably unlikely to be available except in areas where historical data have been collected for other purposes.

Contaminants can cause changes in the genetic composition of natural populations by induced mortality. The genetic diversity can be reduced indirectly by bottlenecking and subsequent loss of genetic variation or by selection. The latter option only applies when there is a complete or partial resistance against specific contaminants occurring. Selection for a certain genotype, which is resistant or copes best with the contaminant, is likely. As it is unlikely—although not impossible—that resistance is based on a single gene and locus, mostly additive effects will be the reason for improved performance and fitness. Hence, finding molecular markers to describe those genotypes can be difficult for both possibilities. Classical laboratory experiments as used, e.g., in plant research, crossing and back-crossing a resistant and non-resistant line/strain and performing bulk-segregant analysis would be a possibility in the case of a single locus resistance. For a multi-locus resistance, this approach is very time consuming and the prospect for success is bad. In the field, it is much more complicated, if not impossible, to design comparable experiments. The main problem is the background noise of natural variation within the genome.

The genetic effect most likely caused by contaminants is the loss of genetic diversity through induced mortality. All of the methods, which can be used to track changes in the genetic composition of populations have been thoroughly discussed by this group in previous reports and will not be repeated here. Sustainable methods are: Allozymes, microsatellites, minisatellites, RFLPs, AFLPs, RAPDs and direct sequencing. When using these markers to show loss of diversity, the same criteria apply to these methods as if they would be used in regular population genetic analyses and were discussed detailed in previous WGAGFM reports.

Co-dominant markers can be used for studying the diversity of exposed populations, as a common observation in populations under contaminant stress is the occurrence of a higher number of heterozygous loci per individual (HLWI) than in control populations (Roark and Brown, 1996; Kopp *et al.,* 1992) – as common as the observation of heterozygote deficiency (Battaglia *et al.,* 1980; Benton *et al.,* 1994; Keklak *et al.,* 1994). The high heterozygosity levels are explained by heterosis, the link between heterozygosity level and fitness (Kekklak *et al.*, 1994); deficiencies can be explained by a bottleneck model where populations are reduced by contaminant stress.

Allozymes are proteins, so alterations at the DNA level that do not result in amino acid changes, are not detected. Sometimes bands scored as the same allele may actually represent multiple alleles so that allozyme data generally underestimate the actual DNA variation. Past work on allozymes showed that there are shifts in gene frequencies within populations exposed to contaminants (Gillespie and Gutterman, 1989, 1993; Roark and Brown, 1996; Nevo *et al.,* 1987), but the response often varies among sites. These findings alone are not sufficient evidence of contaminant effects, as the differences in population structure may reflect subdivision of populations caused by other factors. Most observations suggest that these findings are indirect consequences of selection rather than single locus selection. The main disadvantages of allozyme analyses for this purpose is the fact that it is unlikely that one of the limited number of investigated proteins has a direct effect on resistance or is closely linked to it. So when it comes to the effect on specific parts of the DNA, e.g., certain genes, then other criteria for the choice of a marker system are important.

Mini- and microsatellites are better suited for this purpose as they are widely distributed through the genome. Because of the possibility to score a high number of loci, the likelihood of finding linkages to genes under selection is higher than for allozymes. The establishment of a linkage map for zebrafish, salmon, and oyster based on microsatellites supports this.

RAPD and AFLP analyses are methods which allow the screening of many more loci within the genome in a relatively short time and in an inexpensive way. Therefore, these methods are most suitable when looking for markers closely linked to genes under selection by the exposure of populations to certain contaminants. Theodorakis and Shugart (1997, 1998) discovered RAPD bands that were positively correlated to fecundity in contaminant-exposed fish. They also found a ten-fold higher heterozygosity level for the allozyme locus NP (nucleoside phosphorylase) (Theodorakis and Shugart, 1998; Theodorakis *et al.,* 1999). In contrast, restriction fragment length polymorphism (RFLP) analyses are only suitable when there is already some evidence that a certain part of the DNA is under selection. Then specific restriction enzymes can show different patterns for a specific allele.

To overcome most of the difficulties involved in studies on the influence of contaminants on genetic patterns of populations, Belfiore and Anderson (2001) proposed a generalised ideal study design with four major premises: 1) an adequate sample size should be chosen, to include several sites for each treatment type to account for the lack of uniformity of *in situ* “treatments”; 2) to incorporate site or tissue chemistry, along with other appropriate measures, such as biomarker responses; 3) to estimate contaminant exposure in order to convincingly assign treatment effects; and 4) to verify alternate hypotheses that may explain observed patterns.

The last premise points to the major difficulty one has to deal with when detecting changes in the genetic composition of a population suspected to be caused by contaminants. It is very difficult to exclude all the possible alternative hypotheses which could explain the changes. The question “how much variation reflects selection and how much reflects neutral processes” (Belfiore and Anderson, 2001), which is the question posed by the discussion on genetic patterns in populations (Beardmore, 1980; Nevo *et al.,* 1983; Nevo, 1990; Montgomery *et al.,* 2000), has not been solved yet.

Recommendations:

- Research to investigate the effects of contaminants on fish/shellfish by molecular genetic methods should be encouraged;
- When designing a field study on the effect of contaminants on populations, an adequate sample size has to be chosen;
- The feasibility of the field study should be checked taking into account population migrations and gene flow which could mask any possible effects of contaminants;
- Several contaminated sites should be included and also several control regions to account for the lack of uniformity of contamination;
- The contaminant concentrations should be measured at the site and in the tissue, to estimate contaminant exposure in order to convincingly assign treatment effects;
- Alternate hypotheses that may explain changes in populations have to be verified.

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2.5 Minimal kinship breeding strategy for preserving genetic diversity in hatcheries (ToR e)

Position paper by R. Doyle and P. O'Reilly, adopted by the WGAGFM in Dartmouth, 2002.

A gene bank has been established by the Canadian Department of Fisheries and Oceans, in collaboration with other agencies and institutions, to try to save the unique salmon gene pools in the inner Bay of Fundy, Canada. The hatchery breeding programme is based on a selection procedure (minimal kinship selection) which is designed to minimize the loss of genetic diversity, including quantitative genetic diversity (Doyle *et al.,* 2001). A minimal-kinship criterion calculated from microsatellite data is used to select subsets of breeders which represent the maximum number of founder lineages (i.e., carry the fewest identical copies of ancestral genes). The procedure differs from marker-assisted selection (MAS) in that the markers are used to identify whole lineages or "extended families" rather than chromosome segments carrying particular QTLs. Thus, minimal-kinship selection conserves the diversity of quantitative trait loci involved in adaptation to the hatchery and natural environments, not just the diversity of microsatellites themselves.

Hatchery broodstocks used for genetic conservation or aquaculture may have lost much of the genetic diversity which existed in the ancestral (wild) gene pool. This is especially likely when the fish used to initiate a broodstock population are closely related to each other. Kinship among founders causes the first generation born in the hatchery to diverge markedly from the ancestral gene pool, because related founders carry excess copies of the same genes and a deficiency of other, usually rare, genes. When an initial loss of diversity caused by founder consanguinity has occurred, the usual hatchery strategies for long-term retention of diversity, such as spawning as many fish as possible, reducing the variance of family size and equalizing the sex ratio, will not be able to recover it.

These routine diversity-conserving practices are appropriate when a hatchery has been running for a sufficient number of generations to have reached equilibrium, in the sense that every fish has the same probability of carrying a gene from any particular founder. They will also be appropriate at the beginning of a hatchery programme if the founding broodstock is already in equilibrium, in the sense that each founder has the same probability of carrying any particular gene from the ancestral population. The breeding strategy needs to be modified in the early generations, however, if the founding broodstock is so small that it is non-representative of the ancestral population and is dominated by a few lineages. This is, unfortunately, precisely the situation which is likely to trigger a genetic conservation programme.

Even when hatchery breeding has continued for some generations, e.g., for stock supplementation, the concern about the genetic quality of the stock may be relatively recent. The breeders used to start hatchery programmes decades ago

are often known to have been related to each other. McAndrew *et al*. (1992) cite anecdotal reports of hatchery stocks originating from a single mating pair. In salmonids it often happens that there are very few animals left in the wild population by the time captive breeding or hatchery supplementation begins (Utter, 1998). These remnant wild animals may already be related to each other. Taggart *et al.* (2001), for example, have uncovered an abundance of interlocking half-sib relationships among Atlantic salmon ova collected from spawning redds in a Scottish stream.

The mating strategy recommended to hatcheries has usually been guided by the concept of effective population number, N_e, which is an easily understood theoretical construct when it is applied to a population which has enjoyed a constant size and demographic structure for several generations. Maximization of effective population number N_e by equalizing the number of offspring of all matings is strongly recommended to hatchery managers (e.g., Tave, 1993, 1995), and is in fact adopted whenever possible to reduce inbreeding and genetic erosion.

The generally accepted objective in other areas of genetic conservation, such as zoos (Ebenhard, 1995; Rodriguez-Clark, 1999), is to recover some of the diversity in the ancestral population by *compensatory mating*, in which lineages that are under-represented in the founders are mated preferentially so as to increase their contribution. “One of the primary goals of any captive-breeding program is the maximization of founder allele survival” (Hedrick and Miller, 1992). “The goal of maintaining genetic diversity is equivalent to selecting pairings that maximize the retention of the founder’s diversity” (Lacy *et al.,* 1995). The usual strategies for long-term maximization of N_e in a fish hatchery, which equalize the genetic contribution of all breeders, will merely propagate the distortion caused by a non-representative sampling of the ancestral gene pool.

The consensus is emerging that the minimal kinship (MK) procedure for choosing breeders (Ballou and Lacy, 1995; Lacy *et al.,* 1995; Montgomery *et al.,* 1997; Caballero and Toro, 2000), which in every generation minimizes the overall level of coancestry in the population, is currently the best available procedure for reducing the genetic erosion of captive populations due to founder lineage bias and drift. The practice of MK mating requires pedigree analysis and consequent decisions about every mating. Mating in MK managed populations is neither random nor equalized, even when the objective is solely to minimize random changes in gene frequencies.

The difficulty of applying MK in the early generations in hatcheries is that the pedigrees of the founders are not available. Doyle *et al.* (2001) have, however, shown that it is possible to base MK selection on estimates of the relatedness between pairs of individuals as calculated from microsatellite data, in lieu of pedigrees.

The coancestry of a pair of individuals is defined by the kinship coefficient f_{ij}, the probability that two alleles taken at random from each animal will be identical by descent from a common ancestral gene (Falconer and Mackay, 1996, p. 85). The mean kinship of the *i*th individual mk_i is the average of the kinship coefficients between that individual and all living individuals including itself:

$$mk_i = \frac{\sum_{j=1}^{N} f_{ij}}{N}$$

in which N is the number of living animals in the population. Animals with low mk_i are likely to carry genes which are not otherwise represented in the population and are thus particularly valuable in maintaining, or recovering, genetic diversity. Animals with high mk_i are likely to be carrying genes which have multiple copies elsewhere in the population. If hatchery resources are limited, these high mk_i animals are relatively dispensable. It has been shown by Ballou and Lacy (1995) that a strategy for choosing mated pairs that minimizes the average mk_i (the MK selection strategy) maximizes the retention of genetic diversity. Doyle *et al.* (2001) used Ritland's $\hat{r}$ estimator to calculate the mean relatedness of each animal relative to the other fish in its generation and population (Ritland, 1996a, 1996b).

Other estimators of relatedness have been developed, e.g., Queller and Goodnight (1989), Wang (2002), but their utility in the present context has not yet been investigated.

The N_e-maximizing approach to genetic conservation in hatcheries is (usually implicitly) based on the random effects, variance-component viewpoint of Weir and Cockerham (1984). This viewpoint cannot be applied consistently from beginning to end of a hatchery programme if the hatchery founders consist of sub-subpopulations or lineages that are non-representative, and that must be individually identified and managed in the early generations. At this point, the model differs dramatically from the hierarchical, random-effects, population genetic model, and the implied optimal hatchery procedures differ dramatically from the N_e-maximizing strategy implied by the hierarchical population genetic model.

McAndrew *et al.* (1992), along with many others, have emphasized the importance of using representative founders in a hatchery, if such founders exist. When sufficient numbers do not exist, there may be uncomfortable trade-offs in choosing a genetic conservation strategy. In Finland, for instance, one of the primary aims of an Arctic charr stocking programme has been to maintain the genetic integrity of natural populations, which is accomplished by only stocking fish produced from lake-specific broodstocks (Primmer *et al.,* 1999). In at least one instance (Lake Saimaa), very few wild spawners could be caught and the decision was made to use these fish, and risk the loss of genetic variation, rather than import breeders from another lake. The tradeoff was genetic diversity *vs.* local genetic adaptation. The genetic variation is now low in Lake Saimaa and several aspects of viability and fecundity are also unusually low, although Primmer *et al.* (1999) are careful to note that there is no proof that inbreeding has been the problem. In any case, this is the type of situation in which MK selection might increase diversity and reduce divergence from ancestral populations without importing fish, even when few founders are available.

The goal of conservation in hatcheries should be to maximize the retention of the genetic diversity which existed in the last (wild) generation to which the random-effects population genetic model can be credibly applied. The objective is *not* limited to preserving the diversity of the breeders that are collected to found the hatchery. Owing to the possibility of consanguinity and non-random sampling of the remnants of a dying population, the early generations of the conservation programme should aim to increase the genetic diversity in later generations above that in the founders. This is a crucial difference between MK-minimizing strategy and the N_e-maximizing strategy which is generally recommend to hatchery managers.

The concern in a hatchery is often not genetic conservation *per se* but the accumulation of inbreeding, i.e., loss of individual heterozygosity and possible decrease in individual fitness. This is related to N_e, in the simple equilibrium case, by the equation:

$$F_t = 1 - \left[1 - \left(1/2N_e\right)\right]^t \ .$$

The fixation index F_t in this equation is a useful predictor of the mean inbreeding in later generations of a hatchery (with constant size and demographics) when the imbalance of founder contributions has either been corrected by MK selection or has spread itself equally over all living descendants. However, it is an overly optimistic measure of the rate of population-level inbreeding in the early generations when founder contributions are grossly unequal.

The average mean kinship of the parents will become the average inbreeding of the next generation if mating is random but will be less than this if MK selection is employed. The drift of a captive population away from its ancestral genetic constitution can be considered either a sampling process or an inbreeding (consanguineous mating) process, because both the among-subpopulation variance and the homozygosity within subpopulations increase as a result of finite population size.

The calculation of the relatedness estimator $\hat{r}$ requires that allele frequencies be independently known and free from appreciable sampling error (Ritland, 1996b). As a practical matter, these frequencies will usually be obtained from the sample of individuals for which the relatedness is being determined, possibly from the founding broodstock itself. Nevertheless, the option exists to provide frequency data from another source. In a captive breeding programme, the logical alternative source of frequency information is the ancestral population, which might have yielded data at some time in the past while the population was still large (as in brown trout in Denmark, where in some rivers the genetic composition of the current population of brown trout, heavily influenced by stocking, is rather different from the ancestral composition as found by analysing archived scale samples (Hansen *et al.,* 2000). The animals brought into the captive breeding programme when the population has declined nearly to extinction might be too few, or too

unrepresentative, to provide the required independent and error-free data for the estimation of $\hat{r}$. Archived scale samples, which are often available and which can yield usable microsatellite information (Nielsen *et al.*, 1999; Ruzzante *et al.,* 2001), may be usable for this purpose.

We have observed (Doyle *et al.,* 2001) that if the sample of the founding generation is so small that it cannot yield reliable allele frequencies, data from the previous generation (i.e., earlier samples from the wild) can, at least in the instance examined in that paper, be used to select broodstock so as to minimize kinship.

Conclusions and recommendations

- Microsatellite data can be used for minimal kinship selection (MK selection) of broodstock in hatcheries. This procedure partially recovers the diversity lost through over-represented and consanguineous founder contributions to a captive gene pool;
- After one or more generations of MK selection, the usual broodstock management procedures (e.g., rotational mating and equalized family size) can be adopted.

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2.6 The possible use of gene array techniques in the detection and quantification of responses in fish to pollution (ToR f)

Based on a position paper by J. Trautner, adopted by WGAGFM in Dartmouth, 2002.

The DNA array technique allows binding of a large amount of denatured DNA fragments—preferably cDNA—to a matrix where each fragment is bound to a specific spot of the matrix. Then this matrix, the chip or array, can be exposed for instance to cDNA (reverse transcribed mRNA) from any tissue. By properly choosing the hybridisation conditions, only complementary sequences will bind to the array. With a specialised detector, a specific binding pattern can be screened qualitatively. With this technique it is possible to analyse a large amount of genes for expression within a single experiment. The main problem lies prior to this step and involves the isolation of genes or DNA segments of interest.

There are two different formats of DNA microarrays, in terms of the property of arrayed DNA sequence with known identity: For the first format, probe cDNA (500~5,000 bases long) is immobilised to a solid surface, such as glass, using robot spotting. Next, it is exposed to a set of targets, either separately or in a mixture. This method, “traditionally” called DNA microarray, is widely considered as developed at Stanford University (Ekins and Chu, 1999).

The second format consists of an array of oligonucleotide (20~80-mer oligos) or peptide nucleic acid (PNA) probes, which is synthesised either *in situ* (on-chip) or by conventional synthesis followed by on-chip immobilisation. The sample DNA is labelled by the incorporation of biotinylised nucleotides. The array is exposed to the labelled sample DNA and the identity/abundance of complementary sequences is determined by staining with florescent dye and subsequent measurement of fluorescence. This method, “historically” called DNA chips, was developed at Affymetrix, Inc. (*GeneChip®*) but many other companies are manufacturing oligonucleotide-based chips using alternative *in situ* synthesis or depositioning technologies.

Gene array techniques have not been used in marine fish yet, only in zebra fish (*Danio rerio*) as a model species (Clark *et al*., 2001). For toxicological research, the keyword is toxicogenomics. The goal of toxicogenomics is to find correlation between toxicants and changes in the genetic profiles of the objects exposed to such toxicants (see

Nuwaysir, 1999). For toxicological research a toxicogenomic approach has already been initiated: First Preclinical Toxicity Application (Toxicology EXPRESS™ database using Gene Logic's Flow-thru Chip™ technology) involving Wyeth-Ayerst Research and Gene Logic.

To transfer such systems to fishes, it is necessary to define and find the genes of interest. Much work has to be invested in setting up cDNA libraries to use this technique. However, once this is done the prospects are good that DNA array technology will provide information for toxicological problems related to fish.

However, considering the problems with the methods listed under ToR (d), it can be suspected that this approach will have similar problems when used for field experiments, rather than laboratory experiments. The expression of genes is affected in so many ways and by so many factors that clear signals seen in a laboratory experiment might be overlaid by other signals occurring in the field. This will make it difficult to establish a clear correlation between contaminant exposure and expression patterns. However, it is worthwhile putting some effort into this new approach. Even if it might not be useful in the field, DNA array technology is a powerful method for laboratory experiments dealing with the basics of the response to contaminants on the gene expression level.

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3 WORKING GROUP BUSINESS

3.1 New WGAGFM Chair

The present Chair was very pleased to announce that Dr Ellen Kenchington, Bedford Institute of Oceanography, Canada (and the host of the meeting) has agreed to take over as new Chair of the WGAGFM when the term of the present Chair runs out in autumn 2002. Ellen has made very significant contributions to WGAGFM during the past six years, and there can be no doubt that it will be in good hands in the years to follow, a sentiment shared by everybody at the meeting.

3.2 Discussion of future activities of the WGAGFM in relation to aquaculture (with representatives from the WGMAFC)

Two representatives, John Castell and Tim Jackson from the Working Group on Marine Fish Culture (WGMAFC), attended the meeting in order to discuss future collaboration between the two working groups. More specifically, this collaboration involves developing standard culture conditions under which strains, stocks, or species might be tested to evaluate their performance. The two working groups agreed to suggest this topic for the ToRs of the 2003 WG meetings in the first instance, in order to identify the specific problems that need to be addressed.

3.3 Discussion of suggestion by WGECO to collaborate in developing practical management options for the conservation of genetic diversity in marine fish and shellfish

WGAGFM discussed a suggestion by the Working Group on Ecosystem Effects of Fishing Activities (WGECO) to collaborate in developing practical management options for the conservation of genetic diversity in marine fish and shellfish of economic importance. WGAGFM found it important to first identify the scope for collaboration by identifying the specific genetic problems (e.g., is it all realistic that low effective population sizes could be a problem in marine populations due to overfishing? How can the issue of selective fishing be addressed, etc.?). Dr Ellen Kenchington (the new WGAGFM Chair) later participated in the meeting of WGECO in order to further discuss this issue.

3.4 Suggestions for WG ToR and meeting place in 2003

During discussions on meeting place in the year 2003, the WG responded positively to a generous invitation from Dr Pierre Boudry, IFREMER/RA, La Tremblade, France, to host the 2003 WGAGFM meeting on 10–12 March 2002.

ANNEX 1: TERMS OF REFERENCE FOR THE 2002 WGAGFM MEETING

2F03 The **Working Group on the Application of Genetics in Fisheries and Mariculture** [WGAGFM] (Chair M. Møller Hansen, Denmark) will meet in Halifax, Canada from 18–20 March 2002 to:

a) update the provisions regarding GMOs in the ICES Code of Practice on Introductions and Transfers of Non-indigenous Organisms and transmit this material to WGITMO;

b) assess and evaluate the utility of interspecific comparisons of population genetic parameters in understanding population structure in fish species;

c) review and report on developments in the use of DNA from archived samples (scales, otoliths bones, etc.) for analysing fish populations;

d) review and report on the utility of molecular genetic methodologies for assessing the biological effects of contaminants on fish and shellfish;

e) review and summarize principles for minimizing diversity loss in the early generations of a captive broodstock;

f) prepare a position paper for the Working Group on Biological Effects of Contaminants on the possible use of gene array techniques in the detection and quantification of responses in fish to pollution.

WGAGFM will report by 20 April 2002 for the attention of the Mariculture Committee, ACME and ACFM.

Supporting Information

Priority:	WGAGFM is of fundamental importance to ICES.
Scientific Justification:	a) The existing Code of Practice needs urgent updating, particlarly with regard to its application to GMOs. This update is a matter of urgency given the necessity to have measures to reduce unwanted consequences of introductions and transfers. b) During the past few years numerous important developments have been made in the statistical analysis of genetic data, in particular microsatellite DNA, for assessing and describing the genetic structure of populations. These new statistical procedures are based on principles such as Markov-Chain Monte Carlo simulation, Bayesian statistics and coalescence theory and will undoubtedly have a profound effect on studies of the genetic structure of fish populations. However, there is so far a lack of understanding of "what to expect" from these procedures, both in comparison to "traditional" population genetics statistics and in relation to life history and other biological features of the studied species. Many marine species are particularly difficult to work with using "traditional" statistics due to weak genetic differentiation among populations, and it would be of interest to know how well the newly developed procedures perform in these cases. c) Analysis of DNA from archived samples, such as otoliths, scales and bones, is a new and very promising development in fish population genetics. This allows for studying the genetic composition of populations over much longer time spans than have previously been possible, to detect genetic changes in populations due to anthropogenic influence (e.g. loss of variability, allele frequency shifts at loci subject to selection), and to determine whether or not populations are indigenous or the result of stocked or escaped farmed fish. The WG finds it is important to evaluate the utility of archived samples in studies of fish populations and to identify possible problems and pitfalls. d) This is a ToR resulting from a suggestion by the Working Group on the Biological Effects of Contaminants (WGBEC). It is suggested to assess the utility of developments in molecular genetics and genomics for studying the biological effects of contaminants on fish and shellfish. The ToR builds partly on previous ToRs at the 2000 and 2001 meetings on endocrine disruptors and selected genes, respectively.

	e) This ToR addresses the difficulty which arises when a gene bank or supplementary broodstock is founded with a few, non-representative survivors of a dying natural population. It is often not clear what is supposed to be conserved by breeding these remnants in captivity. Standard practices for maximising effective population sizes, such as equalizing mating success and fecundities, will merely lock in the initial founder distortion. Preferentially mating some founders more than others in the early generations, e.g., to increase lineage diversity, is likely to be controversial, though it is a procedure that is used in zoos. It is important to highlight and discuss the fundamental differences, rationales and consequences of these different approaches. Also, hatchery procedures, particularly in the initial generations, should be rather different in these two cases. This is an important question in relation to management and conservation of populations and captive broodstocks. At the same time the ToR addresses an issue of importance to the maintenance of quantitative genetic variation in aquaculture stocks, particularly if aquacultural stocks are included in a comprehensive program for genetic conservation of a species. f) WGBEC does not have the appropriate expertise and therefore needs assistance in this question
Relation to Strategic Plan:	Responds to Objectives
Resource Requirements:	None required other than those provided by the host institute.
Participants:	Quantitative and aquaculture-type geneticists are particularly needed.
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	ACME
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.) WGBEC
Linkages to other Organisations:	HELCOM
Cost share	ICES 100%

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ANNEX 4: RECOMMENDATIONS FOR 2003

The Working Group on the Application of Genetics in Fisheries and Mariculture [WGAGFM] (new 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) discuss, review and report on genetic issues related to escapes of farmed marine fish and shellfish;

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

d) discuss and report on genetic aspects of developing standard culture conditions under which different strains of aquacultured species can be tested (collaboration with WGMAFC);

e) discuss 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, ACE, and ACME.

Priority:	WGAGFM is of fundamental importance to the ICES 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, oyster). 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 developments 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 ToR is the result of a request from WGMAFC concerning collaboration on the development of standard protocols for testing aquacultured strains. The first task of WGAGFM will be to identify the specific problems, which will then be addressed in more detail at forthcoming meetings. e) This ToR is the result of a request from WGMAFC 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 the 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:	