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REPORT OF THE

WORKING GROUP ON THE APPLICATION OF GENETICS IN FISHERIES AND MARICULTURE

Leuven, Belgium
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International Council for the Exploration of the Sea

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EXECUTIVE SUMMARY

At its meeting in 2000, the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) dealt with a number of Terms of References (ToRs), which will be briefly summarised below:

a) continue the review of general population genetics topics in fisheries and mariculture, with emphasis on the utilisation of possibilities available through the combination of qualitative and quantitative genetics

This session served mainly to discuss and identify topics for ToRs for the 2001 meeting.

b) review the relevant portion of the chapter on Baltic fish prepared for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994–1998 [HELCOM 2000/3]

This Term of Reference was already treated at the 1999 meeting in Reykjavik. However, HELCOM asked for a more detailed response. In particular, answers were requested to questions such as

- *What percentage of salmon in an area (or river or group of rivers) could be considered to be wild?*
- *Is there interbreeding between wild and reared fish?*
- *What are the implications of the loss or dilution of wild salmon genetic material in the Baltic Sea?*

These and other issues of relevance to conservation genetic management of Baltic salmon were reviewed. The conclusions and recommendations were as follows:

Conclusions

- *Evidence exists that some, although not substantial, changes have occurred in both the diversity levels of the marker genes and inherited life-history traits.*
- *Some changes will be inevitable in the future, too, because artificial reproduction can never be completely the same as natural reproduction.*
- *There is no return to the original state of the Baltic salmon populations, and conservation of genetic diversity should thus be planned onwards from the present situation.*

Recommendations

- *For maintaining genetic diversity, large populations are required and thus it is important to conserve areas where substantial natural reproduction can still take place. The conservation of these areas should be prioritised.*
- *All the present genetic material of the Baltic salmon and all its potential reproduction habitats should be in use for natural reproduction.*
- *For a long-term conservation plan, hatchery stocks should be reintroduced into the wild to make them a viable component of the Baltic salmon evolution.*
- *To retain the larger scale genetic structure, major groupings of populations need to be taken into account. Thus, separate strategies are needed for the Ice Lake and Atlantic lineages within the Baltic Sea.*
- *Stock transfers between the ranges of the Ice Lake and Atlantic lineages should be strictly avoided.*
- *The ranges (distances) of stock transfers within the lineages should be minimised.*
- *Activities causing straying, such as delayed releases and sea releases, should be minimised.*
- *Future monitoring of genetic changes (at least of diversity levels of the marker genes) is recommended.*
- *Studies of changes in the viability (fitness) of the reared populations in the wild are recommended.*

c) review principles for prioritisation of marine finfish and shellfish populations for conservation

This session concerned an as yet unexplored but potentially highly relevant topic, i.e., how to prioritise populations of marine living resources for conservation. In terrestrial and freshwater organisms situations already exist where economic resources are not sufficient to ensure conservation of all populations of a given species, and it is argued that similar situations may occur in marine systems as well. Hence, an objective and scientifically based prioritisation procedure is needed. Such procedures have already been developed, and in the present paper a procedure aimed at prioritising Pacific salmon populations for conservation is suggested to be valid for marine organisms as well.

d) review the status of Artificial Intelligence and Neural Networks as tools in population studies based on input requested from SIMWG

This session reviewed and discussed the use of artificial intelligence and artificial neural networks in genetic studies of fish populations. Basically, these methods can be used for classifying individuals into their population of origin, and empirically they seem to work quite well. The problem with the techniques, however, consists in their poor or absent statistical definitions and lack of knowledge of why the techniques actually work. Novel statistical methods (like assignment tests) are available that are just as efficient and have a clear statistical definition. Artificial intelligence may, however, be useful in situations where genetic and other data (e.g., otolith characteristics, growth data, coloration, environmental data, etc.) are combined.

e) compile an updated list of patents in molecular biology which potentially may interfere with population genetics research

No new patents of relevance were identified.

f) review potential genetic implications of recent research on endocrine disruptors

Knowledge on endocrine disruptors and their observed and anticipated environmental effects was reviewed. Endocrine disruptors may have serious impacts on the genetic composition of populations, mainly by causing sex reversal, resulting in skewed sex ratios, or simply by causing sterility. Both effects may lead to reduced effective population sizes resulting in loss of genetic variability and inbreeding. However, in general, knowledge of the effects of endocrine disruptors is still insufficient to make an overall assessment of the associated genetic risks.

g) review the possibility and feasibility of developing coordinated genetic databases for enhancing understanding of genetic diversity in fish species

A lot of genetic data on various fish species have been generated over the past 25 years. In order to study the genetic population structure of species over large geographical areas it would be useful to be able to combine the results from different studies. Similarly, coordinated genetic databases could be useful to conduct metaanalyses for demonstrating, e.g., natural selection. Consequently, the working group sees a large potential in the establishment of coordinated genetic databases for fish populations. However, this requires careful planning and coordination of efforts and will not be feasible for all classes of genetic markers. WGAGFM recommends the following:

1) Before a genetic database is established a standard battery of suitable genetic markers must be defined.

2) A calibration of markers among laboratories contributing to the database must be conducted.

3) Necessary steps must be taken to ensure a uniform and high quality of the data generated by the different laboratories.

4) WGAGFM finds that it is beyond its scope and capacity to be responsible for establishing and maintaining databases on genetic data. However, in order to contribute to a general harmonisation of the use of microsatellite DNA markers in fish and shellfish population studies WGAGFM has decided to start collecting information on available microsatellite loci in different species and make it available on the WGAGFM website. The Chair will ask specific WGAGFM members to take responsibility for collecting information on specific species, and the progress of the work will be assessed and discussed at the 2001 WGAGFM meeting.

h) review genetic implications of commercial fisheries on deep-water fish stocks

Catches of traditionally exploited marine resources are not likely to increase and therefore the possibility of harvesting other species on new fishing grounds will be investigated intensively in the future. Deep-water species constitute a plausible alternative, with many species already being exploited, either directly targeted or as a by-catch product. It is known that some of the species exhibit very slow growth and reach sexual maturity at a high age. This is likely to affect their reproductive output and potentially makes them vulnerable to extensive harvesting. Furthermore, it is known that the distribution of some of these deep-water species extends into international waters, and they are therefore subject to uncontrolled fishing. WGAGFM concludes that there is a strong need for collecting basic data on the population dynamics and genetic population structure of these species, in order to evaluate the potential effects of fisheries on the species.

Recommendations

1) WGAGFM recommends that high priority be given to research aimed at deep-water fish species, as the situation is at present at a point where exploitation has not yet reached intense levels.

2) Moreover, WGAGFM also recommends the research efforts at this stage be concentrated on fewer species, so that more extensive biological data (general biological features, population dynamics, population genetics) can be obtained from a few species. These species could then serve as model species, both in order to assess the importance of specific biological features of deep-water fishes (such as slow growth) in relation to harvesting and potential depletion of genetic resources, and in order to able to focus later research activities on other deep-water species.

i) explore the question of trade-offs between genetic gain and loss of genetic variability in breeding programmes (how to minimise inbreeding in intense breeding)

In most breeding schemes a balance between genetic gain and inbreeding is sought. New developments in animal breeding schemes are designed to increase genetic gain, but inbreeding rates often increase concomitantly. However, a number of procedures and breeding strategies are available aimed at minimising inbreeding and these are reviewed in the paper. The following recommendations are given:

1) At the set-up stage of a breeding programme it is important to ensure maximum genetic variation in the offspring as it is these which will make up future breeding populations. This can be done by crossing between year/classes/lines/strains. It can also be done by examining all potential broodstock for genetic variation at the DNA level using DNA markers such as microsatellites. If enough variable loci are examined, good estimates of relatedness can be obtained between mating pairs. Pairings can then be planned on the basis of minimum relatedness alone combined with any pedigree information available.

2) Any planned programme should include estimation of predicted inbreeding levels.

3) Efforts should be made to restrict inbreeding using one of the methods outlined above; the best method will depend on the conditions of the programme.

4) In mass selection programmes, records should be kept of broodstock pedigree wherever possible.

5) Records should be kept of rates of inbreeding with each generation of breeding programme.

6) Hatchery/broodstock managers could keep small samples of fin or other tissue in 95 % alcohol from each breeding generation for the purpose of measurements of genetic variation changes as a result of breeding practices. In the case of an outbreak of disease fish farmers could store tissue from their morts and healthy fish, for future reference, to investigate any relationship between susceptibility to disease and inbreeding.

j) prepare updated protocols of fishery and mariculture genetics research in Member Countries, and identify scopes for enhanced international cooperation

As usual, WGAGFM collected information on ongoing research activities. However, it was decided this year to make the information available through the unofficial WGAGFM website instead of including it in the report, as 50–60 printed pages with summaries of numerous ongoing projects are difficult to browse and extract information from.

1 INTRODUCTION

As decided by ICES C.Res. 1999/2:F:03 adopted at the 1999 Annual Science Conference in Stockholm, Sweden, the Working Group on the Application of Genetics in Fisheries and Mariculture [WGAGFM] (Chair: M.M. Hansen, Denmark) met at the Catholic University of Leuven, Belgium, 3–6 April 2000, to deal with its Terms of Reference for 2000 (see Annex 1).

1.1 Attendance and Venue

Twenty persons representing fourteen countries attended the 2000 WGAGFM meeting in Leuven (Annex 2). Countries represented (number of persons in parentheses) were: Belgium (2), Canada (2), Denmark (2), Estonia (1), Finland (1), Germany (2), France (2) Iceland (1), Ireland (1), Norway (1), Poland (1), Portugal (1), Spain (1) and UK (2). As in the six previous years, the representation on the quantitative genetics was lower than on the qualitative genetics side. The official ICES member list for WGAGFM is attached as Annex 3.

The Catholic University of Leuven (represented by Dr Filip Volckaert) offered excellent meeting rooms and facilities for the WGAGFM meeting. Filip Volckaert had made excellent arrangements for the meeting, including an 'excursion' to the European Commission, DG Fisheries in Brussels where details on project applications and selection criteria were presented and discussed. WGAGFM is pleased with his kind hospitality and all his efforts to make our meeting effective and enjoyable. WGAGFM also wishes to thank the staff at the European Commission, DG Fisheries, for their kind hospitality and for arranging a very informative meeting.

1.2 Organization of the Work

Prior to the meeting, specific members (and, in one case, a member of another ICES WG) agreed to prepare position papers related to specific issues in the Terms of Reference, and to chair the respective sessions (in one case the author of a position paper was unable to attend the meeting and the session was chaired by another WGAGFM member). The Chair asked the persons responsible for preparing position papers to send him electronic versions of the papers prior to the WGAGFM meeting. The position papers were subsequently distributed to the meeting participants some days before the meeting. This enabled the participants to read through the papers before the actual presentation of papers at the meeting. During the meeting, the position papers were first presented and discussed in plenary. Thereafter, each topic was discussed in *ad hoc* sub-groups. Position papers were updated according to points raised in the plenary and sub-groups discussions and were finally edited and included in the WGAGFM report.

- M.M. Hansen chaired business and general scientific sessions (ToR point a)
- M.-L. Koljonen chaired "Review the relevant portion of the chapter on Baltic fish prepared for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea" (ToR point b)
- E.E. Nielsen chaired "Principles for prioritisation of marine finfish and shellfish populations for conservation" (ToR point c)
- E. Kenchington chaired "Artificial intelligence and neural networks as tools in population studies" (ToR point d)
- J. Trautner chaired "Potential genetic implications of recent research on endocrine disruptors" (ToR point f)
- M.M. Hansen chaired "Coordinated genetic databases for enhancing understanding of genetic diversity in fish species" (ToR point g)
- R. Castilho chaired "Genetic implications of commercial fisheries on deep-water fish stocks" (based on a position paper by J.D.M. Gordon) (ToR point h)
- P. Boudry chaired "Trade-offs between genetic gain and loss of genetic variability in breeding programmes" (based on a position paper by A. Norris) (ToR point i)
- A. Danielsdottir collected and compiled the National Activity Reports (ToR point j)

[ToR point e) "compile an updated list of patents in molecular biology of interest to population genetics research" was not treated, as no significant new developments had occurred since 1999.]

The session Chairs were responsible for leading the plenary sessions and group work, and for preparing the final report text from their sessions. All members were asked to collect national activity reports from their respective countries beforehand and send them by e-mail to the Chair for inclusion in the report. WGAGFM decided that, as in the five previous years, the preparation of the WGAGFM report should mainly be done by the members present at the meeting. 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 2000

Terms of reference for the 2000 WGAGFM meeting are reprinted in full in Annex 1.

2.1 General Population Genetic Topics Related to Fisheries and Mariculture

This session was scattered throughout the meeting, and served mainly to identify topics for the Terms of Reference for the year 2001.

2.2 Review the Relevant Portion of the Chapter on Baltic Fish Prepared for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994–1998 [HELCOM 2000/3]

(Based on a position paper by Marja-Liisa Koljonen, adopted by WGAGFM in Leuven 2000)

Introduction

This Term of Reference was already treated at the 1999 meeting in Reykjavik. However, HELCOM asked for a more detailed response. In particular, answers were requested to questions such as:

- What percentage of salmon in an area (or river or group of rivers) could be considered to be wild?
- Is there interbreeding between wild and reared fish?
- What are the implications of the loss or dilution of wild salmon genetic material in the Baltic Sea?

These and other issues of relevance to conservation genetic management of Baltic salmon are treated in the following paragraphs.

Wild production, its occurrence and relation to fishing regulation

The number of wild smolts has increased in the Baltic Sea in recent years as a result of large year classes and the regulation of fishing. In 1998 the number of wild smolts was estimated to be about 0.37 million and in 1999, 0.48 million. In 1998 the proportion of wild smolts was about 7.5 % in the entire Baltic Sea, but it is estimated to have increased to about 11 % in 1999 (ICES, 1999).

Table 2.2.1. Atlantic salmon smolt production in the entire Baltic Sea area.

Year	Wild	Hatchery	% Wild
1997	372 000	5 885 000	6.32
1998	483 000	6 423 000	7.52
1999	625 000	5 785 000	10.80

The spatial distribution of the wild fish depends on the geographical location of their spawning sites and their migratory behaviour. On the basis of estimated production in 1999, the two largest wild stocks (Tornionjoki: 97 000 smolts and Kalixälven: 77 000 smolts) together produce 28 % of the total wild production (ICES, 1999). Rivers in the Bothnian Bay area account for about 70 % of all wild production. (The location of rivers and hatcheries with their approximate production levels are given in Koljonen *et al.* (1999), updated production levels are in reports of the WGBAST.)

Two different phylogeographic lineages of Atlantic salmon occur within the Baltic Sea area (Koljonen *et al*., 1999), the older one originating from eastern glacial lake populations, the Ice Lake lineage, and the younger one from Atlantic populations, the Atlantic lineage. Current wild smolt production levels and potential reproduction habitats suggest that the Ice Lake lineage is in greater danger of becoming extinct than is the Atlantic lineage. The populations of the Ice Lake lineage with the oldest and some of the most rare genetic material in the Baltic Sea area are currently to be found in the present salmon stocks of Estonia, Latvia, Lithuania, Russia, and southern Sweden.

In general, the proportion of wild fish is high near the mouths of the wild salmon rivers in early summer and also during the spawning migration in the springtime, usually May, along the Finnish coast, when it can reach even 30 % of total

catches (Koljonen and McKinnell, 1996; Koljonen and Pella, 1997). At the national level, local fishing regulations are imposed on coastal fishery and at the mouths of the wild salmon rivers. Finland and Sweden have delayed the opening date of coastal salmon fisheries in the Gulf of Bothnia to restrict the harvest of the early run, when the proportion of wild salmon is at its highest. The provisions of this regulation were made more stringent as of 1996. There are clear indications that the regulation has been effective, in particular by allowing wild fish to escape from the coastal fishery into the spawning rivers (ICES, 1999).

Genetic effects of hatchery rearing and their evaluation

There is some interbreeding between wild and reared fish, especially when wild stocks are supported by enhancement releases. In those cases, releases are based on river-specific broodstocks to ensure that no non-native genetic material is imported into the wild salmon stocks. The hatchery production is, however, sometimes based on an effective population size that is smaller than that of the wild stock to be supported. The releases as such may then reduce the total genetic diversity of the stocks (Ryman and Laikre, 1991), especially if the number of spawners in the river does not increase due to the releases (Waples and Do, 1994).

The genetic effect of hatchery releases on the scale of the whole Baltic Sea is difficult to evaluate, because very little genetic information is available on the state before releases or before other human impacts (loss of populations and marked crashes in population sizes of the remaining stocks). Even if it were possible to obtain information on genetic changes in some particular cases and insight into changes in the total structure, their importance for the future evolution of the Baltic salmon remains unknown. Genetic changes due to hatchery rearing may occur in both marker gene frequencies, such as allozyme or microsatellite loci, and in quantitative loci that determine life-history traits such as growth rate and age at maturity or migration behaviour. Estimates of changes in both types of traits exist.

The genetic effects of hatchery releases on naturally reproductive stocks depend on several factors. The genetic change caused by gene flow from a different fish stock depends on the genetic difference between the stocks and on the amount of gene flow. The amount of gene flow to each river depends, not only on the total amount of fish released, but usually on the geographical distance between the release site and the river mouth and also on the release methods. The amount of released salmon smolts in the Baltic Sea is very high, about 6 million, and at present about 90 % of this production is based on relatively constant hatchery releases (Table 2.2.1). Smolts are produced either from wild spawners caught at river mouths or from captive broodstocks (usually governmental programmes). Broodstock breeding may take place in either long-term or short-term programmes. In long-term captive breeding, genetic material from the original wild stock is no longer available, and the breeding is based on several successive hatchery generations. In short-term breeding, each broodstock generation is renewed, at least partly, with individuals from a wild population, and only second generation hatchery offspring are released.

The majority of the smolt releases are based on water-court decisions and are obligations on hydro power plant companies to compensate for destroyed spawning habitats and lost salmon catches. Straying rates are higher for sea and delayed releases than for river releases. Owing to closure of the rivers, river releases are not always possible.

The genetic difference between hatchery and wild fish may be caused either by different genetic origin or by hatchery rearing processes (non-random sampling of spawners or selective changes in rearing practices). The genetic effects of hatchery rearing on Baltic salmon stocks have been studied by comparing the genetic differentiation pattern and characteristics of stocks and stock groups of wild and hatchery origin and also the amount of diversity (mean heterozygosity or number of alleles) between the wild and hatchery derivatives of the same river stock. In addition, crossing experiments have been conducted to estimate the genetic change in quantitative traits caused by selective forces in hatchery rearing.

Changes in marker gene frequencies

According to an allozyme study of Baltic salmon stocks throughout their range, it seems that, on a large scale, releases of hatchery fish and losses of several original stocks have caused loss of the isolation-by-distance differentiation pattern (i.e., relationships between geographical distance between river populations and measures of their genetic relationships) that was originally present in the genetic differentiation of the Baltic salmon stocks (Figure 2.2.1, Koljonen *et al*., 1999). Thus, hatchery rearing has caused allele frequency shifts and random changes in historical genetic distances between the stocks.

As measured from 18 allozyme loci studied in populations from the whole Baltic Sea, the total diversity of hatchery stocks ($H_t = 0.054$) is somewhat lower than that of the wild stocks ($H_t = 0.076$). The proportion of between-stock diversity component is 16 % ($G_{st} = 0.161$) for the wild stocks and 6 % ($G_{st} = 0.063$) for the hatchery stocks. The wild stocks are thus, on average, more different from each other than are the hatchery stocks. This is, however, explained

partly by the dissimilar distributions of wild and hatchery stocks among sea areas, and partly by historically different levels of diversity within these areas. Almost all hatchery stocks represented the Atlantic lineage. When hatchery and wild groups from the same area (Gulf of Bothnia) were compared, the diversity level was the same (wild stocks Ht = 0.057, and hatchery stocks Ht = 0.055). The average number of alleles in the wild and hatchery groups of the Baltic salmon was the same (wild 1.7 and hatchery 1.8 for seven polymorphic loci). In general, no dramatic changes in diversity levels of the hatchery stocks could be observed.

The population sizes of hatchery stocks tend to be smaller than those of wild stocks, which creates a risk of losing diversity. The first stage of hatchery rearing is broodstock sampling. Comparison of a wild stock with its derived broodstock showed that in three individual allozyme loci the mean heterozygosity was significantly lower in the broodstock than in the wild stock. In the broodstock sampling, two out of 16 different alleles (12.5 %) in seven polymorphic loci were lost in this particular case. Small hatchery stocks pose a risk to the long-term maintenance of genetic diversity, however, rare alleles were also lost in small wild stocks. For conservation, it is therefore necessary to secure a large number of spawners in both wild and captive populations. Risks in both environments should be considered when planning a conservation strategy.

Figure 2.2.1.a. Relation of estimated gene flow (migrants) to geographical distance for wild stocks alone.

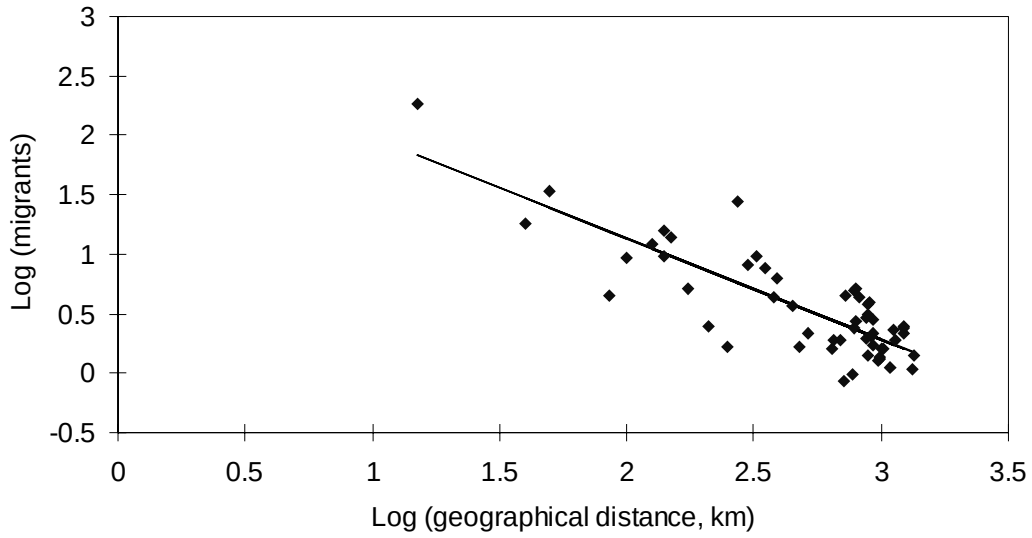
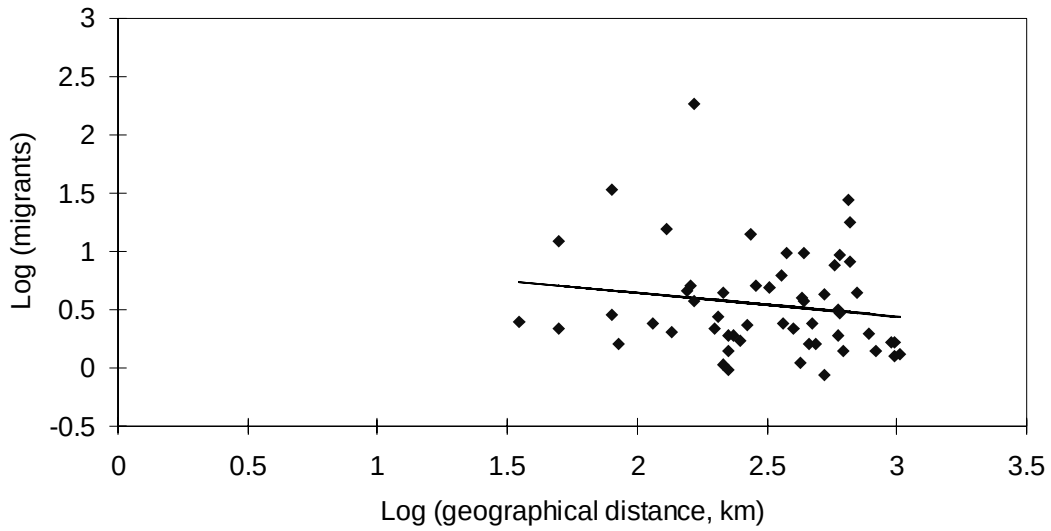


Figure 2.2.1.b. Relation of estimated gene flow (migrants) to geographical distance for hatchery stocks alone.



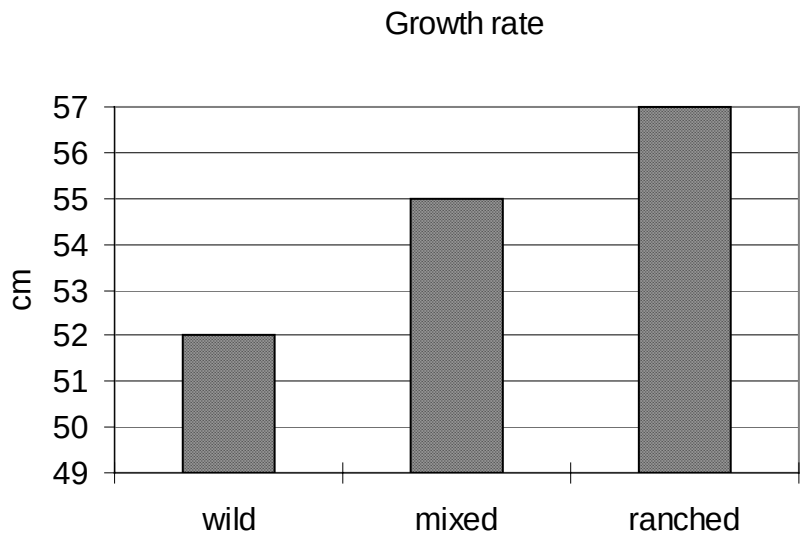
Changes in quantitative traits

A crossing experiment in which offspring of wild and reared parents from the same river stocks were compared revealed some changes in quantitative traits (i.e., traits under both environmental and genetic influence, usually involving several loci). Quantitative genetic traits are directly related to the viability and fitness of the stocks, contrary to the genetic allozyme variation. The growth rate of the offspring of hatchery parents was statistically significantly higher than that of the offspring of wild parents when they were smolts (Table 2.2.2) and also later in the sea (Figure 2.2.2). The growth rate of the hybrid group with wild and reared parents showed intermediate capacity (Kallio-Nyberg and Koljonen, 1997).

Table 2.2.2. Mean length of offspring of wild and reared parents and a mixed parent group as smolts at release**.**

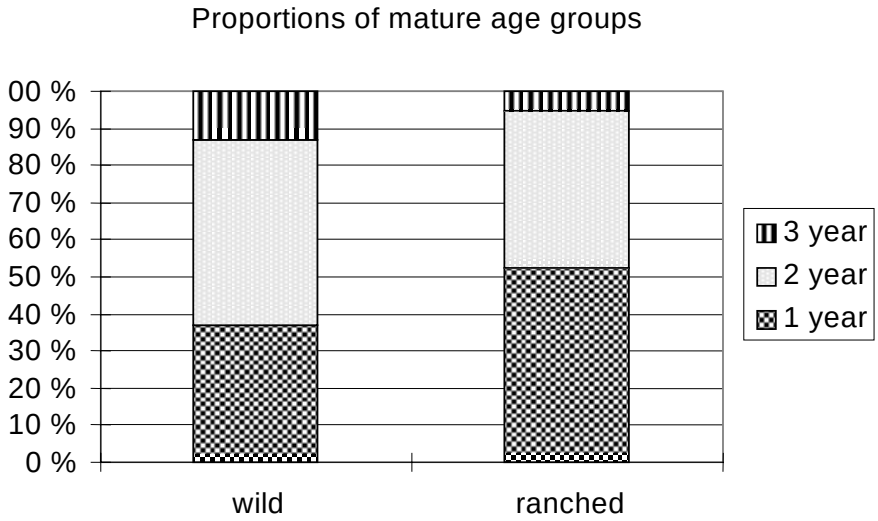
Parent group	Length (cm)	S. E.	n
Wild parents	18.1	2.04	503
Wild and ranched parents	18.9	2.09	441
Hatchery parents	19.7	2.30	1524

Figure 2.2.2. Effect of breeding history on growth rate of Atlantic salmon in the Baltic Sea after second sea growth period (in second winter) measured as length increment since release (Kallio-Nyberg and Koljonen, 1997).



The same crossing experiment showed that the age at maturity of the offspring of the reared parents was lower than that of offspring of the wild parents. Especially the proportion of mature one sea-year old fish, mostly males, was higher in the hatchery group (52 %) than in the wild group (34 %) (Figure 2.2.3).

Figure 2.2.3. Effect of breeding history on the sea-age distribution (1, 2, and 3 sea-winter old fish) of offspring of wild and ranched parents.



Hatchery rearing may include selective factors that might change the genetic composition of quantitative traits. To what extent this has happened is unknown. In the case studied, selection had not been intentionally avoided and the collection of spawners had led to overrepresentation of larger fish in the broodstock, which in turn caused a decrease in average age at maturity and an increase in the proportion of one-year-old mature fish.

The rate of loss of mean heterozygosity was quite low, 0.6 % per generation for the long-term breeding and 1.04 % per generation for the short-term breeding. Neither deviated markedly from the approximate level of 1 %, which is often regarded as an acceptable level of inbreeding (Franklin, 1980; Frankel and Soulé, 1981). The ratio of effective population size Ne to the census number of broodfish Nc varied from 0.24 to 0.48 in short-term breeding, which is more than in wild populations on average (0.11, Frankham, 1995a, 1995b). Captive breeding programmes have also succeeded in maintaining the amount of microsatellite variation fairly high in some otherwise lost populations.

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Conclusions

- Evidence exists that some, but not substantial, changes have occurred in both the diversity levels of the marker genes and inherited life-history traits.
- Some changes will be inevitable in the future, too, because artificial reproduction can never be completely the same as natural reproduction.
- There is no return to the original state of the Baltic salmon populations, and conservation of genetic diversity should thus be planned onwards from the present situation.

Recommendations

- For maintaining genetic diversity, large populations are required and thus it is important to conserve areas where substantial natural reproduction can still take place. The conservation of these areas should be prioritised.
- All the present genetic material of the Baltic salmon and all its potential reproduction habitats should be in use for natural reproduction.
- For a long-term conservation plan, hatchery stocks should be reintroduced into the wild to make them a viable component of the Baltic salmon evolution.
- To retain the larger-scale genetic structure, major groupings of populations need to be taken into account. Thus, separate strategies are needed for the Ice Lake and Atlantic lineages within the Baltic Sea.
- Stock transfers between the ranges of the Ice Lake and Atlantic lineages should be strictly avoided.
- The ranges (distances) of stock transfers within the lineages should be minimised.
- Activities causing straying, such as delayed releases and sea releases, should be minimised.
- Future monitoring of genetic changes (at least of diversity levels of the marker genes) is recommended.
- Studies of changes in the viability (fitness) of the reared populations in the wild are recommended.

2.3 Principles for Prioritisation of Marine Finfish and Shellfish Populations for Conservation

(Based on a position paper by Einar Eg Nielsen, adopted by WGAGFM in Leuven, 2000)

Introduction

The conservation of biological resources has been on the public agenda for several decades. As a consequence, a vast amount of scientific literature has been produced, discussing various aspects of protection of biodiversity at different hierarchical levels. Initially, the main theme was the protection of species, but with the growing body of evidence of within-species differences both on an individual and population level, the awareness of the need for protecting intraspecific diversity has risen (for example: Soulé, 1980; Frankel and Soulé, 1981; Moritz, 1994). This is also the case in the framework of fisheries biology (see, for example, Ryman, 1991; Ryman and Utter, 1987; Ryman *et al*., 1995). For terrestrial and freshwater systems, the knowledge of highly diversified populations, the threatened status of many populations of various species, and limited resources to divert to the protection of them, have in many instances left managers with a need to prioritise populations for conservation. Therefore, there has been a general need for scientifically based tools to assist in this prioritisation process. The initial models to aid decision making were generally developed for species, not populations, and were based on extinction risk alone evaluated using PVA (Population Viability Analysis, see Mace and Lande, 1991 and references therein). Recently, however, with the growing body of genetic data for naturally structured populations, attempts have been made to score biological consequences of extinction, that is, to assign "value" to populations as well (Given and Norton, 1993; Allendorf *et al*., 1997; Laikre, 1999). Thus, the outcome of the process can be seen as a two-dimensional plot with risk on one axis and conservation

value on the other (Allendorf *et al.*, 1997). In the case of Pacific salmon, for which it was initially developed, this method has gained some support, although there has also been criticism (Wainwright and Waples, 1998).

So far, no attempts have been made to apply the method to marine fish and shellfish. In fact the question is does this method have any relevance to marine species? There is a general belief (misconception?) that the oceans are too large and too resilient for human-caused extinctions of populations and species (see Culotta, 1994; Ryman *et al*., 1995; Malakoff, 1997, for a general discussion). Additionally, most marine species show less differentiation among populations than freshwater and anadromous species (Ward *et al*., 1994). In other words: Why prioritise among populations that are not very different and that we can never endanger? The objectives of this paper are: 1) to evaluate the relevance of prioritising marine finfish and shellfish populations, and 2) to provide examples of modifications of the prioritisation procedure developed for salmonids to fit marine organisms with different biological characteristics.

Is it relevant at all?

Until recently, marine conservation biology was almost exclusively a story of saving large marine mammals and birds (Culotta, 1994). The conventional wisdom was that humans could not have any severe impact on most marine life: "The sea was just too big and deep—and its inhabitants too numerous, prolific, and widespread—for humans to leave that kind of permanent biological scar" (from Malakoff, 1997). However, during the last decade several examples of man-induced extinctions at sea have appeared (see Culotta, 1994; Malakoff, 1997). Still, the number of extinctions is relatively small compared to terrestrial and freshwater systems. Whether this is a fact, or an artefact of the difficulties of studying marine biological systems, and having to prove "that something is no longer there", remains to be resolved. Still there are some biological features of marine organisms that distinguish them from other species. As mentioned previously, the level of genetic differentiation among populations of marine organisms has generally been found to be less than that of freshwater and anadromous species (Ward, 1994). The reason for this has been ascribed to a general lack of physical boundaries in the sea leading to a large potential for dispersal of, particularly, the enormous amounts of pelagic eggs and larvae commonly produced by marine organisms. The lack of evidence could, however, also have other causes. First of all, population genetic theory predicts that the large effective populations sizes generally found for marine organisms (but see also Hedgecock *et al*., 1992) make them less susceptible to genetic drift (Waples, 1998). This means that even though populations have been effectively separated for thousands of years, one would expect to find no (or at most low levels of) differentiation (Ryman *et al*., 1995). Furthermore, population genetics of marine organisms (with sufficiently variable markers) is still in its infancy. Until now, focus has been mainly on the commercially most important species, which by definition have very large population sizes. Even in these, several studies have demonstrated significantly differentiated populations (Mork *et al*., 1985; Jörstad *et al.*, 1991; Ward *et al.*, 1994; Ruzzante *et al*., 1999). As more and more population genetic studies of marine organisms are published, and focus is not only directed towards highly mobile ocean dwelling pelagic species, we will most likely find numerous species with highly differentiated populations. A study by Swaby and Potts (1990) of rare marine fish in Great Britain gives a good indication of that (Table 2.3.1). Of 165 fish species classified as rare more than one third have localised distributions, that is, restricted distributions often clearly defined by habitat or geographical limits.

Table 2.3.1. Distribution of 165 uncommon British marine fishes (modified from Swaby and Potts, 1990).

Distribution	**Percentage**
Extensive	0
Widespread UK	6.1
Restricted UK	32.7
Localised	**35.2**
Single population	3
Indeterminate	3.6
Occasional vagrant	19.4

In summation, even though genetic differentiation has generally been found to be smaller in marine species than in freshwater and anadromous species, there are still many marine species that have been shown to have significantly differentiated populations. It is very likely that many others will be found in the future, since the majority of likely candidates have not yet been studied, or not studied with the proper population genetic tools.

The establishment of the fact that there are indeed highly divergent populations is, however, only one side of the problem. The other is to verify if any of these populations are likely to disappear or lose substantial amounts of genetic

diversity. To illuminate this, it is important first to look at the potential threats to populations of marine organisms. Thorne-Miller (1997) has produced a list of threats to marine biodiversity (modified):

1) Over-exploitation (overfishing, overhunting and aquaculture);
2) Physical destruction of habitat (coastal development, fishing activities, marine mining);
3) Chemical pollution (nutrients, toxins);
4) Introduction of exotic species;
5) Global atmospheric change.

Potential impact and illustrative examples of each of the threats can be found in Thorne-Miller (1997).

The ultimate impact of these threats is the extinction of species, and if marine species can become extinct it follows that populations can as well. There are very few examples of extinct or endangered marine species, and among these marine mammals and birds are the most abundant (see Culotta, 1994). Several marine biologists claim, however, that that is an artefact caused by the difficulty of proving that an organism is gone for good. A very illustrative example of that is the story of the emerald sea slug (see Malakoff, 1997), which was discovered in the early 1970s in the Indian River lagoon on the Atlantic coast of Florida. At that time it was relatively common but coastal development diminished the habitat of the slug (sea-grass) and it has not been seen since 1982. Still, since it has planktonic larvae, it could, potentially, be found in other suitable areas, where nobody has been looking for it, and therefore it is not recognised as an extinct species. This species was found in one of the best studied coastal areas in the world, which leaves the potential for many similar unnoticed/uncertain extinctions. It is highly likely that the loss of intraspecific diversity is much more common.

In summation, at present more and more marine organisms from various taxa are being placed on the IUCN red lists including finfish and shellfish (Malakoff, 1997). This indicates that the seas are not immune to human threats. If we are capable of threatening species, we most certainly are capable of wiping out populations or reducing numbers to levels that will result in substantial loss of genetic variation. Since we have already seen that populations of marine organisms can have highly divergent populations, it is possible that we cannot conserve them all and prioritisation becomes an issue. Nevertheless, it is not likely to be equally relevant for all species.

Relevance of applying prioritisation schemes to different groups of finfish and shellfish

Compared to land, the sea harbours a more diverse assemblage of organisms. This can be seen in the number of phyla represented. While there are 28 major groups of organisms on land, 43 can be found in the sea. Until now about 275 000 species have been described, but the estimated number of marine inhabitants is likely to be several million (see Malakoff, 1997). Within such a diverse group there are, naturally, many biological differences that are likely to have great importance for the conservation of individual species. Likewise, it is not likely that the prioritisation of populations for conservation will apply equally well to all marine finfish and shellfish species. However, three major groupings in relation to threat and life history can be readily identified.

Classical Marine Organisms: These have large population sizes, high fecundity, pelagic larvae and wide distributions. Most commercially important finfish and shellfish (such as small tuna species, cod, herring, haddock, scallops and blue mussels) belong to this group. These species have also been regarded as having limited population structure, with most genetic variability distributed within populations, although the use of new genetic markers has challenged this conventional wisdom (see Ruzzante (1999) for examples).

These species have generally been regarded as "safe" in a classical conservation genetic context (such as the 50–500 rule, see Frankel and Soulé, 1981). Even though it is possible to overexploit them to the point that it is no longer profitable to fish for them, population sizes are still very large. Beverton (1990) reviewed the ten largest population crashes for small marine pelagic fish, and found that even in the worst case (Icelandic spring-spawning herring) the lowest estimated census size was more than one million individuals. Although very large differences between census and effective population size have been suggested for marine organisms (Hedgecock *et al*., 1992), it is not likely that the effective size of most populations of these species will reach critical levels in a genetic sense. However, it should also be stressed that considerable qualitative differences can be detected between populations that have not achieved genetic isolation, and it may be *highly desirable* to preserve all such populations both for conservation and commercial interests. This is in contrast to species where the among-population variation is high (e.g., salmonids), or where the species has a metapopulation evolutionary strategy of loss and recolonisation of populations. In these, the accepted philosophy is that we should target certain populations for conservation while accepting the loss of others (Allendorf *et al*., 1997). However, species with fewer, larger populations may undergo changes and/or loss of genetic diversity at the gene level that may not translate into a dramatic change in the census or effective population. The major reasons for this

is that directional selection is much more efficient in large than in small populations (Crow and Kimura, 1970) and secondly, large populations are able to retain more variation than small ones.

This loss of genetic diversity may have consequences to the ecosystem that are just as severe as or even worse than the loss of single populations in a highly structured species. For example, in the instance of commercially exploited marine species, fishing has been shown to have a selective influence—altering important life history traits such as age- and size-at-maturity and even spawning time. While some of the morphometric responses to fishing are known to be plastic, changes in life history traits, such as age-at-maturity, appear to be irreversible. Another example of a fishing practice which has the potential to reduce genetic diversity while maintaining a high census population is the targeting of one sex over the other as is done for the American lobster and snow crab. In species with XY systems, large deviations from a 1:1 sex ratio will reduce effective population sizes. Further, in cases where the mating behaviour requires size specific pairing (as in many crustaceans), fishing only one sex could also alter the ratio of effective to census population size. Both of these traits are amenable to the development of performance measures, with associated criteria, against which to evaluate the status of the population. Finally, for sessile invertebrates which depend on external fertilisation (e.g., sea scallops, abalone), fishing may destroy the fine-scale structure within the population also altering the ratio of effective to census population size. In extreme cases, this can produce an Allee effect (Allee, 1938) where the population size reaches a critical threshold, below which average reproduction per individual is limited and the population goes into an extinction vortex, as seen with white abalone off the coast of California (Davis *et al*., 1996). Therefore, we may experience large changes in the genetic diversity and associated qualitative traits of marine fish and shellfish populations, with concordant effects both on ecosystems and economics, long before the populations reach critically low values of effective population sizes or experience population crashes.

If we put these species into a prioritisation context developed for salmonids, most populations are expected to have low scores with regard to both "threat" and "value", and prioritisation should be of limited relevance. Nevertheless, going through the process of prioritisation could be of value for illuminating the diversity within the species, and for identifying potentially vulnerable populations.

"Grizzlies of the Oceans" (Anonymous, 1991): These are predatory species of intrinsic rareness (Ryman *et al*., 1995) and generally with low reproductive rates. In this group we find many of the large sharks, marlins and some tunas, but also large marine mammals (whales) and some birds. Because of their specific biological features they are vulnerable to overexploitation and several species, such as the bluefin tuna are already considered endangered (Safina, 1993). They are particularly vulnerable as they often prey on commercially important species and are caught as by-catch. Genetic differences between populations have been observed in some species (Broughton and Gold, 1997; Gaida, 1997; Gardner and Ward, 1998), but in general population genetic information is limited. However, there are indications of population structure for many of these species. For instance many large sharks are coastal, which suggests that gene flow among populations could be restricted. For these species prioritisation according to "threat" and "value" could prove highly relevant and useful. The major problem with respect to prioritisation of populations within this group of marine organisms is the difficulty in getting access to genetic data to delineate population structure due to their rareness.

Localised Species: A third group is composed of species with low dispersal abilities inhabiting "islands" in the sea, such as specific coastal habitats, seamounts or coral reefs. These species often have a patchy distribution very similar to freshwater species in rivers and lakes. By their distribution and dispersal abilities, they are candidates for possessing the most differentiated populations of marine organisms and indeed highly divergent populations have been found (Waples, 1987; Shulman, 1998). At the same time, their patchy distribution and subsequent small effective population size make them prone to local extinction. As with the previous group, the prioritisation process will be highly valuable here, in particular as the coastal areas, seamounts and coral reefs are known to be threatened marine habitats (Thorne-Miller, 1997). Therefore, many populations are expected to disappear.

In conclusion, there can be benefits of applying the prioritisation process to a wide range of marine finfish and shellfish populations, including species that we normally consider invulnerable to human activities. However, most benefit and practical use of the process is found for species that are naturally rare, patchily distributed and with low dispersal ability inhabiting areas that are likely to be influenced by human activities (coastal areas, coral reefs, etc.). For classical marine organisms, conserving genetic diversity and monitoring changes to qualitative traits within populations should be of primary concern in order to avoid ever reaching the point where effective population sizes are so small that they are amenable to prioritisation using the scheme developed for salmonids.

Modifications of the prioritisation procedure for marine organisms

As can be seen from the schemes in Table 2.3.2 and Table 2.3.3, the basic process of prioritisation relies on two different components. Each population is first evaluated according to risk of extinction, and subsequently an evaluation of biological consequence of extinction is undertaken. For both schemes the risks of extinction are modified versions of

an initial procedure described by Mace and Lande (1991). The method was developed to provide a scientific way of assigning species and populations to the IUCN categories (critical, endangered, vulnerable). The authors aimed specifically at providing a method which was simple, flexible, appropriate, objective and temporal. Much emphasis was put on only applying scientific data and not incorporating socio-economic interests. Here we have chosen to use the modified scheme from Laikre (1999) as a basis for extinction risk evaluation in marine fish, since it also considers populations with an Ne larger than 500, which was also included in the original version by Mace and Lande (1991). However, this only builds on the scale already established at the critical end; that is, it does not change the numbers associated with the critical and endangered categories. For the "grizzlies" and "localised species/populations" we feel for the most part that these criteria are applicable (but see comments on Table 2.3.3). However for classic marine species the numbers do not correlate with the level of threat. By this we mean that marine populations may become critically endangered at population sizes that are an order of magnitude greater than the ones identified in the table; by the time a population reaches the levels identified it is almost certain to be extirpated in the near future.

The biological impact of catastrophic population crashes or of declining populations is well reflected in the prioritisation schemes for "grizzlies" and "localised species/populations". Their biological features or distribution means that severe population declines will affect reproductive success. However, many "classic marine species" are adapted to large natural fluctuations in populations, and in some cases they exhibit a metapopulation structure where loss of populations and subsequent recolonisation occurs naturally. In the first instance, tracking the deviation of the minima from the long-term average population sizes, or temporal differential in the minima is more critical than reacting to the rate of change. In the case of metapopulations the natural loss of the subpopulation is part of the evolutionary process and the critical question is whether the rate of loss is influenced by man and if so to what extent. Further, in order to facilitate the evaluation of large numbers of populations under threat, the prioritisation schemes do not weight the measures and require two or more of the measures to apply within a threat category. In practice, for marine organisms, it would seem logical to weight the measures with changes to effective population size or ratio of effective population size: census population size having greater influence than changes in population numbers.

We suggest that the following performance measures be considered when monitoring populations of Classic marine organisms:

Sex Ratio Deviations from 1:1

Significant changes in Age- or Size-at-Maturity (Selection Differential)

Reduction in the Number of Spawning Populations

Additionally for sessile marine invertebrates:

Critical density (Number/M^2) for spawning success

Uniform vs. contagious distribution pattern?

And if measurable:

Changes in the ratio of effective population size:census population size

For evaluation of the biological consequence of extinction, we suggest use of a modified (generalised) version of the scheme produced by Allendorf et al. (1997). Very few changes have been made for this general scheme (Table 2.3.3), except for removing the question about introductions. As in the original scheme, a positive answer is assigned one point. Compared to the scheme proposed by Laikre (1999) we have not included the socio-economic value of populations. The reason for this is that to estimate the socio-economic value of a population and compare that to the cost of doing something to protect it is a very complex procedure, which normally is out of the hands of biologists (see Lackey, 1994).

Table 2.3.2. Criteria for assessing the level of risk of extinction**.**

	Risk of extinction			
	Very high	**High**	**Moderate**	**Special concern**
Probability of extinction using PVA*	50 % within 5 years or two of the following	20 % within 20 years or one very high risk criterion or two of:	5 % within 100 years or one high risk or	Historically present believed or known to still exist but no current data Action: Build data set from which risk level can be established
*This method produces high levels of uncertainty around estimates of extinction within 0–1000 years and so is not effective at resolving these three categories with any degree of confidence.				
Effective population size per generation Total population size*	Ne < 50* or N<250*	Ne<500* or N<2500*	Not applicable Not applicable	Run size of population strength estimate Demographic data
*Assumes that Ne is approximately 1/5 of Nc. This may not be true for many marine species and can be influenced by selective fishing (see comments above)	*May be relevant numbers for Grizzlies and Localised species but too small for Classic marine species populations some of which have become very highly threatened with Nc in the millions and billions	*May be relevant numbers for Grizzlies and Localised species but too small for Classic marine species populations (see adjacent)		
Population decline*	Precipitous decline*	Chronic decline or depression*	Decline apparent or probable	such as proportion that spawn at each age
*Many marine organisms experience natural population fluctuations and have adapted to them. It is important to detect changes to the normal pattern as opposed to reacting to a decline per se.	*Relevant for Grizzlies and Localised species that are not biologically adapted to recover from population losses or where threshold levels for reproductive success are crossed	*Relevant for Grizzlies and Localised species that are not biologically adapted (see adjacent). May represent loss of genetic diversity in Classic species if a selective response		
Catastrophe, rate and effect*	Order of magnitude decline within one generation	Smaller but significant decline	Not applicable, stocks rate at least high risk	adult survival between spawning Genetic data
*As above. Also, species with metapopulation strategy may require populations to be lost and recolonised for evolution to proceed.	*Relevant for Grizzlies and Localised species that are not biologically adapted to recover from population losses or where threshold levels for reproductive success are crossed	*Relevant for Grizzlies and Localised species that are not biologically adapted (see adjacent). May represent loss of genetic diversity in Classic species if a selective response		

Table 2.3.3. Biological consequences of extinction.

Biological consequences of extinction	Yes	No
Genetic and evolutionary legacy:		
Does the population have high genetic divergence?		
Does the population exist in an unusual habitat?		
Does the population possess unusual life history traits?		
Does the population possess unusual morphological traits with a genetic basis?		
Has the population been long isolated geographically?		
Has the population avoided any severe bottlenecks in the past?		
Does the population occur at the extreme range of the species?		
Ecological legacy:		
Is this population a member of a native assemblage that is unusual or rare?		
Does this population occur in an unusual or unique biogeographical province?		
Are adjacent and nearby populations of the same species extinct, declining or relictual?		
Are numerous other aquatic species in the same area extinct, declining or relictual?		
Would protecting the habitat of the population play an umbrella role, encouraging recovery of other imperilled populations in the area for which limited data are available?		

It is beyond the scope of this paper to test the relevance of individual questions, for several populations and for a number of species, within each of the previously defined biological groups. However, from a very small "test run" on a few familiar species (cod, herring, turbot) inhabiting the sea areas around Denmark the questions seem to make sense. The relevance of the questions or the generality of the scheme is probably not the largest problem in the application of the method. Potentially, the general lack of data, and the large variance of data among populations, could be the most severe limitations for application of the method to marine finfish and shellfish populations. This has already been pointed out as a major source of bias for the pacific salmonids (Allendorf *et al*., 1997; Wainwright and Waples, 1998) of which much more is known compared to marine organisms.

Conclusions

Prioritisation of marine finfish and shellfish populations for conservation is likely to become an issue in the future with the current speed of marine habitat degradation and overexploitation of many marine organisms. Prioritisation of populations can be a valuable tool for conservation of a wide range of marine organisms with highly different biological characteristics. The combined procedure of evaluation of extinction risk and biological consequence of extinction described for freshwater and anadromous species can easily be modified to fit marine species and is particularly well suited to "Grizzlies of the oceans" and "Localised species" and even for "Classic marine species" with well-differentiated populations. For the majority of marine species that fit the Classic pattern, loss of genetic diversity within populations is considered to be underestimated in the present prioritisation schemes. The main obstacle for employing the procedure is, however, the general lack of genetic and ecological data for marine populations. Further, considerable changes to the ecosystem and to the yield of commercial species can occur through genetic-based changes in qualitative traits of marine species without endangering the integrity of the species.

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2.4 Artificial Intelligence and Neural Networks as Tools in Population Studies

(Based on a position paper by Ellen Kenchington, adopted by WGAGFM in Leuven 2000)

Introduction

Artificial neural networks (ANN) are mathematical models designed to mimic human brain learning and pattern recognition activity. They are powerful in their ability to generalise from examples and adapt to new situations and to extract information from noisy data (e.g., Potter *et al*., 1994; Lek and Guegan, 1999). ANNs are especially useful when applied to problems whose solution is too complex to model but for which there are many examples of the known outcome; neural networks create their own solutions through exposure to many examples of correct solutions. While there are now more than 20 different types of models described as ANN, learning capability is a common feature to all.

Function and algorithms

A neural network learns by adaptively changing the interconnection strengths between the “neurons”. This can be done in an unsupervised or a supervised way. In unsupervised learning the network learns to classify the examples by recognising different patterns in the data. Only inputs are given to the network. In supervised learning, a supervisor outside the network compares the outputs of the network with the desired outputs, using a set of training data, and makes adjustments to the connections in the network. The training element is achieved by modifying weights on the internal connections within the network. The goal of the training session is to correctly learn the stimuli so that in the future, when a particular pattern or a slightly distorted version of one of the stimuli is presented, the system will classify it properly (Sheppard and Gent, 1991).

The algorithms used to implement the classification vary. One of the oldest and simplest is the perceptron algorithm (Rosenblatt, 1962) which is based on a linear model. In ANN terminology it is referred to as "two-layered", i.e., a set of input patterns (input layer) is mapped directly to a set of output patterns (output layer). Weight adjustments are made by penalising the weights of the wrong node and rewarding the weights of the correct node, until the decision function of the correct node is satisfied.

This relatively simple model was further developed to a semi-linear feed-forward net with backpropogation of error (Rumelhart *et al*., 1986). This network is similar to the perceptron model except for the presence of hidden layers of nodes. These "hidden layers" give the network a greater ability to acquire arbitrary non-linear mappings and to generalise from input data (cf. Potter *et al*., 1994; Malmgren and Nordlund, 1997). There are many ANN architectures that utilise various numbers of hidden layers.

The most difficult neural networks to build are those that recognise spatio-temporal patterns. Adapting neural networks that deal explicity with the recognition of time-varying signals buried in a great deal of noise requires an ability to: 1) learn new spatio-temporal patterns without destroying any of the information concerning the previous spatio-temporal patterns; 2) respond quickly both during training and recall; and 3) generalise well (Field *et al*., 1990). Software packages are available which employ choices of calculation and weighting functions (e.g., Neural Desk by Neural Computer Sciences; Brainmaker by California Scientific Software; Beagle by Warm Boot Ltd., 1987; and from http://www.applied-maths.com).

Applications of ANNs

ANNs have been used in a variety of ecological applications ranging from classification of sonar and acoustic information (e.g., Field *et al.*, 1990; Alexandrou and Pantzartzis, 1990) through predictions of paleotemperatures (Malmgren and Nordland, 1997) and marine mammal call discrimination (Potter *et al*., 1994) to biological monitoring programmes (Walley and Fontama, 1998). However, applications in the biological sciences are still relatively rare compared to other fields. However, recently a whole issue of the journal *Ecological Modelling* (120 (2–3), 1999) has been devoted to the use of ANN in the ecological sciences. In the context of population genetics ANN could be applied to genetic data using allele frequencies for a set of loci as the input data to identify species or population of origin. There are some examples of this use of ANN in the literature. Withler *et al.* (1994) used data from a multilocus minisatellite probe combined with ANN to discriminate farmed and wild chinook salmon (*Oncorhynchus tshawytscha*). Even though discriminatory power was high (approximately 90 % of all individuals classified correctly) similarly high power was achieved using discriminant analysis. Aurelle *et al*. (1999) used microsatellite analysis in combination with ANN to discriminate between wild and stocked, domesticated brown trout (*Salmo trutta*) and report that discrimination was up to 95 % successful. However, they did not compare the performance of ANN to that of statistical procedures, such as assignment tests. Finally, Potter *et al.* (1991, 1994) applied ANN to the characterisation of North American and European salmon using morphometric variables through the ICES Working Group on North Atlantic Salmon.

Even though the examples of discriminating fish from different populations using molecular markers suggest that ANN is a useful procedure for this purpose, there seems to be little advantage to taking this approach over existing methodology. In fact, because it can be difficult to determine how ANNs formulate predictions, their direct application to genetic data may be inappropriate. Recent developments in the use of assignment tests (Cornuet *et al.,* 1999) make this type of statistical procedure the preferred option for identifying the population of origin of individuals. First, the assignment of individuals is based on clearly defined principles. Second, even with low to moderate genetic differentiation (F_{ST} of 0.05–0.10) and given a sufficient number of microsatellite loci (approximately 10) assignment success close to 100 % can be achieved (Cornuet *et al.,* 1999). Conversely, ANN may be very useful in applications where genetic data are combined with various other data sources (e.g., life history traits, temperature, salinity) to predict ecosystem responses.

Recommendation

Neural networks show promise for applications where genetic data are one of several data sources used to make a classification. However, there appears to be little advantage in using this method to classify organisms when all of the input data are genetically derived. In this instance neural network assignations are inferior to current statistical procedures that are based on clearly defined principles.

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2.5 Update of Patents in Molecular Biology of Interest to Genetics Research

No new patents were identified that could be of interest and importance to genetics research

2.6 Effects of Endocrine Disruptors on the Genetics of Aquatic Organisms

(Based on a position paper by Jochen Trautner, adopted by WGAGFM in Leuven 2000)

Introduction

Endocrine disruptors are chemical substances interacting with hormone regulatory systems or acting as hormones themselves. An enormous amount of chemicals are produced and widely used for a broad range of human purposes. Most of these substances are released to the environment and a growing number is suspected to act as endocrine disruptors. There is already evidence from laboratory experiments that some endocrine disruptors lead to a shift in sex ratio to one or the other sex in fish and there is also reason to believe that some may cause sterility. The purpose of this paper is to summarise the scientific literature and assess the potential effects of endocrine disruptors on the genetic diversity of fish populations. However, it should be noted that the general topic of endocrine disruption, literature and current research programmes are reviewed by the ICES Working Group on Biological Effects of Contaminants (WGBEC), and more detailed information can be found in their WG reports.

Definition of endocrine disruptors

Even though this has been suspected for a long time (Dodds and Lawson 1938), it has only recently been demonstrated that some environmental chemicals exhibit hormonal effects. These so-called "endocrine disruptors" (EDs) are defined as "exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes" (Kavlock *et al.*, 1996) or as "an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine functions" (EC, 1997). Given this broad definition it is clear that there are many ways how EDs can act and that a wide range of chemicals is assigned to this group. Several endocrine disrupting chemicals have been identified by now and many others are suspected to act as EDs. A detailed list is given in the receptor database (http://impact.nihs.go.jp/RDB.html) where receptors and possible receptor binding chemicals are listed.

EDs include both naturally occurring estrogens produced by plants (phytoestrogens) (Verdeal and Tyan, 1979), fungi (mycoestrogens), and vertebrates (estrogen hormones), and synthetic chemicals (i.e., xeno-estrogens). In aquatic environments a number of EDs occur. Examples of "natural" EDs include 17β-estradiol, estrone, estriol, and synthetic EDs include biodegradation products of alkylphenol, polyethoxylates, polychlorinated biphenyls (PCBs) and pesticides such as DDT, chlordecone, methoxychlor and synthetic estrogens as ethinyl estradiol, nonylphenol. The most intensively studied EDs are estrogen-mimicking, i.e., they bind to estrogen receptors. Most of the current research programmes concentrate on the identification of endocrine disruptors and the magnitude of their endocrine activity in certain species.

One of the most well-known examples of the effects of EDs is the case of alligators in Florida. As a result of an extensive pollution incidence from a chemical plant alligators living in Lake Apopka, Florida, were exposed to the estrogenic pollutants dicofol, DDT and its metabolites, DDD, DDE and chloro-DDT. After this incident alligator populations in the lake declined and increased mortality was observed among eggs and newly hatched alligators. Also, adolescent females had severe ovarian abnormalities and exhibited blood estrogen levels two times higher than normal. The male juvenile alligators were feminised: they had smaller than normal penises, abnormal testes and higher estrogen levels and lower testosterone levels in their blood than normal males of the same age. It was concluded that EDs had not only killed developing eggs but also altered the embryo's endocrine system (hormone levels and sexual development), which severely limited the alligator's ability to reproduce, resulting in a further population decline (Guillette *et al.*, 1994; Guillette, 1995).

Effects of endocrine disruptors on aquatic organisms

Bony fishes are considered especially vulnerable to endocrine disruption because of the unique plasticity of sex differentiation; apart from genetic factors environmental factors such as temperature, salinity, pH, density, etc., could play a role in sex determination. For this same reason fishes are also considered well suited as indicators of endocrine disruption (Arcand-Hoy and Benson, 1998).

Various publications are available on the effects of EDs in fish (Ankley *et al.*, 1998) (some examples include fathead minnow (Tyler *et al.*, 1999), rainbow trout (Jobling and Sumpter, 1993; Carlson and Williams, 1999), Atlantic salmon (Fairchild *et al.*, 1999), mosquitofish (Bortone *et al.*, 1989; Bortone and Davis, 1994), flounder (Janssen *et al.*, 1995; Lye *et al.*, 1997; Allen *et al.*, 1999), medaka (Gronen *et al.*, 1999; Edmunds *et al.*, 2000) and carp (Kuhlmann, pers. comm.). However, not only finfish are affected by EDs; Depedge and Billinghurst (1999) summarise and discuss the ecological significance of endocrine disruption in marine invertebrates and describe several cases where endocrine disruption is likely. Examples include imposex in molluscs (masculinisation of female molluscs exposed to the anti-fouling agent TBT (tributyltin)), ovotestis formation in lobster and vitellogenin induction in crab. They conclude that invertebrates may be just as vulnerable to endocrine disruption as vertebrates.

The most frequently observed effect of EDs is feminisation. This phenomenon is estimated through high levels of vitellogenin in the blood of male fish measured by means of radioimmunoassay and ELISA (Tyler *et al.*, 1999). Vitellogenin is a female egg-yolk protein, which is not naturally found in the male organism.

In the UK extensive studies on fish exposed to sewage effluents have been made. Male fish living near municipal sewage outlets had both male and female sex characteristics and their livers produced vitellogenin (Purdom *et al.*, 1994; Harries *et al.*, 1995, 1996). The fish living close to the sewage outlet had severe abnormalities, whereas the fish living further downstream had less severe symptoms. Several different chemicals, especially the alkylphenols, the breakdown products of chemicals found in detergents and plastics, were suspected of causing the feminising effects.

In the case of rainbow trout exposed to low concentrations of 17β-estradiol in the diet, vitellogenin levels in blood plasma of immature male and female were equally high (Carlson and Williams, 1999).

Common carp exposed to nonylphenol, used in a variety of man-made products such as detergents, colours and clothes, showed a shift in sex ratio towards the female sex with a ratio of 65 % female, 19 % male, 11 % hermaphrodite, and 5 % with no gonad development compared to a 50/50 ratio in the control (Kuhlmann, personal communication).

The opposite effect of feminisation, i.e., masculinisation, has also been described as a result of EDs. Examples include masculinisation of female mosquitofish (more specifically development of gonopodiums) when exposed to the effluents of a pulp mill (Bortone *et al,.* 1989) and inconsistent or extremely limited development of oocytes in female rainbow trout when exposed to Aroclor 1260 (Matta *et al.*, 1998).

Potential genetic effects of EDs on the individual

The effects of estrogen-like endocrine disrupting chemicals on the individual genome are summarised by Roy *et al.* (1998). Diethylstilbestrol (DES), a contraceptive, has been demonstrated to alter the genome via numerical chromosome changes (aneuploidy). DES is also capable of producing instability by producing mutational changes in both the mitochondrial and nuclear genome through obstruction of replication. DES and estrone can also cause a reduction in telomer length (telomeric loss) leading to instability of chromosomes. Chromatid and chromosome breaks have also been shown to be induced by EDs. Finally, at the protein level it has been demonstrated that DES quinone can inhibit RNA and DNA polymerases and increase some transcription-related proteins. Most of these findings are from organisms other than fish, but there is no reason to assume that the same effects could not occur in fish.

Effects of EDs at the population level

At the population level the effects of EDs in fishes are poorly studied and understood. Fairchild *et al*. (1999) have suggested that declines of Atlantic salmon populations could be due to EDs, but no specific mechanisms have been proposed. However, in general it is an issue of concern that a change in sex ratio or reduced reproductive success will lead to a decline in effective and absolute population size and consequently to a reduction of genetic variability. Even though no empirical demonstrations are available of this, there is evidence that EDs may cause a shift in sex ratio, as mentioned previously. However, despite the fact that several substances have been identified to act like estrogens, shifting genetically male organisms towards phenotypic females, in the case of fishes it has never been confirmed in the literature what portion of phenotypic females is in fact composed of genotypic males. This is mostly due to the fact that no appropriate screening systems are available (see 1999 WGAGFM Report, "The gender of fish"). In the case of laboratory studies this problem can be overcome by the use of unexposed control groups, whereas shifts in sex ratios of natural populations assigned to high ED concentrations will remain speculative and might be caused by other mechanisms that are currently not understood.

EDs in the marine environment

Most of the findings on the effects of EDs are from laboratory experiments or freshwater environments. Feminisation and complete sex reversal to functional male or female organisms are likely to be rare in marine environment due to the lower concentrations of EDs compared to concentrations found in rivers and used in laboratory experiments. Contaminated estuaries may, however, be an exception to this. A study of flounder from the Mersey estuary in the UK, where 20 % of all males had oocytes in their testes, could provide indications in that direction (Allen *et al.*, 1999).

Recommendations

It must be concluded that very little is known on the potential effects of endocrine disrupting substances on individuals and populations of aquatic organisms. WGAGFM recommends the following:

1) In order to determine the effect of endocrine disruptors on the phenotypic sex of finfish/shellfish, more research concerning the naturally underlying sex determining mechanism is needed.
2) In those cases where endocrine disruption has been demonstrated in individuals in the wild, genetic analysis of exposed populations should be considered to gain evidence for the putative loss of genetic diversity.

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2.7 Coordinated Genetic Databases for Enhancing Understanding of Genetic Diversity in Fish Species

(Based on a position paper by Michael M. Hansen, adopted by WGAGFM in Leuven 2000)

Introduction

Large coordinated databases accessible via the internet are becoming increasingly common in the biological sciences. In molecular biology databases, such as GenBank and EMBL, have been established that contain huge numbers of DNA sequences. In the fisheries sciences there are also several examples of databases containing, for instance, environmental and oceanographical data (see the ICES homepage, www.ices.dk).

In fish population genetics there is a large potential in establishing coordinated databases containing DNA sequence data and, in particular, allelic frequency data. If several groups contribute to a genetic database on one or several species it will be possible to obtain much larger data sets than any single research group could accumulate on its own. The data could then be used for several kinds of large-scale analyses. In particular, studies of large-scale genetic population structure often require many samples from geographically diverse regions. It may be a difficult task to cover the range of distribution of a species within just one study and in particular in the case of marine species it may simply be too costly to obtain the number of samples required. Mixed-stock analysis/Genetic stock identification is a specific kind of technique that requires an extensive amount of baseline data, and coordinated genetic databases would be particularly useful in this context. However, genetic databases could also be useful for other kinds of problems. For instance, databases on variation in QTLs (quantitative trait loci) or loci directly subject to selection (such as MHC) could be used to demonstrate the presence of natural selection and local adaptations in populations.

Some examples already exist where researchers have compiled data from many published studies and conducted large-scale or meta-analysis of data. As an example, Garcia-Marin *et al*. (1999) used allozyme data from a number of published studies on brown trout to infer phylogeographical patterns within the species. Another example is a study by Woodwark *et al.* (1992) where the collection of a database of allozyme data from a number of different species allowed for testing the neutral theory of evolution.

However, even though the establishment of coordinated genetic databases could become a useful tool in population genetics research on populations of aquatic organisms, there are also a number of potential pitfalls that need to be considered. The following description of points and problems to consider is based mainly on experiences obtained within the framework of the "Concerted action on identification, management and exploitation of genetic resources in brown trout (*Salmo trutta*)—TROUTCONCERT", EU FAIR CT97 3882, where one of the objectives was to collect published and unpublished genetic "raw data" and make the data available through the internet on the following website: www.qub.ac.uk/bb/prodohl/TroutConcert/TroutConcert.htm.

Genetic markers suitable for databases

Not all genetic markers are suitable for genetic databases. This concerns in particular RAPDs, AFLPs and multilocus minisatellite DNA fingerprinting. In the case of RAPDs the technique is very sensitive towards the specific PCR conditions and it may be difficult to reproduce results both within and, particularly, among laboratories. In general, the banding patterns obtained by RAPDs, AFLPs and multilocus fingerprinting are probably too complex to allow for comparisons of results among laboratories. The most suitable and relevant genetic markers for genetic databases are

- allozymes (a large amount of allozyme data has already been generated for many species);
- microsatellites;
- DNA sequences, both nuclear and mtDNA;
- RFLP data, based on small DNA segments (such as PCR-amplified segments);
- SNPs (single nucleotide polymorphisms). Combined with the use of microarray chips this is likely to become a standard method in the future.

Harmonisation of the use of markers

Genetic databases are obviously of little use if the contributors to the databases do not use the same loci and markers. Allozyme electrophoresis is normally based on more or less the same loci. However, in the case of DNA markers and particularly microsatellites it may in fact be a major problem to ensure that the same loci are screened by different laboratories. For many species a large number of microsatellite loci have been developed, and they can often be used in related species as well. For instance, in Atlantic salmon it is possible to find more than 100 well-functioning and polymorphic loci, just by scanning through GenBank. In marine species like cod and turbot there are now also > 20 loci available. Given that most population studies so far are based on something between 5 and 20 microsatellite loci this results in a large number of possible combinations of sets of loci. Therefore, it is obvious that a standard set of loci needs to be defined that all laboratories agree to use, either fully or at least in part. As some loci may work well in some laboratories, but not in others it requires some testing of loci in different laboratories before a standard set of microsatellite markers can be defined. Some examples of standard sets of microsatellite loci can be viewed at the internet addresses:

www.ri.bbsrc.ac.uk/cdiv_www/homepage.htm (cattle) and www.qub.ac.uk/bb/prodohl/TroutConcert/TroutConcert.htm (brown trout). The brown trout web page also contains information about a standard set of primers and restriction enzymes for mtDNA PCR-RFLP analysis.

Calibration of markers

Except for sequence data, all allele designations must be considered relative, rather than absolute. For instance, a microsatellite allele found to be 150 bp long in one laboratory may be scored as a 155 bp allele in another laboratory. Several factors may be responsible for this, such as the use of different equipment and use of different kinds of external and internal size markers. Allele designations at allozyme loci are typically based on relative mobilities of alleles, i.e., mobility relative to the "most common allele", the mobility of which is set to 100. However, the relative mobility of alleles may differ among laboratories due to slight differences in the electrophoretic buffers used. Further, what is the "most common allele" in one population may not be the most common allele in other populations. Finally, in the case of RFLP analysis the estimated lengths of restriction fragments are often not precise and it may not be valid to compare data from different studies based solely on fragment lengths reported in the literature.

The solution to these problems is to conduct a calibration of markers. This could be done in two ways:

1) All involved laboratories analyse a set of individuals, a "standard sample", using all the loci that are intended for inclusion in the genetic database.

2) Alternatively, individuals representing all alleles or haplotypes identified by different research groups could be analysed in one single laboratory in order to establish differences and apparent similarities between alleles.

Due to the high variability at microsatellite loci, option 2 is probably not feasible for this class of markers.

In the TROUTCONCERT concerted action calibrations were undertaken both for PCR-RFLP analysis of the mtDNA ND-1 segment and for microsatellite loci. The calibration of mtDNA markers followed option 2. The results of the calibration showed that restriction morphs observed in different laboratories but assumed to be similar to or different from restriction morphs observed in other laboratories were in fact similar or different.

The calibration of microsatellite markers followed option 1, i.e., DNA was extracted from 5 individuals representing three phylogeographical races and aliquots of the DNA were distributed to all laboratories in the concerted action that work with microsatellites. The results showed that absolute sizing of microsatellite alleles without actually sequencing the alleles is completely unreliable. There were examples of allele size estimates that differed by 13 bp. However, the results were consistent in terms of size differences between alleles at a locus, except for some scoring errors (see the section on quality control).

Data input format

In order to extract the full information from a genetic database it is necessary to have the data set in as detailed a format as possible, i.e., information on the genotype of each individual is required. Many statistics, such as estimation of linkage disequilibrium and unbiased estimators of genetic differentiation (e.g., Weir and Cockerham, 1989), require information on genotype frequencies. Some statistics, like assignment tests (Paetkau *et al.*, 1995), are specifically based on individual multilocus genotypes. It is therefore necessary to define a data input format which allows for identifying the genotype of each individual. GENEPOP (Raymond and Rousset, 1995) is presently one of the most widely used program packages. The input file format for this program can be used directly for a number of other program packages and the files can easily be transformed into other file formats. The GENEPOP file format would therefore appear to be a good choice for input format for a genetic database on nuclear loci.

Unfortunately, in the TROUTCONCERT concerted action it was the experience that many “old” allozyme data sets consisted only of allele frequency data; the genotypic data had not been kept. This may be an important limitation in general if it is the intention to establish genetic databases based on old and new allozyme data sets.

Quality control

If a genetic database has been established, a standard set of loci has been selected and a calibration of markers has been conducted it is still important to be aware of the quality of the data. Scoring error, or more specifically its magnitude, is an issue that is rarely considered and discussed in the literature. However, considering that many microsatellite loci, especially in marine fishes, exhibit high levels of polymorphism with 20–40 or even a higher number of alleles, and given that they are separated by only 2, 3 or 4 bp, depending on the type of microsatellites used, it is obvious that scoring error is a problem that cannot be neglected, particularly in the case of a genetic database, where many research groups depend on the quality of the data. The calibration of microsatellite markers conducted within the framework of TROUTCONCERT showed that in 6 out of 8 loci there were scoring errors. However, in this case scoring was made more difficult by the fact that the “standard sample” consisted of individuals from other phylogeographical races than the laboratories were used to work with. Consequently, allele sizes were in several cases outside the expected size range and were accidentally overlooked.

Even though it may not be possible to completely avoid scoring errors it would at least be useful to minimise and know the magnitude of the problem. Procedures to assure a high quality of data could consist in regularly distributing test samples to all laboratories contributing to the database. The results could then be compared and possible scoring errors identified. Also, within laboratories subsamples of individuals that have already been analysed should occasionally be analysed once more in order to verify that the results are reproducible.

Conclusions and Recommendations

WGAGFM sees a large potential in the establishment of coordinated genetic databases for fish populations. However, this requires careful planning and coordination of efforts and will not be feasible for all classes of genetic markers. WGAGFM recommends the following:

1) Before a genetic database is established a standard battery of suitable genetic markers must be defined.

2) A calibration of markers among laboratories contributing to the database must be conducted.

3) Necessary steps must be taken to ensure a uniform and high quality of the data generated by the different laboratories.

4) WGAGFM finds that it is beyond its scope and capacity to be responsible for establishing and maintaining databases on genetic data. However, in order to contribute to a general harmonisation of the use of microsatellite DNA markers in fish and shellfish population studies WGAGFM has decided to start collecting information on available microsatellite loci in different species and make it available on the WGAGFM website. The Chair will ask specific WGAGFM members to take responsibility for collecting information on specific species, and the progress of the work will be assessed and discussed at the 2001 WGAGFM meeting.

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Information about "standard batteries" of genetic markers for brown trout and calibration of genetic markers can be found on the TROUTCONCERT web page at http://www.qub.ac.uk/bb/prodohl/TroutConcert/TroutConcert.htm and in the final report of the concerted action, which can be downloaded from http://www.dfu.min.dk/ffi/consreport/index.htm.

2.8 Genetic Implications of Commercial Fisheries on Deep-water Fish Stocks

(Based on a position paper by John D.M. Gordon, Scottish Association for Marine Science, Oban, Scotland, UK, modified and presented by Rita Castilho, adopted by WGAGFM in Leuven 2000)

Introduction

This working document is a compilation of information on stock structure of deep-water fishes from (a) the reports of the ICES Study Group on the Biology and Assessment of Deep-sea Fishery Resources (SGDEEP), (b) the author's, by no means comprehensive, bibliography on deep-water fishes, and (c) personal contacts.

SGDEEP includes the following commercial or potentially commercial species in its remit. However, only those indicated by an * have been considered in any detail:

Alepocephalus bairdii	Baird's smoothhead
**Aphanopus carbo*	Black scabbardfish
**Argentina silus*	Argentine, greater silver; smelt
**Beryx splendens*	Golden eye perch
**Beryx decadactylus*	Red bream, alfonsino
**Brosme brosme*	Tusk
Chimaera monstrosa	Rabbitfish
**Coryphaenoides rupestris*	Roundnose grenadier
Epigonus telescopus	Big eye, deep-water cardinal fish
Helicolenus dactylopterus	Bluemouth
**Hoplostethus atlanticus*	Orange roughy
Hoplostethus mediterraneus	Silver roughy
Lepidopus caudatus	Silver scabbardfish
Macrourus berglax	Roughhead grenadier
**Molva molva*	Ling
**Molva dypterygia*	Blue ling
Mora moro	Mora
Pagellus bogaraveo	Red (= blackspot) seabream
**Phycis blennoides*	Greater forkbeard
Polyprion americanus	Wreckfish
Trachyrhynchus trachyrhynchus	Roughnose grenadier
Sharks, various	
Chaceon (Geryon) affinis	Deep-water red crab
Aristeomorpha foliacea	Giant red shrimp

The main shark species caught in deep-water fisheries are:

Centrophorus granulosus	Gulper shark
**Centrophorus squamosus*	Leafscale gulper shark
Centroscyllium fabricii	Black dogfish
**Centroscymnus coelolepis*	Portuguese dogfish
Centroscymnus crepidater	Longnose velvet dogfish
Dalatias licha	Kitefin shark
Deania calcea	Birdbeak dogfish
Etmopterus princeps	Great lantern shark
Etmopterus spinax	Velvetbelly
Scymnodon ringens	Knifetooth dogfish

Some other species, which might be considered as deep-water species, are within the remit of other ICES Study or Working Groups:

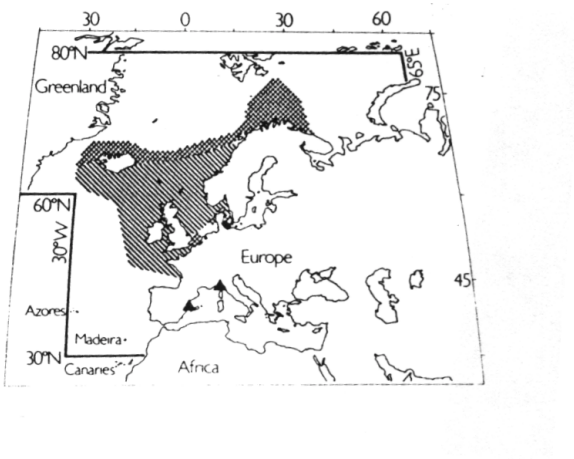
Micromesistius poutassou	Blue whiting
Reinhardtius hippoglossoides	Greenland halibut
Sebastes spp.	Redfish

In addition, there are other species that are fished on the continental shelf but whose distribution extends into deeper waters. This group includes hake (*Merluccius merluccius*), anglerfish (*Lophius* spp.), megrim (*Lepidorhombus* spp.), and conger eel (*Conger conger*).

Summarised information from ICES SGDEEP reports

(1) Extract of general section on stock identity from the report of the ICES Study Group on the Biology and Assessment of Deep-sea Fishery ResourcesICES CM 2000/ACFM:8/Section 6.3 Stock Identity.

The Study Group was not aware of any current results on stock identity of the main deep-water species. Two EC DGXIV study contracts and one EC FAIR contract will provide new data on stock identity. The study contracts involve (1) both DNA and otolith microchemistry of the black scabbardfish in the eastern Atlantic and (2) a study of seasonal aspects of deep-water demersal fish at the Azores which includes work on stock discrimination. The FAIR project is investigating the use of otolith microchemistry for stock discrimination of roundnose grenadier, *Nezumia aequalis*, *Helicolenus dactylopterus* and hake.



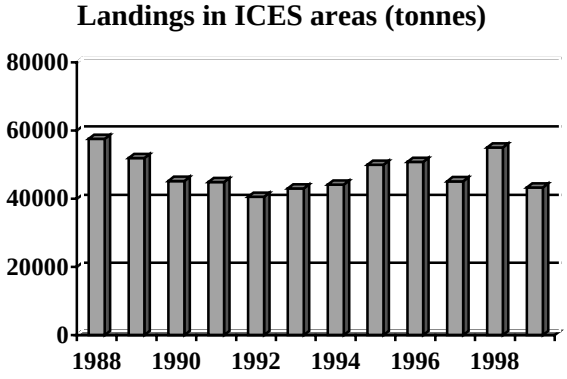
A study on the morphometrics of the black scabbardfish has been completed as part of the EC BASBLACK Project (Carvalho *et al.*, submitted).

A study on the genetics of the stocks of *Macrourus berglax* is in press (Katsarou and Naevdal, in press).

In the Pacific, previous studies have shown that there are several genetic populations of orange roughy in New Zealand and Australian waters. The differences between Atlantic and Pacific samples were of the same order of magnitude as between the Pacific samples (Smith, 1986). The results of new comparisons between the Pacific and the Atlantic are in press and were not available to the Study Group. No results on stock structure in the North Atlantic are available.

(2) Ling (*Molva molva*)

From SGDEEP 1998 report (ICES CM 1998/ACFM:12)



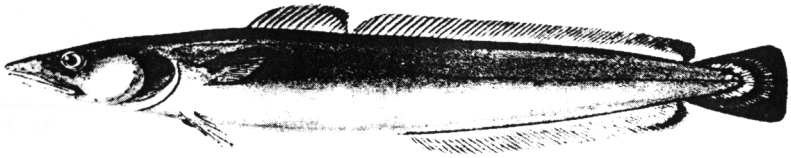
Relevant historical and new information has recently been presented and discussed in reports of Norwegian and Nordic projects (Bergstad and Hareide, 1996; Magnússon *et al.*, 1997). Ripening adult ling and ling eggs have been found in all parts of the distribution area of the ling, but the banks to the west and north of Scotland and around Iceland and the Faroes seem to be the most important spawning areas. There may well be egg and larval drift among all these areas, probably with a net northward and eastward transport. Nothing is known about subsequent migrations within the area of distribution. In recent Norwegian studies of enzyme and haemoglobin frequencies, characters with sufficient variation to study spatial differences could not be found (Bergstad and Hareide, 1996). There is currently no evidence of genetically distinct populations within the ICES area. However, ling at widely separated fishing grounds may still be sufficiently isolated to be considered management units, i.e., stocks between which exchange of individuals is limited and has little effect on the structure and dynamics of each unit. Since no quantitative data on migration exist, it is, however, unclear which of the many fishing areas have units satisfying the criteria of stocks. It is tentatively suggested that Iceland (Va), the Norwegian Coast (II), and the Faroes and Faroe Bank (Vb) have separate stocks, but that the existence of distinguishable stocks along the continental shelf west and north of the British Isles and the northern North Sea (Sub-areas IV, VI, VII and VIII) is less probable.

From SGDEEP 2000 report (ICES CM 2000/ACFM:8)

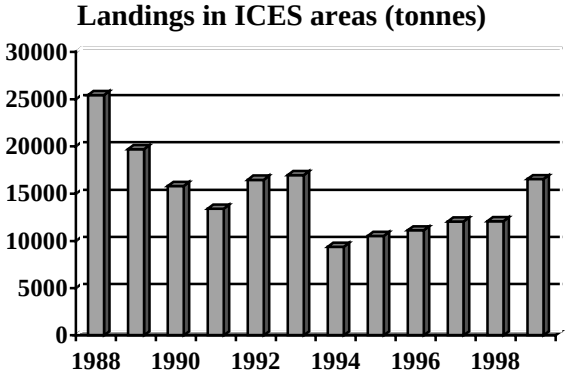
No new information on stock separation was available. Relevant data were presented and discussed in reports of recent Norwegian and Nordic projects (Bergstad and Hareide, 1996; Magnússon *et al.*, 1997) and summarised in the 1998 report of the Study Group (ICES CM 1998/ACFM:12). There is currently no evidence of genetically distinct populations within the ICES area. However, ling at widely separated fishing grounds may still be sufficiently isolated to be considered management units, i.e., stocks between which exchange of individuals is limited and has little effect on the structure and dynamics of each unit. It was suggested that Iceland (Va), the Norwegian Coast (II), and the Faroes and Faroe Bank (Vb) have separate stocks, but that the existence of distinguishable stocks along the continental shelf west and north of the British Isles and the northern North Sea (Sub-areas IV, VI, VII and VIII) is less probable.

(3) Blue ling (*Molva dypterygia)*

From SGDEEP 1998 report (ICES CM 1998/ACFM:12)

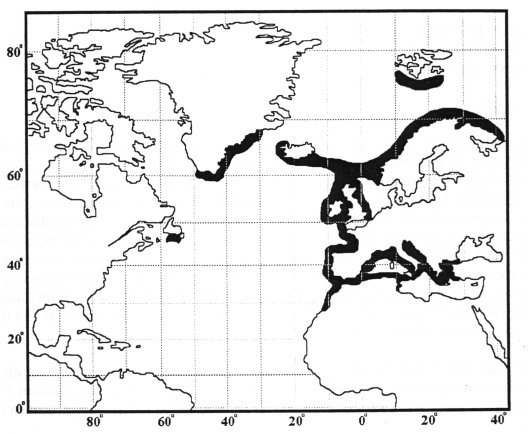


Biological investigations in the early 1980s suggested that at least two adult stocks were found within the area, one in Sub-area XIV and Division Va with a small component in Vb, and another in Sub-area VI and adjacent waters in Division Vb. However, the observations of spawning aggregations in each of these areas and elsewhere suggest further stock separation. This is supported by differences in length and age structures between areas as well as in growth and maturity. Egg and larval data from early studies also suggest the existence of many spawning grounds. The conclusion must be that the stock structure is uncertain within the areas under consideration. For practical purposes the blue ling in Divisions Va and Vb and Sub-area VI, respectively, were treated as three separate units.



From SGDEEP 2000 report (ICES CM 2000/ACFM:8)

Biological investigations in the early 1980s suggested that at least two adult stock components were found within the area, a northern one in Sub-area XIV and Division Va with a small component in Vb, and a southern one in Sub-area VI and adjacent waters in Division Vb. However, the observations of spawning aggregations in each of these areas and elsewhere suggest further stock separation. This is supported by differences in length and age structures between areas as well as in growth and maturity. Egg and larval data from early studies also suggest the existence of many spawning grounds. The conclusion must be that the stock structure is uncertain within the areas under consideration.

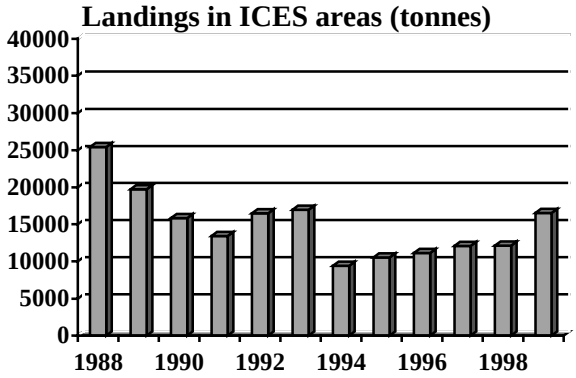


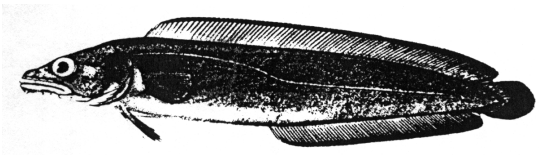
However, in this year's assessment, based on similar trends in the CPUE series from Division Vb and Sub-areas VI and VII, the blue ling from these areas were treated as one unit.

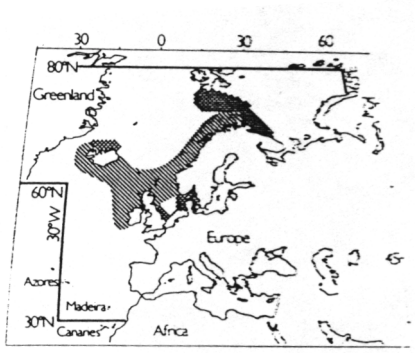
(4) Tusk (*Brosme brosme*)

From SGDEEP 1998 report (ICES CM 1998/ACFM:12)

Ripening adult tusk and tusk eggs have been found in all parts of the distribution area, but the banks to the west and north of Scotland, around the Faroes and off Iceland, as well as the shelf edge along mid- and north Norway seem to be the most important spawning areas (Magnússon *et al.* 1997). Nothing is known about migrations within the area of distribution. In recent Norwegian studies of enzyme and haemoglobin frequencies no geographical structure could be found, hence it was concluded that tusk in all areas, at least of the Northeast Atlantic, belong to the same gene pool (Bergstad and Hareide, 1996). As discussed for ling, widely separated fishing grounds may support separate management units, i.e., stocks. It is tentatively suggested that Iceland (Va) and the Norwegian coast (I and II) have self-contained units, while the separation among possibly several stocks to the north and west of the British Isles is less clear.







From SGDEEP 2000 report (ICES CM 2000/ACFM:8)

No new information was reported.

(5) Argentine (*Argentina silus*)

From SGDEEP 1998 report (ICES CM 1998/ACFM:12)

Icelandic life history studies suggest that a separate stock might exist in Division Va. Irish investigations on stock discrimination in Sub-areas VI and VII are inconclusive. A study by Ronan *et al*. (1993), using morphometrics (box truss analysis) and meristic measurements, suggests that populations from the north of Sub-area VI and the south of Sub-area VII form either end of a shape cline with fish in intermediary populations exhibiting a mixture of northern and southern morphologies. Norwegian investigations in Divisions IIa, IIIa, and IVa appear to show two separate populations in the winter but in the summer the species is widely distributed (see also Section 5.1.10 of ICES CM 1996/Assess:8).

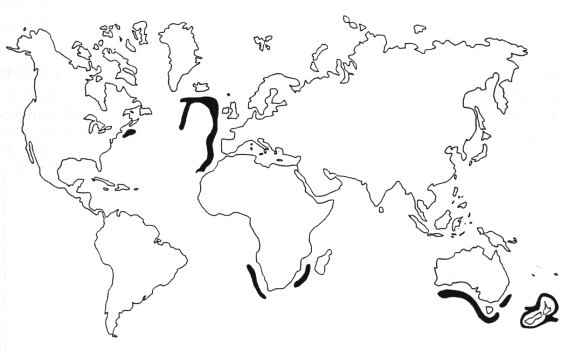
From SGDEEP 2000 report (ICES CM 2000/ACFM:8)

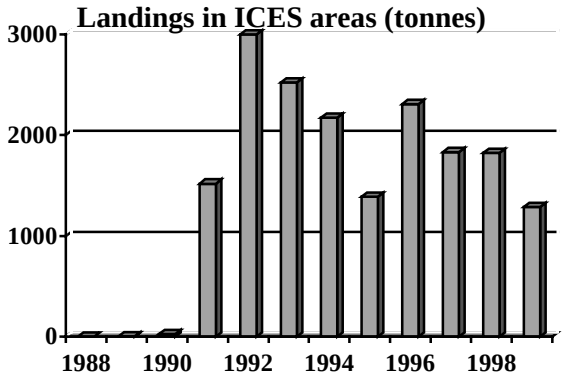
No new information was reported.

(6) Orange roughy (*Hoplostethus atlanticus*)

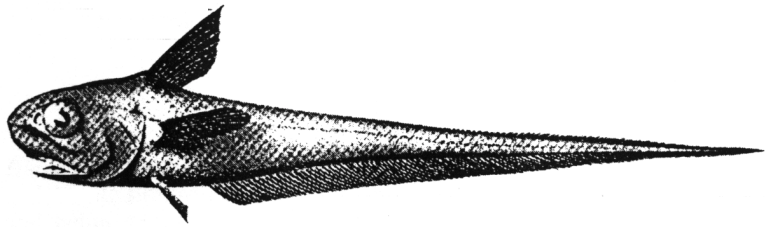
From SGDEEP 1998 report (ICES CM 1998/ACFM:12)

The fishing grounds so far discovered in the North Atlantic have appeared to support relatively small aggregations of fish, usually associated with seamounts and other topographical features. Whether or not these are independent populations is not known. However, with time, the probability of finding, in the northern Atlantic, stocks comparable in size to the stocks exploited in the south Pacific is decreasing.





(7) Roundnose grenadier (*Coryphaenoides rupestris*)



From SGDEEP 1995 report (ICES CM 1995/Assess:4)

There is still uncertainty as to whether the roundnose grenadier in the north Atlantic originate mainly from the known spawning grounds in the central north Atlantic or whether there are several discrete populations. However, the fact that at Iceland spawning is known to take place, nursery areas have been located and all sizes and maturity stages of roundnose grenadier have been observed indicates that there is probably a self-sustaining stock in this area.

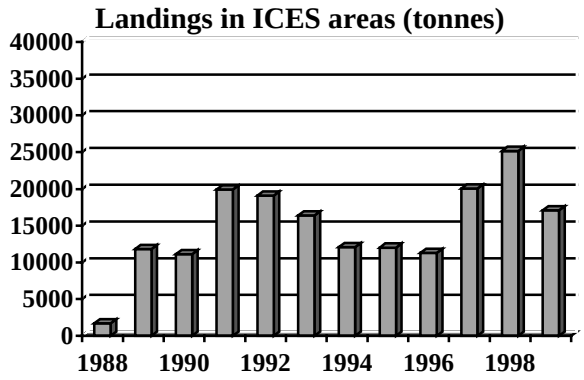
The subject of stock identification in roundnose grenadier is likely to remain controversial until either the genetic polymorphisms of sufficiently large numbers of fish can be studied or some means can be found of marking and recapturing grenadiers.

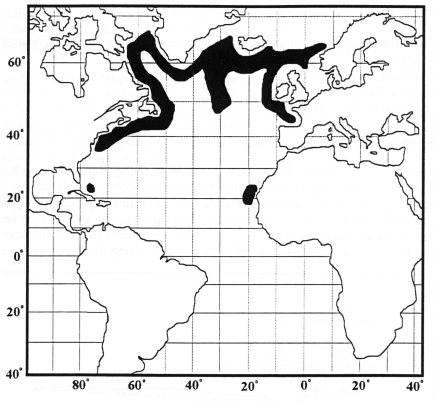
From SGDEEP 2000 report (ICES CM 2000/ACFM:8)

The issue of roundnose grenadier stocks was discussed in the 1994 Study Group Report (ICES CM 1995/Assess:4) and there are no new data on this topic.

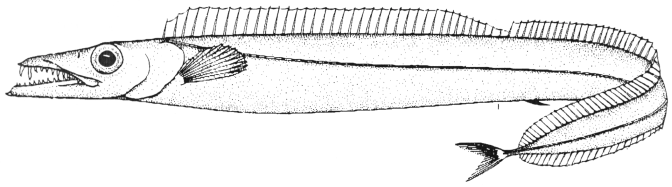
Roundnose grenadier in Sub-areas II (Norwegian fjords) and III (Skagerrak) may represent separate stock(s) due to the physical boundary of the Wyville Thomson Ridge and fjord sills. For other populations, the stock structure remains unclear.

The Study Group carried out assessment for Division Vb and Sub-areas VI and VII combined implicitly considering these areas as a stock unit for this species. Sub-area XII was not included because catches in that area include catches from the Mid-Atlantic ridge and from the western part of Hatton Bank. They cannot be re-allocated properly to each of these areas which are likely to support rather separated stocks units. Moreover, catch in Sub-area XII are likely to be significantly under-reported (see above).



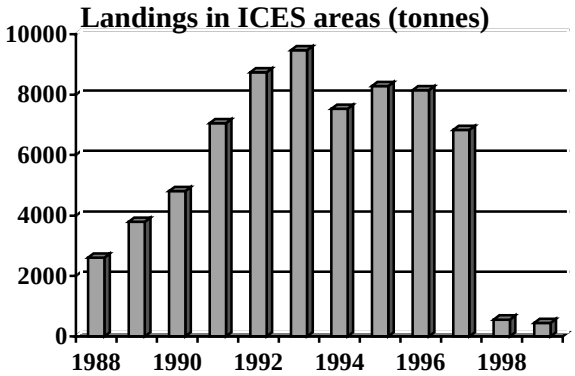


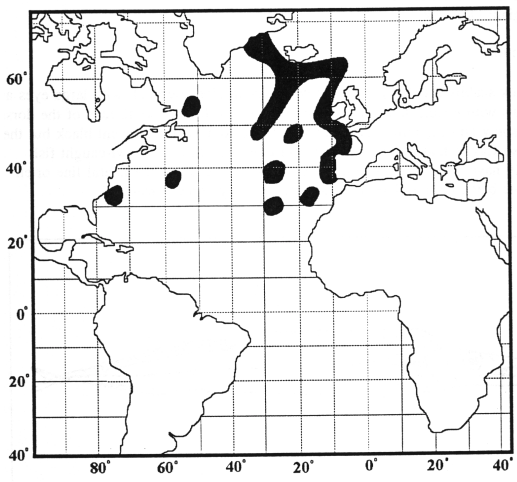
(8) Black Scabbardfish (*Aphanopus carbo*)



From SGDEEP 2000 report (ICES CM 2000/ACFM:8)

Research into stock discrimination is being carried out in the BASBLACK project. These studies involve genetics (DNA), otolith micro-chemistry and morphometric analyses. The work on the first two is progressing but there are no results as yet. The analysis of morphometric data



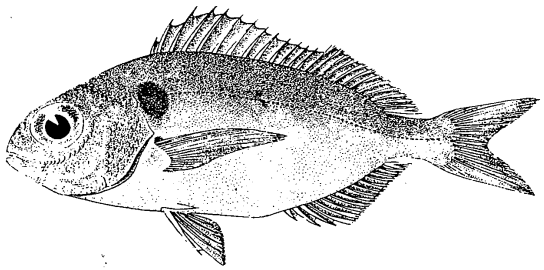


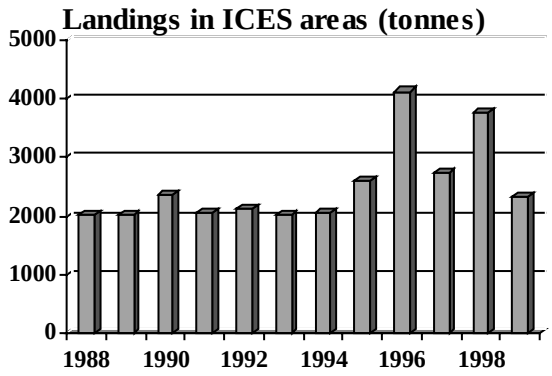
collected in three different regions of the Northeast Atlantic: west of Scotland, off the Portuguese mainland (Sesimbra) and around Madeira, revealed that a significant component of among-group differences is assigned to length. Specimens from Scotland are considerably smaller in size than those from Madeira and Sesimbra (Carvalho, Figueiredo, and Reis, submitted). These results should be treated with caution since the procedure used did not allow for an evaluation of discrimination among samples from regions where the specimens vary in size. This is intrinsically linked with the

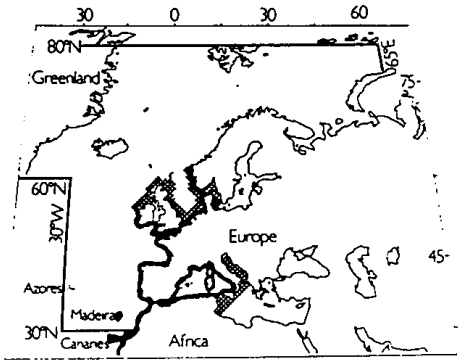
problem of the selection of shape discriminators independent of size in order to partition out the effects of growth (Humphries *et al.*, 1981).

The working hypothesis is that there is one stock extending from Faroe Islands to Madeira. However, there is as yet no strong evidence to support this hypothesis. Some of the results from BASBLACK, namely length distribution and reproductive behaviour, are suggestive of large- or small-scale migratory processes of components of the population.

(9) Red (blackspot) seabream (*Pagellus bogaraveo*)







From SGDEEP 2000 report (ICES CM 2000/ACFM:8)

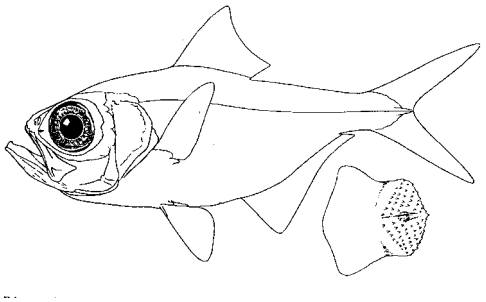
Information on red (blackspot) seabream, *P. bogaraveo*, has been split into three different components, as referred to in the 1996 and 1998 Reports (ICES CM 1996/Assess:8; ICES CM 1998/ACFM:12):

- *P. bogaraveo* in Sub-areas VI, VII and VIII;
- *P. bogaraveo* in Sub-area IX;
- *P. bogaraveo* in Sub-area X (Azores region).

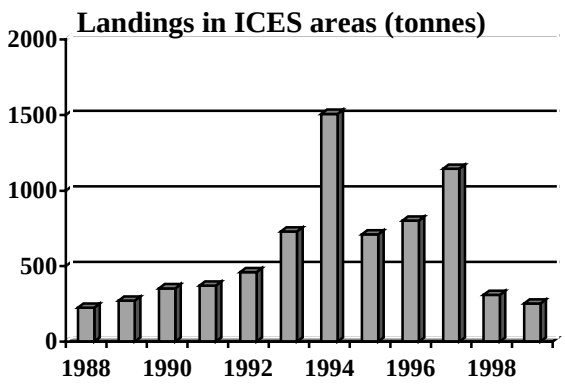
This separation does not pre-suppose that there are three different stocks of *P. bogaraveo*, but it offers a better way of recording the available information. In fact, the inter-relationships of the red seabream from the Sub-areas VI, VII, VIII and the northern part of Division IXa, and their migratory movements within these sea areas have been confirmed in the past by tagging methods (Gueguen, 1974; ICES CM 1996/Assess:8). Studies on possible links between red seabream of the Azorean region with the southern Sub-area IX, Sahara Bank and Sub-areas VI-VII-VIII and the northern part of Division IXa have not yet been carried out and would be welcome.

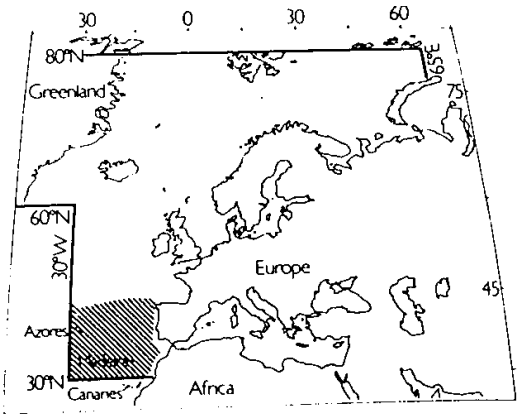
Other information

(1) Alfonsino (*Beryx splenens*)



Galice Hoarau of the Department of Marine Biology, Biological Centre, RUG (g.hoarau@biol.rg.nl) has been working mainly on a population of *Beryx splendens* from New Caledonia using mitochondrial DNA, nuclear introns and DALP. A paper is in press. The main results suggest that the taxon *B. splendens* consists of two sibling species, one with a worldwide distribution and another that seems to be endemic to New Caledonia.





Beryx splendens is also being investigated by Alex Rogers of the University of Southampton, UK (adr2@mail.soc.soton.ac.uk). This work is being carried out as a contribution to the EC DGXIV Study Contract 97/081 *Seasonal changes in biological and ecological traits of demersal and deep-water fish species in the Azores.* Amplification and sequencing of the mitochondrial control region DNA (D-loop mtDNA) has been successful.

(2) Red (blackspot) seabream (*Pagellus bogaraveo*)

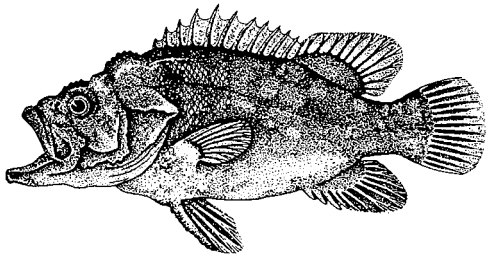
Alex Rogers has also investigated *Pagellus bogaraveo* as a contribution to the EC DGXIV Study Contract 97/081. Amplification and sequencing of the mitochondrial control region DNA (D-loop mtDNA) has been successful. An enriched genomic library has been constructed for *P. bogaraveo* and from this library over 20 microsatelites have been probed, cloned and sequenced.

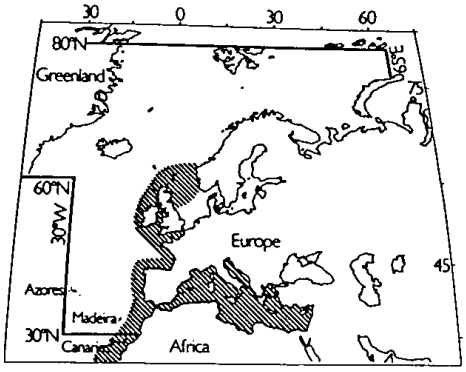
(3) Orange roughy (*Hoplostethus atlanticus*)

Catherine Oke of the Evolutionary Biological Unit, Department of Genetics, Latrobe University, Australia (genco@latrobe.edu.au) has published a primer in molecular ecology (Oke *et al.*, 1999). She is currently working on microsatellites from across the entire global range.

Other published papers on the genetics of orange roughy include Elliot *et al.* (1994), Ovenden *et al.* (1989), Smith *et al.* (1996, 1997), and Smolenski *et al.* (1993).

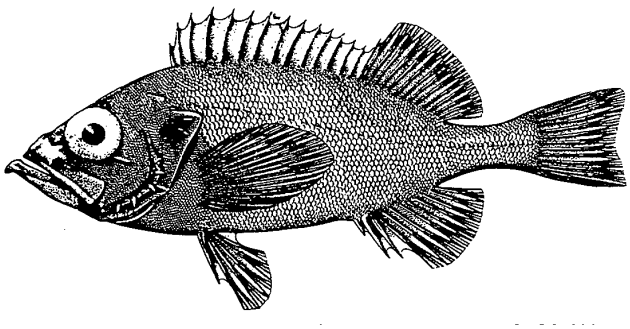
(4) Wreck fish (*Polyprion atlanticanus*)

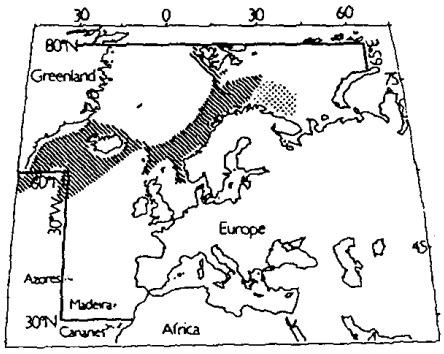




The genetics of the wreckfish have been studied by Sedberry *et al.* (1996).

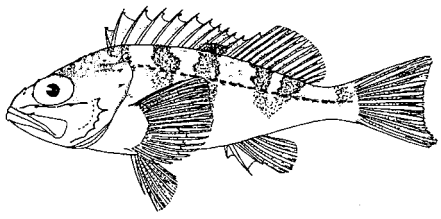
(5) Redfish (*Sebastes* spp.)

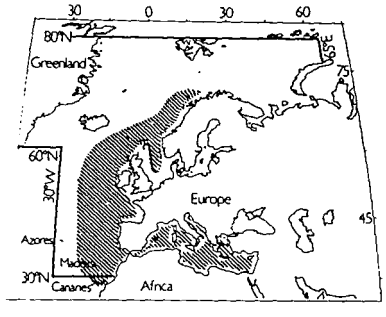




The genetics of deep-water redfish have been investigated by Johansen *et al.* (1997a, 1997b) and Sundt and Johansen (1998).

(6) Bluemouth (*Helicolenus dactylopterus*)





The genetic variation in the family Scorpaenidae was studied by Johansen *et al.* (1993). The samples of bluemouth were collected around Shetland and the Faroe Islands in 1990 and analysed by starch gel electrophoresis and isoelectric focusing of haemoglobin and tissue enzymes. Intraspecific variation was low in bluemouth.

McGlade *et al.* (1983) used electrophoresis to separate *H. dactylopterus* from *Sebastes* spp. The samples were caught in the eastern part of the Scotian Shelf, in 1982. Twelve enzymes were diagnostic for *Helicolenus dactylopterus*. *Helicolenus* and *Sebastes* have only two enzyme profiles in common (LDH and PGM).

(7) Roundnose grenadier (*Coryphaenoides rupestris*)

This species has a wide distribution in the North Atlantic and there has been much, often speculative, discussion on the population structure. This has been discussed by Atkinson (1989). Some of the evidence is from Russian work on genetics (Dushchenko, 1988) and parasites (Zubchenko, 1985). The population in the Skagerrak has different length-weight relationships and growth rates and may be an isolated population (Bergstad, 1990)

Conclusions

Catches of traditionally exploited marine resources are not likely to increase and therefore the possibility of harvesting other species on new fishing grounds will be investigated intensively in the future. Deep-water species constitute a plausible alternative, with many species already being exploited, either directly targeted or as a by-catch product. It is known that some of the species exhibit very slow growth and reach sexual maturity at a high age. This is likely to affect their reproductive output and potentially makes them vulnerable to extensive harvesting. Furthermore, it is known that the distribution of some of these deep-water species extends into international waters, and they are therefore subject to uncontrolled fishing. Consequently, there is a strong need for collecting basic data on the population dynamics and genetic population structure of these species, in order to evaluate the potential effects of fisheries on the species.

WGAGFM is aware that population genetics research is being carried out on some deep-sea species, e.g., *Sebastes* spp. on both sides of the Atlantic. However, it acknowledges the lack of general biological information and of the genetic structure of deep-water fish populations and encourages studies that are able to provide insight into these issues.

Recommendations

1) WGAGFM recommends that high priority be given to research aimed at deep-water fish species, as the situation is at present at a point where exploitation has not yet reached intense levels.
2) Moreover, WGAGFM also recommends that research efforts at this stage be concentrated on fewer species, so that more extensive biological data (general biological features, population dynamics, population genetics) can be obtained from a few species. These species could then serve as model species, both in order to assess the importance of specific biological features of deep-water fishes (such as slow growth) in relation to harvesting and potential depletion of genetic resources, and in order to able to focus later research activities on other deep-water species.

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2.9 Trade-offs between Genetic Gain and Loss of Genetic Variability in Breeding Programmes (how to minimise inbreeding in intense breeding)

(Based on a position paper by Ashie Norris, adopted by WGAGFM in Leuven 2000)

Introduction

In most breeding schemes a balance between genetic gain and inbreeding is sought. New developments in animal breeding schemes are designed to increase genetic gain, but inbreeding rates often increase concomitantly. Detrimental effects of inbreeding are: (1) the reduction of additive genetic variance, with reduced rates of response and limits to selection for the traits under selection and other traits; (2) inbreeding depression for the trait under selection; (3) inbreeding depression affecting the general fitness of the animal.

Inbreeding may occur when individual selection is being practised and no pedigrees are available. This is particularly true in aquaculture where fecundity is high and fewer numbers of parents are used. There is also a tendency to use fewer males than females, resulting in unbalanced sex ratios which further increase inbreeding. Where family selection is practised and individuals are marked, the use of Best Linear Unbiased Prediction (BLUP) procedures lead to a higher rate of inbreeding and a larger magnitude of decrease in genetic variation compared to less accurate selection procedures.

Examples of inbreeding depression in fish populations

The effect of three levels of inbreeding (F = 0.25, F = 0.375 and F = 0.5) on survival and growth in rainbow trout was studied (Gjerde *et al.,* 1983). At each level the inbreeding depression for survival of eyed eggs, alevins and fry was highly significant but no linear relationship was found between inbreeding level and inbreeding depression. For growth of fingerlings at 160 days no inbreeding depression was noted. For growth of adults at sea water a significant inbreeding depression was noted for each level. Inbreeding depression was measured in three lines, two being selected over 5 generations for body weight and egg size in rainbow trout (Su *et al.,* 1996). There was a tendency for inbreeding depression for body weight to increase with advancing age from 2.26 % at 364 days to 5.77 % (males) at spawning per 10 % increase in inbreeding.

Pante, Gjerde, and McMillan (no reference) investigated levels of inbreeding depression for body weight at harvest in 6 generations of selected populations of rainbow trout. In this population no half or full sib matings were carried out and no matings that would yield inbreeding coefficients of greater than 12.5 %; average inbreeding levels were all below 10 %. However, in a breeding programme, the rate of inbreeding is more important than the actual inbreeding level as it measures how many more generations can be kept before reaching the critical inbreeding level. The actual average rate of inbreeding was 1.3 % per generation, below the level of 3 % to 5 % found in commercial salmonid farms. The effective population size estimated using the harmonic mean (Falconer, 1989) was 83 giving an average rate of inbreeding of 0.7 %. The effects of inbreeding on growth (body weight: 0.2 % decrease per 1 % increase in inbreeding) were significant in all generations except one.

A 10 % increase in inbreeding coefficient resulted in a delay of spawning age by 0.52 % and a decrease in egg number by 6.10 % (Rye and Mao, 1998). Estimated inbreeding depression for body weight at slaughter ranged from 0.6 % to 2.6 % per 10 % increase in inbreeding coefficient. DeRose (1999) demonstrated that inbreeding depression was higher for life-history traits (closely related to fitness) than for morphological traits (less closely related to fitness) at F = 0.25 (full-sibling mating) using data from many published research papers including papers on rainbow trout. Life-history traits experienced a median reduction of 11.8 % in trait value, whereas morphological traits showed a depression in trait value of approximately 2.2 %. This study also demonstrates the presence of large amounts of dominance variance in fitness traits, which is significant for broodstock improvement. It is also another indication that inbreeding can cause big problems in small populations.

Traits related to fitness (in aquaculture these would be growth) show greater inbreeding depression than other traits (colour, etc.). Perhaps by the time aquaculturists begin to notice development (shape) abnormalities and deformities which they ascribe to inbreeding, they are already losing much more production due to mortality and slow growth than they realise.

From this and other studies it seems that inbreeding depression is not so important in the early juvenile stages but that non-additive gene effects are more important later in life when processes associated with reproduction and somatic functions create more difficult conditions in females.

If inbreeding is associated with fitness traits it would be expected to have an effect on susceptibility to disease. A paper by (Coltman, 1999) assessed susceptibility to parasites by gastrointestinal nematodes in a relatively inbred population of Soay sheep and its relationship to inbreeding (as assessed by microsatellite heterozygosity). The individual sheep that are inbred are more susceptible to parasitism at high population density. More inbred individuals were also less likely to survive, which is due to their increased susceptibility to parasitism. Extension to aquaculture would be that the effect of inbreeding should show up under stress, in those individuals that are most inbred, and would be manifested in mortality from parasites and disease. This could be investigated on working fish farms by a properly interpreted assay of microsatellite heterozygosity.

Predicting inbreeding rates under selection

Frequently, advantages of new breeding programmes are discussed solely in terms of responses to selection with little regard to the effect of selection on inbreeding with inbreeding rate per generation to be simply that of Wright (1931) $(\Delta F_W) = \frac{1}{8M} + \frac{1}{8F}$

where $M(F)$ are the number of males (females) entering the population each generation. This rate of inbreeding, however, is only appropriate for randomly mated populations with Poisson distributed variances of family size and is therefore not appropriate for a population under selection. Inbreeding can also be considered in terms of effective population size which has an inverse relationship with inbreeding, $\Delta F = \frac{1}{2} N_e$, here also, selection is assumed to be absent.

Inbreeding can also be estimated after selection has occurred from pedigree information. In a given generation, *t*, the rate of inbreeding $\Delta F = (F_t - F_{t-1}) / (1 - F_{t-1})$ can easily be calculated from pedigree information after selection has occurred but not predicted before selection. Robertson (1961) first considered prediction of inbreeding in a selected population. His argument was based on the variance of the change in gene frequency.

$$\frac{N}{N_E} = 1 + Q^2 i^2 \rho \qquad \text{(Equation 1)}$$

where

Q = a measure of relative selective advantage;
N = number of full sib families;
i = intensity of selection;
ρ = intra-class correlation of selection criteria between sibs.

Wray and Thompson (1990) also developed a method for the prediction of rate of inbreeding for populations with discrete generations. This equation relates rate of inbreeding to long-term additive genetic contributions between ancestors and descendants.

$$N_e \approx \frac{2N}{\mu_r^2 + \sigma_r^2} \qquad \text{(Equation 2)}$$

Denominators are the mean and variance of long-term contributions from ancestors to descendants. This relates to Wright's (1931) expression of population size where the mean and variance are now for generation family size.

$$N_e \approx \frac{4N}{\mu_s + \sigma_s^2} \qquad \text{(Equation 2a)}$$

These formulas allow us to predict rates of inbreeding in closed populations of full- and half-sib families undergoing selection and can be extended to populations with a hierarchical mating structure with different numbers of males and females as is normal in aquaculture.

Reducing the rate of inbreeding in a selection programme

Increased rates of inbreeding as the result of selection decisions have a negative effect on future genetic response through reduction in future genetic variance and a negative impact on future performance if inbreeding affects the selected trait. The use of estimated breeding values (EBV) based on BLUP under an animal model is likely to result in higher rates of inbreeding than under mass selection. A number of methods have been proposed to attain high rates of genetic response with moderate or low inbreeding. Some of these methods are described below.

- **Biased heritability—**The use of selection indices or animal model BLUP increases the correlation of predicted breeding values of relatives that are candidates for selection. This leads to an increase in the variance of sizes of families of selected individuals and a reduction in the effective population size. This reduction is greatest when heritability is small as then most weight is given to family mean in predicting breeding value. Biased heritability involves the use of false high heritability in the genetic evaluation (Grundy *et al.,* 1994).
- **Minimum coancestry**—The average pairwise coancestry coefficient between males and females in the selected group is minimised. For a number of selected individuals a computer program can be written that chooses among all possible combinations of mates to achieve minimum coancestry (Toro *et al.,* 1988).
- **Adjusted EBV**—To adjust estimated breeding values for the relationship with breeders already selected. For example, the best male is selected and then each remaining candidate is ranked according to a combination of its own EBV and its relationship with the male already selected (Quinton and Smith, 1995).
- **Minimum variance of contributions—**matings with minimum variance of the contributions. In each generation *t*, the contributions of genes from ancestors in previous generations to descendants in generation *t* are calculated. Matings in generation *t* are made such that the sum of the variances of the contributions from the ancestors in the previous generations to the descendants in generation *t* + 1 are minimised (Caballero *et al.,* 1996).
- **Compensatory mating—**in this system selected individuals of each sex are ordered according to the total number of selected full and half sibs (including the individual) with males given a weight Nf/Nm that of females to reflect their contributions. Then, males with the highest ranking are mated to females with the lowest, in sequence. This is a system of mating between individuals from the largest selected families to individuals from the smallest. The theory behind compensatory mating is that the number of offspring contributed by a parent (k_i) can be partitioned into a number expected from its breeding value (g_i) plus a random deviate (d_i). Changes in gene frequency due to the inherited g_i are correlated in successive generations, whereas those in d_i are not. If animals from large families (high k_i) are mated to small families (low k_i) a negative correlation is induced between g_i and d_i because the pair contribute the same *k* subsequently, thereby partly or completely counteracting the cumulative effect of selection in the term Q (Equation 1) and reducing rates of inbreeding. Compensatory mating can also be explained in terms of Equations 2 and 2a. After several generations, the long-term contributions of genes from an ancestor stabilise and are the same for all individuals in the population, with the values differing between ancestors. Thus, the variance of contributions increases with time until an asymptotic value is reached ($S^2_{c,\infty}$), to which the rate of inbreeding is related,

  $$\Delta F = (1 + S^2_{c,\infty}) / 4N \qquad \text{(Equation 3).}$$

  Rates of inbreeding can therefore be reduced by reducing the variance of long-term contributions (by using a correction to the above equation) (Grundy *et al.,* 1994).

The best system from all of the above will depend on the conditions and restrictions of the selection programme. Quinton and Smith (1995) compared a number of these methods using stochastic simulation. They found that no method of selection was best over all conditions and that the best system will depend on the factors involved, such as population size, heritability, rate of inbreeding tolerated and number of generations, family size, etc. In this study the optimum response-inbreeding "front" was attained using the methods of biased heritability and adjusted EBVs. These two methods had a significant effect on the rate of inbreeding with a small decrease in genetic gain. They also found that the same result could be found by simply increasing the number of sires selected.

In an investigation by Caballero *et al.* (1996) minimum variance of the contributions, compensatory mating, minimum coancestry and compensatory mating combined with minimum coancestry were compared to random mating of selected parents. All systems produced a significant reduction in the rate of inbreeding relative to random mating and generally had little effect on the response to selection (for heritability = 0.4 the loss of response was minimal or non-existent both in the short term (generation 5) and even less in the long term). Compensatory mating was found to be more effective in reducing rates of inbreeding in situations with low existent rates of inbreeding or high population sizes. Minimum coancestry or even avoidance of full sib matings were more effective under high inbreeding, such as BLUP selection, low heritability and small population size. Combining coancestry and compensatory procedures generally gives the

largest reduction in inbreeding. This also has the advantage that the mating rule is simple and does not require linear programming.

The difference between compensatory mating and minimum coancestry should be stressed: compensatory mating is based on the idea of cancelling the cumulative process of drift by making all new families identical with respect to the total contribution of their ancestors, minimum coancestry is based on choosing the less related pairs using the information in the pedigree. Thus, compensatory mating is a method for "looking into the future" while minimum coancestry is a method for "looking to the past".

Compensatory mating can also be combined with the use of biased heritability in BLUP selection and when this is done both methods have approximately additive effects in reducing rates of inbreeding (Grundy *et al.,* 1994). Biased heritability and compensatory mating were compared to mating using the true heritability and random mating. Using $h^2 = 0.4$ (instead of 0.2) reduced rates of inbreeding by approximately 25 % for a range of cases. Compensatory mating alone reduces rates of inbreeding by 15 % on average, when both procedures are combined rates of inbreeding are about 40 % lower. This suggests that rates of inbreeding can be reduced by using heritability estimates that exceed their true value or by reducing the weight given to the family mean in the selection index

Comments: use of biased heritabilities mainly reduces the variance of family size while compensatory mating reduces the cumulative effect of selection on drift over generations so these schemes are complementary to each other.

A more complicated strategy was proposed by Wray and Goddard (1994) who suggested a selection criteria based on balancing the positive contributions of a selected group of parents against the negative contributions to future genetic response (determined by their contribution to inbreeding). In this way selection response is being weighted against future inbreeding. A selection algorithm is used which:

1) Ranks sires on EBV and the best is selected ($n = 1$).
2) For the remaining sires calculates $V_{[n+1]}$ for each sire, which depends on the group of n sires already selected plus the individual sire to be considered (V is our selection criteria and depends on: the proportion of offspring born to each sire and dam, the EBV of each sire and dam and the coancestry among parents).
3) Ranks the sires on their individual $V_{[n+1]}$ values, selects the best sire if ($V_{[n+1]} - V_{[n]} > 0$) then repeats from step 2 ($n = n + 1$).

This algorithm was used to allow a number of sire selection strategies using either a fixed or a variable number of sires each generation. The aim of this selection procedure is to exploit the sires who have become available for selection by chance in the current generation and does not assume that the best group of sires is selected. When simulated over 30 generations of selection on the selection criterion V, higher response and lower levels of inbreeding were achieved than with selection on EBV alone. In summary, the algorithm proposed here uses the selection goal to determine the best balance of selection intensity and inbreeding and then optimises the selection decisions by: deciding the number of sires to be selected, deciding the number of offspring per sire and selecting sires based on their EBV and relationship with other sires.

Villanueva and Woolliams (1997) studied optimum designs of breeding programmes with constrained inbreeding under index and mass selection (only the former considered here). Optimum numbers of parents and index weights are obtained by maximising a single function which combines the expected rates of genetic progress and inbreeding. BLUP selection can be closely approximated by using a selection index including the breeding values of sire and dam and the mean EBV of all dams mated to the sire in addition to information on the individual itself.

Recommendations

1) At the set-up stage of a breeding programme, it is important to ensure maximum genetic variation in the offspring as it is these which will make up future breeding populations. This can be done by crossing between year/classes/lines/strains. It can also be done by examining all potential broodstock for genetic variation at the DNA level using DNA markers such as microsatellites. If enough variable loci are examined, good estimates of relatedness can be obtained between mating pairs. Pairings can then be planned on the basis of minimum relatedness alone combined with any pedigree information available.
2) Any planned programme should include estimation of predicted inbreeding levels.
3) Efforts should be made to restrict inbreeding using one of the methods outlined above; the best method will depend on the conditions of the programme.
4) In mass selection programmes, records should be kept of broodstock pedigree wherever possible.

5) Record should be kept of rates of inbreeding with each generation of breeding programme.

6) Hatchery/broodstock managers could keep small samples of fin or other tissue in 95 % alcohol from each breeding generation for the purpose of measurements of genetic variation changes as a result of breeding practices. In the case of an outbreak of disease fish farmers could store tissue from their mortalities and healthy fish, for future reference, to investigate any relationship between susceptibility to disease and inbreeding.

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2.10 National Activity Reports

Please find the National Activity Reports compiled at the web site http://www.dfu.min.dk/ffi/wgagfmhome/index.htm.

3 WORKING GROUP BUSINESS

3.1 Comments on Working Group Function

The number of participants in WGAGFM meetings seems to have stabilised at around 20, which must be considered very satisfactory. However, the poor representation by quantitative geneticists still remains a problem. The few quantitative geneticists that participated in the meeting will make a further effort to ask other quantitative geneticists to participate in the next meeting.

Whereas the working group function overall must be considered satisfactory the participants in the meeting agreed that some minor changes might be beneficial.

1) WGAGFM decided that there is no need in the present situation to have a "quantitative" and a "qualitative" subgroup, and it was decided not to maintain this subdivision of WGAGFM. However, it is still important to be aware of keeping topics on the agenda of relevance to both quantitative and qualitative genetics.
2) Regarding the national activity reports some concerns were expressed that the work put into collecting this information is not justified by the number of people who actually use the information. Furthermore, the information is not easily accessible on paper in the report, and it would be more easy to browse through if it was made available at the internet. It was therefore decided this year to make the national activity reports available via the internet rather than in the WGAGFM report and to make enquiries to potential users of this information in order to assess to which extent the information is used.
3) In order to increase collaboration and exchange of information among WGAGFM members it was decided to establish groups for preparing and writing future position papers and WGAGFM report sections, rather than having individual persons being responsible for this. However, there will still be one person having the main responsibility for each ToR.

3.2 Suggestions for WG ToRs and Venue in 2001

During discussions on meeting place in the year 2001, WGAGFM responded positively to a generous invitation from Dr Geir Dahle, Institute of Marine Research in Bergen, Norway, to host the 2001 WGAGFM meeting 26–28 March 2001. Concerning Terms of Reference and meeting place for the year 2001, WGAGFM in plenary decided to recommend that:

The Working Group on the Application of Genetics in Fisheries and Mariculture (Chair: Dr Michael Møller Hansen, Denmark) will meet at the Institute of Marine Research, Bergen, Norway, 26–28 March 2001 to:

a) continue the review of general population genetic topics in fisheries and mariculture and identify scopes for enhanced international cooperation;
b) review new developments in the identification of genes of relevance to aquaculture and studies of wild populations;
c) review the importance of different kinds of genetic population structure in relation to human impact;
d) review methods for estimating effective population sizes and/or changes in effective population sizes in anadromous and marine fish populations;
e) review examples where population genetics research has provided important information for the management of marine fish populations;
f) compile information on available molecular markers (mainly microsatellite loci) for use in studies of finfish and shellfish.

3.3 Justifications for Proposed 2001 ToRs

a) WGAGFM is a relatively informal forum where members shall feel free to discuss and update each other on practical and theoretical problems related to genetics of marine species. Experience has shown that there is a need for an open scientific session at the annual meetings, where topics that are not necessarily listed in the Terms of Reference can be enlightened by the competence and experience existing in WGAGFM. Not least have those topics which need competent input from both qualitative and quantitative genetics benefited from these discussions.
b) A number of new important genes have been identified and characterised in species subject to aquaculture. Further, the study of these and other coding genes in wild populations may provide important insight into the

processes of selection and local adaptations. Finally, new screening techniques (so-called microarray chip techniques) are presently being developed that will allow for very fast screening of a huge number of genes, and these techniques are likely to have a profound influence on genetic research on finfish and shellfish. WGAGFM finds it important to provide a review and update on these developments.

c) Different fish species may exhibit different kinds of genetic population structure. For instance, some species are composed of distinct population units, which are subject to more or less frequent extinctions and recolonisations (so-called metapopulations), whereas other species may exhibit very weak population structure. These different kinds of population structures may lead to different kinds of effects and responses as a result of human impact and exploitation. Therefore, WGAGFM finds it important to review this topic.

d) Effective population size is the key parameter determining inbreeding and loss of genetic variability. Estimation of effective population size is therefore of considerable interest in relation to both aquaculture and management of wild fish populations. New statistical developments combined with the use of genetic markers have made it possible to estimate effective population size and genetic bottlenecks. WGAGFM finds it is important to evaluate the use and relevance of these procedures for estimating effective population sizes and bottlenecks in fish populations.

e) Whereas genetic research and techniques in several cases have provided important information for the management and conservation of freshwater and anadromous fish species, there are still relatively few examples of the successful integration of genetics in the management of marine species. WGAGFM finds it important to highlight cases where genetics has provided important results that have been used in the management of marine species in order to draw on experience for future studies.

f) WGAGFM has previously aimed at collecting information about ongoing research projects in ICES Member Countries. However, the number of projects is enormous, and WGAGFM is not sure that the project listings are used by a sufficient number of persons to justify the labour involved in collecting and organising the information. The National Activity reports for 2000 will be made available at the unofficial homepage of the WGAGFM, and persons browsing through the list of projects will be asked to state whether or not the information is useful. The future of the National Activity reports will depend on the outcome of this survey.

WGAGFM feels that it might be more appropriate to target the collection of information at resources that will surely be of need and interest to many researchers. Many primer sets are now available for analysis of microsatellite DNA markers in a number of finfish and shellfish species, but the use of markers is still poorly coordinated. By compiling information on available microsatellite loci in important fish species WGAGFM will make important information available to many researchers and will contribute to the harmonisation of the use of genetic markers.

ANNEX 1: TERMS OF REFERENCE FOR 2000

ICES C.Res. 1999/2:F:03

The **Working Group on the Application of Genetics in Fisheries and Mariculture** [WGAGFM] (Chair: Dr M. Møller Hansen, Denmark) will meet in Leuven, Belgium from 3–6 April 2000 to:

a) continue the review of general population genetics topics in fisheries and mariculture, with emphasis on the utilisation of possibilities available through the combination of qualitative and quantitative genetics;

b) review the relevant portion of the chapter on Baltic fish prepared for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994–1998 [HELCOM 2000/3];

c) review principles for prioritisation of marine finfish and shellfish populations for conservation;

d) review the status of Artificial Intelligence and Neural Networks as tools in population studies based on input requested from SIMWG;

e) compile an updated list of patents in molecular biology which potentially may interfere with population genetics research;

f) review potential genetic implications of recent research on endocrine disruptors;

g) review the possibility and feasibility of developing coordinated genetic databases for enhancing understanding of genetic diversity in fish species;

h) review genetic implications of commercial fisheries on deep-water fish stocks;

i) explore the question of trade-offs between genetic gain and loss of genetic variability in breeding programmes (how to minimise inbreeding in intense breeding);

j) prepare updated protocols of fishery and mariculture genetics research in Member Countries, and identify scopes for enhanced international cooperation.

WGAGFM will report to the ACME before its June 2000 meeting and to the Mariculture Committee at the 2000 Annual Science Conference.

Justifications for the 2000 ToRs

a) WGAGFM is a relatively informal forum where members are free to discuss and inform each other about practical and theoretical problems relating to the genetics of marine species. Experience has shown that there is a need for an open scientific session at the annual meetings, where topics that are not necessarily listed in the Terms of Reference can be expounded upon by the competence and experience existing in WGAGFM; one topic of increasing interest is the combination of qualitative and quantitative genetics.

b) There is a request from HELCOM to evaluate the effects of sea ranching and mariculture on the genetic composition of wild Baltic salmon as part of a chapter on fish for the Fourth Periodic Assessment.

c) With limited resources available for the conservation of marine finfish and shellfish populations, it is important to provide biologically based prioritisation guidelines for managers in order to maximize biodiversity. This has been done for many other species (see Given and Norton, 1993), including freshwater fish (Pacific salmon, brown trout). The general principles of the procedure are to rank populations within a species according to: a) current viability status, and b) biological consequences of extinction (genetic, evolutionary, and ecological). Together, these rankings can serve as a tool for prioritisation of possible action. So far, a formalised list of criteria for prioritisation of marine finfish and shellfish populations has not been produced. Since the marine ecological paradigm is so different from that of freshwater (or terrestrial), all the criteria applied there may not be valid for marine populations or may require some adaptations.

d) WGAGFM has earlier requested information from the Stock Identification Methods Working Group (SIMWG) concerning methodology, in particular, with regards to the current use of Artificial Intelligence and Neural Networks in stock identification. This information will provide the basis for discussions on these methodologies at the WGAGFM meeting in 2000.

e) Further to the discussion on TOR g ('Patenting of gene technology') at the 1999 Reykjavik meeting, WGAGFM feels the need for an updated list of relevant patents.

f) Endocrine disruptors are chemical substances interacting with hormone regulatory systems or acting as hormones themselves. An enormous amount of chemicals are produced and widely used for a broad range of human purposes. Most of these substances are released in the environment and a growing number are suspected endocrine disruptors. There is already evidence from laboratory experiments that some endocrine disruptors lead to a shift in sex ratio in fish and there is also reason to believe that some endocrine disruptors may cause sterility. A

disproportion in sex ratio or increases in sterility may lead to genetic effects such as the reduction of effective population size and the concomitant loss of genetic diversity.

WGAGFM wants to thoroughly review the scientific literature and formulate recommendations for additional research that may be necessary in order to estimate the effects of endocrine disruptors on the genetics of fish/shellfish and to formulate recommendations on how to protect genetic diversity of affected species.

g) Molecular technology is being increasingly used by various laboratories in different countries to characterise genetic diversity in fish species, including the accumulation of DNA sequence data, to understand population structuring and local adaptation. To fully assess broader geographical patterns and structuring across regions, and gain the maximum insight, the results of different research programmes need to be coordinated to allow integrated analysis of combined data sets. The best way to pursue this needs to be considered, including the establishment of common 'core' loci for screening and interlaboratory calibration of genotype assignment.

h) Landings of deep-water fish species from trawl fisheries on the continental slope have increased and fishing pressure on deep-water fish species is likely to continue to increase. The lack of reliable catch and effort data and of biological information on the stocks of deep-water species makes it difficult to establish the stocks' sustainability. There is evidence that deep-water species tend to be slow growers, to mature late, and to be long-lived. Such life-history traits make these stocks particularly vulnerable to exploitation. Moreover, the geographical distribution of deep-water species may extend into international waters and the implications of uncontrolled fisheries in these areas must be considered. WGAGFM feels it is appropriate to review this topic with a view to presenting recommendations for research and management, if possible.

i) Increased rates of inbreeding as the result of selection decisions will have a negative effect on future genetic response through reduction in genetic variance and a negative impact on future performance if inbreeding affects the selected trait. This can be particularly important in small highly selected nucleus populations and when selection is based on breeding values based on best linear unbiased prediction (BLUP) which is likely to result in higher levels of inbreeding than when mass selection is practised. A number of methods have been proposed to attain high rates of genetic response with moderate to low inbreeding. These methods vary from very simplistic approaches such as minimum co-ancestry matings to methods that require quite sophisticated programming techniques. The best method to be used will depend on the population structure, selection intensity, and the heritability of the traits under selection. WGAGFM proposes a review of the best methods to restrict inbreeding as they apply to an aquaculture population under selection.

j) The national activity reports, which are compiled and updated each year by WGAGFM, provide a useful information base for geneticists in ICES Member Countries who are seeking cooperation or information on specific species and/or specific methodologies. This information base also makes it possible to monitor potential changes in research focus within finfish and shellfish genetics throughout the Member Countries.

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ANNEX 4: NATIONAL ACTIVITY REPORTS

The national activity reports are available at the internet address**:**

http://www.dfu.min.dk/ffi/wgagfmweb/Research/research.htm

ANNEX 5: TERMS OF REFERENCE FOR 2001

The **Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM**) (Chair: Dr M. Møller Hansen) will meet at Bergen, Norway from 26–28 March 2001 to:

a) continue the review of general population genetic topics in fisheries and mariculture and identify scopes for enhanced international cooperation;

b) review new developments in the identification of genes of relevance to aquaculture and studies of wild populations;

c) review the importance of different kinds of genetic population structure in relation to human impact;

d) review methods for estimating effective population sizes and/or changes in effective population sizes in anadromous and marine fish populations;

e) review examples where population genetics research has provided important information for the management of marine fish populations;

f) compile information on available molecular markers (mainly microsatellite loci) for use in studies of finfish and shellfish;

WGAGFM will report to the ACME before its June 2001 meeting and to the Mariculture Committee at the 2001 Annual Science Conference.

Priority:	WGAGFM is of fundamental importance to the ICES advisory process.
Scientific Justification:	a) WGAGFM is a relatively informal forum where members shall feel free to discuss and update each other on practical and theoretical problems related to genetics of marine species. Experience has shown that there is a need for an open scientific session of the annual meetings, where topics that are not necessarily listed in the Terms of Reference can be enlightened by the competence and experience existing in WGAGFM. Not least have those topics which need competent input from both qualitative and quantitative genetics benefited from these discussions. b) A number of new important genes have been identified and characterised in species subject to aquaculture. Further, the study of these and other coding genes in wild populations may provide important insight into the processes of selection and local adaptations. Finally, new screening techniques (so-called microarray chip techniques) are presently being developed that will allow for very fast screening of a huge number of genes, and these techniques are likely to have a profound influence on genetic research on finfish and shellfish. WGAGFM finds it important to provide a review and update on these developments. c) Different fish species may exhibit different kinds of genetic population structure. For instance, some species are composed of distinct population units, which are subject to more or less frequent extinctions and recolonisations (so-called metapopulations), whereas other species may exhibit very weak population structure. These different kinds of population structures may lead to different kinds of effects and responses as a result of human impact and exploitation. Therefore, WGAGFM finds it important to review this topic. d) Effective population size is the key parameter determining inbreeding and loss of genetic variability. Estimation of effective population size is therefore of considerable interest in relation to both aquaculture and management of wild fish populations. New statistical developments combined with the use of genetic markers have made it possible to estimate effective population size and genetic bottlenecks. WGAGFM finds it is important to evaluate the use and relevance of these procedures for estimating effective population sizes and bottlenecks in fish populations. e) Whereas genetic research and techniques in several cases have provided important information for the management and conservation of freshwater and anadromous fish species, there are still relatively few examples of the

	successful integration of genetics in the management of marine species. WGAGFM finds it important to highlight cases where genetics has provided important results that have been used in the management of marine species in order to draw on experience for future studies. f) WGAGFM has previously aimed at collecting information about ongoing research projects in ICES Member Countries. However, the number of projects is enormous, and WGAGFM is not sure that the project listings are used by a sufficient number of persons to justify the labour involved in collecting and organising the information. The National Activity reports for 2000 will be made available at the unofficial homepage of the WGAGFM, and persons browsing through the list of projects will be asked to state whether or not the information is useful. The future of the National Activity reports will depend on the outcome of this survey. WGAGFM feels that it might be more appropriate to target the collection of information at resources that will surely be of need and interest to many researchers. Many primer sets are now available for analysis of microsatellite DNA markers in a number of finfish and shellfish species, but the use of markers is still poorly coordinated. By compiling information on available microsatellite loci in important fish species WGAGFM will make important information available to many researchers and will contribute to the harmonisation of the use of genetic markers.
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
Linkages to other Committees or Groups:	SIMWG
Linkages to other Organisations:	HELCOM