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Report of the Stock Identification Methods Working Group (SIMWG)

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Contents

Executive summary

The Stock Identification Methods Working Group met at the Öregrund Institute of Coastal Research, Sweden, from the 21 – 23 June 2010.

The agenda originally comprised eight Terms of Reference:

a) Liaise with ICES working groups and study groups dealing with stock identification issues and provide technical reviews to these groups and SCICOM;

b) Review and report on new advances in stock identification methods as they develop, and new results that are relevant to ICES work;

c) Consider the available multidisciplinary studies in Stock Identification, and produce a first draft "SIP" (Stock Identification Protocol) for the integration of results from multiple disciplines;

d) Review the scientific resources and tools available to ICES for investigating stock structure and determining appropriate management units, including technologies, sampling programmes, laboratories;

e) Identify limitations and gaps in the scientific capacity of ICES for investigating stock structure and determining appropriate management units, including technologies, sampling programmes, laboratories;

f) Consider stock identification methods used for non-fish biology (e.g. marine mammals) and whether any lessons may be learned for fish stock assessment;

g) Develop terms of references based on a work plan for the next two years, which complement the objectives of the ICES science plan;

h) Express expert advice on the NEAFC request to ICES regarding additional review of the stock structure of *Sebastes mentella* in the Irminger Sea and adjacent areas, with specific consideration of NEAFC documents AM 2009/23 and AM 2009/29-rev1.

All matters were given full consideration, and the present report delivers the outcome of the discussions and the reviewing work conducted during and after the meeting.

It quickly became apparent that ToR d) and e), as they stand now, represent a mighty task to be accomplished by a handful of scientists within SIMWG. While at a surface level it is possible to outline strengths and weaknesses within the ICES community, if these need to be quantitatively assessed in detail, across all associated institutions, it is necessary to get a much larger portion on the ICES community involved. Some ideas are proposed and discussed in this report, but no systematic analysis of resources and limitations is conducted.

The work for ToR c) represents the most novel aspect of recent SIMWG activities, and it will be continued into 2011.

The text of ToR h) has also been sent directly to ACOM and other groups, within ICES, that have interest in the redfish stock issues.

Overall, SIMWG members feel that there may be more ICES working groups which would welcome SIMWG feedback, than currently perceived. We therefore encourage all Working Group Chairs, and SCICOM and SGSUE to clearly request for SIMWG feedback on specific Stock ID issues. Although the working capacity of SIMWG is relatively limited in terms of personnel and financial support, we are committed to contribute with expert evaluations, compatibly with the available time.

SIMWG is expected to meet by correspondence in 2011.

1   ToR a) - Liaisons with other ICES groups

Over the past three years, SIMWG were asked to express expert feedback on the status of stock structure in beaked redfish (*Sebastes mentella*) in the Irminger Sea and adjacent areas. In 2009, SIMWG reviewed the stock status of blue whiting (*Micromesistius poutassou*) and examined issues relevant to Elasmobranchs. In 2008, SIMWG discussed stock structure in MoU species, wide ranging sharks and rays, and Atlantic herring west of the British Isles. Since 2007, advances in stock identification of deep sea fish were also monitored.

Although there is evidence that the SIMWG advice was well received among the groups of interest (WGDEEP, SGRS, WGNEW, WGEF, SGHERWAY, WKREDS, etc.) and ACOM, we believe that several more Working Groups may be interested in receiving feedback from SIMWG, and that improved communication in the future can considerably facilitate exchange of ideas within the ICES community.

2   ToR b) – Advances in stock identification methods and results relevant to ICES work

2.1   Overview

The array of different stock identification methods has been widely summarized since the publication of the Cadrin *et al.* (2005) book, and since then, new methods have been introduced (Moen *et al.,* 2008; Beacham *et al.,* 2010) and the ecological processes influencing others are being unravelled (Brown and Severin, 2009; Vignon and Morat, 2010). This constantly enhances the capacity of the toolkit available for Stock ID.

This section is an overview of recent (2009–2010) advances in stock identification methods and results relevant to ICES work and therefore is a continuation of the corresponding section in the 2009 SIMWG report. It is, however, important to note that this is not a complete review of all research conducted in this field but rather an attempt to cover what SIMWG deems to be the most relevant to ICES. Both peer-reviewed material and some results from the "grey literature" are reviewed.

A literature research of references published between 2009–01–01 and 2010–06–10 in the database of Aquatic Science and Fisheries Science, using search criteria based on the keywords "population or stock" and "identification or structure or complex", revealed that genetic based methods are the most commonly employed for stock identification, with microsatellite markers being employed in about half of the published studies.

2.2   Otoliths and other hard structures

Similar to previous years, otolith- and scale-based stock identification studies published during 2009 and 2010 primarily focus on microchemistry and morphometry/shape analyses.

2.2.1   Microchemistry

The elemental composition of otoliths and scales has been useful with regards to many aspects of the stock identification process, providing information on past and present locations and environmental history. Spatio-temporal variation in the elemental composition of otoliths of southern bluefin tuna (*Thunnus maccoyii*) in the Indian Ocean for example, and its ecological implications, were studied by Wang *et*

al. (2009). They compared fish collected at the feeding and spawning grounds and found that elemental composition can be used to study time of ontogenetic shift between larval to juvenile stages and to reconstruct environmental history during past migrations. Elemental composition of otolith cores of adults from feeding and spawning grounds were similar and consistent with the hypothesis of one spawning population. On the issue of identifying natal areas, Walther and Thorrold (2009) reported interannual variability of otolith isotopes and elemental ratios from annual cohorts of juvenile American shad (*Alosa sapidissima*) in three rivers. The results emphasize the importance of quantifying signatures for each cohort separately in order to successfully identify juvenile origins.

On the medium scale, otolith microchemical analyses by Schuchert *et al.* (2009) on Patagonian hoki (*Macruronus magellanicus*) indicated mixed-stocks of Pacific and Atlantic origin around Chile, Argentina and the Falkland Islands.

Several papers report fine-scale differences in otolith microchemistry, indicating subpopulations of North Sea fish, e.g. cod (*Gadus morhua*, Svedäng *et al.*, 2010), haddock (*Melanogrammus aeglefinus*, Wright *et al.*, 2010) and sole (*Solea solea*, Cuveliers *et al.*, 2010). The study by Limburg *et al.* (2010) on coastal cod off Norway also suggests that Bromine could be used as stock marker.

Gao *et al.* (2010) found stable Oxygen and Carbon isotope ratios as well as the relationship between δO^{18} and δC^{13} as successful tools to identify spawning stocks or subpopulations of several groundfish along the US Pacific coast. The study of Schloesser *et al.* (2010) used the same isotopes to predict natal origin of bluefin tuna (*Thunnus thynnus*) with high classification success (84% cross-validated). Similarly, Newman *et al.* (2010) studied stock structure of grey mackerel (*Scomberomorus semifasciatus*) off northern Australia, and Steer *et al.* (2010) investigated Southern garfish (*Hyporhamphus melanochir*) populations off South Australia. Both studies provided evidence that current management units may need to be revised in the light of their findings.

More novel advances in the use of elemental composition of calcified structures include the study by Adey *et al.* (2009) in which scale microchemistry was successfully used to identify the origin of wild and farmed Atlantic salmon, *Salmo salar* from sites throughout Scotland. Furthermore, Kuroki *et al.* (2010) validated transgenerational mass marking of viviparous brown rockfish (*Sebastes auriculatus*) by injecting strontium into female fish, demonstrating the potential to standard application of this new technique.

Importantly, Brown and Severin (2009) found, when reviewing profiles of Sr:Ca from 81 species of freshwater, diadromous and marine fish, that water Sr:Ca is the main factor affecting otolith Sr:Ca variation for freshwater and diadromous fish but not for marine fish.

An increasing number of studies used two stock ID markers simultaneously, e.g. otolith microchemistry and shape (roundnose grenadier, *Coryphaenoides rupestris*, Longmore *et al.,* 2010), otolith Strontium isotopes and microsatellite DNA (Chinook salmon, *Oncorhynchus tshawytscha*, Barnett-Johnson *et al.*, 2010 and Miller *et al.*, 2010), and otolith Strontium and body morphology (Arctic charr, *Salvelinus alpinus*, Loewen *et al.*, 2010).

2.2.2 Morphology

Oliveira *et al.* (2009) showed significant discrimination among fish groups from different geographic origins based on shape and allometric relationships in sagitta otoliths of the banded croaker, *Paralonchurus brasiliensis*, along the coast of Rio de Janeiro State. Differences in otolith morphology were coupled to variation in growth and nutrition through the variation in particular environmental conditions. Similarly efficient was the use of otolith shape analysis (Elliptic Fourier descriptors) to discriminat stocks of black scabbardfish (*Aphanopus carbo*) in the Northeast Atlantic (Farias *et al.*, 2009). The areas around the Azores, Madeira and off mainland Portugal separated well (91–98% correct classification).

Twenty-one morphological and meristic characters among 13 representative localities across the Indo-Pacific region were used to investigate population structure in *Anguilla marmorata* (Watanabe *et al.*, 2009), with the total number of vertebrae (NV) showing the greatest variation among the characters examined. The differences in NV among regions in conjunction with population genetic results of this species indicate at least four populations (North Pacific, Micronesia, Indian Ocean and South Pacific), which also are consistent with the ocean current patterns in each region and the evolution and migration loops of each spawning population.

Meristic characters were more efficient than morphometric ones to discriminate between Turkish populations of the Atlantic horse mackerel *Trachurus trachurus*, although in this case, also morphometrics indicated a small degree of separation among locations (Bektas and Belduz, 2009).

Morphology and morphometry of lapillus otoliths have also been used to distinguish between different ecomorphological groups of Silurifom fish (Volpedo and Fuchs, 2009).

The integrated use of morphology, genetics and life history revealed that two distinct species have been erroneously confused since the 1920s under the single scientific name *D. batis*. These happen to represent two critically endangered and spatially segregated taxa (Griffiths *et al.*, 2010): the blue skate, *D. flossada*, and the flapper skate, *D. intermedia*, and urgent management actions are needed to protect these long-lived fish, which have been severely affected by commercial trawling for decades.

2.3   Genetics

The vast majority of studies of population structure have used microsatellite markers, either alone (cf. Bott *et al.*, 2009, Danacher and Garzia-Vazquez 2009, Yoon *et al.*, 2009, Beacham *et al.*, 2009, Israel *et al.*, 2009, Teel *et al.*, 2009, White *et al.*, 2010a, Shubina *et al.*, 2009, Eldridge *et al.*, 2009, Garibkhani *et al.*, 2009, Nielsen *et al.*, 2009a,) or together with MtDNA (Lynch *et al.*, 2010, Muths *et al.*, 2009, Karlsson *et al.*, 2009, Sala-Bozano *et al.*, 2009). Only few studies are using other types of genetic markers such as allozymes (Chlaida *et al.*, 2009).

2.3.1   Microsatellites

Microsatellites generally reflect more recent demographic events, while mtDNA depicts more ancient historical processes; this is discussed in Sala-Bozano *et al.* (2009), using the striped sea bream, *Lithognathus mormyrus*, in the Mediterranean as a case study. MtDNA and microsatellites often complement each other. For example, earlier mtDNA studies of European grayling (*Thymallus thymallus*) have shown that grayling populations in Finland, Estonia and Russia belong to a single lineage and exhibit high differentiation at small geographical scale despite this, microsatellite analysis re-

vealed the existence of three regional clusters within Finland (Swatdipong *et al.,* 2009). In addition, Swatdipong *et al.* (2009) come up with a new tool to prioritize populations for conservation based on the relative strength of different evolutionary forces (migration and drift) shaping the gene pool.

Combining microsatellites and MtDNA can also reveal sex biased differentiation, which was shown for Swordfish (*Xiphias gladius*) in the southwest Indian Ocean (Muths *et al.*, 2009).

In few cases a more multidisciplinary approach is used, combining microsatellites with tagging (Tatarenko *et al.,* 2010) or parasites (Sala-Bozano *et al.,* 2009), or even multiple non-genetic markers (Higgins *et al.,* 2010, Miller *et al.,* 2010).

In green sturgeon (*Acipenser medirostris*), microsatellites have successfully been used for mixed-stock analysis in Lake Michigan (Bott *et al.,* 2009) as well as for stock composition in estuarine populations along the Pacific coasts of the USA (Israel *et al.,* 2009).

Several microsatellite-based studies have helped unveiling previously unrecognized patterns of stock structure in several species, including megrim species (*Lepidorhombus whiffiagonis* and *Lepidorhombus boscii*) across the Northeast Atlantic and the Mediterranean (Danacher and Garzia-Vazquez (2009), the walleye pollock (*Theragra chalcogramma*) in the Bering Sea (Shubina *et al.,* 2009), the pikeperch (*Sander lucioperca*) in the Caspian Sea (Gharibkhani *et al.,* 2009), the olive flounder (*Paralichthys olivaceus,* Kim *et al.,* 2010) and Pacific cod (*Gadus macrocephalus*, Cunningham *et al.,* 2009) in the Northwest Pacific; as well as the European anchovy (*Engraulis encrasicolus*) in the Bay of Biscay (Zarraonaindia *et al.,* 2009) and the European sprat (*Sprattus sprattus*) between the Northeastern Atlantic Ocean and the Baltic Sea (Limborg *et al.,* 2009). These new results can play an important role in revising current management strategies for these stocks.

Although microsatellites are assumed to be neutral genetic markers, allowing inference on the processes of random genetic drift and gene flow, in some cases they can be linked to a functional locus that is under the influence of natural selection ("hitchhiking selection") and can therefore be informative of adaptive processes. Recent advances in statistical approaches and investigative philosophies have resulted in an increasing number of studies that identify non-neutral microsatellite loci, and treat them separately from the others in order to gauge information on potential influence of selective processes on the studied populations. Recent examples include the roundnose grenadier (*Coryphaenoides rupestris*), in which one of 16 microsatellites used showed markedly higher values of differentiation, possibly linked to depth (White *et al.,* 2010b); and Atlantic herring (*Clupea harengus*), in which allelic frequencies at one microsatellite locus appeared to be associated with changes in salinity (Andre *et al.*, 2010).

2.3.2 mitochondrial DNA

Despite the generally acknowledged better suitability of microsatellites for demographic processes whose time-scales can be of interest to management, mtDNA continues to have some use in stock identification studies. Species in which mtDNA has recently helped unveiling patterns of spatial structure include the rockfish *Sebastes miniatus* (Hyde and Vetter 2009) and the emperor *Lethrinus miniatus* (van Herwerden *et al.*, 2009).

The use of mtDNA in conjunction with nuclear markers, such as microsatellites and AFLPs (Karlsson *et al.*, 2009; Liu *et al.*, 2009; Lynch *et al.*, 2010) are to be considered more reliable, given the often contrasting signals yielded by different classes of markers (Sala-Bozano *et al.*, 2009). Particular caution should be used when even the application of several markers fails to detect any significant pattern of structure (Cardenas *et al.*, 2010); the lack of differentiation in these cases should not automatically lead to a conclusion of panmixia, and further approaches should be considered.

2.3.3   Mitochondrial DNA, 'barcoding' and mixed fisheries

Recently, mtDNA has offered great opportunities for species identification in mixed fisheries and in seafood identification (Farrell *et al.*, 2009; Chapman *et al.*, 2009; Miller and Mariani, 2010; Barbuto *et al.*, 2010). Given the ease and availability of reliable non-specific protocols applicable to thousands of species (Ward *et al.*, 2009), it is reasonable to suggest that such techniques be more frequently applied in the case of many problematic mixed fisheries in ICES areas, such as those involving gurnards (*Trigla spp*), sandeels (*Ammodytes, Hyperoplus*), hakes (*Merluccius spp*), monkfish (*Lophius spp*), redfish (*Sebastes spp*), scabbardfish (*Aphanopus sp*), skates (*Dipturus spp*), rays (*Raja spp*), smooth-hounds (*Mustelus spp*).

2.3.4   Single Nucleotide Polymorphisms (SNPs)

SNPs have now been established as additional stock identification tools for the future (Nielsen *et al.*, 2009b; Beacham *et al.,* 2010), and some basic information exists on their power and accuracy, comparatively to microsatellites: Beacham *et al.* (2010) showed that six microsatellites proved as efficient as 43 SNPs, and that, in order to replace the suite of 14 microsatellites currently used for screening sockeye salmon (*Oncorhynchus nerka*) populations, there is the need of 75–79 SNPs for stock identification and a need of over 120 SNPs for individual assignment to population of origin. However, the use of a mixed array of microsatellites and SNPs also appears to be a valuable and effective strategy. It should also be noted that SNP are polymorphisms that can be both neutral and adaptive, and that a cautious approach to marker choice should always be applied (Nielsen *et al.*, 2009b). Over the forthcoming years, large amounts of SNP-based results will be communicated and published as an output of the FishPopTrace European consortium (https://fishpoptrace.jrc.ec.europa.eu/home), which will likely provide a benchmark for future applications of SNPs in commercial marine stocks.

2.3.5   Theoretical considerations

Sampling scheme can have an important affect on the result of population genetic studies. For example, when using larval samples to infer population structure, a bias is introduced, as the researcher is only sampling successful breeders and this may be biased towards certain families. Goldberg and Waits (2010) have quantified this error and point to a solution using sibship analysis to reduce this bias. Another problem pointed out by Nielsen *et al.* (2009a) is that without cautious consideration of biased samples of individuals and loci, apparent microgeographical patterns of spatial genetic differentiation could be caused by sampling of non-randomly distributed individuals or, as said above, using marker loci presumed neutral, but that may be under hitch-hiking selection.

The inclusion of ocean currents in modelling larval exchange among sites, using a 'seascape genetics' approach, has been shown to have the potential to significantly alter interpretations of spatial stock structure (White *et al.*, 2010b). Furthermore, particle tracking simulations suggest that the timing of release and position of early life

stages in the water column substantially influence the direction and extent of connectivity among stocks (Ashford *et al.*, 2010).

One fundamental aspect that should be recognized is the existence of a vast array of molecular genetic markers, which differ from each other in so many significant ways. This should prompt stock identification scientists to refuse the traditional philosophy of grouping these diverse methods under the umbrella term of "genetics". Instead, it would be most useful if the broader community of fisheries scientists, including those without a genetic background, could routinely embrace the distinction between 'neutral' and 'adaptive' markers, as well as the dichotomy between nuclear (bi-parentally inherited) and mitochondrial (maternally inherited) genes.

2.4 Parasites, tagging, Life histories

The use of parasites as biological tags has not recorded significant advances since 2009 (Lester and Mackenzie, 2009), although it is worth documenting the successful use of multivariate discriminant analysis of parasitic fauna in the identification of three different stocks of the Brazilian whitemouth croaker, *Micropogonias furnieri* (Luque *et al.*, 2010). Moreover, Sala-Bozano *et al.* (2009) have also demonstrated how a judicious interpretation of the patterns of parasitic infestations can offer a valuable supplementary tool to unravel spatial population structure; especially where/when microsatellite and mitochondrial markers offer slightly discordant results.

Partial migration has been revealed in European eel using tagging (implants and PIT tags) (Imbert *et al.*, 2010). Comparison of results from tagging and microsatellites in the green swordtail fish (*Xiphporus helleri*) revealed a significant genetic differentiation between fish from different creeks despite extensive migration; this was explained by high genetic drift due to small population sizes and highly skewed paternity (Tatarenko *et al.*, 2009).

Stock identification can also benefit from the analysis of life history traits, such as spatial/temporal variation in ovarian maturation, used to identify two stocks in Argentine hake (*Merluccius hubbsi*) off the brazilian coast (Vas-Doz-Santos *et al.*, 2009), or length-at-age in *Liza agentea* and *Myxus elongatus* in two temperate Australian estuaries (Kendall *et al.*, 2009). Furthermore, in an experimental set-up, it was shown that size at maturity differed between cod from different parts of the North Sea (Harald *et al.*, 2010).

## 3   ToR c) – Development of a 'Stock Identification Procedure' for the integration of multiple markers

Although the stock identification process is widely recognized as a fundamental step for the purposes of assessment and management, several stocks, including a number of high profile ones within ICES areas, are currently managed without regard to their true biological structuring (Reiss *et al.*, 2009). This is partly due to the difficulties associated with determining the most biologically realistic pattern of structure, based on a multitude, and constantly refining, array of stock identification methods.

Sometimes different markers produce slightly different results, and these are of great interest to scientists, but may represent an undesirable obstacle for management advice and policy-making, as complex results require longer time for interpretation. Traditionally, stock identification scientists either tend to "trust" results obtained with their preferred techniques or, when multiple results from several methods are available, a qualitative assessment is carried out, and a most likely/parsimonious scenario – often somewhat subjective - is provided (Abaunza *et al.*, 2008; Sala-Bozano *et al.*, 2009; Cadrin *et al.*, 2010; Andre *et al.*, 2010).

A valid alternative would be to employ a quantitative approach, in which variables obtained using different methods and techniques are analysed statistically within the same framework, in order to achieve a more objective result, as partially shown by Higgins *et al.* (2010). A standardized, quantitative, integrated approach would have a number of significant advantages, but also some caveats.

The advantages include an increased objectivity, a common statistical framework familiar to researchers with different backgrounds, a more direct, neat response for managers and policy-makers, and the possibility to be employed in other conservation scenarios, beyond fisheries. However, before this method can be proposed with some confidence, a number of issues need to be resolved. Firstly, data obtained from remarkably different disciplines can have very different forms, and this will require careful standardization. Different multivariate analyses might be employed according to the main question that needs to be addressed. For example, ordination techniques could be used to explore the dataset and generate hypotheses (Principal Coordinate Analysis with Gower's similarity index, for example, allows both continuous and categorical variables to be included in the same analysis); multivariate permutational techniques, such as ANOSIM and PERMANOVA could be used to address question such as "are fish in these different areas forming independent units?"; Discriminant Function Analysis could be employed to address the question "can I assign a fish to a most likely putative stock or area of origin?". Obviously there will be situations in which certain variables will be much more responsible than others in identifying the pattern, and this also merits further consideration.

Initially, we are using a dataset based on population sampled and partially analysed in Sala-Bozano *et al.* (2009), in which populations of striped sea bream, *Lithognathus mormyrus*, were sampled over two consecutive years from five different areas in southern European coasts (Cadiz, Atlantic; Malaga, Alboran; L'Estartit, Balearic; Sabaudia, Tyrrhenian; Duce, Adriatic). Each specimen has been examined for infestation of 13 parasitic species, mtDNA, nine microsatellites, growth constant, maturation size, size at sex-change, sex-ratio and biometric condition factor. Preliminary analyses show a very high classification rate (between 94 and 100% depending on the area of origin) (Figure 1).
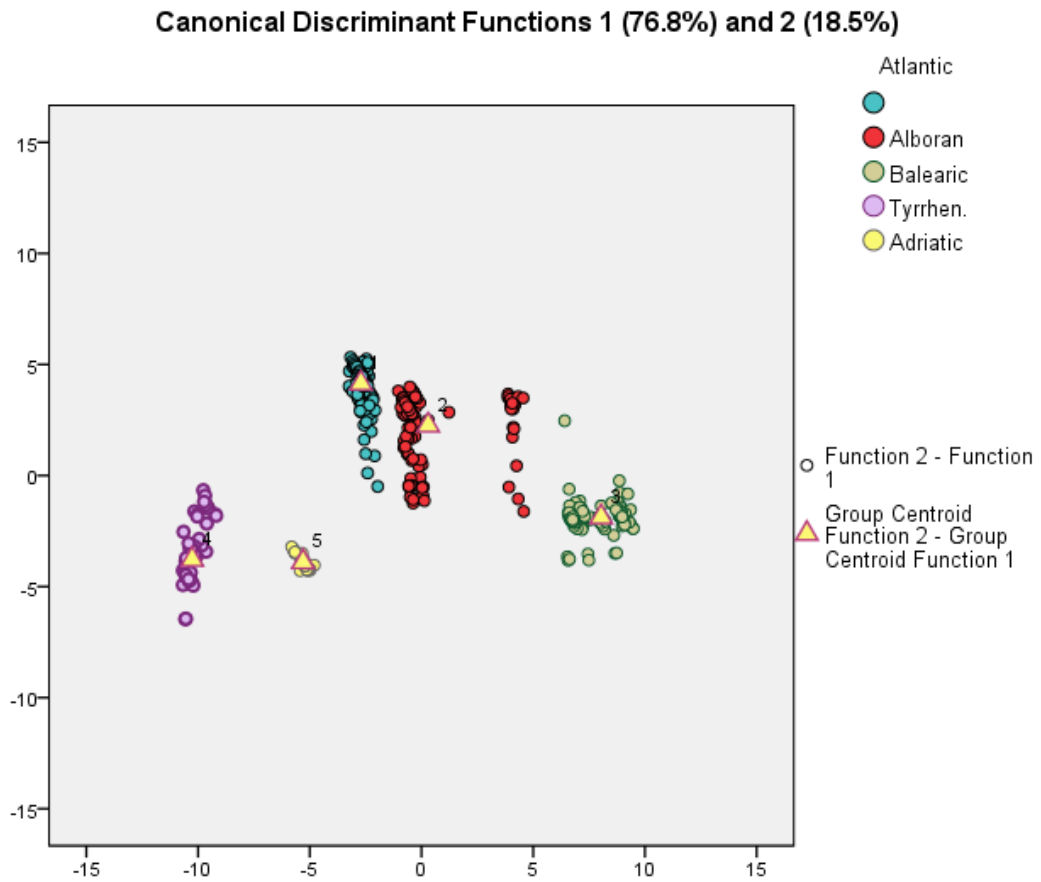
Figure 1. Individual striped sea bream plotted in the space identified by the first two canonical discriminant functions.

Using PERMANOVA, interannual variation was shown to contribute to a much smaller extent (<5%), compared to variation accounted for by location (over 90%). Figure 2 illustrates the relationship among specimens as synthesized in a Principal Coordinate Analysis.
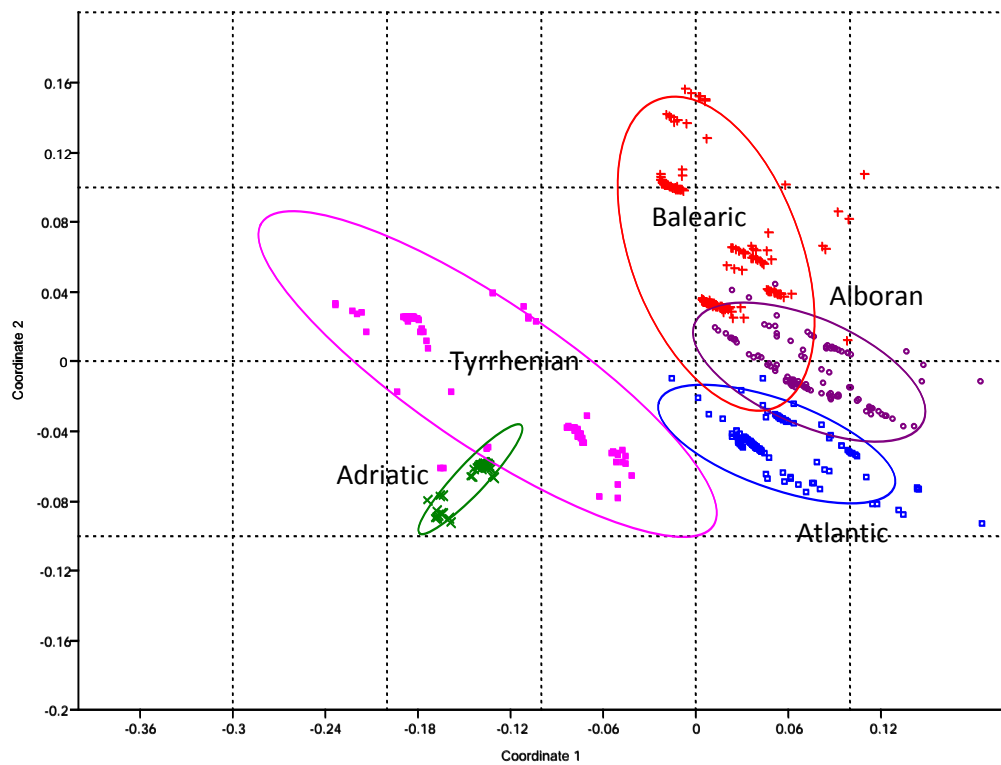
Figure 2. Individual striped sea bream plotted in the space identified by the first two Principal Coordinates (together explaining over 93% of the variance).

There remains much to be done before this approach is refined and applicable. Robustness, consistency and data standardization are priority issues to be tackled. Furthermore, we hope to have the opportunity to test the approach using another dataset from another species. The target is to have completed this work for inclusion in the next SIMWG report in 2011.

4 ToR d) and e) – Review the scientific resources and tools available to ICES – as well as the limitations and gaps in scientific capacity – for investigating stock structure

A full assessment of all the existing laboratories, researchers, survey/monitoring campaigns, currently available to the ICES community for the purpose of Stock identification, is a difficult and time-consuming task; so is, consequently, the identification of limitations and gaps in knowledge.

In terms of resources and capacity, ICES can benefit from a vast number of nationally and internationally funded institutions and consortia, and the services of thousands of expert scientists, between Europe and North America. Among these, many routinely employ some of the key tools for stock ID, although the limitation of financial resources prevents ICES from directly endorsing groups of scientists with specific stock ID tasks as they become of priority. However, ICES can to some extent influence the drafting of EU Framework programme themes, which in recent years have supported several studies on fish stock identification. One complication within the ICES community is that several scientists involved in expert groups and committees do not work for their reference governmental research institutes, but are employees of universities and other independent research institutions, whose priorities do not necessarily cover the ICES remits. Therefore, the capacity of ICES – and its limitations – can only be gauged if an exhaustive census is conducted, under the umbrella of SCICOM.

A proposed approach could be that SIMWG drafts a questionnaire addressing all the key aspects relevant to Stock ID requirements. SCICOM could then circulate the questionnaire among all the official national governmental institutions (e.g. RIVO, DIFRES, IMR, MARINE INSTITUTE, Cefas, Ifremer, etc.), who would then nominate a person, or a group of people, responsible to complete the questionnaire, based on information that they can obtain within their own national remits. The questionnaires are then returned to SCICOM, who forward them back to SIMWG to be compiled, analysed and the results reported. It is hoped that SCICOM can take this idea in consideration, as it there is no other conceivable way by which SIMWG can collate this type of information and report on the matter.

5   ToR f) – Stock identification-like approaches in non-fish biology

Marine mammals, especially Cetaceans, are priority targets for marine conservation. The use of neutral and adaptive genetic markers for population identification in these species is no more advanced than it is in fisheries applications (and fish also have the advantage that much larger and more accessible sample sizes can be obtained); thus, it is unlikely that fish population genetics may benefit from methodological advances in marine mammal science. Other methods for studying population movements and distribution in cetaceans, such as acoustic and photographic approaches, are unlikely to represent usable tools in fish stock identification.

Methods from landscape genetics – which tend to use the "Isolation by Distance" model as a "null hypothesis" of connectivity, and contrasting other features (oceanographic, geomorphologic, physico-chemical, etc.) against empirical genetic data, with the aim to identify the landscape (seascape) features that account most for the observed spatial pattern (Frantz *et al.*, 2010) can be, and are being, of great use in studying marine fish populations (White *et al.*, 2010b). This approach might not directly find applications in the identification of stock boundaries, but it can detect features that disrupt gene flow locally, and therefore become a useful approach to understand the processes affecting patterns of structure and come to aid in the design of Marine Protected Areas.

6 ToR h) – Further comments on beaked redfish, *Sebastes mentella*, stock structure in the Irminger Sea and adjacent areas

SIMWG reviewed the NEAFC documents AM 2009/23 (Makhrov, 2009) and AM 2009/29rev1 (Bakay *et al.*, 2009) in the light of the WKREDS report (ICES, 2009) and its subsequent publication in Cadrin *et al.* (2010).

6.1 Document AM 2009/23

This document focuses on genetic analyses and contains several general issues and a number of detailed/technical aspects that were considered and discussed.

General issues

The argument of <u>temporal stability</u> in genetics is quite important when the magnitude of noise associated with the genetic markers employed approaches the genetic signal contained in the dataset. Allelic frequencies of enzyme loci (allozymes) generally exhibited strong differences between "shallow oceanic" and "deep sea" *S. mentella* and although it would be preferable to obtain long-term temporal studies (which would take several decades, considering the high longevity of redfish), they are very consistent with several studies using other genetic methods over the last 15 years. Collective results therefore yield a picture two distinct groups, approximately separated along a depth transect, consistently obtained over a period of 15 years (which – although ageing of *S. mentella* is problematic – likely included age-structured samples belonging to different cohorts, as inferred by the relatively broad size distributions of samples).

Several arguments in the review refer to <u>natural divergent selection</u> in different depth zones or age groups.

It should be noted that there are two different levels of adaptive considerations relative to *S. mentella*: one refers to a proposed reconstruction of Pleistocenic events that might have led to genetic and phenotypic divergence between populations, and the other refers to alternative hypotheses proposed by various authors in order to interpret patterns of allelic frequencies at some allozyme loci. These are remarkably different arguments – which are examined and discussed in different papers and with different purposes – and should not be mixed. The Russian Federation document, instead, tends to "cherry-pick" concepts and theories at will, and extracting sentences from various papers, using them outside the context. To clarify: the recent study by Stefansson *et al.* (2009) is not presenting data on selection related to redfish moving to deeper layers on a day-to-days basis, or on a life cycle basis, but rather refers to <u>evolutionary time-scales</u>, with adaptations possibly resulting from different selection pressures in different refuge habitats during the Pleistocene.

Secondly, and very importantly, invoking the potential effect of disruptive selection between "shallow oceanic" and "deep sea" *S. mentella* does not weaken the view that different stocks or population units exist. In fact, it strengthens it. The <u>usefulness of genetic markers that are subject to selection</u> has been confirmed (Hauser and Carvalho 2008, Hemmer-Hansen *et al.*, 2007, Nielsen *et al.*, 2009c, Zane 2007), as they might reflect local adaptation processes and genetic differentiation among locally adapted populations which should definitively be considered as Evolutionary Significant Units (ESU) and consequently as different Management Units. A good example of such a genetic marker is the *Pan*-I locus in Atlantic cod, which presents different allele frequencies according to latitude and/or temperature gradients (Case

et al., 2005; Sarvas and Fevolden 2005a, b; Pampoulie *et al.,* 2006; Skarstein *et al.,* 2007; Westgaard and Fevolden 2007; Pampoulie *et al.,* 2008a; Pampoulie *et al.,* 2008b; Wennevik *et al.,* 2008). This process may occur at some allozyme loci in S. mentella, such as MEP, which shows divergent frequencies above and below the 500m depth mark. On the other hand, the significant differentiation observed at several microsatellite loci – none of which has been so far shown to be under selection – provide evidence of restricted gene flow between "shallow oceanic" and "deep sea" S. mentella. Thus, collective evidence from allozymes and microsatellites cannot justify an interpretation of panmixia, or "single stock" in S. mentella in the Irminger Sea.

Detailed (technical) points:

The <u>selection hypothesis</u> referred to in the Russian review is based on the "Sisyphean Cycle hypothesis" published by George Williams in 1975 (Sex and evolution, Princeton university Press) and later discussed by Milton (1997) in his book "Selection in natural Populations". Milton tried to test if the "Sisyphean Cycle hypothesis" was applicable to genetic data, suggesting that heterozygotes will be consider as Sisyphus in Williams' theory. He then analysed available data on all taxa and all biological (including genetic data) to assess the veracity of this hypothesis. He concluded that "In highly fecund species, selection is predicted to favour highly heterozygous individuals", but he also says in the same page, few lines above: "Admittedly, much of the scenario of the Sisyphean Cycle is <u>speculative</u>, but I hope that this speculation will <u>inspire empirical test of hypothesis</u> concerning the affect of protein polymorphisms on whole plant and animal physiology, the mechanizm of fitness determination,…. and the intensity of natural selection." Although selection has been suggested to favour heterozygote individuals (heterozygosity-fitness correlations and theory [HFCs]), very few empirical studies on wild populations have been able to prove this theory. In particular, the singular consideration of the MEP allelic frequencies, which, according to the Russian scientists would be resulting from selection on older fish going deeper (but still maintaining a cohesion, generation after generation, within the same stock as the shallow water fish), presents many fallacies and contradictions from an evolutionary point of view: 1) the allelic frequencies, in many independent studies, are largely different between "shallow oceanic" and "deep sea", which means that there either is a positive, divergent selective process that maintains this difference, and therefore we cannot assume the existence of a single stock, or 2) alternatively, we would have to assume that the vast majority of the deep sea fish with the most common MEP allele at that depth would fail to successfully reproduce, resulting in the juveniles having predominantly the other allele, which, later in life would decrease again – presumably as a result of selective mortality in the older deep sea fish – which would in turn have to re-establish the frequencies of what becomes again a rare allele in the deep sea. This type of interpretation assumes two opposing selective forces at two different stages of the life cycle, which would represent a definitely Sisyphean and speculative process with rather dubious evolutionary gain. Additionally, this puzzling cycle would still not explain why strong genetic differences are still maintained at neutral microsatellite loci.

The document also argues that "genetic differences between the samples collected at different depths can be explained not only by selection, but also by the different <u>intensity of vertical migration</u> in fish with different genotypes. This phenomenon was described for genotypes of protein-coding genes in daphnia *Daphnia pulex* (Weider, 1984)…". It should be noted that two <u>very distinct organisms cannot be compared directly</u>, simply due to the specificity of their life cycle. *Daphnia* have a very complex reproduction system, which includes parthenogenesis, sexual reproduction and rest-

ing eggs. Resting eggs can be dormant for thousands of years. When they awake from dormancy, one can believe that the vertical migration or the genetic composition of a local population has drastically changed due to the emergence of genes that had been dormant for thousands years and were not under the effect of the current selection regime (see Vanoverbeke, 2001). In Weider's paper quoted by the Russian Federation it is also stated that "vertical migration pattern may result from <u>clonal succession</u> as well as from <u>individual clonal variation</u>", which are two processes that would be inappropriate to liken to the redfish case.

The <u>RAPD</u> analysis referred to in the review was one of the first studies done, and many subsequent genetic studies confirmed this preliminary study. <u>AFLP</u> techniques are also not easily repeatable; even so, data obtained with these techniques do not appear to be in contrast to all other studies using a wide array of genetic markers. Most importantly, Cadrin *et al.* (2010) discuss cautiously all these aspects, essentially synthesizing the outcomes of the WKREDS (ICES, 2009) workshop, in which the Russian scientists participated and during which they agreed with the main conclusions of the group.

6.2   Document AM 2009/29rev1

This document provides a list of arguments that had been already taken into <u>full consideration</u> at WKREDS (ICES, 2009). The comments in document AM 2009/29rev1, however, mostly appear to <u>deviate</u> from the deliberations at WKREDS and are partly <u>inconsistent or contradictory</u>: For example, the lack of differences in occurrence of pigmented spots on the redfish skin and muscular melanin had been used as indications for the single-stock theory, but the document notes that these "criteria cannot be used to identify stock structure" and later states that pigmented spots are "reliable natural markers". Moreover, the argumentation is largely based on parasitological results, and there are obvious limitations in the data presented, e.g. the lack of statistical significance for the prevalence of some of the parasites in Table 4 and of *Sphyrion lumpi* in Table 5, which clearly deviates between depth layers and areas.

Largely, the presented arguments have already been considered at WKREDS, and the points reiterated in document AM 2009/29rev1 do not offer any substantial evidence in support of a single panmictic stock in *S. mentella* in the Irminger Sea and adjacent areas. SIMWG believes that available publications and data had been thoroughly examined at WKREDS and that the conclusions reached by that exercise – now also synthesized in the official scientific literature (Cadrin *et al.*, 2010) – still remain the most parsimonious and the most realistic interpretation for the case of *S. mentella*. Participants from the Russian Federation had participated in WKREDS and agreed with its outcome at the end of the meeting. SIMWG does not see any new, consistent, robust, convincing scientific framework, contained in the latest documents submitted to NEAFC, for considering *S. mentella* in the Irminger Sea and adjacent waters as a single panmictic unit; thus, SIMWG finds no plausible reason for rejecting the agreed outcome of WKREDS 2009.

Although SIMWG fully supports the recommendations of WKREDS, we are aware that more studies should be conducted still, in order to learn more about the population biology of *S. mentella*, and obtain precious information that could also be useful for management. In particular, the following aspects might be worth tackling:

- Some new targeted statistical analyses could help: analysis by smaller depth 'layers', such as 100- or 200m-intervals, or by "age intervals" (e.g. 0–5; 6–10, 11–15… years).

- Consider aspects of fisheries-induced evolution: for example, it could be interesting to see if differences in size-at-maturation (over the period of recent exploitation) can be identified between "shallow oceanic" and "deep sea" components. The ICES Working Group on Fisheries-Induced Evolution (WGEVO) could take this issue into consideration perhaps in 2011.

- Calculate sex ratios by length-class and test for differences between 'shallow' and 'deep' *S. mentella*, in order to investigate potential effects of sex-biased fishing pressure.

- Use simulations to examine mechanizms of neutral genetic divergence: under which temporal (generational), and effective population size scenarios would it take to reach an observed F_{ST} of nearly 0.02, in presence of moderate to low gene flow?

- Future studies should also consider genetic analysis of juveniles from off Greenland. Do their allelic frequencies resemble the "shallow" or the "deep" type? Are they a mixture?

- Variation in the rhodopsin gene (which confers adaptation to dim light, cf. Sivasundar and Palumbi, 2010) should be assessed to see if it fits with: a) the "divergence with depth" hypothesis, and b) the recent evolutionary reconstruction provided for the Pacific rockfish.

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Annex 1: List of participants

Name	Email
Mikaela Bergenius	mikaela.bergenius@fiskeriverket.se
Steve Cadrin	steven.cadrin@noaa.gov
Ann-Britt Florin	ann-britt.florin@fiskeriverket.se
Stefano Mariani **(Chair)**	stefano.mariani@ucd.ie
Henrik Møsegaard	hm@dfu.min.dk
Christophe Pampoulie	christophe@duo-nature.com
Christoph Stransky	christoph.stransky@vti.bund.de

Annex 2: Recommendations

RECOMMENDATIONS	FOR FOLLOW UP BY:
1. SIMWG members present at the meeting in Sweden have agreed to have Stefano Mariani serve for another 3-year term as Chair of the Group. In order to guarantee pluralism, a further endorsement will also be sought by the other members who were not present.	SIMWG, SSGSUE, SCICOM
2. It is a unanimous opinion of SIMWG that the documentation provided by the Russian Federation to NEAFC regarding Sebastes mentella does not provide any solid evidence for considering the population units in the Irminger Sea and adjacent areas as a single biological stock.	NEAFC, SSGSUE, SCICOM, ACOM
3. The two main functions of SIMWG are hereby clearly defined: a) SIMWG is available to express expert opinions on matters of Stock Identification on a yearly basis on all stocks and areas of interest to ICES, provided that the Group Chairs clearly express the need for feedback from SIMWG in their Report's recommendations; b) SIMWG will regularly review and collate new developments in Stock Identification methods and will ensure to keep up with the advances in the field to the best of the members' abilities.	SIMWG, SSGSUE, SCICOM, ACOM
4. SIMWG members believe that there should be a more clearly defined communication system between expert groups and SIMWG on matters of Stock Identification. Liaisons between SIMWG and Expert Groups can be optimized if recommendations to involve SIMWG are clearly expressed in annual reports and that SCICOM directly forwards these requests to SIMWG.	SIMWG, WGCHAIRS, SSGSUE, SCICOM, ACOM
5. SIMWG members will continue to work on a "Stock Identification Procedure for the Integration of Multiple Methods" and plans to submit a manuscript to an international peer-reviewed journal as a result of this ongoing work, and to implement a description of this approach in the SIMWG Annual Report for 2011.	SIMWG, SSGSUE, SCICOM
6. Consider employing "species-level" identification methods – such as for instance DNA barcoding – for some multispecies fisheries (e.g. Trigla, Ammodytes, Merluccius, Lophius, Sebastes, Aphanopus, Dipturus, Raja, Mustelus).	SIMWG, WGCHAIRS, WGEF, SSGSUE, SCICOM
7. SIMWG members will submit at least one proposal for a Theme Session for the ASC 2011 by 6 September 2010.	SIMWG, SSGSUE, SCICOM, ACOM

Annex 3: SIMWG terms of reference for the next meeting

The **Stock Identification Methods Working Group** (SIMWG), chaired by Stefano Mariani, Ireland, will work by correspondence in 2011 with the following Terms of Reference:

a) Review and report on new advances in stock identification methods as they develop, as well as new results that are relevant to ICES work;

b) Provide technical reviews and expert opinions on matters of Stock Identifications, as requested by specific Working Groups and SCICOM;

c) Present and illustrate thoroughly a "Stock Identification Procedure for the Integration of Multiple Methods".

d) Review the scientific resources and tools available to ICES for investigating stock structure and determining appropriate management units, as well as the relevant limitations and gaps in the scientific capacity of ICES for carrying out such activities.

SIMWG will report by 31 July, 2011 (via SSGSUE) for the attention of SCICOM and ACOM.

Supporting Information

Priority	Understanding stock structure is a fundamental requirement before any assessment or modelling on a stock level can be contemplated. SIMWG liaises with ICES expert groups and working groups on stock identification issues and continues to review new methods as they develop.
Scientific Justification and relation to Action Plan	Action Plan No 1 – Action 1.2.1: Understand and quantify stock structure of commercially and ecologically important species. [SSGSUE] Stock structure and stock identification have been identified as part of the work programme of the Steering Group on Sustainable Use of Ecosystems (SSGSUE) and SIMWG continues to make progress on the development of its Stock Identification Methodology. After the publication of a book on Stock Identification Methods (2005), SIMWG will now develop practical standardized protocols for the stock identification process, and for the integration of results from multiple disciplines
Resource Requirements	SharePoint website and clear feedback from expert groups, SCICOM and SSGSUE is pivotal for the efficacy of SIMWG.
Participants	10–15
Secretariat Facilities	None
Financial	It should be noted that, being the Chair of this group NOT funded by his National Government (he works for a University), he will not be able to travel to ICES meetings, WGCHAIRS, etc., unless ICES commits to provide travel and accommodation support.
Linkages to Advisory Committees	ACOM
Linkages to other Committees or Groups	WGNEW, WGDEEP, WGEF, WGAGFM. SSGSUE.
Linkages to other Organizations	There are no direct linkages to other organizations.