ICES SIMWG REPORT 2011

SCICOM STEERING GROUP ON SUSTAINABLE USE OF ECOSYSTEMS

ICES CM 2011/SSGSUE:06

REF. SCICOM, ACOM

Report of the Stock Identification Methods Working Group (SIMWG)

By Correspondence in 2011

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

Recommended format for purposes of citation:

ICES. 2011. Report of the Stock Identification Methods Working Group (SIMWG). By Correspondence in 2011. ICES CM 2011/SSGSUE:06. 91 pp.
https://doi.org/10.17895/ices.pub.9072

Contents

Executive summary

The Stock Identification Methods Working Group (SIMWG) worked by correspondence in 2011.

The agenda originally comprised five 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 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;

e) Evaluate any new information relevant to the stock identity of deep-water stocks and to make recommendations to WGDEEP on the geographical composition of stock units where new information is available.

An update on the most significant advances from last year's exhaustive review was briefly provided for ToR a).

In relation to ToR b), the WKBENCH 2011 requested to look into their suggestion that haddock (*Melanogrammus aeglefinus*) in the North Sea and the West of Scotland may form a single demographic unit. HAWG requested a judgement on sprat (*Sprattus sprattus*) stock structure in the Celtic ecoregion. NEAFC and ACOM requested to follow recent developments on *Sebastes mentella* in the Irminger Sea and adjacent areas, with special focus on a new set of documents produced by the Russian federation.

The work for ToR c) will be implemented in a special new section of the second edition of Cadrin *et al.* (2005), which will be published by Elsevier between the end of 2012 and the beginning of 2013.

Tor d) and e) could not be tackled this year. SIMWG intends to dedicate a ToR on deep sea stock structure in 2012.

One year after meeting in person in 2010, SIMWG feel that greater success can be achieved by meeting in person in 2012. The Chair will circulate potential dates and locations in September. A final decision will be made before the end of 2011.

1 ToR a) – Advances in stock identification methods and results relevant to ICES work

Advances in stock identification between 2010 and 2011 have mainly pertained: a) the introduction of Single Nucleotide Polymorphisms (SNPs) as practical genetic tools, for which we refer to the WGAGFM latest report (http://www.ices.dk/workinggroups/ViewWorkingGroup.aspx?ID=164); b) the increased sophistication of computational and modelling approaches to analyse otolith microchemistry data (Mercier *et al*., 2011); c) the increase of interdisciplinary approaches within otolith-based methods, such as trace element coupled by shape analysis (Ferguson *et al*., 2011) and trace element coupled with stable isotope analysis (Longmore *et al*., 2011); and d) the attempt to link parasitic infestation with fish trophic level (Timi *et al*., 2011).

Relevant to this ToR, it is worth mentioning that the Workshop on the Implications of Stock Structure (WKISS) met at ICES Headquarters, 5–7 April 2011, chaired by Lisa Kerr (USA) and Niels Hintzen (The Netherlands) to examine the implications of complex stock structure on fish resources, fisheries, stock assessment and management. This work is seen as a key initiative, as it specifically looks into the consequences of assuming different stock boundaries for the purpose of assessment and management. One major concern is, for instance, the potential for overexploitation of unique spawning components which can potentially result in a loss of productivity, genetic diversity and destabilization of local and regional dynamics. Further, ignoring complex population structure and connectivity may impact the accuracy of stock assessments and effectiveness of management actions. WKISS addressed how simulation modelling can be used, in conjunction with biological information gathered from stock identity projects, to evaluate implications of complex stock structure for provision of reliable advice.

2 ToR b) – Liaisons with other ICES groups and organisations

2.1 Sprat

HAWG requested SIMWG to provide advice on stock structure/number/boundaries in sprat (*Sprattus sprattus*) in the Celtic Ecoregions (sub-areas VI and VII). Unfortunately there is no detailed population structure information in the literature regarding this species in these areas. One recent study by Limborg *et al.* (2009) provides some insights into connectivity patterns in sprat, but only one location in the Celtic Sea was examined. Therefore, SIMWG is in no position to judge the status of spatial structure of sprat in the Celtic ecoregion; however, it should be noted that in Limborg *et al.* (2009) the Celtic Sea population was not significantly differentiated from the German Bight samples, suggesting the potential for large scale homogeneity, at least outside the Kattegat/Baltic areas. New data must be generated for the Celtic Ecoregion, using any appropriate technique, before any view can be expressed on sprat stock structure in this area.

2.2 Haddock

WKBENCH concluded that "... there is independent evidence from research vessel surveys, VMS data and hydrobiological stock models [that haddock from the West of Scotland (ICES area VI) and the North Sea (area IV)] are in fact members of the same biological unit. If this is the case, then treating them as separate in the assessment and management cycle may be inducing unwanted bias and uncertainty...".

SIMWG notes that published information on haddock stock structure, especially in the above-mentioned areas, is extremely limited. Reiss *et al.* (2009) recently suggested that a mismatch between biological evidence and current management strategy existed in the case of haddock in these areas, but they did not uphold a view of a single panmictic unit. Wright *et al.* (2010) looked into habitat use and connectivity in juvenile and adult haddock from these areas, using otolith microchemistry, and, despite unveiling some considerable degree of connectivity, they also showed that during some part of their life cycle, juvenile haddock were localised in separate nursery areas, with high level of discrimination between them. Based on current knowledge SIMWG does not see merit in assuming a single population unit all across areas VI and IV, and rather call for greater efforts in examining stock structure in this highly valuable species, using state-of-the-art techniques and an exhaustive sampling design.

2.3 Redfish (*Sebastes mentella*)

In April this year, scientists from the Russian Federation forwarded three new documents raising methodological concerns and providing new data in the attempt to rebuke the recently re-defined management areas for *Sebastes mentella* in the Irminger Sea. These documents are enclosed (Annex 4). SIMWG received NEAFC and ACOM requests to continue reviewing the stock structure of *S. mentella* in the Irminger Sea and adjacent waters and to independently assess the new Russian documentation and verify its strength in relation to *S. mentella* management areas. The review process was conducted through the use of two independent non-European experts, who had not been previously involved in *S. mentella* management issues. The experts, hereafter referred to as "Referee 1" and "Referee 2", are two experienced fish population biologists, with complementary expertise, based in North America.

Based on the assessments from referees 1 and 2 (which can be perused *in extenso* in Appendix 4 of this report), the new documents by the Russian Federation do not provide sufficient substance to refute Cadrin *et al.*'s (2010) appraisal and certainly do not warrant the Russian assertion that *S. mentella* in the Irminger Sea represents a single panmictic unit.

The following key points are elaborated upon in the extensive referees' reports in Annex 5:

- It is not correct to state that only loci that are not under selection should be considered for fisheries problems. Non-neutral loci should not be ignored; rather, they should simply be interpreted differently from neutral loci, as they provide different information, which could still be relevant for fisheries management.
- A major fallacy pervasively found in several passages of the Russian documents is that one can "prove" the null hypothesis of panmixia. This is not true: one can either reject or fail to reject the null hypothesis, but failure to reject it does not offer proof. In any case, there are abundant data which in fact reject the panmixia hypothesis using several methods (again, reviewed in Cadrin *et al.* (2010).
- An increased infestation of the parasite *Sphyrion lumpi* with depth is explained with a hypothetical migration from the deep Greenland slope water to adjacent pelagial areas, but this is just a hypothesis. However attractive it might be, no data are provided to support this migration hypothesis. Additionally, Cadrin *et al*. (2010) cite a number of papers that show conflicting patterns regarding the prevalence of infestation of *Sphyrion lumpi* by depth.
- Makhrov *et al.* claim that the samples in Danielsdóttir *et al.* (2008) and in Stefansson *et al.* (2009) are not organized into deep and shallow samples, presumably because the samples were originally collected based on phenotype. Cadrin *et al.* (2010) acknowledge this, but also mention that nearly all of the "deep-sea" samples (95%) came from deep water and nearly all of the "oceanic" samples (93%) came from shallow water, and that Stefansson *et al.* (2009) regrouped samples by depth and reanalyzed them, demonstrating differences based on depth.
- Overall consistency of shallow/deep structuring patterns across several years and markers are believed to be sufficient to rule out artifacts due to sampling variability.
- No evidence exists to indicate that there is a causal relationship between *S. lumpi* infestation and fish mortality.
- The new microsatellite data provided in the Russian documentation does not appear consistent with previous microsatellite studies on this species in similar areas; however, we do not believe that the addition of new information that contrasts with existing studies should necessarily refute existing studies. Rather, one should examine what could have caused the differences and which information appears more reliable. In the new Russian data, depth information is sorely lacking and numbers are unsatisfactory: The authors should at least provide the depth range of the trawl (where the samples were collected). Simply stating that the samples were collected below and above 500m is not enough (e.g. 501m is below and 499m is above!). This is a massive limitation that renders the new results

almost uninterpretable. Additionally, sample size is limited, varying between 12 and 31. Table 2 indicates that the results are based on even lower sample sizes and that sample size varies across loci within a given sample. Small sample sizes associated with uneven sample sizes across loci are likely to influence statistical comparisons.

- The data analysis was not sophisticated enough, nor appropriately tailored to the specific questions being addressed (see details in Appendix 4).
- The authors mention that the genetic analyses carried out demonstrate that there is no population structure of *S. mentella* associated with either depth or geographic location in the Irminger Sea. However, the maximum likelihood tree based on Fst values shows some organisation of samples according to depth with only two exceptions: samples B66 and A41.
- Overall, based on knowledge on Pacific *Sebastes* species, population structure, segregation and even speciation associated with depth layers, it is not surprising that similar phenomena were observed also in Atlantic species.

Finally, SIMWG wish to point out that a summarized version of the Russian critique was submitted recently as a comment to the ICES Journal of Marine Science (Makhrov *et al*. 2011), and was rebuked by Cadrin *et al*. (2011). Both short commentaries are due to soon appear in a forthcoming issue of the Journal.

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

In recent weeks, Elsevier has agreed to publish a second edition of Cadrin *et al.*'s (2005) book "Stock Identification Methods", which will update this important work with all the many recent advances in the relevant fields. The book will be co-edited by Steve Cadrin, Lisa Kerr and Stefano Mariani, and the work of the present ToR (as started in last year's report) (ICES, 2010) will be implemented to inform a special section in the new edition of the book, specifically devoted to 'interdisciplinary analyses' in stock identification and conservation biology.

4 References

Cadrin, S. X., Bernreuther, M., Danielsdóttir, A. K., Hjörleifsson, E., Johansson, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigurðsson, T., and Stransky, C. 2010. Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats. *ICES Journal of Marine Science*, 67: 1617–1630.

Cadrin, S. X., Friedland, K. D., and Waldman, J. R. 2005. Stock Identification Methods: Applications in Fishery Science. Elsevier, 736 pp.

Cadrin, S. X., Mariani, S., Pampoulie, C., Bernreuther, Daníelsdóttir, A., Johanssen, T., Kerr, L., Nedreaas, K., Reinert, J., Saborido-Rey, F., Sigurðsson, T., and Stransky, C. 2011. Countercomment on: Cadrin *et al.* (2010) 'Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats'. ICES Journal of Marine Science, 67: 1617–1630. *ICES Journal of Marine Science*, doi:10.1093/icesjms/fsr134.

Danielsdóttir, A. K., Gislason, D., Kristinsson, K., Stefansson, M. O., Johansen, T., and Pampoulie, C. 2008. Population structure of deep-sea and oceanic phenotypes of deepwater redfish in the Irminger Sea and Icelandic continental slope: are they cryptic species? *Transactions of the American Fisheries Society*, 137: 1723–1740.

Ferguson, G. J. (Ferguson, Greg J.)1; Ward, T. M. (Ward, Tim M.)1; Gillanders, B. M. (Gillanders, Bronwyn M.). 2011. Otolith shape and elemental composition: Complementary tools for stock discrimination of mulloway (*Argyrosomus japonicus*) in southern Australia. FISHERIES RESEARCH, Volume: 110, Issue: 1, Pages: 75-83.

ICES. 2010. Report of the Stock Identification Methods Working Group (SIMWG). 21–23 June 2010, Öregrund, Sweden. ICES CM 2010/SSGSUE:04. 33 pp.

Limborg, M. T., Pedersen, J. S., Hemmer-Hansen, J., Tomkiewicz, J., Bekkevold, D. 2009. Genetic population structure of European sprat *Sprattus sprattus*: differentiation across a steep environmental gradient in a small pelagic fish. *Mar Ecol Prog Ser*, 379: 213–224.

Longmore C., Trueman C. N., Neat F., O'Gorman E., Milton J. A., and Mariani, S. 2011. Otolith geochemistry indicates life-long spatial population structuring in a deep sea fish, Coryphaenoides rupestris. *Marine Ecology Progress Series*, 435: 209–224.

Makhrov, A. A., Artamonova, V. S., Popov, V. I., Rolskiy, A. Y., Bakay, Y. I. 2011. Comment on: Cadrin *et al*. (2010) 'Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats'. ICES Journal of Marine Science, 67: 1617–1630. *ICES Journal of Marine Science*, 68: 000-000.

Mercier, L., Darnaude, A. M., Bruguier, O., Vasconcelos, R. P., Cabral, H. N., Costa, M. J., Lara, M., Jones, D. L., Mouillot, D. 2011. Higher predictability of fish nursery areas by otolith microchemistry using non-linear classification methods and optimal element combination. *Ecol Appl*., 21: 1352–1364.

Reiss, H., Hoarau, G., Dickey-Collas, M., Wolff, W.J. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries*, 10: 361–395.

Timi, J. T. 1; Rossin, M. A., Alarcos, A. J., Braicovich, P. E., Cantatore, D. M. P., Lanfranchi, A. L. 2011. Fish trophic level and the similarity of non-specific larval parasite assemblages. *International Journal for Parasitology*, 41: 309–316.

Wright, P. J., Tobin, D., Gibb, F. M., Gibb I. M. 2010. Assessing nursery contribution to recruitment: relevance of closed areas to haddock *Melanogrammus aeglefinus*. *Mar Ecol Prog Series*, 400: 221–232.

Annex 1: List of participants

Name	E-mail
Stefano Mariani (Chair)	s.mariani@salford.ac.uk
Christophe Pampoulie	chrisp@hafro.is
Steve Cadrin	scadrin@umassd.edu
Jean-Marie Sevigny	jean-marie.sevigny@dfo-mpo.gc.ca
Paul Spencer	paul.spencer@noaa.gov
Vladimir Vinnichenko	vinn@pinro.ru
Evgeny Shamray	shamray@pinro.ru
Alexander A. Makhrov	makhrov12@mail.ru
Viktor Popov	popov@pinro.ru
Alexey Rolskiy	rolskiy@pinro.ru
Yuri Bakay	bakay@pinro.ru
Valentina Artamonova	valar99@mail.ru

Annex 2: Recommendations

Recommendations	For follow up by:
1. SIMWG believe there is simply insufficient biological data to inform the status of stock structure of Sprat in the Celtic ecoregion.	HAWG, SSGUE, SCICOM
2. SIMWG feel there is insufficient ground for assuming a single-stock unit of haddock across the North Sea and the West of Scotland. SIMWG advises that more research is necessary on this topic.	WKBENCH, SCICOM, ACOM
3. SIMWG feel that the new documentation provided by the Russian Federation regarding *Sebastes mentella* does not provide solid justification for considering the population units in the Irminger Sea and adjacent areas as a single biological stock.	NEAFC, SSGSUE, SCICOM, ACOM
4. 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
5. 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
6. SIMWG members will submit at least one proposal for a Theme Session for the ASC 2012 by 5 September 2011.	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 organise a physical meeting in 2012, (venue and dates in July 2012 to be confirmed) to:

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) Evaluate any new information relevant to the stock identity of deep-water stocks and to make recommendations to WGDEEP on the geographical composition of stock units where new information is available.

SIMWG will report by 15 August, 2012 (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.
Linkages to other Organizations	There are no direct linkages to other organizations.

Annex 4: Documents forwarded by the Russian Federation on the intra-specific structure of Redfish in the Irminger Sea

New microsatellite data on redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters

by

Daria A. Zelenina, Sergey P. Melnikov, and Nikolai S. Mugue

Russian Federal Research Institute of Fisheries and Oceanography (VNIRO)
17, V. Krasnoselskaya st., Moscow, 107140, Russia
e-mail: dzel67@mail.ru

Summary

We have analyzed 19 samples (total 454 fish) of *Sebastes mentella* throughout the Irminger Sea from different depths (above and below 500 m.) and collected in different years (2005, 2007, 2009). All samples were scored for eleven microsatellite loci, ten of which were used in Stefansson et al., 2009b. Our data indicate the absence of significant genetic segregation based on microsatellite loci between “deep-pelagic” and “oceanic” samples in the Irminger Sea. Samples taken from the same geographic area (but from different depths) manifest more genetic similarities compare with the geographically distant samples from the same depth. In addition, no temporal stability was revealed, and the samples from the same area but taken in different years did not cluster together. The results clearly indicate that while pelagic *Sebastes mentella* has prominent polymorphism revealed by meristick, morphometric, and genetic data (ICES, 2009), neither geographical nor temporal stability in genetic structure from deep- and shallow pelagic zones has been confirmed. The proposed assignment of two management units is not justified and coordinated multi-national study of genetic structure for Sebastes mentella is desired.

Introduction

Since 1996, in view of the ICES recommendations, NEAFC is controlling pelagic fishery in the Irminger Sea and adjacent waters on the basis of one management unit. In 2009, based on the conclusions of the Workshop on Redfish Stock Structure (WKREDS), ICES for the first time recommended to NEAFC two pelagic management units (ICES, 2009). Additionally, it was stated that suggested boundaries of management units effectively delineate the pelagic fishery in the northeast Irminger Sea from the pelagic fishery in the southwest Irminger Sea, with a small portion of mixed-stock catches (Anon., 2009).

The decision to recommend two management units of pelagic redfish instead of one was not unanimous among ACOM members. The Russian Federation could not agree with developing a scientific advice on the management of the Irminger Sea redfish stock because only part of the available scientific data was considered.

In ICES advise the following were noted:

1) Should important new scientific information become available then a future review may be appropriate.

2) The individual Stock Summary Sheets provide descriptions of these stocks Catch advice for the Shallow Pelagic stock and the Deep Pelagic stock can be summed if it is decided to continue with a single management unit for these stocks."

Following the ICES advice, additional researches on genetic structure of redfish using microsatellite method were conducted. That allows estimate scientific relevance of recommendations for introducing new management regime for pelagic redfish fishery in the Irminger Sea and adjacent waters.

Material and methods

Sample collection.

Eighteen samples from the Irminger Sea and one from the area of Rosengarten bank were analyzed. Seven samples were collected at the depth above 500 meter and 12 samples – below 500 m (Table 1). The total number of specimens was 464. The strategy of sample collection was different from that described in the paper of Stefánsson et al., 2009a. In our study samples from depths above 500 m and below 500 m were collected in different regions over the entire area of the Irminger Sea (Figure 1) while in Stefansson's study all "above 500 m" samples originated from the north-eastern part of the sea and samples "below 500 m' are predominantly taken from the South-West part of the region.

DNA extraction and microsatellite analysis.

DNA was extracted from fin clips fixed in 96% ethanol using the Promega Wizard SV 96 Genomic Purification System according to manufacturers. All specimens were scored for 13 microsatellite loci: SEB9, SEB25, SEB31, SEB33, SEB45 (Roques *et al*., 1999); Spi4, Spi6, Spi10 (Gomez-Uchida *et al*., 2003); Smen5, Smen10 (Stefansson *et al*., 2009); Sal1, Sal3, Sal4 (Miller *et al*., 2000). However, due to abundance of null- alleles or unreliability in allele size assessment, two loci (Spi4 and SEB33) were discarded from further analysis

Polymerase chain reactions (PCR) were performed in a reaction volume of 15 µl consisting of 50-100 ng DNA template, 70 mM Трис-HCl (pH 8.6), 16,6 mM $(NH_4)_2SO_4$, 1,8 mM $MgCl_2$, 200 µM each of dNTPs, 1 pM of labeled with FAM, HEX or TAMRA universal (M13) primer, 0,75 pM of M13-tailed forward primer, 4 pM of reverse primer and 0,8 U of Taq-polymerase (Sileks, Russia) in thermocycler PTC-225 Peltier Thermal Cycler (MJ Research). PCR touch-down cycling protocol consisted of denaturation at 94 °C for 1 min followed by 30 cycles (denaturation – 95 °C, 20 s., annealing 25 s at 58 °C -0.2 °C in every cycle, elongation 40 s at 65 °C) followed by additional 7 cycles (20 s at 90 °C, 25 s at 52 °C and 40 s at 65 °C). The last step was designed to insure the proper incorporation

of fluorescent-labeled universal primers into PCR product. Final extension was for 10 min at 70 °C.
PCR products were genotyped with ABI3100 Genetic analyzer, with assistance of GeneMapper software .

Statistical analysis. Genetic diversity (expected (He) and observed (Ho) heterozygosity) and deviations from Hardy-Weinberg expectation (inbreeding coefficient (Fis) and corresponding p-values) were evaluated in GenAlEx6 (Peakall and Smouse, 2006). The same software was used for Principle component analysis performing. Pairwise Fst meanings (Weir and Cockerham, 1984) were calculated in FSTAT program (Goudet, 1995). The ML dendrogramm of genetic similarity of samples based on Fst was calculated with Phylip v.3.69 (Felsenstein, 2009).

Results and Discussion

Genetic variability.
Most samples were in HWE and deviations were not related to particular samples or loci (Table 2). Allelic richness and expected heterozygosity for each locus were similar to data reported, however, observed heterozygosities averaged across “deep” and “shallow” samples were very similar (0,689 and 0.701 respectively) in contrast with the Stefansson et. al., (2009b).

Spatial genetic structure analysis.
Samples analyzed in this study were collected from two regions of the Irminger Sea - North-East region to the North from 59N, and South-West region, located to the south from 59N latitude. In both regions samples were taken from both shallow (above 500m) and deep (below 500 m) water layers. As a reference, sample from Rozengarten bank was also included in the analysis. To reveal the existence of genetic differentiation, pairwise genetic distances based on Fst values were calculated (Table 3). Pairwise Fst ranged from 0 to 0,040 (the highest value was between sample A58 and Rozengarten Bank). Cluster analysis based on among populations genetic distances did not revealed clades with significant (over 70) bootstrap support (Fig.2). There is no evidence for separate clustering of deep and shallow samples. There is some genetic signal for separation among samples based on geographic location. However, two samples from North-East area (A47 and A89, Fig.2) are clearly located within South-West cluster of samples. These data indicate that in case of existence any genetic structure in pelagic population of *Sebastes mentella* this structure does not correspond neither with 59N latitude boundary, nor with depth of 500 meters.
Principal component analysis also does not reveal stable relationship among deep and shallow samples. Three samples from North-East part of the area are located within the South-West cluster.

Temporal genetic structure analysis.

A majority of the samples (13) studied were collected in 2007, but we have also analyzed four samples collected in 2005 and two samples from 2009. We have applied the sampling strategy implicated in Danıelsdottir et al., 2008, where the samples from the same geographic locations were taken in different years. Genetic similarity between geographically and bathymetrically close samples collected in different years would indicate temporally stable genetic structure, while lack of correlation between samples from different years would evidence for dynamic nature of cohort distribution and temporally unstable genetic structure of deep sea redfish. Our results indicate that samples from different years, although collected in the same locations, do not cluster together (Figs 2 and 3). Genetic difference between samples B40 (2007) and B22 (2009) from the same depth and geographic coordinates is one among the highest observed (Fst=0.020) and these two samples are placed in different clusters on the PCA plot (Fig.3). These data indicate that the temporal genetic structure in *S. mentella* population is not stable.

Conclusions

Our study has applied the same methodology and the same genetic markers as in the recent investigations of Stefansson et al., 2009 a, b. The highly overlapping set of microsatellite loci was used to estimate genetic structure on the samples throughout the Irminger Sea. However, we have significantly improved the methodology of the study by including both “deep” and “shallow” samples from every parts of the region (in the abovementioned study all “deep” samples where located at North-East region and all “shallow” samples came from South-West part of the area investigated). Also, we have included samples from three different years (2005, 2007 and 2009). As a result of this study, no scientific grounds for declaring stable genetic segregation of “deep-” and “shallow -pelagic” forms of *S. mentella* in the North Atlantic region and for delineation of management boundaries were found. Our results clearly indicate that genetic polymorphism found in pelagic *Sebastes mentella* (summarized by Cadrin et al., 2010) does not demonstrate spatial or temporal stability and further investigation on genetic structure of *S. mentella* and development of coordinated research program on biology, ecology and genetics of this valuable species is required.

Literature cited

Anon., 2009. ICES Advise 2009, Book 2.

Cadrin, S. X., Bernreuther, M., Danıelsdottir, A. K., Hjorleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigursson, T., and Stransky, C. 2010. Population structure of beaked redfish, Sebastes mentella: evidence of divergence associated with different habitats. ICES Journal of Marine Science, 67: 1617–1630.

Danıelsdottir, A. K., Gıslason, D., Kristinsson, K., Stefansson, M. O., Johansen, T., and Pampoulie, C. 2008. Population structure of deep-sea and oceanic phenotypes of deepwater redfish in the Irminger Sea and Icelandic continental slope: are they cryptic species? Transactions of the American Fisheries Society, 137: 1723–1740.

Felsenstein, J. PHYLIP (Phylogeny Inference Package) version 3.69 Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, WA. 2009

Gomez-Uchida, D., Hoffman, E. A., Ardren, W. R., and Banks, M. A. 2003. Microsatellite markers for the heavily exploited canary (Sebastes pinniger) and other rockfish species. Molecular Ecology Notes, 3: 387–389.

Goudet, J. 1995. Fstat (Version 1.2): a computer program to calculate F-statistics. J. Heredity, 86: 485–486

ICES. 2009. Report of the Workshop on Redfish Stock Structure (WKREDS), 22-23 January 2009, ICES Headquarters, Copenhagen. Diane. 71 pp.

Miller, K. M., Schulze, A. D., and Withler, R. E. 2000. Characterization of microsatellite loci in Sebastes alutus and their conservation in congeneric rockfish species. Molecular Ecology, 9: 240–242.

Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6, 288-295.

Roques, S., Pallotta, D., Sevigny, J. M., and Bernatchez, L. 1999. Isolation and characterization of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei: Scorpaenidae, genus Sebastes). Molecular Ecology, 8: 685–687.

Stefansson, M. O., Sigurdsson, T., Pampoulie, C., Danıelsdottir, A. K., Thorgilsson, B., Ragnarsdottir, A., Gıslason, D., Coughlan, J., Cross, T. F., Bernatchez, L. 2009a. Pleistocene genetic legacy suggests incipient species of Sebastes mentella in the Irminger Sea. Heredity, 102: 514-524.

Stefansson, M. O., Reinert, J., Sigurdsson, T., Kristinsson, K., Nedreaas, K., Pampoulie, C. 2009b. Depth as a potential driver of genetic structure of Sebastes mentella across the North Atlantic Ocean. ICES Journal of Marine Science, 66: 680–690.

Weir, B. S., Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38:1358–1370.

Table 1. Sampling details: sample name, sample size, depth, date and position of capture.

Sample name	Sample size	Depth of capture	Date	Latitude (N)	Longitude (W)
A89	30	Above 500 m	20.06.2005	62º13´	33º20´
B90-1	21	Below 500 m	21.06.2005	62º59´	31º29´
B90-2			21.06.2005	62º43´	30º35´
A108-1	16	Above 500 m	30.06.2005	56º15´	42º27´
A108-2			30.06.2005	56º14´	42º11´
B110	16	Below 500 m	30.06.2005	56º15´	40º55´
B40	21	Below 500 m	25.06.2007	61º42´	29º08´
A41	30	Above 500 m	25.06.2007	62º06´	30º07´
B42	12	Below 500 m	26.06.2007	62º29´	28º20´
B44	19	Below 500 m	27.06.2007	63º12´	31º05´
A47	30	Above 500 m	28.06.2007	61º15´	34º55´
A58	30	Above 500 m	03.07.2007	57º59´	37º30´
B59	25	Below 500 m	03.07.2007	57º50´	37º42´
B61-1	31	Below 500 m	04.07.2007	55º42´	39º45´
B61-2			17.07.2007	56º15´	41º39´
B66	30	Below 500 m	07.07.2007	54º18´	47º39´
A67-1	26	Above 500 m	07.07.2007	54º42´	47º43´
A67-2			13.07.2007	54º15´	46º25´
A68	30	Above 500 m	07.07.2007	56º04´	47º57´
B69-1	20	Below 500 m	07.07.2007	56º11´	48º07´
B69-2			08.07.2007	56º15´	51º04´
Roz88	15	Below 500 m	21.07.2007	62º55´	12º46´
B15	31	Below 500 m	25.06.2009	61º18´	28º22´
B22	31	Below 500 m	01.07.2009	61º43´	29º10´

Table 2. Sample size (N), number of alleles (A), observed (Ho) and expected (He) heterozygosity, fixation index (Fis), test for deviation from Hardy-Weinberg proportions – p-value (HW) and significance of this deviation (ns=not significant, * p<0.05, ** p<0.01, *** p<0.001).

Sample	Variable	Loci										
		Spi10	***Spi6***	***Smen10***	***Sal4***	***Sal1***	***Smen5***	***Sal3***	***Seb9***	***Seb31***	***Seb45***	***Seb25***
A41	**N**	27	30	29	25	22	24	20	14	25	12	27
	A	19	12	10	3	11	11	5	7	8	9	7
	Ho	0.741	0.833	0.931	0.120	0.818	0.708	0.700	0.143	0.320	0.417	0.778
	He	0.844	0.871	0.837	0.185	0.872	0.848	0.716	0.837	0.755	0.809	0.791
	Fis	0.123	0.043	-0.112	0.351	0.062	0.165	0.023	0.829	0.576	0.485	0.017
	HW	0.095	0.291	0.995	0.015	0.306	0.521	0.256	0.000	0.000	0.001	0.904
	Signif	ns	ns	ns	*	ns	ns	ns	***	***	**	ns
A47	**N**	30	30	30	30	29	29	29	26	29	30	29
	A	10	10	9	3	13	10	6	10	10	18	10
	Ho	0.700	0.833	0.867	0.367	0.862	0.828	0.655	0.692	0.690	0.833	0.828
	He	0.652	0.834	0.755	0.384	0.876	0.842	0.627	0.768	0.796	0.880	0.769
	Fis	-0.073	0.001	-0.148	0.046	0.016	0.018	-0.046	0.098	0.134	0.053	-0.076
	HW	0.937	0.437	0.963	0.407	0.393	0.806	0.736	0.969	0.152	0.220	0.452
	Signif	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A58	**N**	30	30	30	29	29	29	30	30	30	29	27
	A	11	8	9	3	13	10	6	9	10	14	7
	Ho	0.567	0.800	0.867	0.345	0.966	0.655	0.600	0.833	0.800	0.897	0.741
	He	0.615	0.813	0.777	0.300	0.882	0.806	0.664	0.811	0.818	0.848	0.800
	Fis	0.079	0.016	-0.115	-0.151	-0.095	0.187	0.097	-0.027	0.022	-0.058	0.074
	HW	0.149	0.885	0.996	0.739	0.049	0.802	0.439	0.552	0.881	0.400	0.467
	Signif	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
A67	**N**	26	26	26	25	23	26	26	18	20	20	22
	A	9	10	7	3	12	9	4	9	7	14	9
	Ho	0.731	0.923	0.731	0.400	0.826	0.808	0.692	0.722	0.550	0.850	0.864
	He	0.733	0.832	0.703	0.460	0.877	0.813	0.630	0.762	0.665	0.860	0.787
	Fis	0.003	-0.109	-0.040	0.130	0.058	0.006	-0.099	0.053	0.173	0.012	-0.097
	HW	0.793	0.942	0.931	0.585	0.554	0.619	0.687	0.922	0.277	0.879	0.686
	Signif	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A68	**N**	30	29	29	30	26	29	30	24	30	23	21
	A	10	8	7	3	11	12	7	11	11	13	10
	Ho	0.700	0.759	0.724	0.300	0.808	0.862	0.700	0.875	0.767	0.696	0.810
	He	0.659	0.823	0.744	0.339	0.866	0.872	0.726	0.794	0.739	0.856	0.802
	Fis	-0.062	0.078	0.026	0.116	0.067	0.012	0.036	-0.102	-0.037	0.188	-0.010
	HW	0.738	0.182	0.161	0.189	0.122	0.034	0.983	0.016	1.000	0.081	0.088
	Signif	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns
A89	**N**	30	29	29	27	27	29	28	19	29	27	18
	A	11	10	7	3	13	10	7	8	11	14	9
	Ho	0.567	0.828	0.862	0.296	0.852	0.690	0.679	0.842	0.724	0.852	0.722
	He	0.653	0.853	0.745	0.318	0.883	0.855	0.673	0.735	0.789	0.861	0.704
	Fis	0.133	0.030	-0.157	0.069	0.035	0.193	-0.008	-0.145	0.082	0.010	-0.026
	HW	0.254	0.862	0.358	0.093	0.836	0.530	0.478	0.849	0.140	0.224	0.945

	Signif	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A108	**N**	10	10	10	15	16	14	16	10	12	14	12
	A	9	8	8	2	13	9	5	6	6	9	6
	Ho	0.800	0.500	0.600	0.200	0.750	0.786	0.563	0.400	0.583	0.786	0.667
	He	0.815	0.745	0.780	0.180	0.895	0.814	0.654	0.605	0.767	0.804	0.660
	Fis	0.018	0.329	0.231	-0.111	0.162	0.034	0.140	0.339	0.240	0.022	-0.011
	HW	0.588	0.035	0.142	0.667	0.709	0.180	0.223	0.004	0.634	0.924	0.566
	Signif	ns	*	ns	ns	ns	ns	ns	**	ns	ns	ns
B15	**N**	23	31	31	30	30	29	29	29	30	20	24
	A	11	11	8	5	11	9	6	8	16	11	7
	Ho	0.565	0.742	0.839	0.233	0.900	0.862	0.931	0.621	0.800	0.850	0.583
	He	0.677	0.868	0.795	0.214	0.863	0.834	0.730	0.760	0.883	0.839	0.760
	Fis	0.165	0.145	-0.055	-0.088	-0.042	-0.033	-0.275	0.183	0.094	-0.013	0.233
	HW	0.678	0.002	0.799	1.000	0.074	0.193	0.474	0.673	0.625	0.642	0.350
	Signif	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
B22	**N**	22	31	31	31	31	28	29	30	31	25	29
	A	12	11	9	4	14	12	6	9	14	18	7
	Ho	0.500	0.774	0.903	0.387	0.968	0.821	0.966	0.733	0.839	0.840	0.793
	He	0.654	0.866	0.823	0.360	0.884	0.864	0.730	0.775	0.886	0.909	0.791
	Fis	0.235	0.106	-0.098	-0.077	-0.094	0.049	-0.322	0.054	0.053	0.076	-0.003
	HW	0.000	0.330	0.945	0.552	0.883	0.030	0.279	0.715	0.479	0.032	0.235
	Signif	***	ns	ns	ns	ns	*	ns	ns	ns	*	ns
B40	**N**	21	21	20	21	17	18	13	14	16	13	20
	A	18	12	8	3	12	9	6	9	6	8	8
	Ho	0.762	0.762	0.700	0.143	0.941	0.833	0.692	0.429	0.125	0.385	0.800
	He	0.847	0.868	0.775	0.214	0.874	0.812	0.746	0.857	0.719	0.805	0.770
	Fis	0.100	0.123	0.097	0.333	-0.077	-0.027	0.071	0.500	0.826	0.522	-0.039
	HW	0.989	0.517	0.571	0.235	0.961	0.917	0.137	0.000	0.000	0.023	0.967
	Signif	ns	ns	ns	ns	ns	ns	ns	***	***	*	ns
B42	**N**	10	11	11	12	9	9	7	5	12	10	9
	A	11	9	6	3	8	8	4	4	5	11	6
	Ho	0.800	0.636	1.000	0.250	1.000	0.556	0.429	0.400	0.167	0.600	0.889
	He	0.800	0.855	0.814	0.226	0.858	0.858	0.643	0.640	0.628	0.880	0.778
	Fis	0.000	0.256	-0.228	-0.108	-0.165	0.353	0.333	0.375	0.735	0.318	-0.143
	HW	0.227	0.070	0.466	0.970	0.143	0.170	0.407	0.451	0.000	0.528	0.366
	Signif.	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns
B44	**N**	17	19	18	19	12	11	8	13	18	13	18
	A	19	10	9	3	9	8	5	9	10	13	9
	Ho	0.882	0.684	0.833	0.158	0.917	0.636	0.625	0.692	0.333	0.385	0.667
	He	0.913	0.860	0.792	0.278	0.826	0.835	0.609	0.852	0.784	0.858	0.770
	Fis	0.034	0.205	-0.053	0.433	-0.109	0.238	-0.026	0.188	0.575	0.552	0.134
	HW	0.584	0.023	0.862	0.008	0.520	0.297	0.034	0.014	0.000	0.000	0.214
	Signif	ns	*	ns	**	ns	ns	*	*	***	***	ns
B59	**N**	23	20	19	22	25	25	25	25	23	18	16
	A	21	8	8	3	14	10	6	10	12	14	9
	Ho	0.870	0.650	0.895	0.364	0.840	0.760	0.680	0.760	0.913	0.889	0.750
	He	0.853	0.834	0.785	0.313	0.894	0.861	0.577	0.758	0.864	0.869	0.762

	Fis	-0.020	0.220	-0.139	-0.162	0.060	0.117	-0.179	-0.003	-0.057	-0.023	0.015
	HW	0.409	0.311	0.880	0.780	0.065	0.464	0.978	0.953	0.168	0.636	0.658
	Signif	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B61	**N**	24	29	30	31	29	24	31	23	27	25	20
	A	10	9	9	4	11	12	6	8	15	14	8
	Ho	0.625	0.759	0.867	0.452	0.897	0.833	0.677	0.696	0.815	0.800	0.650
	He	0.633	0.840	0.823	0.377	0.877	0.864	0.627	0.747	0.853	0.831	0.784
	Fis	0.012	0.097	-0.053	-0.197	-0.022	0.035	-0.080	0.068	0.044	0.038	0.171
	HW	0.024	0.379	0.475	0.853	0.315	0.725	0.021	0.367	0.898	0.213	0.579
	Signif	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
B66	**N**	30	30	30	30	29	30	30	30	25	23	25
	A	10	9	7	3	11	11	5	10	11	14	7
	Ho	0.633	0.900	0.800	0.300	0.897	0.933	0.700	0.767	0.560	0.957	0.760
	He	0.561	0.823	0.726	0.316	0.879	0.836	0.607	0.719	0.692	0.853	0.730
	Fis	-0.130	-0.094	-0.103	0.051	-0.020	-0.117	-0.153	-0.066	0.191	-0.122	-0.042
	HW	1.000	0.878	0.815	0.227	0.079	0.041	0.864	0.987	0.284	0.357	0.975
	Signif	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
B69	**N**	20	20	20	20	19	19	19	18	18	14	15
	A	9	7	8	5	11	12	6	9	8	14	8
	Ho	0.800	0.800	0.750	0.350	0.895	0.842	0.632	0.722	0.667	0.929	0.800
	He	0.729	0.785	0.781	0.346	0.857	0.893	0.615	0.776	0.818	0.898	0.771
	Fis	-0.098	-0.019	0.040	-0.011	-0.044	0.057	-0.027	0.070	0.185	-0.034	-0.037
	HW	0.974	0.976	0.835	0.461	0.570	0.049	0.995	0.979	0.404	0.837	0.824
	Signif	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
B90	**N**	14	21	21	20	19	21	21	14	21	14	14
	A	11	11	9	3	10	10	6	8	11	11	8
	Ho	0.786	0.810	0.810	0.100	0.895	0.857	0.714	0.857	0.905	0.929	0.786
	He	0.870	0.846	0.793	0.184	0.855	0.789	0.711	0.793	0.848	0.865	0.781
	Fis	0.097	0.043	-0.021	0.456	-0.047	-0.086	-0.005	-0.080	-0.067	-0.074	-0.007
	HW	0.181	0.341	0.970	0.042	0.378	0.286	0.939	0.990	0.408	0.928	0.444
	Signif	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
B110	**N**	7	12	16	12	12	13	14	7	13	11	10
	A	6	8	8	2	9	6	6	7	8	12	8
	Ho	0.714	0.833	0.813	0.167	0.750	0.692	0.714	0.571	0.769	0.818	0.700
	He	0.786	0.847	0.859	0.153	0.826	0.784	0.712	0.755	0.781	0.888	0.805
	Fis	0.091	0.016	0.055	-0.091	0.092	0.117	-0.004	0.243	0.015	0.079	0.130
	HW	0.342	0.542	0.007	0.753	0.115	0.584	0.081	0.152	0.367	0.831	0.561
	Signif	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns
Roz88	**N**	14	15	15	15	15	14	14	6	12	13	13
	A	17	9	9	3	11	6	3	5	8	11	6
	Ho	0.786	0.800	0.933	0.333	1.000	0.857	0.643	0.667	0.500	0.923	0.615
	He	0.923	0.824	0.804	0.287	0.893	0.783	0.500	0.667	0.726	0.888	0.660
	Fis	0.149	0.030	-0.160	-0.163	-0.119	-0.094	-0.286	0.000	0.311	-0.040	0.067
	HW	0.104	0.781	0.971	0.896	0.293	0.437	0.498	0.694	0.751	0.085	0.387
	Signif	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 3. Pairwise Fst values for 19 beaked redfish samples. Detailed description of samples is given in Table 1.

	B42	B40	B44	A41	A47	B90	A89	B110	A108	A58	B59	A68	B69	B66	A67	B61	Roz88	B15	B22
B42	0.000																		
B40	-0.004	0.000																	
B44	-0.008	-0.002	0.000																
A41	-0.014	-0.008	-0.008	0.000															
A47	0.019	0.028	0.024	0.021	0.000														
B90	0.018	0.014	0.001	0.010	0.015	0.000													
A89	0.021	0.024	0.027	0.017	-0.003	0.024	0.000												
B110	0.012	0.021	0.013	0.011	0.016	0.004	0.023	0.000											
A108	0.016	0.033	0.028	0.023	0.005	0.017	-0.002	-0.001	0.000										
A58	0.019	0.021	0.025	0.014	-0.001	0.014	0.001	0.014	0.004	0.000									
B59	0.012	0.019	0.012	0.010	-0.001	0.009	0.001	0.007	0.004	0.005	0.000								
A68	0.019	0.027	0.022	0.015	0.008	0.021	0.004	0.006	0.008	0.004	0.007	0.000							
B69	0.005	0.018	0.022	0.005	0.001	0.012	-0.001	-0.003	0.005	-0.001	-0.004	-0.003	0.000						
B66	0.031	0.033	0.033	0.019	0.005	0.025	0.004	0.020	0.007	0.000	0.010	-0.001	0.003	0.000					
A67	0.015	0.017	0.022	0.014	0.001	0.012	-0.001	0.012	0.010	-0.000	0.005	-0.001	-0.004	-0.002	0.000				
B61	0.013	0.009	0.013	0.010	0.004	0.010	0.007	0.007	0.009	0.004	0.006	0.008	0.001	0.007	0.003	0.000			
Roz88	0.023	0.015	0.006	0.012	0.035	0.027	0.032	0.019	0.028	0.040	0.006	0.031	0.020	0.037	0.026	0.023	0.000		
B15	0.014	0.013	0.009	0.008	0.013	0.003	0.013	0.009	0.010	0.010	0.008	0.008	0.008	0.017	0.013	0.005	0.022	0.000	
B22	0.014	0.020	0.025	0.017	0.010	0.017	0.011	0.007	0.017	0.013	0.006	0.011	0.002	0.020	0.015	0.005	0.027	0.002	0.000

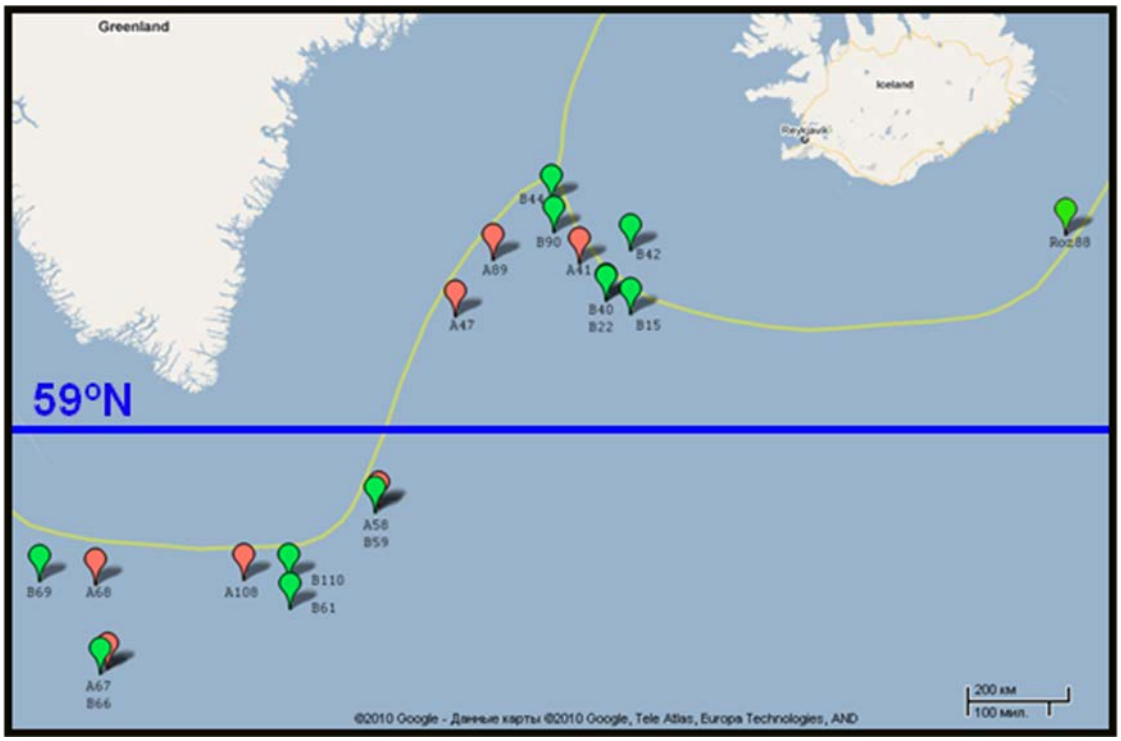

Figure 1. Location of 7 samples taken above 500 m (A##, red signs) and 12 samples taken below 500 m (B##, green signs).

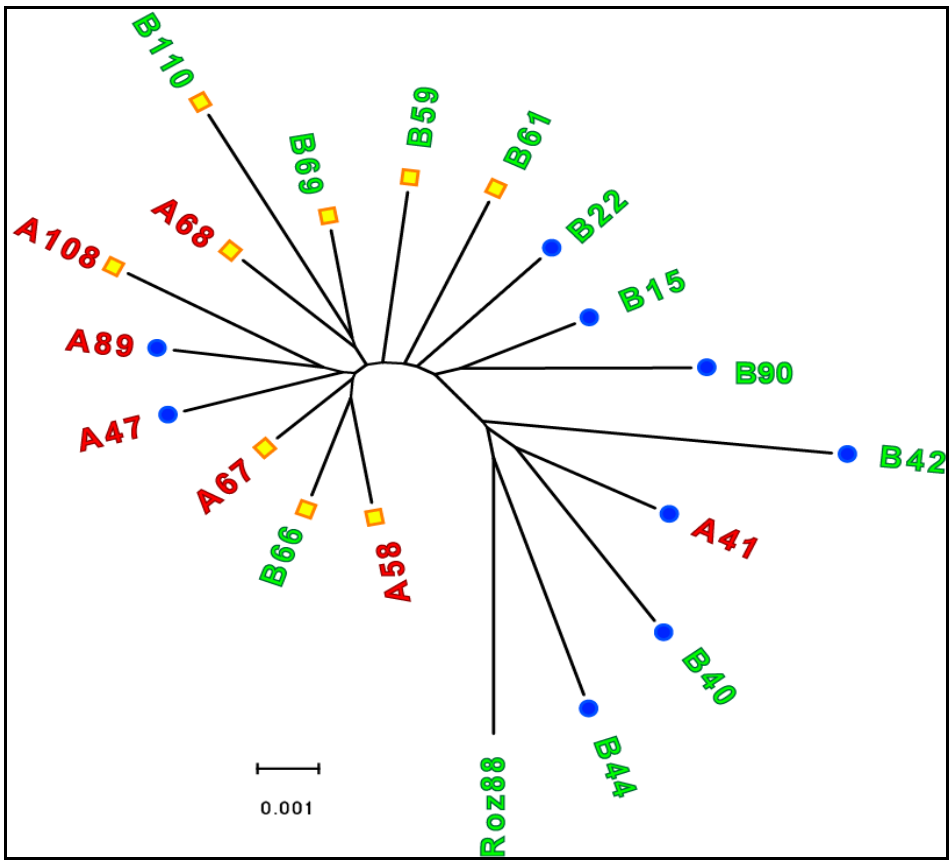

Figure 2. A maximum likelihood tree showing the relationship among 19 samples of *Sebastes mentella*. Samples taken above 500 m (A##, red numbers), below 500 m (B##, green numbers), to the north from 59°N parallel (blue circles) and to the south from 59°N parallel (yellow squares) are indicated.

Figure 3. The Principle component analysis (PCA) plot based on Fst values. Samples taken above 500 m (A##, red labels), below 500 m (B##, green labels), to the north from 59°N parallel (blue circles) and to the south from 59°N parallel (yellow squares) are shown.

The influence of temperature of the surface layer on distribution and pelagic fishery of *Sebastes mentella* in the Irminger Sea and adjacent waters

by

S.P. Melnikov*, A.L. Karsakov, A.P. Pedchenko****

* Russian Federal Research Institute of Fisheries and Oceanography" (FSUE "VNIRO")
17, V. Krasnoselskaya st., Moscow, 107140, Russia
e-mail: melnikov@vniro.ru

**Polar Research Institute of Marine Fisheries and Oceanography (PINRO),
6 Knipovich Street, 183763 Murmansk Russia
e-mail: karsakov@pinro.ru

Introduction

Fishery on *Sebastes mentella* in the Irminger Sea pelagial has been conducted not long ago, since early 1980-s. It was started in the international waters in the north of the Irminger Sea in 0-400m layer. Fishing area was gradually expanding, including 200-miles zone of Greenland, Iceland with increasing depth of fishing to 1000m. During the last decade, a large-scale pelagic fishery on this species was carried out in the Labrador Sea pelagial as well (NAFO Divisions 1F, 2GHJ).

Despite numerous studies, scientists still have no general understanding of populational structure of the species in this area of Atlantic as well as measures required for sustainable harvesting of pelagic aggregations. Since 1996 pelagic fishery on *S.mentella* was controlled on the basis of knowledge on biological identity of pelagic aggregations in all distribution area at all depths. In 2009 participants of WKREDS meeting developed recommendations for introduction of new management regime for pelagic fishery based on the hypothesis of two biological stocks of this species in the pelagial of the Irminger Sea and adjacent waters (Anon., 2009).

Suggested harvesting strategy for two supposed stocks of *S.mentella* mainly based on data on their possible genetic and geographic isolation of two fishing areas in the pelagial of the Irminger Sea and adjacent waters, which have to be reviewed, did not consider extensive information on environmental conditions in different areas and at different depths of *S.mentella* habitat. This information was collected in the course of regular Russian and ICES trawl accoustic sureveys. However, it is a common knowledge that there is a close relationship between biological, oceanological and atmospheric processes in the World Ocean. Additionally, analysis of complex causal relations is more important than direct relationship between climate variations and various biological aspects of hydrobionts (Laevastu, Hela, 1974). Moreover, such

relationship often indicates recurrence of various duration for long-term forecasts of distribution, abundance and catches of commercial fish species (Gershanovich, 1986).

Material and methods

Data on monthly TSL fields at the regular grid nodes were used in the paper from the server of the International Research Institue for climate prediction (IRI) being a part of the Itegated Global Ocean Services System (IGOSS) as initial oceanographic information. Besides, data on air temperature at station Reykjavik (Iceland) and those for regular grid nodes from the server of Climate Prediction Center (CPC) were applied.

Objective use of TSL data in the analysis of oceanographic conditions in this area were proved by studies which estimated conjugation of data massive and water temperature in areas of the North Atlantic and Northern European Basin. According to the results, TSL data demonstrate well main oceanographic processes in different areas of the Northern Basin. Therefore, this base can be further applied in researches, including development of various forecasting models (Karsakov et al., 2000; Ionov, Karsakov, 2009).

IGOSS TSL data massive are mean monthly values of surface temperature in the period 1982-2008 in knots of 1-grade grid in the North Atlantic area. Estimates were made for the Irminger Sea area within 55°30'-63°30'N, 26°30'-50°30'W in April-October (fig.1). In the studied period the domestic fishery of *S.mentella* was conducted in this area, distribution and migration behaviour of this species being dependant on oceanographic conditions. Additionally, the area was conventionally divided into two districts: northern and southern. As a result, three time lags of averaged surface temperature were formed: for the northern district, southern district and all studied area (see fig.1).

To identify and forecast TSL data, the aliquant frequency method was used (Schiekedanz, Bowen, 1977). Applying this method, time sampling can be estimated as a summed finite number of harmonic functions with various frequency and intensity (amplitude) by expansion of time sequence into Fourier series, i.e. approximation of complicated function by weighted sum of simple functions. This estimation suggests trigonometric coefficients which approximate, to a certain extent, distinct cyclic components. Standard statistical methods were applied: correlation and regression analyses. Realized TSL forecasts were estimated according to the “Guidance for estimation of quality of methods and realized hydrological forecasts”(1965).

According to the data of Russian and international trawl-accoustic surveys of stock in summer period 1982-2007, analysis of interannual variability of boundaries and

distribution of *S.mentella* aggregations was conducted. Since the stock estimated by means of the trawl method at depths over 500m has a short series of observations, and in first years was experimental, accoustic estimates above the deep scattering layer at depth under 500m were used.

Results and discussion

Estimation of variations in TSL

Actual data series of TSL is of small length (1982-2008, n=27). It was decided to restore available data on TSL to estimate oceanographic conditions in the area at a long time lag. With this aim, data on air temperature from Reykjavik station were used, where observations have been conducted since early XX century. Correlation rates between variations in air temperature and TSL for three studied areas comprised 0,65-0,70. Applying methods of linear regression, TSL series since 1901 has been restored. Charts describing relations and equations applied in restoring are given in figure 2.

Technical realization of restored TSL values according to the data 1982-2008 was 82-88%, and efficiency in comparison with climate forecast – 23-28%. This suggests the use of restored values of TSL in our studies (Instructions on quality assessment..., 1965).

Resulted from restoration, series of TSL for 1901-2008 were obtained. Preliminary analysis of water temperature in this period revealed significant interannual variations in temperature condition of surface waters. Method by Tereschenko V.V. (Tereschenko et al., 1985) was chosen for quantitative estimation of temperature condition in TSL. In this method mean-square deviation of water temperature was used as a quantitative index (σ_T). Such criterion has been successfuly practised in PINRO studies. The temperature water level was estimated by five-grade scale:

1 – anomalous cold year	$-\Delta T\ ^\circ C > 1{,}5\sigma_T$;
2 – cold year	$0{,}5\sigma_T < -\Delta T\ ^\circ C \leq 1{,}5\sigma_T$;
3 – normal year	$\Box\Delta T\ ^\circ C \leq 0{,}5\sigma_T$;
4 – warm year	$0{,}5\sigma_T < \Delta T\ ^\circ C \leq 1{,}5\sigma_T$;
5 – anomalous warm year	$\Delta T\ ^\circ C > 1{,}5\sigma_T$.

Normalized anomalies and classification of years by V.V. Tereschenko method are given in figure 3.

It should be noted that the temperature level during the period 1901-2008 is similar for all three areas. From 1901 to 1916 there were hardly any tendencies to warming or cooling. Water temparature was close to normal, increasing to the level of warm years (1912 and 1915) and decreasing to the level of cold years (1903 and 1914).

The period of cooling was observed from 1917 to 1927, when TSL in the Irminger Sea corresponded to the category of cold and anomalous cold years (1921 and 1922).

1928-1966 was a long-term period of TSL warming with a local cooling in 1948-1949. In some years water temperature was equal to the level of anomalous warm years. In 1939 and 1941 TSL was the highest for all studied period (1901-2008).

The period of warming was changed by cooling in 1967-1995. Periods with the coldest temperature were 1983-1984 and 1989-1990, when water temperature corresponded to anomalous cold years.

Next period of warming, started in 1996, has been presently continued. The highest TSL was observed in 2005 and 2007. In recent years there is a trend to decrease in TSL in this area. Water temperature decreased from anomalous warm to warm. These variations were mostly distinct in the southern part of the area (see fig.3c).

It is difficult to estimate duration of the current period of warming. On this ground, authors try to forecast TSL for the nearest two years. With this aim, the aliquant frequency method was used. Based on this method, frequency content of primary samples was found. Main local energy-carrying maximums exceeding significance threshold for all three areas (see fig.1) are: 14-15 and 8-9 years. Additionally, the trend component estimated by air temperature with a period of 53 years was used for forecast. These summed recurrencies describe around 60% of TSL variability.

Applying autoforacsting, i.e. extrapolation of found quasi-frequencies, the forecast for two steps ahead was made, i.e. for the period 2009-2010. According to the forecast, TSL in the studied area of the Irminger Sea in the nearest two years will correspond to the level of warm years having a tendency to decrease.

Variation of TSL and distribution of *S.mentella* aggregations

Data analysis of Russian and international trawl accoustic surveys permits studying relationship between climatic processes in the area and spacial distribution of *S.mentella* aggregations in the upper 500m layer of the Irminger Sea and adjacent waters during summer 1982-2007.

During the studied period the interannual variability in geographic boundaries of pelagic aggregations of *S.mentella* with various density was revealed. In the period of anomalous cold years 1982-1984 most aggregations distributed in the north of the Irminger Sea, which southern boundary was on the level of 58°N. As negative anomalies were decreasing, the southern boundary of aggregations gradually moved and by 1992 it had reached 54°N. By that period, dense concentrations of *S.mentella* had moved to the central and southern parts of the Irminger Sea and Eastern Greenland (Pavlov, 1992; Pedchenko et al., 1996). Since 1994 dense concentrations

of *S.mentella* started moving from the Irminger Sea to the NAFO area. In the period of medium and warm years in 1999-2001 the south-western boundary of *S.mentella* area reached 52°N, 50°W, and fish dense concentrations distributed mainly in the NAFO area and, partly, in the southern part of the Irminger Sea (fig. 4, 5, 6).

It should be noted that location of the north-eastern boundary of the area was constant from 1982 on. Resulted from our studies, it was found that increased advection of Atlantic waters by the Irminger current and the rise of water temperature in open areas of the North Atlantic observed since 1990-s, were main reasons of migrating *S.mentella* aggregations from the Irminger Sea area to the NAFO area (Melnikov et al., 2001; Melnikov, 2005, 2006, 2007, 2008; Melnikov, Bakay, 2009; Melnikov, Popov, 2009). Increase in warm waters in *S.mentella* distribution area was illustrated by an isotherm 4°C located at 200m depth. This migration of aggregations provided for a large-scale international fishery in the Labrador Sea pelagial started in 2001.

As the growth of TSL became stable with a steady location of isotherm 4°C, the tendency of adverse migration of *S.mentella* aggregations became evident. According to the data of surveys 2005 and 2007, despite the fact that during these years a part of fish dense concentrations was in the open part of the Labrador Sea (the NAFO area), another one moved to the north-east to the Greenland area.

This was probably caused by other factors, including biological, which alongside with high water temperature during several years, could influence migration of *S.mentella* aggregations. Although these issues are outside the present study, according to the data of autumn surveys of German exploratory fishing vessel “Valter Herving III” in the area of the eastern and western Greenland, during the recent years an important climatic aspect of increased flow of Arctic waters through the Denmark Strait was found. This is related to increased stratification in the upper layer resulted from water desalination: high stratification limits the depth of winter convective interfusion and heat transfer from deep layers to surface. This results in growing ice coverage in freezing waters and long-term ice periods. In view of all these effects, variable conditions in Greenland waters indicate a high probability of the fact that in the nearest future marine climate in the northwestern Atlantic and Labrador Sea will evolve towards water cooling and growth of ice coverage (Stein, Borovkov, 2008).

Variations in TSL and fishery pattern of *S.mentella*

Statistical data of Russian fishery in the pelagial of the Irminger Sea and adjacent waters 1982-2009 were analyzed to find a possible relationship between variations in oceanographic conditions and catches of *S.mentella*. Unlike data of other countries, only Russian data cover all period, seasons, areas, depths of fishery and, on the whole, correspond to the international fishing statistics (Sigurdsson et al., 2006). During the first decade of fishery, the Russian portion of *S.mentella* was 59-100%, and in the following years – 22-52% from the total catch.

A number of main periods of *S.mentella* fishery were identified. Each period had its own fishery pattern (fig.7).

In the period of anomalous cold and cold years in 1982-1988 the fishery was mostly conducted in the north of the Irminger Sea. The season started in late March, when *S.mentella* females formed dense spawning concentrations above slopes of the Reykjanes Ridge at 300-500m depths. In late June-first part of July feeding concentrations were fished at 70-150m depths. Fishing season lasted 4-5 months.

In 1989-1992 when temperature was on the level of anomalous cold and cold years, decrease in fishing efficiency effected spreading of fishing area in the central and southern parts of the Irminger Sea. Duration of the fishing season comprised 4-5 months, like in the previous period.

As cold years were changed by normal in 1993-1998, the fishery in the north of the sea moved to the depths over 600m and covered the eastern Greenland area. Fishing season comprised 7 months, until October.

As the water temperature was growing and normal years were changed by warm, by 1999 the fishing area had considerably spread in the south-western direction, covering Divisions 1F, 2GHJ of the NAFO area. In the following years the fishery started in early April in the north of the Irminger Sea at depths larger than 600-1100m. In May-June aggregations were fished in the same areas being slightly expanded. In July-August the fleet moved to the southern part of the Irminger Sea and further to the NAFO area, where redfish aggregations distributed at 200-500m depths. The portion of *S.mentella* in the north of the Irminger Sea comprised 43-72%, in the southern part and the NAFO area – 28-57%. The fishing season lasted 7 months and ended in early November.

Since 2006, when TSL became stable with decrease in temperature, the fishing area and the catch of *S.mentella* have been gradually decreasing in the southern part of the Irminger Sea and the NAFO area, alongside with a considerable increase in the caught fish in the north of the Irminger Sea. Fishing season became 5 months shorter be means of reduced fishing period in the southwestern area, where *S.mentella* catch had decreased by 2008 to 10,4%. In the north of the Irminger Sea *S.mentella* catch in 400-600m layer grew from 3,6% in 2006 to 25% in 2008. The year of 2009 was the most representative, when the Russian fishery was conducted merely in the north and the central part of the Irminger Sea.

Thus, analyzed during 28 years commercial data indicate a close relationship between areas and depths of *S.mentella* fishery, on one side, and variations in oceanographic conditions in the area of the Irminger Sea and adjacent waters, on the other. During anomalous cold and cold years, the fishery was mainly conducted in the north and central part of the Irminger Sea in the upper 500m layer. As the warming period started, the fishing area was gradually expanding, covering the southern part of the

Irminger Sea and the NAFO area with 0-500m depths. In this period in the north of the sea aggregations were mainly fished at depths over 500m. Stabilization and a certain decrease in TSL caused a gradual reduction in the fishing area and the catch in the southwest. The northern part of the Irminger Sea has become again the main fishing area. There *S.mentella* catch was growing in the intermediate 400-600m layer.

Conclusion

Resulted from restored TSL, significant interannual variations in TSL for the period 1901-2008 were found. Quantitative estimation of TSL permits to identify the following categories of years: anomalous cold, cold, normal, warm, anomalous warm. In view of this classification, two periods of cooling were specified: 1917-1929 and 1967-1995. They were estimated as cold and anomalous cold years by TSL. Since 1966 next warming period was observed with the highest TSL in 2005-2007 and a tendency to their decrease in the following years. According to the forecast, based on the extrapolation of found quasi-frequencies, in 2009-2010 a tendency to decreased TSL will preserve in the area.

In the period of anomalous cold and cold years 1982-1984, *S.mentella* aggregations distributed in the north of the Irminger Sea. In the second part of 1980-s in TSL the feeding area was spreading with decrease in negative anomalies in TSL. By that period, dense concentrations of *S.mentella* had moved to the central and southern parts of the Irminger Sea and Eastern Greenland. Increased volume of warm waters since mid 1990-s in the north of the area caused movements of dense concentrations of *S.mentella* from the Irminger Sea to the NAFO area. Evolution of marine climate in the northwestern Atlantic towards water cooling was of the reason of *S.mentella* reverse migration, observed since 2005 from the Labrador Sea to the northeast, while high positive anomalies preserved in the Irminger Sea.

Variations in water temperature, having impact of distribution of *S.mentella*, influenced pelagic fishery and fishery pattern as well. If in the period of cold years, fishery was conducted in the northern and central parts of the Irminger Sea in the upper 500m layer, in the period of water warming fish migration was followed by increase in the depth of fishing and spreading of fishing area. In anomalous warm and warm years the second fishing area was forming in the southern part of the Irminger Sea and the NAFO area. There feeding and coupling *S.mentella* of small and medium size were fished. Stabilization and a certain decrease in TSL in the north of the Irminger Sea followed by water cooling in the northwestern Atlantic influenced a gradual reduction of fishing area and portion of *S.mentella* in the southwest. As a result, fishery pattern was newly transformed, simillar to the period of moderate and cold years.

Thus, steadily variable environmental conditions result in simultaneous variations in distribution and fishery pattern of *S.mentella*. This should be certainly considered

while developing the strategy of sustainable exploitation of the present species in the pelagial of the Irminger Sea and adjacent waters. Reccurent variations in TSL result in the fact that in different periods pelagic fishery on *S.mentella* may occur in one local area, in one wide area, in two distant local areas and again in one area at constantly variable depths of fishing. Such transformation of fishery pattern makes it problematic to specify any management components for *S.mentella*, which boundaries "... are based on spatial patterns of the fishery" (Anon, 2009b). Thus, suggested WKREDS (Anon., 2009a) boundaries for *S.mentella* management components in the northeast of the Irminger Sea are groundless, from scientific viewpoint, and inefficient, from practical one. These revealed relations and the forecast of oceanographic conditions suggest that in the northern and central parts of the Irminger Sea in the nearest future (like in 1980-s and early 1990-s) harvested will be the part of *S.mentella* aggregations, which fishery was conducted in 2000-s in the NAFO area and southern part of the Irminger Sea.

References

Anon., 2009. Report of the Workshop on Redfish Stock Structure (WKREDS). ICES CM 2009 / ACOM:37.

Gershanovich D.Ye. 1986. Fisheries oceanography. Moscow. “Agropromizdat”. 336 p.

Guidance for estimation the quality of methods and realization of marine hydrological forecasts (instead of 7 and 9 chapters of forecast service manual, section 3, part 3). 1965. Moscow.GIMIZ. 144 p.

Ionov V.V., Karsakov A.L. 2009. Possible forecast of TSL.// Vestnik SPBGU. Series 7. Vyp. 1. P.93-98.

Karsakov A.L., Sentyabov Ye.V., Bochkov Yu.A. 2000. The temperature on the surface of northern Atlantic and a long-term forecasting of abiotic and biotic parameters of the ecosystem in the Northern Basin // Materials of PINRO report resulted from researches in 1998-1999. Part 1. Murmansk: PINRO Press. P.188-199.

Laevastu T., Hela I. 1970. Fisheries Oceanography. Fishing News (Books) LTD., London.

Melnikov S. P. 2005. Candidate’s Dissertation in Biology (PINRO, Moscow). 23p. (in Russian).

Melnikov S. P. 2006. Oceanic Redfish in the Northern Atlantic: Biology and Fishery (PINRO, Murmansk). 127 p. (in Russian).

Melnikov S.P. 2007. The use of information on parameters of the life cycle in the management of *Sebastes mentella* fisheries in the Irminger Sea. ICES CM/L:03, 19 p.

Melnikov S.P. 2008. Modified regime of international management of *S.mentella* stock in the pelagial of the Irminger Sea and adjacent waters: for and against.// Rybnoye hozyaystvo. N5. P. 34-36 (in Russian).

Melnikov S.P., Pedchenko A.P., Shibanov V.N. 2001. Results from the Russian investigations on pelagic redfish (*Sebastes mentella*, Travin) in the Irminger Sea and in NAFO Division 1F. NAFO SCR Doc. 01/20. Ser. № 4388, 20 p.

Melnikov S.P., Bakay Yu.I. 2009. Structure of Aggregations and Principal Populational Characteristics of Redfish *Sebastes mentella* (Scorpaeniformes: Scorpaenidae) in the Pelagic Zone of the Irminger Sea and Adjacent // Journal of Ichthyology, Pleiades Publishing, Ltd., Vol. 49, No. 2, pp. 170–182.

Melnikov S.P., Popov V.I. 2009. The Distribution and Specific Features of the Biology of Deepwater Redfish *Sebastes mentella* (Scorpaenidae) During Mating in the Pelagial of the Northern Atlantic. Journal of Ichthyology, Pleiades Publishing, Ltd., Vol. 49, No. 4, pp. 300–312.

Pavlov A. I. 1992. Candidate’s Dissertation in Biology (PINRO, Moscow). 25 pp. (in Russian).

Pedchenko A.P., Melnikov S.P., Shibanov V.N. 1996. Regularities of feeding concentrations of redfish (*Sebastes mentella*) formation in the Irminger Sea. ICES CM/G:45, 20 p.

Schiekedanz P., Bowen E. 1977. The computation of climatological power spectra.-

Yournal of applied meteorology, - Vol. 16. – 4. P. 359-367.

Sigurdsson Th., Rätz H.-J., Nedreaas K., Melnikov S.P., J. Reinert. 2006. The fishery for pelagic redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters // ICES Journal of Marine Science. № 63. P. 725-736

Stein M., Borovkov V. A. The effect of winter cooling on inter-annual changes of near-bottom water temperatures off Southwest Greenland - a forecast option for bottom water temperatures on half year time scales. 2008. NAFO SCR Doc.08/02, Ser. No. N5485. 12 pp.

Tereschenko V.V., Dvinina Ye.A., Borovaya L.I. 1985. Reference source on water temperature in the Barents Sea. Murmansk. 72p.

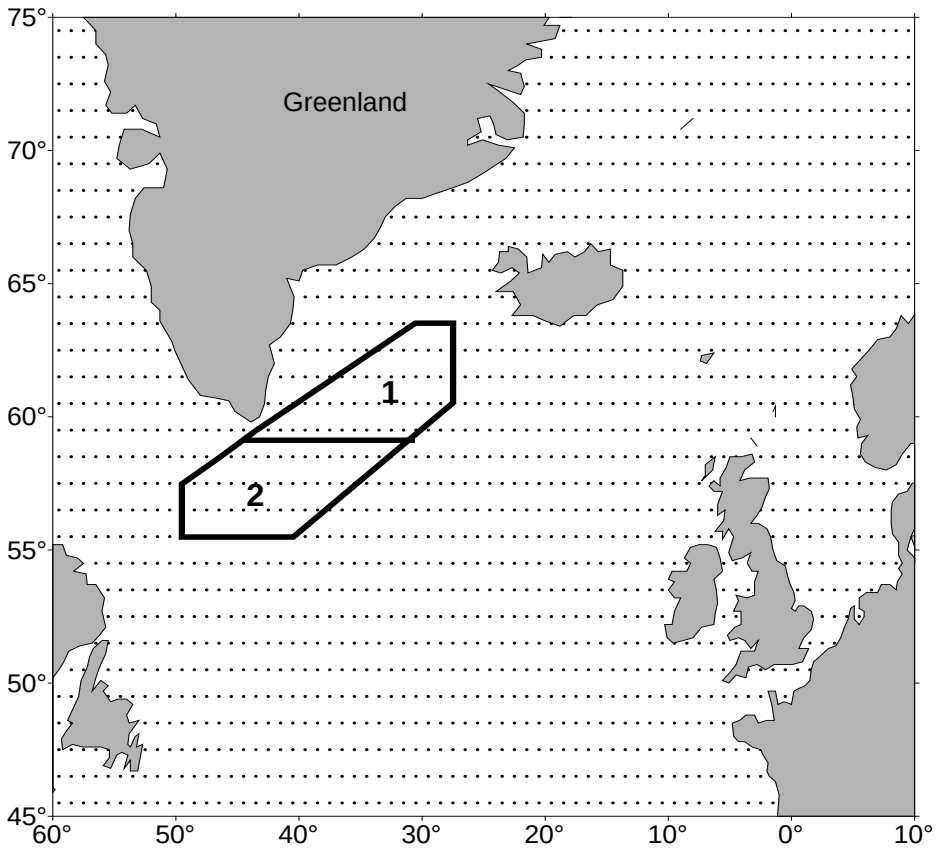

Fig 1. Location of the TSL IGOSS regular grid nodes and boundaries of the studied area: 1– northern area, 2 – southern area.

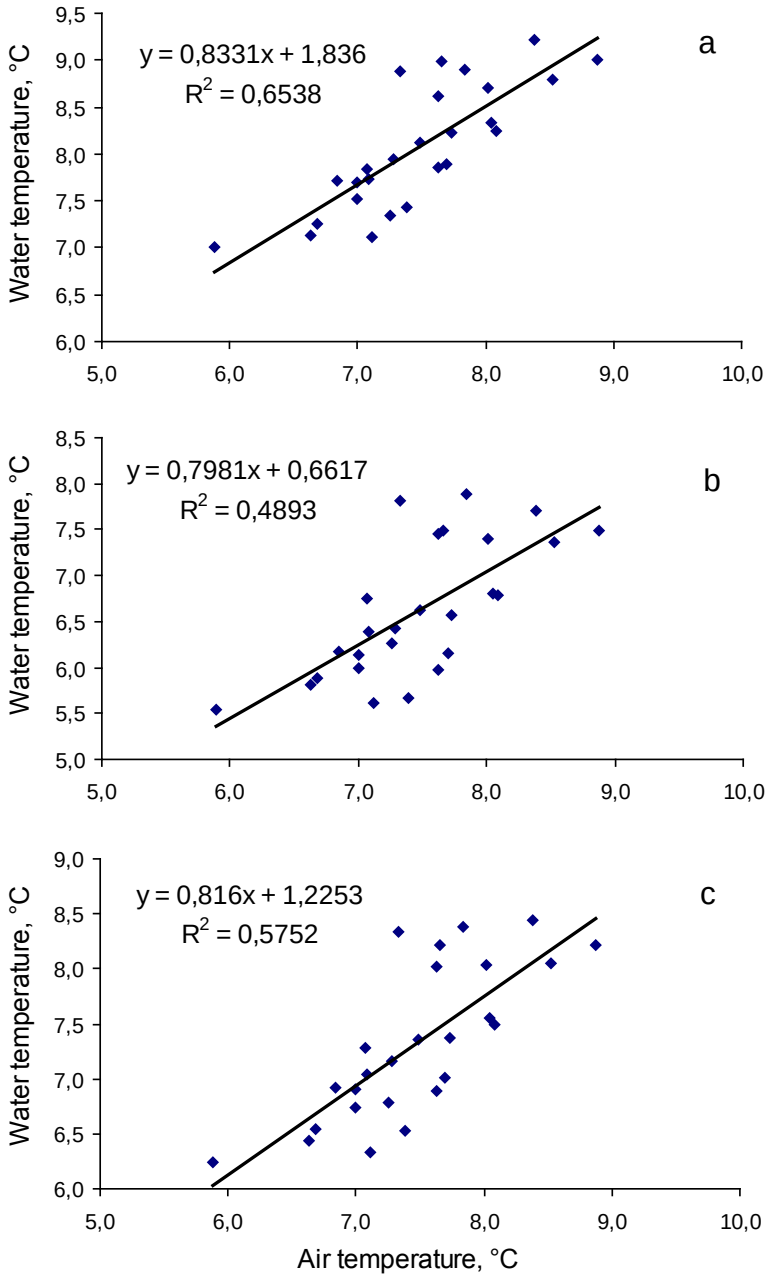

Fig. 2. Charts indicating relation between variations in air temperature at the station Reykjavik and TSL in the Irminger Sea and adjacent waters in 1982-2008: a – northern area; b – southern area; c – all studied area.

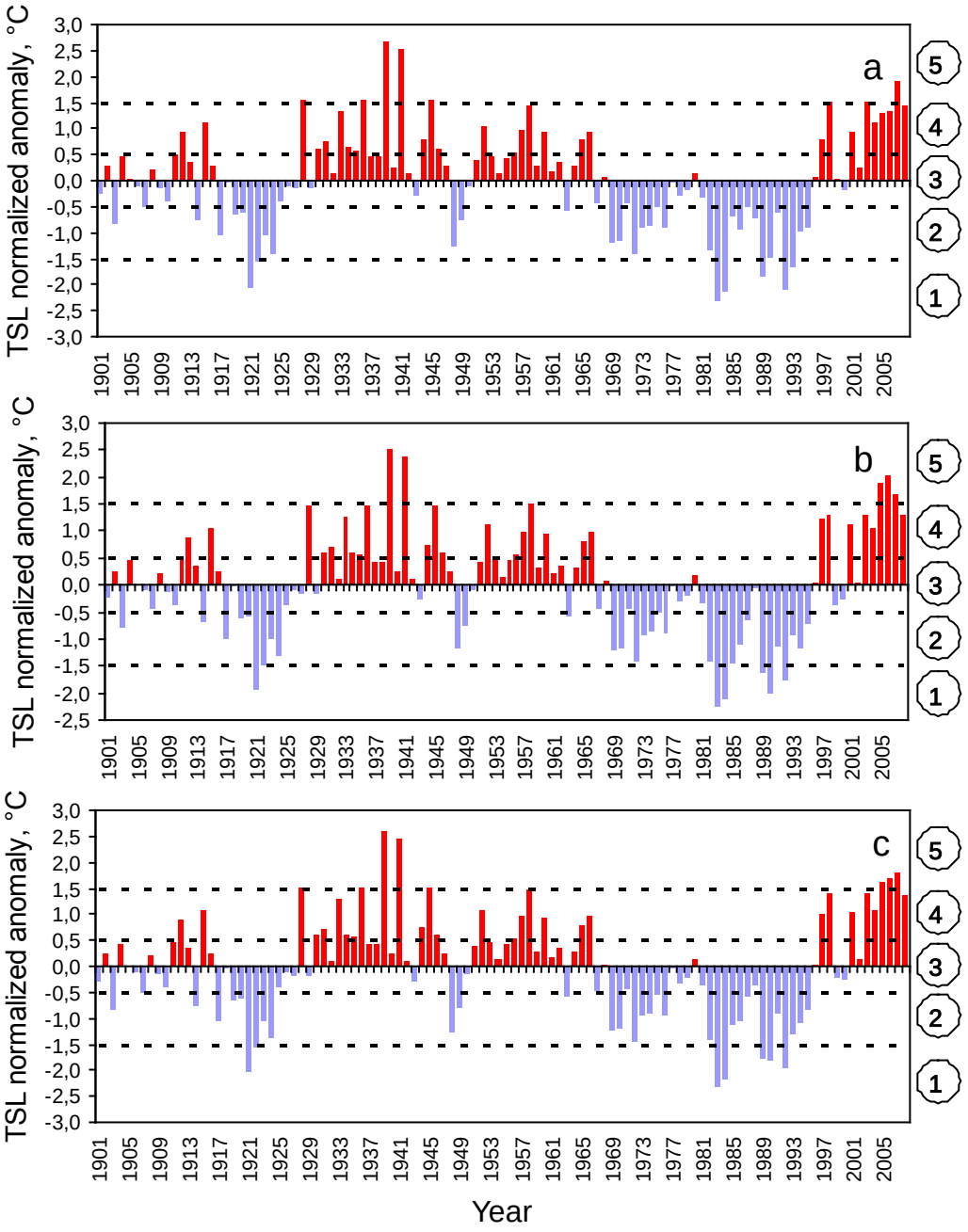

Fig. 3. TSL normalized anomalies in the northern (a), southern (b) area and in all area (c) of the Irminger Sea and adjacent waters in 1901-2008 and categories of years by V.V. Tereschenko classification: 1– anomalous cold, 2 – cold, 3 – normal, 4 – warm, 5 – anomalous warm.

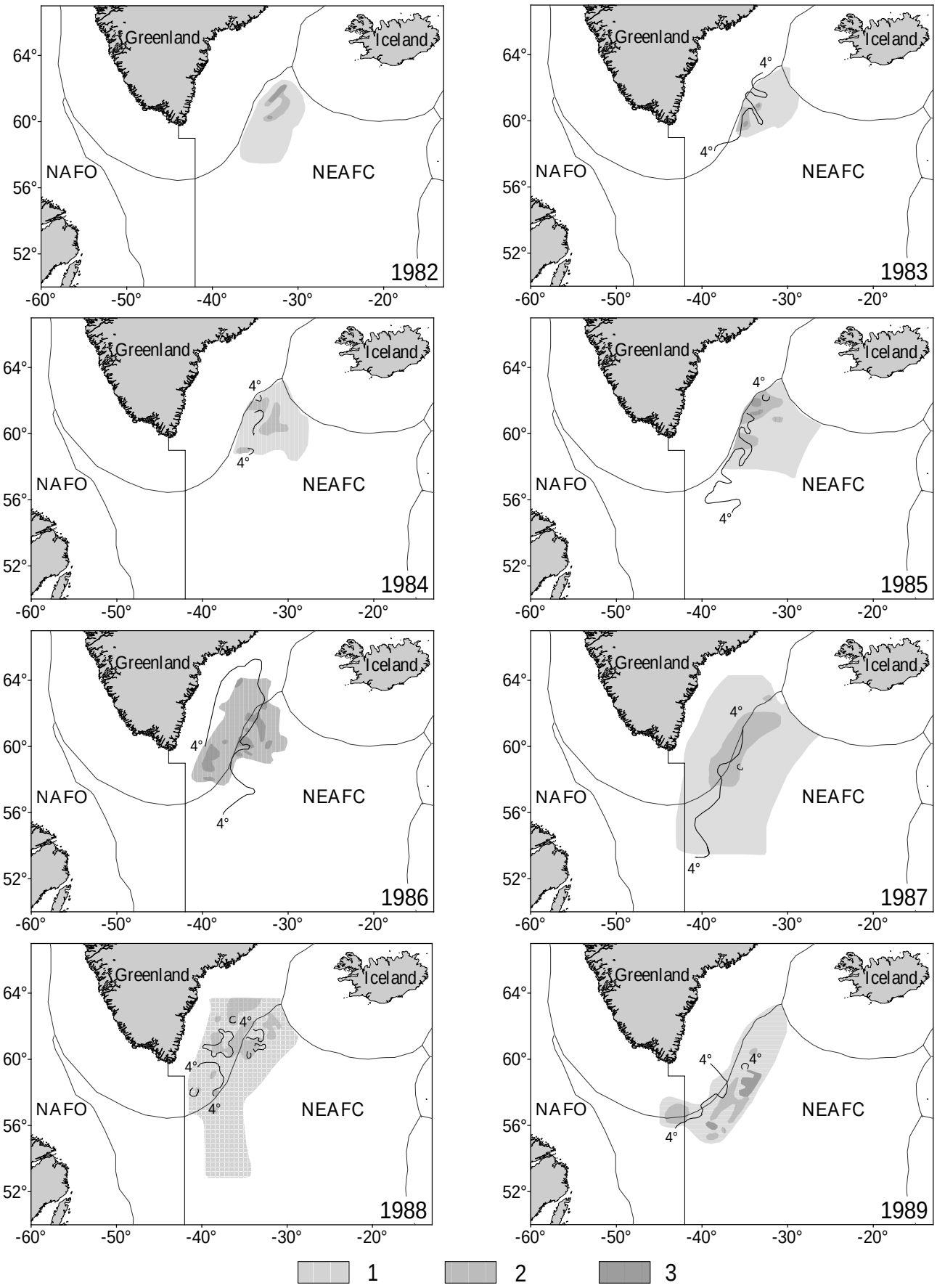

Fig.4. Distribution of redfish aggregations in the pelagial of the Irminger Sea and adjacent waters in 0-500m layer and location of isotherm 4^0 C in 200m layer, resulted from summer trawl accoustic surveys in 1982-1989. Density of concentrations (SA m^2/$mile^2$): 1 – 0-10; 2 – 11-20; 3 – >20.

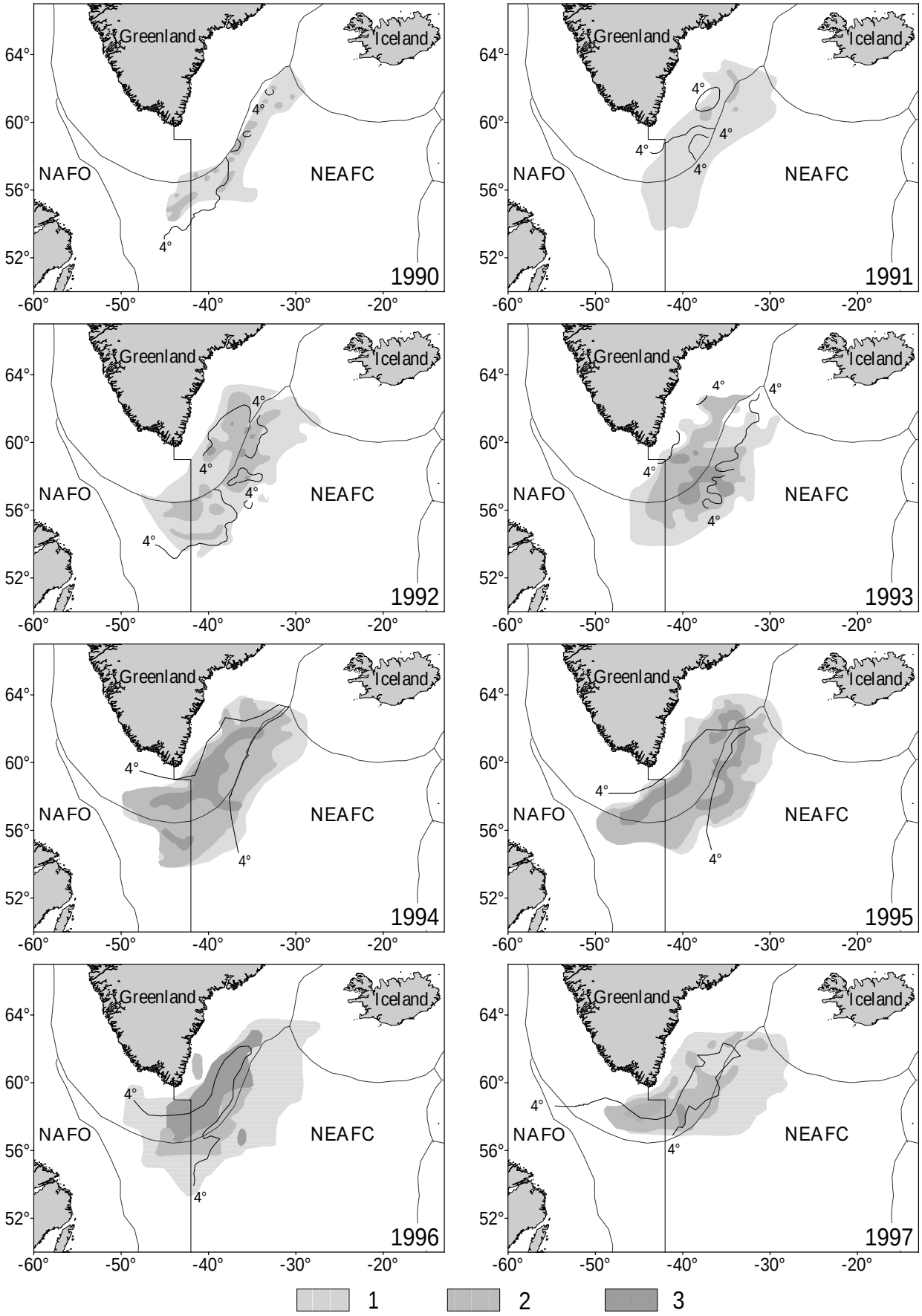

Fig. 5. Distribution of redfish aggregations in the pelagial of the Irminger Sea and adjacent waters in 0-500m layer and location of isotherm 4^0 C in 200m layer, resulted from summer trawl accoustic surveys in 1990-1997. Density of concentrations (SA m^2/$mile^2$): 1 – 0-10; 2 – 11-20; 3 – >20.

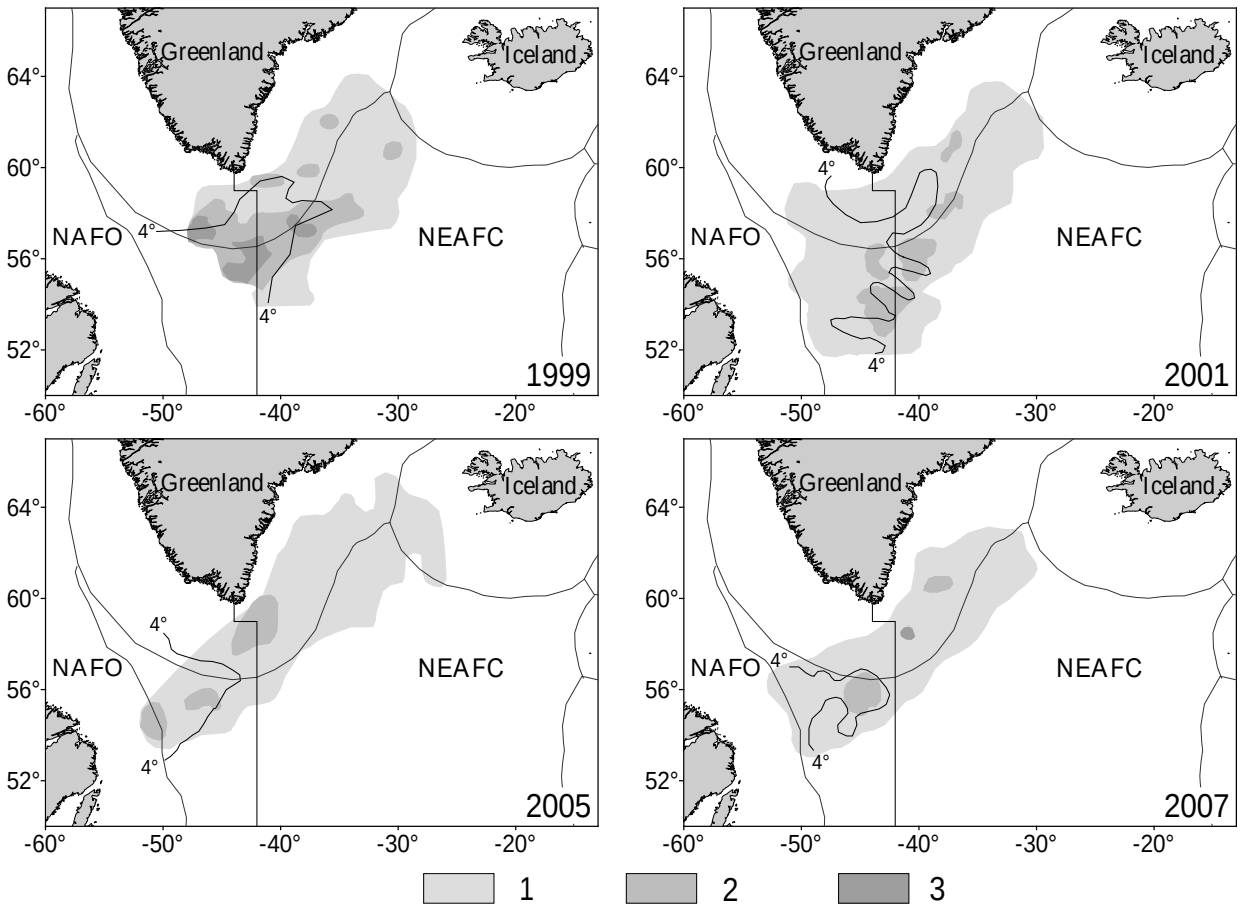

Fig. 6. Distribution of redfish aggregations in the pelagial of the Irminger Sea and adjacent waters in 0-500m layer and location of isotherm 4^0 C in 200m layer, resulted from summer trawl accoustic surveys in 1999-2007. Density of concentrations (SA m^2/$mile^2$): 1 – 0-10; 2 – 11-20; 3 – >20.

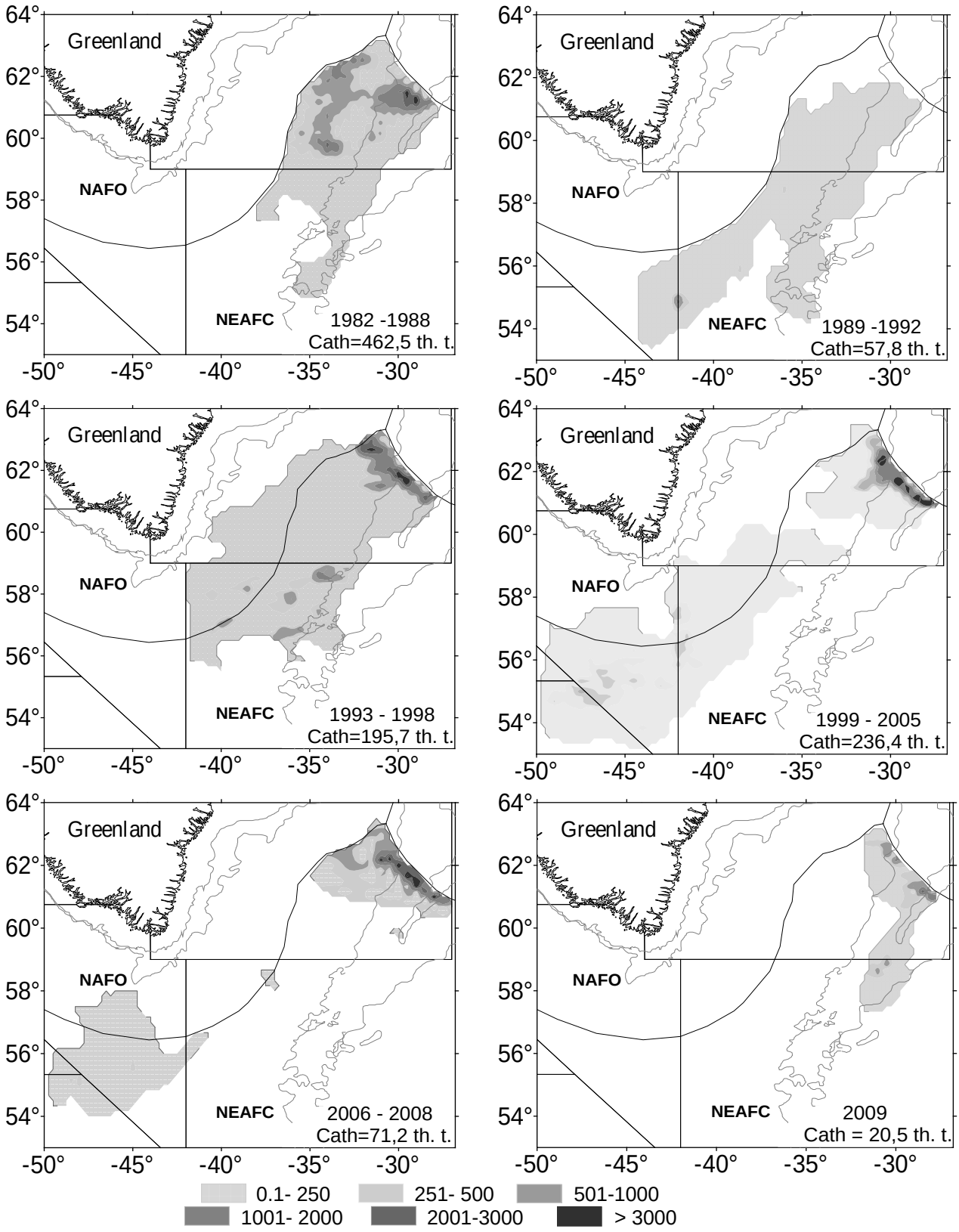

Fig. 7. Fishing areas and the catch (squared t, 10' in latitude and 15' in longitude) of *S.mentella* by Russian vessels in 1982-2009.

Single population of beaked redfish (*Sebastes mentella*) in the Irminger Sea: biological characteristics and dynamics of gene pool

by

Makhrov A.A., Artamonova V.S., Popov V.I.*, Rolskiy A.Y.*, Bakay Y.I.*

A.A. Makhrov, Ph.D. (Biol.)., Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninsky prosp. 33, Moscow, 119071, Russia, e-mail: makhrov12@mail.ru

**Knipovich Polar Research Institute of Marine Fisheries and Oceanography, 6 Knipovich str., Murmansk, 183038, Russia, e-mail: persey@pinro.ru*

A brief overview of the main results of investigations related to ecological and population parameters of beaked redfish *Sebastes mentella* inhabiting the pelagial of the Irminger Sea and adjacent waters is presented. Data from previous genetic studies of composition of *S. mentella* aggregations in that area has been analysed. The results indicate that the conclusion about the two stocks of *S. mentella* inhabiting the Irminger Sea pelagial lacks a methodological substantiation. Data from new genetic investigations of *S. mentella* in that area using allozyme markers are given. Interspecific hybridisation and selection-induced changes in allele frequencies in *S. mentella* from the same cohort were revealed. This explains the slight differences in genotype frequencies observed in *S. mentella* sampled at different depths.

Introduction

Pelagic redfish *S. mentella* of the Irminger Sea and adjacent waters is an important commercial species in the North Atlantic. In the 1950-70s, Russian and foreign scientists discovered *S. mentella* in the pelagial over the oceanic depths outside the slopes of Iceland and Greenland (Templeman, 1959; Rikhter, 1961; Zakharov, 1964; Jones, 1970; Magnusson, 1977 et al.). Since 1981, the USSR had conducted comprehensive studies of *S. mentella* in that area on a regular basis (Noskov et al., 1984; Shibanov et al., 1984; Dushchenko, 1986; Bakay, 1988, 1989; Pavlov, 1988; Pavlov and Galuzo, 1989; Pavlov et al., 1989ab). Since 1982, large-scale Soviet and, later on, international fishery for that species has been conducted. The start of intensive fishery for pelagic *S. mentella* gave rise to discussions about the number of management units and stocks involved in the pelagic fishery, as, with the extension of fishery, their number tended to increase without a proper scientific rationale (Saborido-Rey et al. 2005).

The debate concerning the stock structure of *S. mentella* gained momentum in the mid-90s following the attempt by the Icelandic researchers to identify two types of *S. mentella* in the Irminger Sea pelagial (Magnusson and Magnusson, 1995; Magnusson et al., 1995). In their opinion, redfish dwelling at depth greater than 500 m, that they called “pelagic deep-sea” redfish, is different from that inhabiting the upper 500 m layer, or the “oceanic” redfish, and makes up a separate “type”/stock.

This opinion is based on differences by five criteria: 1) length composition; 2) length-at-maturity; 3) thickness of neck; 4) intensity of red body colour; 5) infestation by copepod *Sphyrion lumpi*, occurrence of pigmented patches on skin and melanin (melanocytes) in muscle tissue.

However, the observed differences in the occurrence of those criteria cannot be used as a basis for stock differentiation, as most of them are related to age and feeding of fish (Bogovski and Bakay, 1989; Bakay, 2000, 2004; Bakay and Melnikov, 2002), methodologically incorrect (Bakay and Karasev, 2001), biased or unreliable (Bakay, 2004). It was also proved that massive maturation of *S. mentella* in the upper and lower layers occurs at similar length/age, while the large distribution range can be accounted for by age-related feeding selectivity (Bakay and Melnikov, 2002).

Genetic studies indicate that the two *S. mentella* types differ by allele frequencies at some loci. There was, however, no definitive explanation of this difference (Nielsen, 2004). The possible reasons discussed in literature were natural selection (Stroganov and Novikov, 2004; Melnikov et al., 2007), the occurrence of two separate stocks (Johansen et al., 2000a, Johansen and Nævdal, 2004), and cohort-related differences in allele frequencies (Schmidt and Trautner, 2004; Schmidt, 2005).

Comprehensive analysis of literature data on the population structure of the Irminger Sea *S. mentella* is given in the overview by F. Saborido-Rey et al. (2005), which uses biological, parasitologic and genetic data to substantiate the single-population (single-stock) concept for redfish inhibiting pelagic waters of the Irminger Sea and adjacent waters. The overview has summarized the first stage in the discussion.

In recent years, papers have been published, where an attempt is made to differentiate *S. mentella* in pelagic waters of the Irminger Sea into two populations, one of which ("Shallow Pelagic") presumably inhabits waters above 500 m depth, and the other one ("Deep Pelagic") is distributed below 500 m. The latter type, however, is "not to be confused with the 'deep-sea' phenotype" (ICES, 2009, p. 51).

At the Workshop on Redfish Stock Structure (WKREDS) arranged by ICES in January, 2009, there was a disagreement among experts over the population structure of *S. mentella* in the Irminger Sea (ICES, 2009). The arguments used by the supporters of differentiating the stock are summarized by S.X. Cardin *et al.* (2010).

This paper aims at making a conclusion on the stock structure and aggregation structure of redfish *S. mentella* living in the pelagial of the Irminger Sea and adjacent waters. To achieve this objective, the following has been done:

- analysis of available information on stock structure of *S. mentella* based on the holistic approach;
- critical overview of methodology and results of genetic studies used to identify types/stocks of *S. mentella;*

- presentation of new genetic data explaining the observed differences between *S. mentella* sampled at different depth.

Methods

The paper gives a brief overview of the main results from ecological and population studies of pelagic redfish *S. mentella* Travin in the Irminger Sea and adjacent waters. As many other researchers, we regard “stock” and “population” as equivalent notions, the definitions of which are given in Saborido-Rey et al. (2005).

For example, Cadrin et al. (2010) describe differentiation methods based on the holistic view and, on the face of it, give rather convincing arguments supporting the existence of several *S. mentella* stocks in the Irminger Sea. However, on closer examination the statement that “Conclusions on biological stocks were based on the most robust and parsimonious view of stock structure that was consistent with the best scientific information available” is not always true.

Like the authors of the mentioned paper (Cardin *et al*., 2010), we are going to examine separately the data obtained by using different methods of population studies and compare the findings from different types of analysis. Given below are the criteria we used to estimate the applicability of different genetic methods to substantiate the existence of two stocks of *S. mentella* in pelagic waters of the Irminger Sea.

Criterion 1. Studied (compared) samples must be collected reliably above and below 500 m depth (ICES, 2009).

Criterion 2. Statistically significant differences in the trait analysed must be found between the two groups of samples (Weir, 1990).

Criterion 3. Allele frequencies at loci used to differentiate the supposed populations (diagnostic loci) must be stable over time (Waples, 1998).

Criterion 4. These diagnostic loci must not be subjected to selection (Beaumont, Nicholas, 1996), at least during the life span of one generation.

Criterion 5. A possibility for interspecific hybridisation to influence allele frequencies at diagnostic loci must be ruled out. This condition is rather important since it was revealed that interspecific hybridization between *S. mentella* and *S. fasciatus* takes place on a large scale in other North Atlantic areas (Roques et al., 2001). Hybrids of *S. mentella* and *S. marinus* are known as well (Altukhov et al., 1968, Nedreaas and Nævdal, 1991; Nedreaas et al., 1994; Nefyodov, 2002).

In this section we would like to focus on the analysis of some points discussed in the paper (Anon., 2010) based on the report of the Stock Identification Methods Working Group, SIMWG (ICES, 2010). This group considered the working documents of the Russian Federation (AM 2009/23 and AM 2009/29-rev 1) submitted to ICES. The report of the WG was used to prepare the ICES response to the request of NEAFC. It should be noted that Russian specialists did not participate in the work of the WG.

Regarding that study, we are deeply puzzled by the statement "The protein loci usually exhibited strong differences and although it would be preferable to obtain long-term temporal studies (which will take several decades, considering the high longevity of redfish), they are not necessary as all the genetic studies that have been performed over the last 15 years revealed a significant differentiation between “Oceanic” and “Deep-sea” *S. mentella* within and between markers (mtDNA, allozymes, microsatellite loci)." No wonder that this statement is not proven by references to any methodical studies, as it runs counter to generally accepted rules of analysis of population structure (Hedgecock, 1994; Waples, 1998). Impossibility to identify the temporal stability of allele frequencies at the genes studied means that it is also impossible to draw the borderline between populations.

The authors of the document (Anon. 2010) make further references to a number of studies which suggest using genetic markers subjected to selection to identify population structure. However, all the mentioned studies refer to selection, as the document (Anon. 2010) specifies it, on “an evolutionary scale”. Here we consider the selection that changes allele frequencies at a certain gene during the life span of one generation, i.e. the one revealed in the population of *S. mentella* in the Irminger Sea (Appendix 1).

Besides, the paper (Anon. 2010) states that "All statistical tests performed during the most recent genetic studies, however, did not bring any evidence on selection acting on the microsatellite loci used". However, there is evidence indicating the influence of selection on four out of nine microsatellite loci studied in redfish (Pampoulie and Danielsdóttir, 2008). Also, according to the study (Anon. 2010), "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. Most of the cases have been found in profound inbred populations". This statement is not supplied by any references. At the same time, Makhrov (2009) gives a reference to the overview (Coltman and Slate, 2003), which provides a collection of facts showing heterozygosity-fitness correlations in wild populations. A number of similar facts, including those obtained when studying large wild populations of fish, is given in the monograph by Altukhov (2006) translated into English.

To demonstrate the possibility of genotype influence on migration behaviour in marine species, Makhrov (2009) used the example with *Daphnia pulex.* The paper (Anon. 2010) gives the following objection: "It should be noted that two very distinct

organisms cannot be compared directly, simply due to the specificity of their life cycle". We analysed literature data and showed that the influence of genotype on behaviour is also known in some fish species. It is interesting that authors of the paper (Anon. 2010) are acquainted with one of the publications referring to such facts (Pampoulie et al., 2008).

It should be emphasized that the study (Anon. 2010) considers only a part of arguments given in the paper by Makhrov (2009). In particular, it is neglected that most authors supporting the presence of differences between the groupings of *S. mentella* in the Irminger Sea, use another differentiation criterion than that used in the document (ICES, 2009). Besides, the document (Anon. 2010) does not take into account data on the existence of interspecific redfish hybrids in the Irminger Sea. Therefore, we have to repeat some arguments by Makhrov (2009) in this paper.

Results

Parasitological investigation of *S. mentella.*

Parasitological investigations have revealed a complete similarity of parasite fauna and level of infestation by most parasite species for *S. mentella* from the upper and the lower layers. Similar level and specific features of infestation by the parasitic copepod *Sphyrion lumpi*, which (including the traces of parasite invasion old cephalotoraxes) is considered as a reliable natural tag in population studies (Bakay, 2000, 2004; Bakay and Karasev, 2001), was observed for redfish from both layers. Absolute predominance of traces of infestation by *S. lumpi* at > 500 m depth indicates that fish migrated to deeper layers from the shallower mesopelagial inhabited by the mesopelagic species *S. lumpi* (Squires, 1966). Less frequent occurrence of individuals with pigment patches at greater depths is caused by age-specific changes in fish and obvious abnormality of this phenomenon for *S. mentella*. Comparison of depth-related frequency of occurrence of pigment patches in fish of different length and age suggests that redfish aged 17 years and older migrate from the upper layer to the deeper waters (Bogovski and Bakay, 1989; Bakay and Melnikov, 2008).

Parasitological studies of *S. mentella* show that there is no geographical/spatial variability of parasite fauna of that species between the six study areas in the pelagial of the Irminger Sea and adjacent waters (Melnikov and Bakay, 2009a). Also, no reliable geographical differences were revealed in the level and specific features of invasion by *S. lumpi*, frequency of occurrence and body sites where pigment patches, reliable natural tags for *S. mentella*, were located. The data obtained demonstrate the unity of pelagic *S. mentella* aggregations over the entire distribution range in the Irminger and Labrador Seas.

Close similarity of parasite fauna of *S. mentella* observed at different sites of the southeastern slope/shelf of Greenland and the Irminger Sea pelagial indicates the close links between these fish and their belonging to a single population.

Composition of parasite fauna of *S. mentella* in these areas shows that maturing redfish migrate from shallower to deeper areas on the Greenland slope and to the pelagial of the Irminger Sea. The latter direction is obviously the main one in summer. This is confirmed by a massive primary invasion of pelagic *S. mentella* maturing for the first time by *S. lumpi* (Melnikov and Bakay, 2009b). Infrequent occurrence of *S. lumpi* on *S. mentella* in deeper (600-1100 m) waters on the Greenland slope can be caused by redfish migration to the adjacent pelagial or by the settling of some fish from pelagic aggregations there. The latter in proved by the presence of pigment patches on the body of some fish, which is typical of *S. mentella* from the pelagial.

Genetic diversity

Genes coding proteins (allozymes and hemoglobin)

The paper (Cadrin et al., 2010) cites: "*The Faroese redfish project found differences in allozyme frequencies between shallow samples (the southwest Irminger Sea, the western Iceland shelf, north and east of the Faroes, and off Norway) and deep samples (the northeast Irminger Sea, the eastern Iceland slope, and southwest of the Faroe Islands*; ICES, 2005)".

However, the chapter describing the Faroese redfish project in the paper (ICES, 2005) do not contain the required more detailed description of the findings or a reliable sampling method, but indicates that the genetic analysis was performed by colleagues from other countries (Torild Johansen, Department of Fisheries and Marine Biology, Bergen, Norway and Anna Kristin Danielsdottir, Marine Research Institute, Population Genetics Laboratory, Reykjavik, Iceland). The findings from the papers by T. Johansen and K. Danielsdottir as well as other papers cited in the overview (Cadrin et al., 2010) are analysed below.

Criterion 1. The paper by Johansen et al. (2000a) compares two groupings/"types" of *S. mentella* (deep-sea and oceanic). However, half of 10 deep-sea type samples (five samples) were collected at less than 500 m depth that is indicative of non-compliance with the requirements of criterion 1 for identifying two groups of *S. mentella* (ICES, 2009).

Danielsdottir et al. (2008) have also analysed *S. mentella* individuals, preliminary separating them into the two groupings by morphological traits, which did not satisfy the requirements of criterion 1 (ICES, 2009). Thus, the data in that paper cannot be used to support the hypothesis about the existence of two *S. mentella* stocks in the Irminger Sea (above and below 500 m).

Allele frequencies at loci coding proteins within the samples taken in the Irminger Sea above and below 500 m were only compared in the papers by G. Novikov et al. (2006) and S. Melnikov et al. (2007).

Criterion 2. In the paper by G. Novikov et al. (2006), no differences in allele frequencies between *S. mentella* samples collected at different depths were described. S. Melnikov et al. (2007) indicate that in 2002, the frequencies of *MEP-2*100* allele in the samples taken above and below 500 m were virtually the same (0.550 and 0.572). In other years, there were some differences, but they were comparable with differences between the samples collected in different years (*MEP-2** in Russian papers corresponds to *MEP-1** in the papers by researchers from other countries – Anon. 2004).

In the paper (Johansen et al., 2000a), significant differences between the samples by allele frequencies at loci coding malic enzyme (*MEP-1**) and hemoglobin (*HB-2**) were found. In the paper by Danielsdottir et al. (2008), differences between samples in frequencies of the *MEP-1** allele were indicated. Differences in allele frequencies at other loci were small. However, the authors did not test any significance of differences by separate loci.

In the methodological paper (Balloux and Lugon-Moulin, 2002, p. 157), a test is described that permits to establish if the borderline between the populations was drawn correctly: "*As long as samples from the same deme are pooled together, no significant change in F_{IS} is expected. However, when a sample from a different breeding unit is incorporated in the pooling strategy, a significant increase in F_{IS} should occur*".

Data in the paper (Danielsdóttir et al., 2008) permit to perform such a test. Mean F_{IS} for the samples of "deep-sea" *S. mentella* phenotype is 0.182 and for the samples of "oceanic" phenotype it is 0.178. The F_{IS} value for the pooled sample is 0.163, i.e. when the two groupings are pooled, no increase but rather some decrease in F_{IS} is observed. This means that the two groupings of samples in question do not belong to different populations of *S. mentella*.

Criterion 3. The paper by T. Johansen et al. (2000a) does not show the temporal stability of the revealed differences, which is the necessary condition for identification of the population structure. It should be noted that Melnikov et al. (2007) identified considerable year-to-year differences in allele frequency between the samples.

Danielsdóttir et al. (2008) have not revealed any significant genetic differences between the samples collected in the three consecutive years. This period is definitely too short for such a long-lived species like beaked redfish with a life span that can exceed 30 years (Stransky et al., 2005; ICES, 2006). It should be noted that many samples studied in the paper (Danielsdóttir et al., 2008) exhibit significant heterozygote deficiencies, i.e. they were genetically heterogeneous, most probably due to the presence of individuals of different age.

Criterion 4. The authors of the paper (Melnikov et al., 2007) showed gradual depth-dependent changes in allele frequency at the *MEP-2** locus in *S. mentella*. The presence of a clinal genetic variation related to depth does not support the hypothesis about the existence of two *S. mentella* populations but rather reflects the selection observed in each cohort as maturing fish move to deeper layers of the Irminger Sea (Bakay and Melnikov, 2002, 2008). This assumption is in full accordance with the aforementioned biological data.

The selection hypothesis is criticized in the paper by Danielsdóttir et al. (2008, p.1733): "*If this hypothesis is true, then the loss of so many individuals would result in reduced genetic variability, which would be detected by measurement of* A_R. *However,* A_R *was higher in the deep-sea phenotype than in the oceanic phenotype, rendering this life cycle hypothesis unlikely*". However, selection often increases genetic variability, for example, a frequently observed selection in favour of heterozygous individuals (review: Hansson and Westerberg, 2002).

The selection hypothesis is also discussed by S.X. Cadrin et al. (2010, p.1621): "*Assuming that most spawning would be achieved by the larger, older fishes in the deep layer, there is no reasonable explanation for the maintenance of high frequencies in the juveniles of the alleles that are selected against after the movement to the deeper layer. Thus, variation at the MEP locus between "oceanic" and "deep-sea" phenotypes is more parsimoniously explained as the result of adaptation to different environments by two diverging populations*". However, age-specific selection when the direction of selection can change with age is a well-known phenomenon (Mitton, 1997, and references in this paper) which is quite likely to occur in *S. mentella.*

Moreover, genetic differences between the samples of *S. mentella* collected at different depths can be explained not only by selection, but also by different intensity of vertical migration in individuals with different genotypes. This phenomenon was described for other species (Ehrman and Parsons, 1981). In particular, in Atlantic cod (*Gadus morhua* L.) carriers of different alleles of a protein-coding locus show different vertical migration patterns (Pampoulie et al., 2008). In Atlantic salmon (*Salmo salar* L.), individuals carrying different genotype of the *MEP-2** locus have different duration of feeding at sea (Jordan et al., 1990). Direct evidence of selection changing allele frequencies at *MEP-2** locus during the life span of one generation of *S. mentella* is given in our new paper (Artamonova et al., Appendix 1).

Criterion 5. The diversity in protein-coding loci observed in the samples from the Irminger Sea can be explained, to a large degree, by the occurrence of hybrids of *S. mentella* and other species of the genus *Sebastes* inhabiting this area in the samples. Here, for example, the *MDH-2*30(20)* allele, which is typical of *S. viviparus* and *S. fasciatus*, was found. In some years, this allele was found in 5% of *S. mentella* (Anon. 2004; Novikov et al., 2006). A. Makhrov et al. provided new data

on hybridization between *S. mentella* and one of these species in the Irminger Sea (Appendix 2).

It is virtually impossible to find hybrids of *S. mentella* and *S. marinus* using allozyme markers. However, the facts of hybridization of these species in the Irminger Sea, which were revealed using other genetic markers, are given below. Such hybridization can particularly affect the frequency of the *IDHP-1*60* allele, which is more often found in *S. marinus* than in *S. mentella* (Anon. 2004).

The results from our analysis are summarized in Table 1. The conclusion is as follows: available data the on diversity of protein-coding loci cannot be used to substantiate the hypothesis about the existence of two redfish stocks in the Irminger Sea – below and above 500 m depth – because the requirements of the majority of the criteria used to analyse population structure of species have not been met in the papers analysed by us. Moreover, these criteria are not fully complied with in any of the papers analysed.

Diversity of haplotypes of the mitochondrial DNA

The paper (Cardin et al., 2010) cites data on the diversity of mitochondrial DNA (mtDNA) of *S. mentella* presented in two papers (Schmidt, 2005; Ingimarsdóttir, 2008). Here we shall analyze the main results and methodology of these studies and their compliance with the requirements for the criteria used to study population structure.

Criterion 1. In the study by C. Schmidt (2005) the diversity of the mitochondrial gene, ND3, is investigated. This author studied 3 small (12-15 fish) samples from each of the two hypothetical groupings of *S. mentella* (deep-sea and oceanic) collected in 1996-2001. It should be noted that these groupings of *S. mentella* were differentiated according to morphology (Magnusson and Magnusson, 1995) rather than by depth as suggested in the ICES document (2009). Therefore, the results from this paper cannot be used to substantiate the hypothesis about two (< 500 m and > 500 m) *S. mentella* populations in the Irminger Sea.

S. Ingmansdóttir (2008) compared haplotype frequencies of the mitochondrial gene cyt *b* in the samples of *S. mentella* collected in 2006 and 2007 in < 400 m and > 500 m depth in the Irminger Sea.

Criterion 2. Though S. Ingmansdóttir (2008) has found a significant difference in haplotype frequencies between the samples, this difference is very small. It should be noted that the mitochondrial DNA fragment coding for subunit 16S ribosomal RNA was found to be identical in *S. mentella* individuals caught in the Irminger Sea at 300 and 500 m depth (Sundt and Johansen, 1998).

Criterion 3. No temporal stability in the observed differences has been revealed in the both papers by S. Ingmansdóttir (2008) and C. Schmidt (2005).

Criterion 4. There are numerous literature data on the adaptive role of mtDNA polymorphism in fishes (review: Brown, 2008). In particular, the differences are shown between haplotype frequencies in mtDNA in migratory and resident Atlantic salmon *Salmo salar* L. which obviously belong to the same population (King et al., 1993). This observation suggests that differences in genotype frequencies between samples can be related to migration intensity of different genotype carriers. It cannot be ruled out that differences in genotype frequencies in redfish sampled at different depth can be caused by more frequent migrations of fish carrying certain genotypes to deeper layers.

The paper by S. Ingimarsdóttir (2008) contains an interesting observation concerning nonrandom distribution of different mtDNA haplotypes in the samples of *S. viviparus*, collected at different depth. In our opinion, this observation can indicate that carriers of different mtDNA haplotypes adapt to living at different depths. This means that frequencies of those haplotypes can be subjected to selection and therefore cannot serve as markers of population differentiation in aquatic organisms.

Criterion 5. The paper by Cadrin et al. (2010) indicates that the use of the mitochondrial DNA to differentiate redfish populations of the genus *Sebastes* is limited due to their frequent interspecific hybridisation. It should be added that this limitation also applies to other genetic markers.

Data in the paper by Johansen and Nævdal (2004) suggest that data on the diversity of the mitochondrial DNA are almost inapplicable for the studies of population structure of the Irminger Sea *S. mentella*. In this paper, the sequence of mtDNA, including ND3/ND4 and 12S/16S genes, is studied. Together with common haplotype for the “deep-sea” and “oceanic” samples, the haplotype that occurs in the “deep-sea” sample but does not occur in the “oceanic” sample was revealed. However, this haplotype is typical of *S. marinus* and its carriers are, most probably, hybrids of *S. mentella* and *S. marinus*.

Data about genetic diversity of “giant” redfish (or “giants”), presented by C. Schmidt (2005), are of particular interest. Some of those fish had mitochondrial haplotypes typical of *S. marinus*, other fish, of *S. mentella*, while some individuals had unique (original) haplotypes. The author correctly concludes that “giants” appear due to hybridization of the two redfish species. The assumption about the hybrid origin of the “giants” was also put forward in some previous works (Kotthaus, 1961; Altukhov and Nefyodov, 1968). Young “giants” certainly occur in the Irminger Sea, and the admixture of such fish can therefore affect allele frequencies in *S. mentella* samples.

Thus, in accordance with the methodological recommendations (Hedgecock, 1994; Waples, 1998), data on mtDNA haplotype frequencies in the Irminger Sea *S. mentella*

samples cannot be used to identify the population structure as temporal stability of those frequencies has not been shown and there are data suggesting the depth-dependent selection in favour of some haplotypes. Moreover, interspecific hybridisation (genus *Sebastes*) most probably significantly affects mtDNA haplotype frequencies in *S. mentella* in the Irminger Sea and adjacent waters.

RAPD and AFLP methods

The paper by Cadrin et al. (2010, p. 1621) correctly indicates that "*RAPDs produce results that may not be repeatable and are no longer considered to be a reliable approach for testing population-structure hypotheses", "AFLP results are not always repeatable among laboratories* ..." and " *both RAPD and AFLP are considered to be more exploratory than confirmatory for stock identification studies*".

RAPD-based studies (Johansen et al., 1997; Johansen and Dahle, 2004) do not meet the majority of the criteria used to investigate population structure. Two small samples of *S. mentella* (20 and 28 ind.), collected in 1995 at different depth (120-190 m and 620-700 m) in the Irminger Sea, were studied in the papers. Statistical significance of frequencies of bands observed in the RAPD analysis between the samples and temporal stability of the differences were not tested. A possible role of selection and interspecific hybridization in differences of allele frequencies was not studied.

Two samples of “oceanic” and two of “deep-sea” *S. mentella* from the Irminger Sea were used in AFLP analysis (Schmidt, 2005). However, fish were selected for analysis by their morphological features rather than by ICES criteria (ICES document, 2009). Genetic differences were revealed between the samples studied, but there was also temporal instability of their genetic structure. A possible role of selection and interspecific hybridization in differences of allele frequencies was not studied. Therefore data obtained in the cited work are not suitable for identification of the *S. mentella* population structure.

Thus, data derived by RAPD and AFLP methods and presented in the mentioned papers (Johansen et al., 1997; Johansen and Dahle, 2004; Schmidt, 2005) cannot be used to substantiate the hypothesis that there are two *S. mentella* stocks in the Irminger Sea (> 500 m and < 500 m stocks), because the authors of these studies do not follow important methodological recommendations (Waples, 1998).

Microsatellites

In the paper by Cadrin et al. (2010, p.1623), the *S. mentella* stock inhabiting depths below and above 500 m in the Irminger Sea is separated “based primarily on microsatellite information”. The data are presented in the papers (ICES, 2005; Schmidt, 2005; Pampoulie and Danielsdóttir, 2008; Stefansson et al., 2009).

However, the paper (Joensen, 2002; ICES, 2005) describing the results from the Faroese Redfish Project contain no detailed information about microsatellite diversity but state that the microsatellite analysis was carried out by Anna Kristin Danielsdóttir, Marine Research Institute, Population Genetics Laboratory, Reykjavik, Iceland. Presented below are the results of the other three studies.

Criterion 1. Two small "deep-sea" and "oceanic" *S. mentella* samples (14 and 15 ind.) from the Irminger Sea were studied in the paper (Pampoulie and Danielsdóttir, 2008). However, fish were selected for analysis of these redfish groups by their morphological traits rather than by ICES criteria (ICES, 2009).

M. Stefansson et al. (2009) studied the genetic structure of the two groups of samples of *S. mentella* inhabiting the Irminger Sea collected at depths below and above 500 m.

C. Schmidt (2005) studied allele frequencies at microsatellite loci in *S. mentella* from some North Atlantic areas. It was shown that two samples from deeper layers (640 and 700 m) of the central Irminger Sea differ from the group of samples collected in other Atlantic areas (among those samples there was one from the southern Irminger Sea, 159 m depth).

Criterion 2. The paper (Pampoulie and Danielsdóttir, 2008) does not indicate whether the genetic differences among the samples from the Irminger Sea are significant, but states that these differences are very small. In the two other papers small yet significant differences in allele frequencies at microsatellite loci between the samples compared were revealed.

Criterion 3. According to data by Stefansson et al. (2009), differences between the groups compared were stable. However, the second paper indicated temporal instability of frequencies of microsatellite loci that can, according to the author (Schmidt, 2005), explain genetic differences observed between the Irminger Sea *S. mentella* samples. In the third paper (Pampoulie and Danielsdóttir, 2008), temporal stability of allele frequencies was not tested.

Criterion 4. Literature contains abundant evidence indicating that many microsatellites have very important adaptive value. They contribute to the formation of DNA structure and DNA recombination and replication, serving as very important components in the gene activity regulation system (review: Chistiakov et al., 2006). Moreover, one of the papers (Pampoulie and Danielsdóttir, 2008) contains evidence of selection affecting four out of nine microsatellite loci in redfish (genus *Sebastes*).

Criterion 5. Investigations of microsatellites provide additional evidence of existence of a great number of interspecific hybrids of the genus *Sebastes* in the Irminger Sea. A considerable number of individuals in the samples collected at greater depths in the Irminger Sea have genotypes which are unusual for *Sebastes*

mentella. Several of these admixed genotypes revealed a proportion of membership in the "*S. viviparus* cluster" or in the "*S. fasciatus* cluster" (Schmidt, 2005).

In one of the two *S. mentella* samples from the Irminger Sea analysed in the paper by Pampoulie and Danielsdóttir (2008), it was impossible to identify 20% of the individuals as any of existing redfish species or “deep-sea” and “oceanic” *S. mentella*. It is assumed that some of these individuals are hybrids of *S. mentella* and *S. marinus.*

Meanwhile, even a relatively small admixture of hybrid individuals can explain the slight differences in the frequencies of microsatellite loci between some samples of Irminger Sea *S. mentella*. Hybrids are frequently found at greater depths. This can explain an increased diversity of alleles observed in the samples taken in this area, mentioned by Stefansson et al. (2009).

Thus, none of the cited studies of microsatellite loci can be used to substantiate the hypothesis that pelagic waters of the Irminger Sea are inhabited by two populations (stocks) of *S. mentella* (< 500 m and > 500 m depth). There is convincing evidence of temporal instability of allele frequencies in microsatellites, and of the impact of selection and interspecific hybrids on these frequencies. Thus, according to the mentioned methodological recommendations (Hedgecock, 1994; Waples, 1998), data on allele frequencies of microsatellite loci in the samples of the Irminger Sea *S. mentella* cannot be used to identify its population structure.

Conclusions from analysis of genetic diversity

None of the cited papers describing the genetic structure of *S. mentella* in the Irminger Sea provides data that can be used to substantiate the hypothesis that there are two pelagic populations (stocks) of redfish (< 500 m and > 500 m) in the Irminger Sea (Table 1). In many of the papers, fish were selected for the identification of population by morphological criteria rather than by depth, which is methodologically unacceptable.

For allozymes, microsatellites and mtDNA haplotypes, there is evidence of selection (in AFLP and RAPD analyses, the possibility of selection was not tested). For allozymes, microsatellites and the AFLP method, there is evidence of temporal instability of allele frequencies (temporal stability of haplotype genetic differences in mtDNA has not been studied and tested; neither was it tested in RAPD analysis). A precautionary approach to interpretation of genetic data is also needed due to the occurrence of interspecific hybrids of the genus *Sebastes* and “giant” redfish of hybrid origin in the Irminger Sea.

Phenotype diversity

Phenotype features are successfully used for stock identification provided there is time stability. Nonetheless, this method has a number of limitations. In particular,

phenotype differences do not necessarily imply different genotypes (Clayton, 1981). Such differences may be caused by environmental conditions (Lindsey, 1964; Todd et al., 1981).

After the beginning of extensive harvesting of pelagic *S. mentella* Icelandic scientists made an attempt to differentiate between oceanic and deepwater redfish on the basis of phenotype traits.

In the frames of the EU Redfish project 2000-2004 (Anon, 2004) great attention was paid to phenotype diversity of *S. mentella*. In particular, in a comprehensive practical study by D. Garabana (2005) a morphometric examination of North Atlantic redfishes was carried out. The results allow for a conclusion that pelagic *S. mentella* phenotypes described by Icelandic scientists do not have any morphometric differences. Morphometrically, both phenotypes belong to a single population: "No morphometric differences were found among the two *S. mentella* phenotypes described in the Irminger Sea. The pelagic component of *S. mentella* living in the Irminger Sea is morphometrically a single unit".

Besides, the mentioned EU Redfish project involved the analysis of diversity of shape/structure for otoliths of *S. mentella* from different North Atlantic areas (Stransky, 2002, 2005). As a result, fish from three large marine areas were identified: 1) Flemish Cap and Davis Strait, 2) Greenland Shelf, Irminger Sea, Icelandic Slope, and Faroe Islands, and 3) Barents Sea. The study included comparison of *S. mentella* samples collected at < 500m and > 500 m depth, which did not reveal any difference between the samples (Stransky, 2002).

For identification of *S. mentella* stocks in the Irminger Sea a comparatively new method, the analysis of fatty acid profile, was applied. This permitted Norwegian scientists to put forward the hypothesis about four *S. mentella* stocks living in the North Atlantic (Joensen and Grahl-Nielsen, 2004). However, as correctly noted by Cadrin et al. (2010, p. 1624), this method must be applied with caution "in the context of stock identification". There is data that fatty acid composition in cod is influenced by genotype (Grahl-Nielsen, 2005), but this does not imply that the environmental impact can be ruled out. Given below is the discussion of its use to identify stock structure of *S. mentella* (Joensen and Grahl-Nielsen, 2004).

The study (Joensen and Grahl-Nielsen, 2004) of fatty acid composition of the Irminger Sea *S. mentella* compared (alongside samples from other North Atlantic areas) two samples (20 fish each) collected at > 500 m and < 500 m depth. Significant differences in fatty acid composition were found between *S. mentella* from two samples. According to Joensen and Grahl-Nielsen (2004), the IR2 sample that makes up a separate cluster was collected at 220-300 m depth. However, Grahl-Nielsen (2005, p. 262) states that the same IR2 sample making up a separate cluster was collected at 650-800 m depth.

Temporal stability of the revealed differences was not analysed. However, as shown in the methodological paper (Grahl-Nielsen, 2005, p. 266), "we need to sample fish from different populations at intervals, preferably during an entire year, to see how stable the fatty acid profile is under shifting dietary regimens and during changes in other environmental factors, such as temperature." Susceptibility of analysed criteria to selection was not tested either.

One of the two compared samples of *S. mentella* from the Irminger Sea was collected in June, unlike all the other samples collected in August-November. Besides, half of fish in that sample were spent (in other samples the portion of spent fish did not exceed 5%). To substantiate the use of that sample in the analysis, H. Joensen and O.Grahl-Nielsen (2004, p. 121) argue that "lipid levels in white muscle were not significantly different among ovary maturation stages (MacFarlane et al., 1993)". However, MacFarlane et al., (1993, p. 396) did not analyse data on lipid levels in muscle of other *Sebaste*s species (*S. flavidus,* the Pacific species) at all stages of female maturation. Besides, these authors found variations in lipid levels in muscle tissue between the months. In particular, lipid content in June was estimated as ca.40 mg/g for females and ca.50 mg/g for males, while in August, it was ca. 20 mg/g for both sexes.

Joensen and Grahl-Nielsen (2004) discuss the possible impact of diet on fatty acid composition, but they do not produce any evidence of the absence of such dependence. However, diet composition of *S. mentella* in the Irminger Sea at > 500 m and < 500 m depth show considerable differences (Saborido-Rey et al., 2005; Bakay and Melnikov, 2002, 2008).

The above arguments demonstrate the obvious invalidity of fatty acid composition for identification of stock structure of the Irminger Sea *S. mentella.*

Conclusions of phenotype diversity analysis

As shown in this paper, the analysis of phenotype diversity does not permit to identify two or more populations of *S. mentella* in the Irminger Sea due to high ontogenetic plasticity of all phonotype traits studied (life-history traits, morphology and fatty acid content).

One can agree with Cadrin et al. (2010, p. 1624): "Although interpretation of phenotypic traits is somewhat subjective, i.e. can be validly interpreted in several ways, all information on phenotypic variability is consistent with our perception of genetic stocks." It should be, however, added that data on diversity of phenotypic traits are similarly in line with the concept of a single stock of *S. mentella* in the Irminger Sea and adjacent waters.

Discussion

Thus, the results of long-term biological investigations of *S. mentella* in the Irminger Sea, including ecological and parasitological data, indicate the presence of a single population of that species in the study area. A number of well known facts (absence of geographical and oceanographic barriers, single spawning area, single nursery area etc.) disprove the assumption about several reproductively isolated stocks.

Small differences in allele frequencies at some loci between the samples of the Irminger Sea *S. mentella* that were taken as evidence of occurrence of two populations in this area are well explained by hybridization between *S. mentella* and other species of genus *Sebastes*. Hybrids mainly occur in deeper waters of the Irminger Sea among other large marine fishes, and “giant” redfish (“giants”) appear to be represented predominantly by hybrids (Schmidt, 2005). Large sizes of hybrids are probably the result of heterosis. It should be noted that individuals representing intermediate forms of *S. mentella* and *S. marinus* in terms of morphology were already revealed in the beginning of redfish study in the Irminger Sea (Zakharov, 1964).

The increased frequency of hybrids in deeper waters of the Irminger Sea can explain the differences in allele frequencies at some loci between the samples collected above and below 500 m and high allele diversity in deep-sea samples (Danielsdóttir et al., 2008; Stefansson et al., 2009). It is very likely that the frequency of hybrids in the Irminger Sea population varies from year to year and this may lead to changes in allele frequencies in some genes over time.

Selection most probably affects genotype frequencies at a number of loci. For some loci, such an influence is discussed (Melnikov et al., 2007; Pampoulie and Danielsdóttir, 2008). Selection may change allele frequencies as well as heterozygosity. Temporarily and spatially varying selection results in temporal instability of genotype frequencies.

Temporal instability occurring due to temporally varying hybridisation and selection, as well as probably due to gene drift is attributed to a Wahlund effect observed in the aggregate of samples of *S. mentella* from the Irminger Sea (Danielsdóttir et al., 2008).

Thus, all the genetic traits of *S. mentella* in the Irminger Sea can be explained by hybridization, selection and probably by gene drift within the single population.

Studies of phenotypic traits of *S. mentella* in the Irminger Sea show that these traits are either characterised by considerable plasticity that denies their use as population markers (fatty acid profile) or are unsuitable for stock identification (morphological traits).

Conclusions

Genetic differences observed between several samples of *S. mentella* collected at different depth are likely to be linked to the presence of interspecific (genus *Sebastes*) hybrids in some of them, to selection at some loci and, probably, to gene drift. Therefore, those differences cannot disprove the concept about a single stock of *S. mentella* inhabiting that area. The analysis of a large array of data on phenotype diversity in that stock does not contradict this conclusion.

References

Altukhov, Y. 2006. Intraspecific Genetic Diversity. Monitoring, Conservation and Management. Berlin, Heidelberg: Springer-Verlag. 438 p.

Altukhov, Y., Nefyodov, G., and Payusova, A. 1968. Thermostability of isolated muscle in determining the taxonomic relationship of the marinus- and mentella-types of the redfish (*Sebastes*). Research Bulletin ICNAF. № 5: 130-136.

Anon. 2004. Population structure, reproductive strategies and demography of redfish (Genus *Sebastes*) in the Irminger Sea and adjacent waters (ICES V, XII and XIV, NAFO 1). REDFISH QLK5-CT1999-01222 Final Report. 318 p.

Anon. 2010. Stock identification of Irminger Sea redfish. ICES. ACOM Consultations (Nantes). Doc. 4.2.

Bakay, Y. 1988. Application of results from parasitological investigations in redfish (*Sebastes mentella* Travin) populational structure studies. ICES Doc. CM/G:35, 14 p.

Bakay, Y. 1989. On infestation of marine redfishes (*Sebastes* genus) of the North Atlantic by the copepod *Sphyrion lumpi* (Krøyer, 1845). Proceed. Workshop "*Sphyrion lumpi*". Güstrow. GDR. P.29-36.

Bakay, Y. 2000. Parasites and pigmented patches as indicators of intraspecific structure of *Sebastes mentella* in the Irminger Sea. ICES Doc. CM/Z:06, 15 pp.

Bakay, Y. 2004. On the issue of unity of redfish *Sebastes mentella* fishable stock in the Irminger Sea and adjacent waters in the light of parasitological data. Working document № 3.5. SGSIMUR Meeting. 31 p. (www.ices.dk).

Bakay, Y., and Karasev, A. 2001. Registration of ectolesions of redfish from *Sebastes* genus in the North Atlantic (methodical guidelines). NAFO SCR Doc. 01/27. № 4401, 8 pp.

Bakay, Y., and Melnikov, S. 2002. Vertical structure of *Sebastes mentella* concentrations in the pelagic open part of the Irminger Sea. NAFO SCR Doc. 02/10, № 4611, 21 pp.

Bakay, Y., and Melnikov, S. 2008. Biological and ecological characteristics of deepwater redfish *Sebastes mentella* (*Scorpaeniformes: Scorpaenidae*) at different depths in the pelagial of the Irminger Sea. Journal of Ichthyology, 48, № 1: 68-80.

Balloux, F. and Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. Molec. Ecol., 11: 155-165.

Beaumont, M. and Nichols, R. 1996. Evaluating loci for use in the genetic analysis of population structure. Proc. R. Soc. Lond. B. V. 263: 1619-1626.

Bogovski, S. and Bakay, Y. 1989. Chromatoblastomas and related pigmented lesions in deepwater redfish, *Sebastes mentella* (Travin), from North Atlantic areas, especially the Irminger Sea. Journal of Fish Diseases, 12: 1-13.

Brown, K. H. 2008. Fish mitochondrial genomics: sequence, inheritance and functional variation. Journal Fish Biology, 72: 355-374.

Cadrin, S. X., Bernreuther, M., Daníelsdóttir, A., Hjörleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigurðsson, T., and Stransky, C. 2010. Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats. ICES Journal of Marine Sciences. V. 67. Issue 8: 1617-1630.

Chistiakov, D. A., Hellemans, B., and Volckaert, F. A. 2006. Microsatellites and their genomic distribution, evolution, function and application: A review with special reference to fish genetics. Aquaculture, 255: 1-29.

Clayton, J. W., 1981. The stock concept and the uncoupling of organismal and molecular evolution. Can. Journ. Fish. Aquat. Sci., 38: 1515-1522.

Coltman, D. W., and Slate, J. 2003. Microsatellite measures of inbreeding: a meta-analysis. Evolution, 57. № 5: 971-983.

Daníelsdóttir, A. K., Gíslason, D., Kristinsson, K., Stefansson, M., Johansen, T., and Pampoulie, C. 2008. Population structure of deep-sea and oceanic phenotypes of deepwater redfish in the Irminger Sea and Icelandic continental slope: are they cryptic species? Transactions of the American Fisheries Society, 137: 1723–1740.

Dushchenko, V. V. 1986. Polymorphism of NADP-dependent malatedehydrogenase in *Sebastes mentella* (Scorpaenidae) from the Irminger Sea. Journal of Ichthyology, 27: 129-131.

Ehrman, L., and Parsons, P. A. 1981. Behavior genetics and evolution. McGraw-Hill. New York.

Garabana, D. 2005. The genus *Sebastes* Cuvier, 1829 (Pisces, Scorpaenidae) in the North Atlantic: Species and stock discrimination using traditional and geometric morphometrics. PhD Thesis, University of Vigo, Spain. 306 pp.

Grahl-Nielsen, O. 2005. Fatty acid profiles as natural marks for stock identification. *In* Stock Identification Methods. Application in Fishery Science, pp. 247-271. Ed. by S. X. Cadrin, K. D. Friedland, and J. Waldman. Elsevier Academic Press, Amsterdam. 719 pp.

Hansson, B., and Westerberg, L. 2002. On the correlation between heterozygosity and fitness in natural populations. Molecular Ecology, 11: 2467-2474.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? Genetics and Evolution of Aquatic Organisms. (Beaumont A. R., ed.). London. p. 122-134.

ICES. 2005. Report of the Study Group on Stock Identity and Management Units of Redfishes (SGSIMUR). ICES Document CM 2005/ACFM:10.

ICES. 2006. Workshop on Age Determination of Redfish (WKADR). ICES Document CM 2006/RMC: 09.

ICES. 2009. Report of the Workshop on Redfish Stock Structure (WKREDS). ICES Document CM 2009/ACOM: 37.

ICES. 2010. Report of the Stock Identification Methods Working Group (SIMWG). ICES Document CM 2010/SSGSUE: 04.

Ingimarsdóttir, S. 2008. Species and population differentiation in the North Atlantic *Sebastes*. A study of mtDNA variation. MSc thesis. University of Iceland. 52 pp.

Johansen, T., Daníelsdóttir, A.K., Meland, K., and Nævdal, G. 1997. Studies on the relationship between deep-sea and oceanic *Sebastes mentella* in the Irminger Sea by the use of haemoglobin, allozyme analyses and RAPD. ICES Document CM 1997/HH:13.

Johansen, T., Daníelsdóttir, A., Meland, K., and Nævdal, G. 2000a. Studies of the genetic relationship between deep-sea and oceanic *Sebastes mentella* in the Irminger Sea. Fisheries Research, 49: 179-192.

Johansen, T., Nævdal, G., Daníelsdóttir, A., and Hareide, N. 2000b. Genetic characterization of giant *Sebastes* in the deep water slopes in the Irminger Sea. Fisheries Research, 45: 207-216.

Johansen, T., and Dahle, G. 2004. Discrimination among species of the genus *Sebastes* in the North Atlantic by random amplified polymorphic DNA. Sarsia, 89: 478-483.

Johansen, T. and Nævdal, G. 2004. Population genetic studies of *Sebastes mentella* in the North Atlantic with emphasis on the stock complex in the Irminger Sea. Working Document of the SGSIMUR meeting 31.08-03.09. 25 p. (www.ices.dk)

Joensen, H. 2002. Discrimination among fish species and stocks by multivariate analysis of fatty acid profiles in selected tissues. Doctoral dissertation, University of Bergen.

Joensen, H., and Grahl-Nielsen, O. 2004. Stock structure of *Sebastes mentella* in the North Atlantic revealed by chemometry of the fatty acid profile in heart tissue. ICES Journ. Mar. Sci., 61: 113-126.

Jones, D. 1970. Food, parasites and the reproductive cycle of pelagic redfish (*Sebastes mentella* Travin) from weather station Alfa in the North Atlantic. Bull. Mar. Ecology, 6, № 9: 347-370.

Jordan, W., Youngson, A., and Webb, J. 1990. Genetic variation at the malic enzyme-2 locus and age at maturity in sea-run Atlantic salmon (*Salmo salar*). Can. Journ. Fish. Aquat. Sci., 47: 1672-1677.

King, D., Hovey, S., Thompson, D., and Scott, A. 1993. Mitochondrial DNA variation in Atlantic salmon, *Salmo salar* L., populations. Journ. Fish Biol., 42: 25-33.

Kotthaus, A. 1961. Contributions to the race problem in redfish. Rapports et Proces-Verbaux des Reunions, 150: 42-44.

Lindsey, C. C. 1964. Temperature-controlled meristic variation in the paradise fish, *Macropodus opercularis* (L.). Can. Journ. Zool., 32: 87-98.

MacFarlane, R. B., Norton, E. C., and Bowers, M. J. 1993. Lipid dynamics in relation to the annual reproductive cycle in yellowtail rockfish (*Sebastes flavidus*). Can. Journ. Fish. Aquat. Sci., 50: 391–401.

Magnusson, J. 1977. Pelagic redfish in the Irminger Sea. Distribution and abundance. ICES Doc. CM /H: 43, 10 pp.

Magnusson, J., and Magnusson, J. V. 1995. Oceanic redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters. Scientia Marina, 59, № 3-4: 241-254.

Magnusson, J., Magnusson, J. V., and Sigurdsson, T. 1995. On the distribution and biology of the oceanic redfish in March 1995. ICES Doc. CM/G:40, 14 pp.

Makhrov, A. A. 2009. Critical analysis of data on genetic differences between two groupings of redfish (*Sebastes mentella*) in the Irminger Sea. NEAFC Annual Meeting. Doc. AM/23, 10 pp.

Melnikov, S., Shibanov, V., Stroganov A., and Novikov, G. 2007. On the issue of biological and genetic studies of *Sebastes mentella* in the open areas of the North East Atlantic. ICES Doc. CM/L:02, 25 pp.

Melnikov, S., and Bakay, Y. 2009a. Structure of Aggregations and Principal Population Characteristics of Redfish *Sebastes mentella* (Scorpaeniformes: Scorpaenidae) in the Pelagic Zone of the Irminger Sea and Adjacent Waters. Journal of Ichthyology, 49, No. 2: 170-182.

Melnikov, S., and Bakay, Y. 2009b. Recruitment of stock of deepwater redfish *Sebastes mentella* (Scorpaeniformes: Scorpaenidae) in the pelagial of the Irminger Sea and adjacent waters. Journal of Ichthyology, 49, No. 8: 650-661.

Mitton, J. 1997. Selection in Natural Populations. Oxford Univ. Press. 240 pp.

Nedreaas, K., Johansen, T., and Nævdal, G. 1994. Genetic studies of redfish (*Sebastes* spp.) from Icelandic and Greenland waters. ICES Journal of Marine Sciences, 51: 461-467.

Nedreaas, K., and Nævdal, G. 1991. Genetic studies of redfish (*Sebastes* spp.) along the continental slopes from Norway to East Greenland. ICES Journal Marine Science, 48. No. 2: 173-186.

Nefyodov, G. 2002. On the hybrid origin of the west Greenland population of the goldish redfish. Problems of Fisheries, 3. No. 1: 65-72 (In Russian, English summary).

Nielsen, E. 2004. Review of the genetic data of *Sebastes mentella* in the Irminger Sea and adjacent waters. SGSIMUR Work. Doc. No. 8. 4 pp. (www.ices.dk)

Noskov, A., Romanchenko, A., and Pavlov, A. 1984. Abundance of larvae and assesment of the spawninf redfish (*Sebastes mentella* Travin) stock of the Irminger Sea for 1982. ICES Doc. CM/G: 24, 16 pp.

Novikov, G., Stroganov, A., Shibanov, V., and Melnikov, S. 2006. Biological and genetic characteristics of redfish *Sebastes mentella* of the Irminger Sea and adjacent waters. Deep Sea 2003: Conference on the Governance and Management of Deep-sea Fisheries. Rome: FAO. Part 2: 66-76.

Pampoulie, C., and Danielsdóttir, A. 2008. Resolving species identification problems in the genus *Sebastes* using nuclear genetic markers. Fisheries Research, 93: 54-63.

Pampoulie, C., Jakobsdóttir, K., Marteinsdóttir, G., and Thorsteinsson, V. 2008. Are vertical behaviour patterns related to the pantophysin locus in the Atlantic cod (*Gadus morhua* L.)? Behaviour Genetics, 38: 76–81.

Paradies, G., Petrosillo, G., Paradies, V., Reiter, R., and Ruggiero, F. 2010. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. Journ. Pineal. Res., 48: 297-310.

Pavlov, A. 1988. Distribution and behaviour of beaked redfish (*Sebastes mentella* Travin) on the Reykjanes Ridge based on observations from the underwater vehicle “Sever-2” // Abstracts of the reports from All-Union Conference “Biological resources of the thalassobathyal marine zone”. Moscow, p. 54-55 (in Russian).

Pavlov, A., and Galuzo, A. 1989. Assessment of redfish (*Sebastes mentella*) stock in terms of conception of populational homogenety of the cpecies in the North Atlantic. ICES Doc. CM/G:18, 11 pp.

Pavlov, A., Gorelov, A., and Oganin, I. 1989. Larval distribution, eliminates indices spawning stock assessment for beaked redfish (*Sebastes mentella* T.) in the Irminger Sea in 1984-1988. ICES Doc. CM/G:16, 21 pp.

Pavlov, A., Mamylov, V., Noskov, A. et al. 1989. Results of the USSR investigations of *Sebastes mentella* Travin in 1981-1988 (ICES Subareas XII, XIV). ICES Doc. CM/G:17, 25 pp.

Rikhter, V. A. 1961. Are there aggregations of redfish in the pelagial? // Scientific and Technical Information Bulletin of the Baltic Research Institute of Marine Fisheries and Oceanography, 12:19-20 (in Russian).

Roques, S., Sevigny, J-M., and Bernatchez, L. 2001. Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example. Mol. Ecol., 10: 149-165.

Saborido-Rey, F., Garabana, D., Stransky, C., Melnikov, S., and Shibanov, V. 2005. Review of the population structure and ecology of *Sebastes mentella* in the Irminger Sea and adjacent waters. Reviews in Fish Biology and Fisheries, 14: 455-479.

Schmidt, C. 2005. Molecular genetic studies on species and population structure of North Atlantic redfish (genus *Sebastes*; Cuvier 1829). PhD thesis, University of Hamburg. 303 pp.

Schmidt, C., and Trautner, J. 2004. Molecular genetic studies of species and population structure of North Atlantic redfish (genus *Sebastes*). Work. Doc. SGSIMUR Meeting. 60 pp. (www.ices.dk).

Shibanov, V., Gorelov, A., and Oganin, I. 1984. Some results of researches into the biology of redfish (*Sebastes mentella*) in the Irminger Sea. ICES Doc. CM/G:79, 16 pp.

Shibanov, V., and Melnikov, S. 2004. Population status and identity of fishable stock of redfish *Sebastes mentella* in the pelagic Irminger Sea and adjacent waters. Work. Doc. SGSIMUR Meeting. 33 pp. (www.ices.dk)

Stefansson, M. Ö., Reinert, J., Sigurðsson, P., Kristinsson, K., Nedreaas, K., and Pampoulie, C. 2009. Depth as a potential driver of genetic structure of *Sebastes mentella* across the North Atlantic Ocean. ICES Journal of Marine Science, 66: 680-690.

Stransky, C. Otolith shape analysis of Irminger Sea redfish (*Sebastes mentella*): Preliminary results// NAFO, SCR Doc. 02/35. Ser.No. N4646. – 2002. - 7 pp.

Stransky, C., Gudmundsdóttir, S., Sigurdsson, T., Lemvig, S., Nedreaas, K., and Saborido-Rey, F. 2005. Age determination and growth of Atlantic redfish

(*Sebastes marinus* and *S. mentella*): bias and precision of age readers and otolith preparation methods. ICES Journal of Marine Science, 62: 655–670.

Stroganov, A., and Novikov, G. 2004. Study of genetic characteristics of the redfish *Sebastes mentella* Travin from the Irminger Sea and adjacent waters. Work. Doc. SGSIMUR Meeting. 46 pp. (www.ices.dk).

Sundt, R. C., and Johansen, T. 1998. Low level of interspecific DNA sequence variation in the mitochondrial 16S rDNA in North Atlantic redfish *Sebastes* (Pisces, Scorpaenidae). Sarsia, 83. No. 5: 449-452.

Templeman, W. 1959. Redfish distribution in the North Atlantic. Journ. Fish. Res. Board Can. № 120: 167.

Todd, T. N., Smith, G., and Cable, L. 1981. Environmental and genetic contributions to morphological differentiation in ciscoes (*Coregoninae*) of the Great Lakes. Can. Journ. Fish. Aquat. Sci., 38: 59-67.

Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. The Journal of Heredity, 89. № 5: 438-449.

Weir, B. S. 1990. Genetic data analysis. Methods for discrete population genetic data. Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers. 400 pp.

Zakharov, G. 1964. Redfish above the ocean depths. ICNAF Res. Bull. № 1: 39-42.

Table 1. Compliance of the results of genetic diversity studies with established criteria. YES – compliance, NO –contradiction, ? – no data available

Markers	Publications	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5
Protein-coding genes	Johansen et al., 2000a	**NO**	YES	?	**NO**	**NO**
	Novikov et al., 2006	YES	**NO**	?		
	Melnikov et al., 2007	YES	**NO**	**NO**		
	Danielsdóttir et al., 2008	**NO**	YES?	YES?		
Mitochon-drial DNA	Schmidt, 2005	**NO**	YES	?	**NO?**	**NO**
	Ingimarsdóttir, 2008	YES	YES	?		
RAPD analysis	Johansen and Dahle, 2004	YES	?	?	?	?
AFLP analysis	Schmidt, 2005	**NO**	YES	**NO**	?	?
Micro-satellites	Schmidt, 2005	YES	YES	**NO**	**NO**	**NO**
	Pampoulie and Danielsdóttir, 2008	**NO**	?	?		
	Stefansson et al., 2009	YES	YES	YES		

Appendix 1

*MEP-2*60* allele frequency correlation with invasion by copepod *Sphyrion lumpi* in beaked redfish (*Sebastes mentella*)

Artamonova V.S., Bakay Y.I*., Makhrov A.A., Popov V.I*., Rolskiy A.Y*.
A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences,
*Knipovich Polar Research Institute of Marine Fisheries and Oceanography

Frequency of allele *MEP-2*60* in *Sebastes mentella* heavily infested by *Sphyrion lumpi* is considerably and significantly higher than in fish with low infestation by that parasite or in healthy fish. This fact does not comply with the hypothesis about the presence of two stocks (types) of *S. mentella* in the Irminger Sea (above and below 500 m).

Introduction

In the 1990s, Icelandic scientists made an attempt to identify two types (forms) of *S. mentella* in the Irminger Sea pelagial, the "oceanic" and the "pelagic deep-sea" redfish. “Oceanic *S. mentella*” was supposed to live in a wide depth range from 50 to 700 m, while “pelagic deep-sea *S. mentella”*, only at depths greater than 500 m. The Icelandic researchers based their opinion about the existence of two *S. mentella* types on five differentiation criteria: 1) intensity of red body colour; 2) length composition; 3) length-at-maturity; 4) thickness of neck; 5) infestation by copepod *Sphyrion lumpi*, occurrence of pigmented patches on skin and in muscle (ICES, 1998).

However, it was established that these characteristics could not be used to differentiate between the stocks, as the occurrence of most of them depends on age and feeding of *S. mentella*, and in some cases these differences are unreliable. In particular, it was shown that traces of infestation by copepod *Sphyrion lumpi* (old cephalotoraxes of parasites) are also widespread in individuals caught in the Irminger Sea at 600-900 m depth. This proves that those fish originally inhabited shallower areas where they were infested by this mesopelagic parasite (Bakay, 2000; Bakay and Melnikov, 2002, 2008).

Frequency of protein coding genes was compared to prove that the two types of redfish *S. mentella* in the Irminger Sea belong to different stocks. Significant differences were detected in allele frequency at certain genes that were most pronounced at malic enzyme coding locus. For instance, samples of “deep sea” *S. mentella* demonstrated lower allele *MEP-1*60* frequency than “oceanic” samples (Johansen et al., 2000; Danielsdóttir et al., 2008).

However, other studies (Melnikov et al., 2007) showed that frequency of allele *MEP-2** locus changes gradually with depth (*MEP-2** in Russian papers corresponds

to *MEP-1** in foreign studies – Anon. 2004). It is assumed that the gradual variation in allele frequency disagrees with the hypothesis of two stocks, being suggestive of the selection in every *S. mentella* cohort as older fish migrate to deeper waters of the Irminger Sea.

Another hypothesis has recently been launched by S.X. Cardin et al. (2010, p. 1621): "variation at the MEP locus between oceanic and deep-sea phenotypes is more parsimoniously explained as the result of adaptation to different environments by two diverging populations".

It is noteworthy that this hypothesis disagrees with the gradual change in the frequency of this locus with an increase of depth inhabited by *S. mentella*, as shown by Melnikov et al. (2007).

This paper presents the fact testifying to the adaptive nature of polymorphism at *MEP-2** locus and shows that this fact is incompatible with the hypothesis about two populations (stocks) of *S. mentella* inhabiting the Irminger Sea.

Materials and methods

S. mentella samples were collected in 2007 during the cruise of R/V "Smolensk" as part of international trawl and acoustic survey to assess *S. mentella* stock in the pelagial of the Irminger Sea. Data on sampling time and location are shown on the table 1, the sampling area (Fig. 1). We used only samples collected at <500 m depth as, by S.X. Cadrin et al. (2010), 500 m depth is the conditional line separating the two "stocks".

Biological examination was carried out immediately after catching. Number and location of copepod *Sphyrion lumpi* was recorded on every *S. mentella* individual (both live parasites and traces of their presence, old cephalothoraxes that fish obviously tend to carry till the end of its life, were counted) (Bakay and Karasev, 2001). For further genetic analysis white muscle samples were taken, frozen and stored at -25°C till processing.

To investigate polymorphism of malic enzyme-coding locus (*MEP-1**), 10% starch gel electrophoresis was employed with buffer system CAME (Clayton and Tretiak, 1972). Allele frequencies in samples were compared using $\chi 2$ criterion and Monte-Carlo method (Roff and Bentzen, 1989) using CHIRXC software (Zaykin and Pudovkin, 1993).

Results and discussion

The distribution of *MEP-2** locus genotype in all investigated samples did not differ significantly from the Hardy-Weinberg distribution. Allele frequencies in samples did not differ significantly, being therefore pooled for interpretation purposes.

Comparison of groups with different levels of infestation by copepod *S. lumpi* indicates that heavily infested fish demonstrate a considerably higher *MEP-2*60* allele frequency than individuals with low infestation level (Table 2). The difference is highly significant ($p<0.005$).

Therefore, individuals with the presence of *MEP-2*60* allele are more susceptible to copepod *S. lumpi* infestation and, presumably, show higher mortality rate as the result of infestation. This can account for the reduction of frequency of this allele in *S. mentella* samples with depth.

The described fact disproves the hypothesis of two populations (stocks) of *S. mentella* inhabiting the pelagial of the Irminger Sea. The presence of two populations would involve lower frequency of *MEP-2*60* allele in the "oceanic" population which is more susceptible to infestation with copepod *S. lumpi*, and should have demonstrated selection against this allele.

As *MEP-2*60* frequency is lower in samples from deeper areas of the Irminger Sea, the sampled fish apparently survived the selection and became resistant to copepod *S. lumpi* at depths <500 m. As noted above, another proof of their stay at <500 m depth is the detection of traces of infestation by copepod *S. lumpi*.

It is noteworthy that facts of correlation between parasite infestation and fish genotype at protein-coding loci were reported previously (review: Golubtsov, 1988). Such correlation is justified as agents of disease are among major motive forces of evolution of living organisms forcing them to develop different adaptive mechanisms at the genetic level (Altizer et al., 2003).

References

Altizer, S., Harvell, D., and Friedle, E. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology and Evolution, 18. № 11: 589-596.

Anon. 2004. Population structure, reproductive strategies and demography of redfish (Genus *Sebastes*) in the Irminger Sea and adjacent waters (ICES V, XII and XIV, NAFO 1). REDFISH QLK5-CT1999-01222 Final Report. 318 pp.

Bakay, Y. 2000. Parasites and pigmented patches as indicators of intraspecific structure of *Sebastes mentella* in the Irminger Sea. ICES Doc. CM/Z:06, 15 pp.

Bakay, Y., and Karasev, A. 2001. Registration of ectolesions of redfish from *Sebastes* genus in the North Atlantic (methodical guidelines). NAFO SCR Doc. 01/27. № 4401. 8 pp.

Bakay, Y., and Melnikov, S. 2002. Vertical structure of *Sebastes mentella* concentrations in the pelagic open part of the Irminger Sea. NAFO, SCR Doc 02/10, № 4611. 21 pp.

Bakay, Y., and Melnikov, S. 2008. Biological and Ecological Characteristics of Deepwater Redfish *Sebastes mentella* (*Scorpaenidae*) at Different Depths in the Pelagial of the Irminger Sea. Journal of Ichthyology, 48: 68–80.

Cadrin, S. X., Bernreuther, M., Daníelsdóttir, A., Hjörleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigurðsson, T., and Stransky, C. 2010. Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats. ICES Journal of Marine Sciences. V. 67. Issue 8: 1617-1630.

Clayton, J., and Tretiak, D. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. Journ. Fish. Res. Board Can., 29: 1169-1172.

Daníelsdóttir, A. K., Gíslason, D., Kristinsson, K., Stefansson, M., Johansen, T., and Pampoulie, C. 2008. Population structure of deep-sea and oceanic phenotypes of deepwater redfish in the Irminger Sea and Icelandic continental slope: are they cryptic species? Transactions of the American Fisheries Society, 137: 1723–1740.

Golubtsov, A. 1988. Intrapopulation variability in animals and protein polymorphism. Moscow, Nauka Press, 168 pp. (in Russian).

ICES. 1998. Report on the Study Group on Redfish Stocks (SGRS). ICES Document CM 1998/G: 03.

Johansen, T., Daníelsdóttir, A., Meland, K., and Nævdal, G. 2000. Studies of the genetic relationship between deep-sea and oceanic *Sebastes mentella* in the Irminger Sea. Fisheries Research, 49: 179-192.

Magnusson, J., and Magnusson, J. V. 1995. Oceanic redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters. Scientia Marina, 59, № 3-4: 241-254.

Melnikov, S., Shibanov, V., Stroganov, A., and Novikov, G. 2007. On the issue of biological and genetic studies of *Sebastes mentella* in the open areas of the North East Atlantic. ICES Doc. CM/L:02, 25 pp.

Roff, D. A., and Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: x2 and the problem of small samples. Mol. Biol. Evol., 6, № 5: 539-545.

Zaykin, D. V., and Pudovkin, A. 1993. Two programs to estimate Chi-square values using pseudo-probability test. J. Heredity, 84: 152.

Table 1. Characteristics of *Sebastes mentella* samples under investigation 2007.

Sample (transect) number	Sample collection date	n	Latitude	Longitude	Depth of trawling, m	Avrg. length, cm	Avrg. weight, g
1 (38)	24.06.2007	1	60°46′ N	27°03′ W	0-500	46	1126
2 (39)	24.06.2007	3	61°11′ N	28°16′ W	400-500	45	1029
3 (41)	25.06.2007	30	62°06′ N	30°07′ W	400	40	731
4 (47)	28.06.2007	30	61°15′ N	34°55′ W	270-310	34	460
5 (36)	02.07.2007	30	59°27′ N	36°08′ W	250-290	35	507
6 (58)	03.07.2007	30	57°59′ N	37°30′ W	260-280	36	560
7 (55)	02.07.2007	7	60°13′ N	34°47′ W	230-260	36	543

Table 2. *MEP-2*60* allele frequency in *Sebastes mentella* samples with different number of copepod *Sphyrion lumpi*

Number of *Sphyrion lumpi* (with old cephalotoraxes) on one fish	Number of *S. mentella* individuals examined	*MEP-1*60* allele frequency
0	64	0.336
1	30	0.317
2 and more	37	0.540

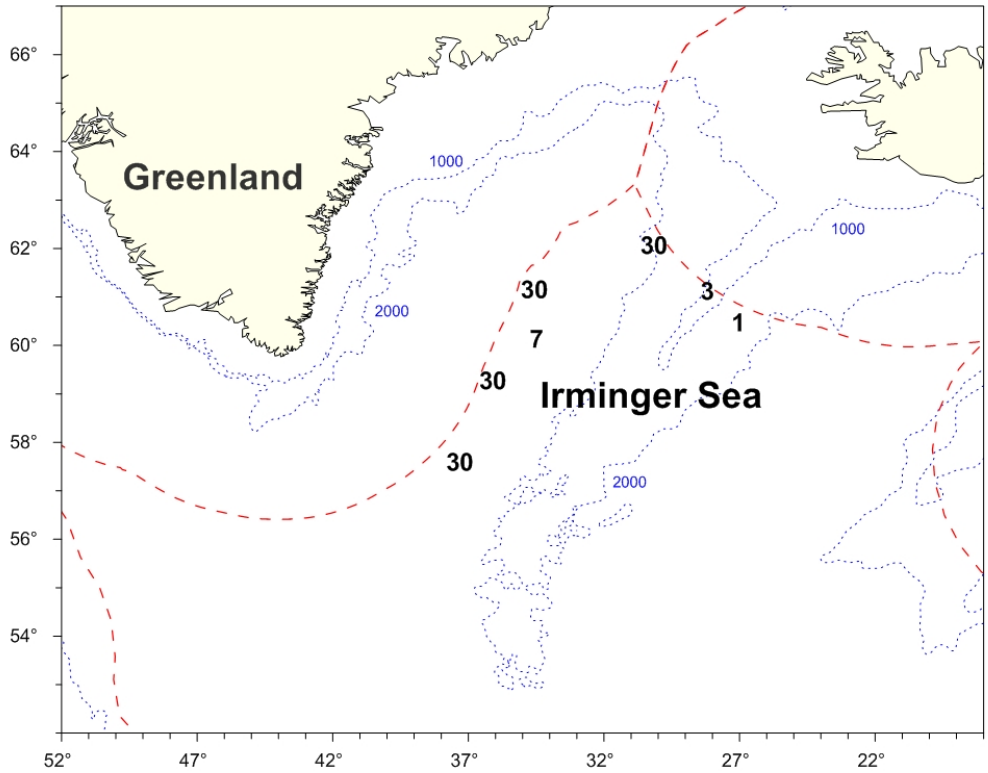

Fig. 1. Sampling location and number of samples *Sebastes mentella* for genetic analysis 2007.

Appendix 2

Redfish (genus *Sebastes*) hybridization in the Irminger Sea and its relevance for the assessment of population structure

Makhrov A.A., Artamonova V.S., Popov V.I.*, Rolskiy A.Yu.*, Bakay Yu.I.*
A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences,
*Knipovich Polar Research Institute of Marine Fisheries and Oceanography

Introduction

One of the most interesting model objects for research of biological forms development processes is the so called “flocks” of related forms occurring in isolated areas (Cristescu et al., 2010). Formation of such structures is to a large extent due to hybridisation (Herder et al., 2006, and references in this paper). Description and investigation of such formations in large water bodies permits the extrapolation of previously established correlations to a broad spectrum of objects and apply fundamental knowledge to resolve practical problems. One of the promising research objects are viviparous redfish of genus *Sebastes*. In the study by V. Barsukov (1986) these fish were for the first time used as a model object to study evolution. He described different groups of redfish inhabiting different depths.

Genus *Sebastes* in the North Atlantic is represented by four species, *S. marinus*, *S. mentella*, *S. viviparus* and *S. fasciatus* (Hureau and Litvinenko, 1984). In recent years, scientists’ attention has been focused on the deepwater Irminger Sea, where, more than half a century ago, different researchers discovered aggregations of redfish which was commonly thought to live near the bottom on the continental slopes (Zakharov, 1964; Magnusson et al., 1965; Jones, 1968). Foreign researchers assumed the presence of two populations of redfish in the Irminger Sea (“deep-sea” and “oceanic”) and even two “emerging” species of the genus *Sebastes*. Arguments in favour and against this hypothesis are summarized in reviews (Saborido-Rey et al., 2005; Cadrin et al., 2010), referring to a large array of biological, morphological, parasitological and genetic data.

Unfortunately, these papers disregard the possibility of the presence of hybrids of already known redfish species in the Irminger Sea. Meanwhile, the very first research of redfish in the Irminger Sea detected transition forms of *S. mentella* and *S. marinus* (Zakharov, 1964). The study of microsatellites in three small samples of *S. mentella* from the Irminger Sea revealed that 20% (3 individuals) in one of the samples qualified neither as beaked redfish nor other redfish species. Presumably two of the individuals are hybrids of *S. mentella* and *S. marinus* (Pampoulie and Danielsdóttir, 2008).

The research focused on interspecies hybridization in the North-West Atlantic redfish revealed that a considerable part of individuals in the area are hybrid forms of

S. mentella and *S. fasciatus* (Roques et al., 2001). S.X. Cadrin et al. (2010) use it as the basis for identifying *S. mentella* in this area as a specific stock. It is interesting that in one of the previous studies a number of local *S. mentella* populations were identified because of incorrect species identification (this paper was criticised by Barsukov (1986).

We searched for interspecies hybrids in quite representative samples of redfish collected in the Irminger Sea that were identified by external morphological characteristics as *S. mentella*. Our objective was to study the role of hybridisation in the development of the gene pool of *S. mentella* in the Irminger Sea. The results were used to resolve some theoretical (studies of form development) and practical (stock structure analysis) tasks.

Materials and methods

Samples of *S. mentella* (131 specimens) were collected in 2007 during the cruise of the Russian R/V "Smolensk" within the framework of an international trawl and acoustic survey aiming to assess *S. mentella* stock in the pelagial of the Irminger Sea and in 2010 during the commercial cruise "Obelyai" (Fig. 1). The sample series of *S. fasciatus* (30 specimens) was collected in August 2010 during the cruise of vessel "Melcart-2". Data on time and location of sample collection, their quantity, and biological characteristics of the examined individuals collected in 2007 are shown in Appendix 1 which also describes the methodology of electrophoresis. Similar data for sample series, which were collected in 2010 are given in table 1.

To identify hybrids of *S. mentella* and *S. fasciatus* in accordance with the recommendations (Payne, Ni, 1982; Rubec et al., 1991), the malate dehydrogenase ferment coded at *MDH-1** loci (expressed in muscle only) and *MDH-2** (expressed in muscle and liver) was analysed. Different alleles are fixed or close to fixation at locus *MDH-2** in both *S. mentella* and *S. fasciatus*.

The analysis of this locus allows to identify hybrids of *S. mentella* and *S. viviparus* as both *S. fasciatus* and *S. viviparus* have fixed or prevalent same allele in *MDH-2**. Unfortunately, allozyme analysis does not permit to discriminate between *S. fasciatus* and *S. viviparus,* neither does it allow an unmistakable identification of *S. mentella* and *S. marinus* hybrids (Johansen, 2003).

Results and discussion

The analysis of *S. mentella* samples, which were collected in 2007, indicated two carriers of *MDH-2*67* allele, that is characteristic of *S. mentella* and *S. fasciatus,* heterozygotes of *MDH-2*67/100* (this allele frequency for all samples in average was 0.0153). At *MDH-1**, two heterozygotes, carriers of *MDH-1*23* and *MDH-1*161*, were found (frequency of each allele being 0.0076).

In redfish samples collected in 2010 one fish with *MDH-2*67/100* was registered. Frequency of this allele in all samples averaged 0.0136. Besides, one heterozygote *MDH-1*23/100* was identified in those samples.

The allele that is characteristic of *S. viviparus* and *S. fasciatus* was previously identified in *S. mentella* samples from the Irminger Sea in T. Johansen (2003) and referred to as *MDH-2*30*, while Russian researchers refer to this allele as *MDH-2*20*. Carriers of this allele are present in samples collected in different years and at different depths of the Irminger Sea. In 2000, average frequency of this allele in samples collected at >500 m was estimated as 0.05 (Novikov et al., 2006).

Mating of redfish *S. mentella* with three other species of genus *Sebastes* is quite likely to occur on the shelf/slope of Greenland and Iceland, where, along with abundant species *S. marinus* and *S. mentella*, also occur *S. fasciatus* (Hureau and Litvinenko, 1984) and *S. viviparus* (Johansen et al., 2002; Bakay, 2008; Bakay and Grudnev, 2009), as well as *S. mentella* from pelagic aggregations which migrate to those areas in summer and autumn (Melnikov et al., 2005; Melnikov and Bakay, 2006, 2009), including males ready for mating (Melnikov and Popov, 2009).

Apparently, fish heterozygous by *MDH-2** locus are hybrids of *S. mentella* and *S. viviparus*. *S. viviparus* was occasionally observed in the Irminger Sea (Hureau and Litvinenko, 1984; Johansen, 2003; Pampoulie and Danielsdóttir, 2008). Only 18 individuals of *S. fasciatus* were sampled in that area (Hureau and Litvinenko, 1984).

Furthermore, as mentioned above, hybrids of *S. mentella* and *S. marinus* are reportedly present in considerable amounts in the Irminger Sea. Therefore, interspecies hybridization poses quite significant influence on the genetic composition of *S. mentella* in the Irminger Sea and should be taken into account while assessing its population structure.

Genetic data are used as the basis for distinguishing the two (“shallow-pelagic” and “deep-pelagic”) stocks of *S. mentella* in the Irminger Sea (review: Cadrin et al., 2010). However, all differences in gene frequencies between samples from different depths are well accounted for by hybridization (with the exception of allele frequencies of the *MEP-2** gene linked to selection for susceptibility to copepod *Sphyrion lumpi* (Artamonova et al., Appendix 1).

Thus, the mtDNA chain including ND3/ND4 and 12S/16S was assessed by Johansen and Nævdal (2004). Alongside the haplotype common for both “deep-sea” and “oceanic” samples, the study indicated the presence of the haplotype in “deep-sea” samples that was not observed in “oceanic” samples. However, the latter haplotype is typical of *S. marinus* and, quite likely, it is carried by hybrids of *S. mentella* and *S. marinus*.

*IDHP-2*60* allele is more frequently observed in samples of “deep-sea” redfish than in “oceanic” redfish. However, this allele is more frequently observed in *S. marinus* than in *S. mentella* (Johansen, 2003) and its carriers are quite possibly the hybrids of these two species. Minor differences in allele frequencies at microsatellite loci demonstrated by samples from the Irminger Sea areas with different depths as well as a higher diversity of alleles at these loci in samples collected from >500 m, may be accounted for by a higher level of occurrence of hybrid redfish in deeper waters.

According to biological and parasitological data, the Irminger Sea pelagial is inhabited by a single stock of *S. mentella*. With age fish gradually migrate to deeper areas (review: Saborido-Rey et al., 2005). It is quite probable that, being sterile, hybrids live longer than ordinary redfish. That is why the occurrence of hybrids is higher among older and larger fish that inhabit deeper waters of the Irminger Sea.

The highest occurrence of hybrids was observed among the so called “giant” redfish occurring on the Reykjanes Ridge. They are morphologically closer to *S. marinus* yet have specific otolith and gill raker morphology (Kotthaus, 1961). In terms of serum albumin frequency, “giants” occupy an intermediate position between *S. marinus* and *S. mentella* (Altukhov and Nefyodov, 1968) and a considerable part of them are sterile (Altukhov et al., 1968). Hemoglobin spectrum of most giants resembles that of *S. marinus*, yet some patterns are characteristic specifically of this form (Johansen, 2003), whereas certain individuals have fractions typical of *S. mentella* (Nefedov, 1970). The electrophoresis patterns of some proteins found in the giants resemble those of *S. marinus* (Johansen, 2003) while other protein patterns resemble those of *S. mentella* (Nefedov, 1970). Some of these fish have a mitochondrial haplotype characteristic of *S. marinus*, others had that of *S. mentella* or unique haplotypes (Schmidt, 2005). One individual was, after microsatellite assessment, clustering with *S. viviparus* (Pampoulie and Danielsdottir, 2008).

It can be concluded that *S. mentella* population in the Irminger Sea represents an interesting case of a drastic intensification of evolutionary processes under the environmental conditions that are new for the species. This population demonstrates a significant level of interspecies hybridization and intense selection. However, the existing data do not contain the evidence of the existence of two different stocks of *S. mentella* in pelagic waters of the Irminger Sea.

References

Altukhov, Ju. P., Artemjeva, K., Borisova, I., and Nefyodov, G. 1968. Immunological analysis of serum proteins of redfish in connection with maturation. Research Bulletin ICNAF, № 5: 44-48.

Altukhov, Ju. P., and Nefyodov, G. 1968. A study of blood serum protein composition by agar-gel electrophoresis in types of redfish (genus *Sebastes*). Research Bulletin ICNAF, № 5: 86-90.

Bakay, Y. 2008. Parasite fauna of little redfish *Sebastes viviparus*. Materials of the 4th All-Union Congress of the Parasitological Society of the Russian Academy of Sciences "Parasitology in the 21st century: challenges, methods, solutions". V. 1. St.Petersburg. p.42-46 (in Russian, English summary).

Bakay, Y., and Grudnev, M. 2009. Myxosporidian (*Myxozoa: Myxosporea*) fauna of redfish of genus *Sebastes* in the North Atlantic. Parazitologia, 43, № 4: 317-329 (in Russian, English summary).

Barsukov, V. 1986. On species and speciation. System of species integration. Vilnius: 162-212. (in Russian, English summary).

Cadrin, S. X., Bernreuther, M., Daníelsdóttir, A., Hjörleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigurðsson, T., and Stransky, C. 2010. Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats. ICES Journal of Marine Sciences. V. 67. Issue 8: 1617-1630.

Cristescu, M., Adampwicz, S., Vaillant, J., and Haffner, D. 2010. Ancient lakes revisited: from the ecology to the genetics of speciation. Molecular Ecology, 19: 4837-4851.

Herder, F., Nolte, A., Pfaender, J., Schwarzer, J., Hadiaty, R., and Schliewen, U. 2006. Adaptive radiation and hybridization in Wallace's dreamponds: evidence from sailfin silversides in the Malili lakes of Sulawesi. Proceedings of the Royal Society. B, 273: 2209-2217.

Hureau, J-C., and Litvinenko, N. 1984. Scorpaenidae. Fishes of the North-eastern Atlantic and the Mediterranean. V. 3. Paris: 1211-1229.

Johansen, T. 2003. Genetic study of genus *Sebastes* (redfish) in the North Atlantic with emphasis on the stock complex in the Irminger Sea. Thesis for the partial fulfilment of the Dr. Scient. Degree: University of Bergen.

Johansen, T., Danielsdottir, A., and Nævdal, G. 2002. Genetic variation of *Sebastes viviparus* Krøyer in the North Atlantic. J. Appl. Ichthyol., 18: 177-180.

Johansen, T., and Naædal, G. 2004. Population genetic studies of *Sebastes mentella* in the North Atlantic with emphasis on the stock complex in the Irminger Sea. Work. Doc. of the SGSIMUR meeting, 25 pp. (www.ices.dk)

Jones, D. H. 1968. Angling for Redfish. ICNAF, Spec. Publ., Nr. 7(1): 225-240.

Kotthaus, A. 1961. Contributions to the race problem in redfish. Rapports et Proces-Verbaux des Reunions, 150: 42-44.

Magnusson, J., Magnusson, J. V., and Hallgrimsson, I. 1965. The "Aegir" redfish larvae expedition to the Irminger Sea in May 1961. Rit Fiskideildar, 4, № 2: 4-86.

Melnikov, S., Bakay, Y., Makeenko, G., and Kalashnikova, M. 2005. Structure of *Sebastes mentella* concentrations in demersal and pelagic fisheries in the Reykjanes Ridge area. ICES, NWWG, Work. Doc.

Melnikov S., and Bakay, Y. 2006. Biological and ecological substantiation of management measures for redfish fishery off Iceland. Rybnoe khozyaistvo, 1:48-50 (in Russian).

Melnikov, S., and Bakay, Y. 2009. Recruitment of stock of deepwater redfish *Sebastes mentella* (*Scorpaeniformes: Scorpaenidae*) in the pelagial of the Irminger Sea and adjacent waters. Journal of Ichthyology, 49, No. 8: 650-661.

Melnikov, S., and Popov, V. 2009. Distribution and biology of beaked redfish *Sebastes mentella* (Scorpaenidae) during the mating season in the North Atlantic pelagial. Journal of Ichthyology, 49, No. 3: 341-353.

Novikov, G., Stroganov, A., Shibanov, V., and Melnikov, S. 2006. Biological and genetic characteristics of redfish *Sebastes mentella* of the Irminger Sea and adjacent waters. Deep Sea 2003: Conference on the Governance and Management of Deep-sea Fisheries. Rome: FAO. Part 2: 66-76.

Pampoulie, C., and Danielsdóttir, A. 2008. Resolving species identification problems in the genus *Sebastes* using nuclear genetic markers. Fisheries Research, 93: 54-63.

Payne, R., and Ni, I-H. 1982. Biochemical population genetics of redfishes (*Sebastes*) of Newfounland. Journal Northwest Atl. Fish. Sci., 3: 169-172.

Roques, S., Sevigny, J-M., and Bernatchez, L. 2001. Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example. Mol. Ecol., 10: 149-165.

Rubec, P., McGlade, J., Trottier, B., and Ferron, A. 1991. Evaluation of methods for separating of Gulf of St. Lawrence beaked redfishes, *Sebastes fasciatus* and *S. mentella*: malate dehydrogenase mobility patterns compared with extrinsic gasbladder muscle passages and anal fin ray counts. Can. J. Fish. Aquat. Sci., 48. No. 4: 640-660.

Saborido-Rey, F., Garabana, D., Stransky, C., Melnikov, S., and Shibanov, V. 2005. Review of the population structure and ecology of *Sebastes mentella* in the Irminger Sea and adjacent waters. Reviews in Fish Biology and Fisheries, 14: 455-479.

Schmidt, C. 2005. Molecular genetic studies on species and population structure of North Atlantic redfish (genus *Sebastes*; Cuvier 1829). PhD Thesis: University of Hamburg. 303 pp.

Table 1. Characteristics of *Sebastes. mentella* samples under investigation 2010.

Sample (transect) number	Sample collection date	n	Latitude	Longitude	Depth of trawling, m	Avrg. length, cm	Avrg. weight, g
1 (2)	01.05.2010	15	61˚35′N	28˚83′W	670-740	41	811
2 (4)	02.05.2010	15	61˚37′N	28˚78′W	670-750	41	868
3 (8)	05.05.2010	10	61˚37′N	28˚68′W	590-800	39	705
4 (15)	09.05.2010	20	61˚67′N	29˚05′W	870-900	42	917
5 (34)	28.05.2010	12	61˚62′N	28˚97′W	657-717	40	858

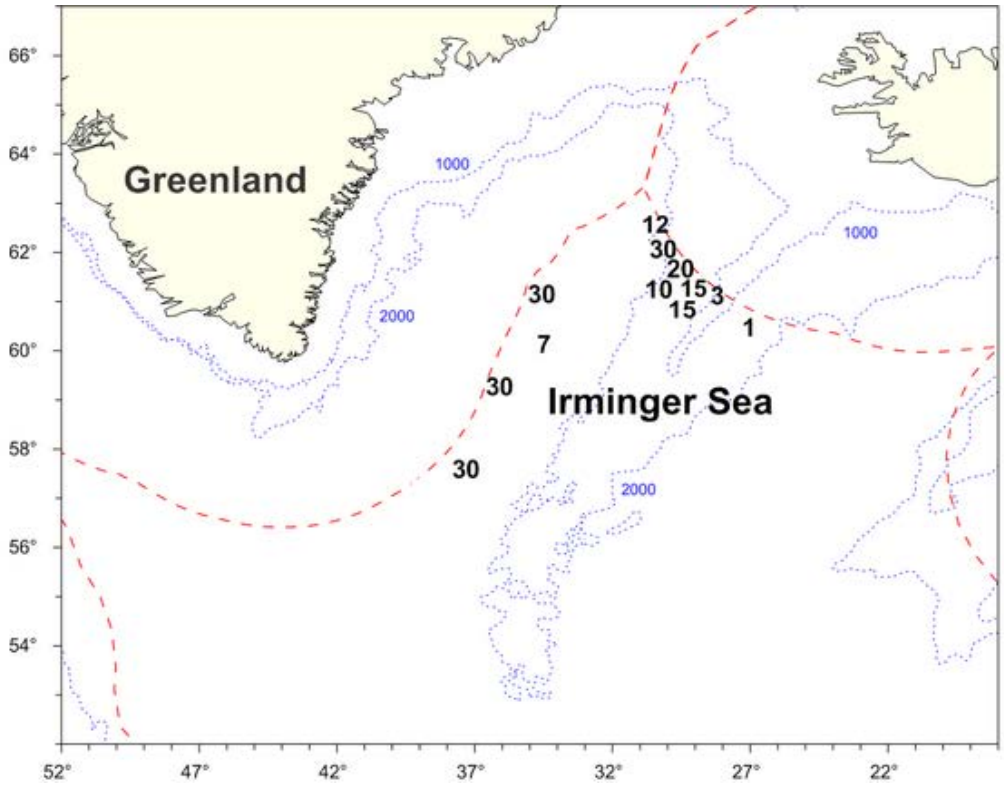

Fig. 1. Sampling location and number of samples of *Sebastes mentella* for genetic analysis 2007, 2010.

Annex 5: Original reviews of the Documents forwarded by the Russian Federation on the intra-specific structure of Redfish in the Irminger Sea

Referee #1:

Summary

These documents have been submitted to ICES for consideration redefining spatial management units of *Sebastes mentella* in the Irminger Sea, which have recently changed from a single management area to two areas roughly corresponding to different depth zones (as a proxy of geographical areas). The bulk of the information is contained in Makhrov *et al*., which contains a review and reinterpretation of the information used to define the management units (summarized in Cadrin *et al.* 2010), and the introduction of new genetic information. Makhrov *et al.* review information pertaining to geographic distribution and life-cycle, genetic diversity; and phenotypic diversity. Genetic information is evaluated with respect to five criteria: 1) samples should be available above and below 500 meters; 2) statistical differences must be demonstrated; 2) indications of genetic structure must be temporally stable; 4) the loci analyzed must be not be subject to selection; and 5) the genetic analyses must not be influenced by hybridization. Makhrov *et al.* conclude that "there is a single population/stock of this species in the Irminger Sea and adjacent waters".

Although the guidance I received suggested that my review should be analogous to reviewing a manuscript submitted for a journal, the task is more complex. Makhrov *et al.* can be viewed as a "rebuttal" to Cadrin *et al*. (2010), and both of these papers are broad reviews that summarize large bodies of work. Because Makhrov *et al.* presents a methodological critique and reinterpretation of many papers, a comprehensive review would require a detailed examination of each these papers. In addition, a detailed review would also be required of additional papers not cited by Makhrov *et al.* that were considered when defining the spatial management areas. In fact, a full review of these issues appears to have motivated the meeting of the SIMWG. I bring this up only to indicate that the scope of my comments here cannot be considered as a comprehensive review of the topic, and are largely based on the three Russian documents, Cadrin *et al*. (2010), and some of the papers on microsatellite analyses.

The primary issue appears to be whether the methodological issues raised by Makhrov *et al.* and the introduction of new data, cast reasonable doubt on the spatial management units established from the original data analysis. In my opinion, the issues raised by Makhrov *et al.* do not rise to this level due to a number of reasons, including: 1) many of the critiques raised by Makhrov *et al.* are acknowledged and considered by Cadrin *et al.* (2010); 2) some other critiques can be reasonably explained; and 3) some of the most influential papers cited by Cadrin *et al.* (2010) are not addressed by Makhrov *et al*. In the end, several types of data exist that do not support the one-stock hypothesis of Makhrov *et al.* and are not explained by the methodological issues raised. I begin with some general comments and then make some more detailed comments on the manuscripts.

General comments

Given my experience with Alaska rockfish, it does not surprise me that rockfish stocks could segregate by depth. Although the depth range shown for *S. mentella* is larger than we have seen for any given Alaska species, we have observed that various

species of rockfish occupy distinct depth zones along the Alaska continental shelf ranging from less than 100m to deeper than 500 m. A particularly relevant example is the blackspotted rockfish (*Sebastes melanostictus*) and rougheye rockfish (*Sebastes aleutianus*). It has long been observed that two colour morphs of fish referred to as "rougheye rockfish" existed. Analyses of several types of data, including allozynes (Hawkins *et al.,* 2005), mitochondrial and microsatellite DNA (Gharrett *et al.,* 2005), and morphometric and meristic characteristics (Orr and Hawkins, 2008) confirmed that fish referred to as "rougheye rockfish" actually contained two different species, with blackspotted rockfish now recognized. In addition, there is an indication that the two species may segregate by depth (Gharrett *et al.,* 2007), although this is not conclusive and the limited data available could represent confounding with gear type and habitat differences. It should be noted that the ranges of the two species overlap broadly, and are often found in close proximity to each other, so the processes that have lead to speciation are a current area of research.

I would disagree that only loci that are not under selection should be considered for fisheries problems. For example, there may be areas where gene flow occurs between areas but adaptive divergence arises to due selection, and in these cases studying loci under selection may provide valuable information. Conover *et al.* (2006) argue that selection pressure can maintain adaptive variation in marine environments and maintaining adaptive variation should be considered a management goal, and Hauser and Carvalho (2008) demonstrate local adaptation in Atlantic cod. Thus, it is not that non-neutral loci should be ignored, but rather that these loci provide different information that neutral loci and could still be relevant for fisheries management.

Makhrov *et al.* appear to largely emphasize casting doubt on previous genetic tests rather than evaluating the single-stock hypothesis in light of all the available information. The emphasis on reproductive isolation and genetic tests corresponds to an "evolutionary paradigm" (Waples and Gaggiotti, 2006) in which populations are viewed as groups of interbreeding individuals that transfer genetic information between generations. In contrast, an "ecological paradigm", in which populations are defined with respect to the demographic interactions between co -occurring individuals (Waples *et al.,* 2008), may be the most suitable to fisheries management goals. The critical issue is that what might be considered demographically independent stocks under the ecological paradigm may not represent completely reproductively isolated stocks under the evolutionary paradigm. It is well known in genetics that only very small interchange of spawners could prevent reproductive isolation (Waples *et al.,* 2008), but still allow essentially independent populations. This is relevant to this review because the approach taken in these papers seems to be that structure is assumed not to exist unless it can be demonstrated by the genetic analyses. The focus on statistical analyses of genetic data may lead to errors in not recognizing underlying demographic stocks.

A related point is that genetic stocks typically have weak power because the ability to detect the small amounts of spawner interchanges required for connectivity is usually quite limited. In a typical genetic test consisting of a null hypothesis of panmixia and an alternative hypothesis of not panmixia, a non-significant result does not necessarily imply that panmixia is occurring, but only means that we there is not enough evidence to reject the hypothesis of panmixia. This is a subtle point that relates to the general hypothesis testing framework – one can either reject or fail to reject the null hypothesis, but one cannot "prove" the null hypothesis. This is especially true in marine fisheries genetics because the lack of power to distinguish between meaningful migration rates is exacerbated at high effective population sizes. This results in

"one-sided" genetic tests in which a significant result may clearly indicate the presence of more than one stock, but a non-significant result cannot exclude the possibility of more than one stock. If fact, the asymmetry and limitations of genetic hypothesis testing led Waples *et al.* (2008) to suggest the holistic approach of considering non-genetic data. Makhrov *et al.* conclude that several types of genetic data cannot be used to support the presence of multiple stocks, and have an overall conclusion of that the data "indicate the presence of a single population". Although this overall conclusion is ostensibly from both genetic and non-genetic data, it appears to be heavily based on the genetic data with relatively little considerable of what would be the most sensible configuration of management areas based on all the available data.

Detailed comments

Doc. 1) "*Single population of beaked redfish (Sebastes mentella) in the Irminger Sea: biological characteristics and dynamics of gene pool*" by Makhrov *et al.*

Geographic distribution and life cycle of S. mentella

Depth-related changes in pigment patches are hypothesized to occur from ontogenetic movement from the shallow pelagic to the deeper pelagic. However, Cadrin *et al*. (2010) cite studies that indicate that data on pigment patches show considerable annual variability, and this is not addressed by Makhrov *et al.*

Makhrov *et al.* refer to "absolute predominance" of infestation of the parasite *Sphyrion lumpi* in water > 500m [presumably the Irminger Sea?], but infrequent occurrence of this parasite in deep waters of the Greenland slope. These data are explained by ontogenetic movement from shallow to deep water in the Irminger Sea, and migration from the deep Greenland slope water to adjacent pelagial areas. However, data are not cited to support this migration hypothesis. Additionally, Cadrin *et al*. (2010) cite a number of papers that show conflicting patterns regarding the prevalence of infestation of *Sphyrion lumpi* by depth.

An important point here is that Cadrin *et al.* (2010) cite studies that indicate the age readings of *S. mentella* are unreliable, so the hypothesis of ontogenetic movement into the deep water cannot be evaluated.

Allozymes and haemoglobin

Cadrin *et al.* (2010) and Makhrov *et al.* agree that some earlier studies may not have properly distinguished between deep and shallow samples, and that interpretation of allozymes can be hindered by hybridization.

The most recent and extensive allozymes study appears to be Danielsdóttir *et al.* (2008), which analysed a large number of fish and loci. Makhrov *et al*. claim that the samples in this study are not organized into deep and shallow samples, presumably because the samples were originally collected based on phenotype. Cadrin *et al.* (2010) acknowledge this, but also mention that nearly all of the "deep-sea" samples (95%) came from deep water and nearly all of the "oceanic" samples (93%) came from shallow water. I found this level of correspondence between phenotype and depth to be satisfactory, especially given the consistency of the results of this study with other studies, and role of this study in an interdisciplinary framework on stock structure.

Danielsdóttir *et al.* (2008) also have data from three years (1995–1997) and do not show temporal variability, but this is interpreted by Makhrov *et al*. as too short of a time period to demonstrate temporal variability given the longevity of *S. mentella*.

However, Makhrov *et al.* offer no advice on what would be a suitable length of years to demonstrate temporal stability. In my opinion, the important issue here is that the analyses should reflect genetic differences and not sampling variability. I found the existence of consistent results from three years of samples to be sufficient to rule out artifacts due to sampling variability, particularly given the numerous other genetic studies.

Danielsdóttir *et al.* (2008) show differences for the MEP allele, consistent with other allozyme studies, and Makhrov *et al.* hypothesize that this result could reflect a selection gradient that occurs over depth and movement of older fish to deep water. As Cadrin *et al.* 2010 state, a selection gradient would generally imply some degree of local adaptation that should be considered in the management process. However, Makhrov *et al*. do not present data supporting ontogenetic movement that underlies the proposed selection process, and as mentioned above, there is some question regarding the reliability of age-reading methods.

Microsatellites

Makhrov *et al*. mention that Pampoulie and Danielsdóttir (2008) based their analysis on phenotypic classification of samples, but do not mention that the samples were regrouped by depth and reanalyzed by Stefansson *et al.* (2009a). According to Cadrin *et al*. (2010), this latter analysis demonstrated microsatellite differences based on depth.

Temporal stability is one of the reasons Makhrov *et al*. use to claim that the microsatellite data cannot be used. The 3 microsatellite studies cited by Makhrov *et al*. show a range of information on temporal stability, with Schmidt (2005) claimed to have shown temporal instability. Assuming that the Schmidt (2005) data is temporally unstable and cannot be used (which I cannot fully evaluate because I do not have access to this study), other microsatellite studies cited in Cadrin *et al.* (2010) have samples that have been collected over many different years and cohorts (see below), and show seemingly consistent results.

Roques *et al.* (2002) – samples from 1993, 1995-1998

Stefansson *et al*. (2009b) – samples from 1995-1996, 1999-2002

Pampoulie and Danielsdóttir (2008) – samples from 1996–1997, 2001

Although some studies may show temporal instability (see also the paper introduced by Zelenina *et al.*), this does not account for the existence of several studies which provide consistent results over several years of sampling.

Makhrov *et al.* state that the microsatellite data is influenced by hybridization, with a "considerable number" of the samples of Schmidt (2005) reflecting hybridization. The inability of Pampoulie and Danielsdóttir (2008) to classify some individuals to the "deep-sea" or "oceanic" types is attributed to hybridization. However, this attribution was not made in Pampoulie and Danielsdóttir (2008), who discuss hybridization in the context of misclassifying species. It does not seem unusual to me that phentotypic appearance may not always correspond to genotypes of two different stocks of the same species. Although this may be an issue for a geneticist to consider, it seems that variability in phenotypic characteristics could be caused by factors other than hybridization. In any event, Pampoulie and Danielsdóttir (2008) are able to estimate misclassification rates results from a Bayesian analysis applied to multiple loci, so their analysis accounts for the detection of hybrids.

Phenotypic diversity

Makhrov *et al.* begin this section by stating that phenotype differences do not necessarily imply different genotypes, and could be caused by environmental conditions. While this is true, the emphasis on whether phenotypic traits strongly imply genotypes and reproductive isolation somewhat misses the utility of this information in a fisheries management context. As Cadrin *et al.* (2010) state, temporally stable phenotypic differences do imply limited mixing, and differences in life-history characteristics important for stock productivity can be especially important for management.

Makhrov *et al.* summarize Garabana *et al*. (2005) as concluding that pelagic *S. mentella* phenotypes do not have any morphometric differences. Considering that phenotypes are often defined by observable properties, this sentence seems odd and could be clarified. Cadrin *et al.* (2010) describe the Garabana *et al.* (2005) study as indicating deep-sea and oceanic phenotypes, but morphological characteristics could not accurately classify individuals.

Makhrov *et al*. concludes that the phenotypic data "does not permit to identify two or more populations" due to ontogenetic plasticity. However, I did not find any discussion of age-related variation in phenotypic traits in their text. An important consideration is the temporal stability of phenotypic traits, but this point is not addressed by Makhrov *et al.*

*Appendix 1: MEP-2*60allele frequency correlation with invasion by copepod Sphyrion lumpi in beaked redfish (Sebastes mentella)*

Appendix 1 contains data from shallow water samples indicating that the degree of infestation of *S. lumpi* corresponds to a higher frequency of the MEP allele. The conclusion is that the decline in the MEP allele with depth results from a higher mortality on the fish with the MEP allele and high infestation rates. This argument depends on ontogenetic movement to deeper water, but as Cadrin *et al*. (2010) point out, age-reading methods are evidently in dispute. Also, the relationship between *S. lumpi* and fish mortality have not been established. Finally, a theory that depends on ontogenetic movement between shallow and deep areas is not consistent with the microsatellite data.

Appendix 2: Redfish (genus Sebastes) hybridization in the Irminger Sea and its relevance for the assessment of population structure

The objective of Appendix 2 is to study the role of hybridization in the development of the gene pool of *S. mentella* in the Irminger Sea, and it is claimed that "all differences in gene frequencies between samples from different depths are well accounted for by hybridization" with the exception of the MEP gene.

The influence of hybridization on the allele frequencies has been acknowledged by Cadrin *et al*. (2010), and the influence of hybridization on the IDHP locus would be expected to be small given this is only one of many loci examined. The existence of hybridization between *S. mentella* and *S. marinus* has been identified by Pampoulie and Danielsdóttir (2008) and a multiloci analysis was used to identify hybrids.

New microsatellite data on redfish (Sebastes mentella) in the Irminger Sea and adjacent waters by Zelenina et al.

This paper presents microsatellite analyses of samples collected in 2005, 2007, and 2009 in the Irminger Sea. Samples from the same area, but different depths, were similar to each other. Temporal variability was also observed, as samples from the same area in different years were different from each other.

Without being a geneticist, it is difficult for me to compare the methods and results of this study to the several other microsatellite studies cited by Cadrin *et al.* (2010) and account for the differences. I would note, however, that the addition of new information that contrasts with existing studies would not necessarily refute existing studies. Assuming the differences could not be explained with a close examination of the methodology, the addition of contrasting information would indicate, at most, some uncertainty in the interpretation. The relevant question is then assessing which hypothesis of stock structure is most likely given all the available information.

The influence of temperature of the surface layer on distribution and pelagic fishery of Sebastes mentella in the Irminger Sea and adjacent waters by Melnikov et al.

This paper demonstrates that the distribution of the Irminger Sea stock and fishery has moved over time based on temperature, and argues that this temporal variation poses problems for defining management areas based on the spatial pattern of the fishery.

Cadrin *et al.* (2010) notes that the distribution of *S. mentella* in the Irminger Sea has varied over time with variations in temperature, and makes many of the same observations as this paper. The spatial fishery data was used to define spatial management units in order to separate the deep pelagic fisheries from the shallow pelagic fishery and minimize mixed-stock catches. If spatial management areas identified in Cadrin *et al*. (2010) do not efficiently achieve this, then it seems other management boundaries could be considered. It is important to note that the issue here is not stock structure, but identifying management areas that conserve the identified stocks. If the fishery spatial pattern is not helpful for this task, one could perhaps rely solely on the survey spatial pattern.

References

Cadrin, S. X., Bernreuther, M., Danielsdóttir, A. K., Hjörleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigurdsson, T., and Stransky, C. 2010. Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats. ICES J. Mar. Science, 67: 1617-1630.

Conover, D. O., L. M. Clarke, S. B. Munch, G. N. Wagner. 2006. Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. Journal of Fish Biology, 69: 21–47.

Danielsdóttir, A. K., Gislason, D., Kristinsson, K., Stefansson, M. O., Johansen, T., and Pampoulie, C. 2008. Population structure of deep-sea and oceanic phenotypes of deepwater redfish in the Irminger Sea and Icelandic continental slope: are they cryptic species? Transactions of the American Fisheries Society, 137: 1723–1740.

Garabana, D. 2005. The genus Sebastes Cuvier, 1829 (Pisces, Scorpaenidae) in the North Atlantic: species and stock discrimination using traditional and geographic morphometrics. PhD thesis, University of Vigo. 306 pp.

Gharrett, A. J., A. P. Matala, E. L. Peterson, A. K. Gray, Z. Li, and J. Heifetz. 2005. Two genetically distinct forms of rougheye rockfish are different species. Trans. Am. Fish. Soc., 134: 242-260.

Gharrett, A. J., A. P. Matala, E. L. Peterson, A. K. Gray, Z. Li, and J. Heifetz. 2007. Distribution and population genetic structure of sibling rougheye rockfish species. Pages 121-140 In J. Heifetz, J. DiCosimo, A.J. Gharrett, M.S. Love, V.M. O'Connell, and R.D. Stanley (eds.) 2007. Biology, assessment, and management of North Pacific rockfishes. Alaska Sea Grant College Publication AK-SG-07-01, University of Alaska Fairbanks.

Hauser, L., and G. R. Carvalho. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish and Fisheries, 9: 333–362.

Hawkins, S. L., J. Heifetz, C. M. Kondzela, J. E. Pohl, R. L. Wilmot, O. N. Katugin, and V.N. Tuponogov. 2005. Genetic variation of rougheye rockfish (*Sebates aleutianus*) and shortraker rockfish inferred from allozymes. Fish. Bull., 103: 524-535.

Orr, J.W., and S. Hawkins 2008. Species of the rougheye rockfish complex:resurrection of Sebastes melanostictus (Matsubara, 1934) and a redescription of *Sebastes aleutianus* (Jordan and Evermann, 1898) (Teleostei: Scorpaeniformes). Fish Bull., 106: 111-134.

Pampoulie, C., and Danielsdóttir, A. K. 2008. Resolving species identification problems in the genus Sebastes using nuclear genetic markers. Fisheries Research, 93: 54–63.

Roques, S., Sevigny, J-M., and Bernatchez, L. 2002. Genetic structure of deep-water redfish, *Sebastes mentella*, populations across the North Atlantic. Marine Biology, 140: 297–307.

Schmidt, C. 2005. Molecular genetic studies on species and population structure of North Atlantic redfish (genus Sebastes; Cuvier 1829). PhD thesis, University of Hamburg. 303 pp.

Stefansson, M. O., Reinert, J., Sigurðsson, T., Kristinsson, K., Nedreaas, K., and Pampoulie, C. 2009a. Depth as potential driver of genetic structure of *Sebastes mentella* across the North Atlantic Ocean. ICES Journal of Marine Science, 66: 680–690.

Stefansson, M. O., Sigurðsson, T., Pampoulie, C., Danielsdóttir, A. K., Thorgilsson, B., Ragnarsdottir, A., Gıslason, D., *et al*. 2009b. Pleistocene genetic legacy suggests allopatric incipient species of *Sebastes mentella* in the Irminger Sea. Heredity, 102: 514–524.

Waples, R. S., and Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology, 15: 1419–1439.

Waples, R.S., A. E. Punt and J. M. Cope. 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish and Fisheries, 9: 423–449.

Referee #2:

Comments on the various documents prepared by the Russian Federation on the intra-species structure of redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters.

New microsatellite data on redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters
by Daria A. Zelenina, Sergey P. Melnikov, and Nikolai S. Mugue

This study based on the analyses of 11 microsatellite loci on 19 samples concludes that *S. mentella* is not structured as a function of depth in the Irminger Sea.

The authors reject the stock structure proposed on the basis that:

- Their analyses did not reveal significant genetic difference between the deep pelagic samples (i.e. caught below 500 m) and the oceanic samples (i.e. caught above 500 m);
- Samples collected at the same geographic sites but at different depth are more similar to each other than samples collected at different sites at the same depth;
- Their analyses detect no temporal stability of genetic characteristics as the samples collected in the same area but in different years do not cluster together.

It is very difficult to determine if the results support or not the authors claim that there is no population structure according to depth in the Irminger Sea. Indeed, some key information is not presented by the authors. There are probably technical problems associated with the analyses of microsatellite markers. These ambiguities are summarized below.

Information on samples

A better description of the samples analysed would help with the interpretation of the results. Even if depth is of critical importance in the assessment of *S. mentella* stock structure in the Irminger Sea this information is not provided. The authors should at least provide the depth range of the trawl (where the samples were collected). Simply stating that the samples were collected below and above 500m is not enough (e.g. 501 m is below and 499 m is above). Furthermore, the scientific literature on these stocks shows that there is overlap in the depth distribution of the two *S. mentella* stocks. Without information about the depth at which the samples were collected it is not possible to conclude that the samples come from only one stock.

Size of the fish should also be provided. It may be assumed that the size of the fish will have an impact on the depth distribution of the different redfish stocks. Besides, when comparing temporal samples of redfish, information on individual size is important. Indeed considering the longevity of redfish, the same year-class might be sampled repeatedly over time.

Other sampling considerations

Sample size is limited varying between 12 and 31. Table 2 indicates that the results are based on even lower sample sizes and that sample size varies across loci within a given sample. Small sample sizes associated with uneven sample sizes across loci are likely to influence statistical comparisons. In such situation, individual variability becomes a significant source of variation in sample comparisons.

DNA amplification

Why using M13 considering that every primers have already been developed? Could this approach have generated some artefacts and technical problems which would explain the uneven sample size (N) across loci within sample and the need to discard Spi4 and Seb33 from the analyses? Examination of the data in Table 2 suggests that there may have been some technical problems with loci *Seb9*, *Seb31* and *Seb45* given the strong deficit in heterozygote observed for the sample A41 and B44. Significant deficits were observed at 5 loci for sample B44.

Statistical analyses

The approach adopted for data analyses could have been more sophisticated. Beside the basic statistics describing the samples characteristics the authors have limited the analyses to PCA, pairwise Fst comparisons and ML tree. Other model-based clustering method such as the one described in STRUCTURE (Hubisz *et al.*, 2009 and references therein) can and, in fact, has been used to infer population structure of different species including redfish.

It is not clear how the tree presented in Figure 2 was generated (i.e. which module of the Phylip software was used? Was it *Contml* based on a maximum likelihood approach or *Neighbour* based on a clustering approach). The number of bootstrap should be given in the "Methods" section and bootstrap values should by present on Figure 2.

Results and interpretation

Table 2:

- Should provide the overall genetic characteristic values for each sample.
- Does the symbol A represent the number of alleles (as stated in the legend) or allelic richness (as mentioned in the "Results and Discussion" section)? Considering the different sample size, allelic richness should be presented.
- The legend suggests that the probability thresholds have not been corrected for multiple comparisons (e.g. Bonferroni).

Table 3. Significant tests should be indicated.

Figure 1. A different symbol could be used for each sampling year.

Figure 2. At least the highest bootstrap values should be indicated.

Figure 3. The proportion of total variation should be given for each axis. Are the scales of both axes in the same unit?

The authors mention that the genetic analyses carried out demonstrate that there is no population structure of *S. mentella* associated with either depth or geographic location in the Irminger Sea. However, the maximum likelihood tree based on *F*st values (Figure 2) shows some organisation of samples according to depth with two exceptions: samples B66 and A41. Sample B66 is clustered with the samples above 500m and was collected very close to sample A67. Could sample B66 be a sampling artefact? Same applies to sample A41 that is the only sample collected above 500m in an area where all other samples were collected below 500m. As mentioned previously, the authors should at least provide the depth range of the trawl.

Temporal variation

As mentioned above, information on individual size should be considered when comparing temporal samples of redfish since the same year-class might be sampled repeatedly over time.

The approach used to assess temporal structure is not convincing. An AMOVA could have been done, but the sampling design limits this option since most samples (i.e. 13 out of 19) were caught in 2007.

The authors claim that the temporal genetic structure is not stable. This statement is mostly based on samples (B40 and B22) collected below 500m in 2007 and 2009 at the same site. The authors claim that the pairwise *F*st value of 0.20 between these samples is among the highest observed. Is it significant? There are many values that are equal to or higher than 0.20 in Table 3. The authors should have provided the probability values of the pairwise differentiation in Table 3. Pairwise *F*st values can be quite small but still significant. Simply mentioning that these comparisons are not significant is not enough.

In conclusion, the document, as presented, does not make a significant contribution to the debate regarding the *S. mentella* stock structure in the Irminger Sea and adjacent waters. It certainly does not present data that would refute the stock structure described in other population genetic studies such as those of Stefánsson *et al.* 2009a, b.

Reference cited

Hubisz M. J., Falush, D., Stephens, M., and Pritchard, J. K. 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Res., 9: 1322–1332

Stefansson, M. O. Reinert, J., Sigurðsson, Th., Kristinsson, K., Nedreaas, K., and Pampoulie, C. 2009a. Depth as a potential driver of genetic structure of *Sebastes mentella* across the North Atlantic Ocean. ICES Journal of Marine Science, 66: 680–690.

Stefansson, M. O., Sigurdsson, Th., Pampoulie, C., Danıelsdottir, A. K., Thorgilsson, B., Ragnarsdottir, A., Gıslason, D., Coughlan, J., Cross, T. F., and Bernatchez, L. 2009b. Pleistocene genetic legacy suggests incipient species of *Sebastes mentella* in the Irminger Sea. Heredity 102: 514–524.

The influence of temperature of the surface layer on distribution and pelagic fishery of *Sebastes mentella* in the Irminger Sea and adjacent waters by S.P. Melnikov, A.L. Karsakov, A.P. Pedchenko

In their document, the authors describe the evolution of *S. mentella* distribution and redfish fisheries patterns as a function of temperature anomalies. The temperature of the surface layer is used to describe the oceanographic conditions prevailing in the Irminger Sea. They show that variation in water temperature influence redfish distribution in the upper 500 m and therefore fishing patterns as well.

The authors suggest that changes in oceanographic conditions and therefore in the distribution redfish aggregations will complicate the development and application of management components in the Irminger Sea. They contend that the stock structure proposed by WKREDS is groundless from a scientific point of view and inefficient from a practical one.

There is evidence (from other studies) of the existence of redfish stocks in the Irminger Sea. This study does not provide new information on *S. mentella* stock structure in the Irminger Sea as no criterion for stock identification is presented. It does not demonstrate that the management units as defined by WKREDS are inefficient. The existence of some overlap in the distribution of the biological stocks was recognized when the management units were defined and that a monitoring of the mixed-stock catches in the Irminger Sea was needed (Cadrin *et al*., 2010).

Reference cited

Cadrin, S. X., Bernreuther, M., Daníelsdóttir, A. K., Hjörleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigurðsson, T., and Stransky, C. 2010. Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats. ICES Journal of Marine Science, 67: 1617–1630.

Single population of beaked redfish (*Sebastes mentella*) in the Irminger Sea: biological characteristics and dynamics of gene pool by Makhrov A.A., Artamonova V.S., Popov V.I., Rolskiy A.Y., Bakay Y.I.

The objectives of the authors of this document are to draw conclusions on *S. mentella* stock structure in the Irminger Sea and adjacent waters. To achieve this objective, they (1) present an analysis of the available information on stock structure of *S. mentella* based on the holistic approach, (2) carry out a critical overview of methodology and results of genetic studies used to identify types/stocks of *S. mentella* and (3) present new genetic data aiming to explain the observed differences between *S. mentella* sampled at different depth as the result of selection and hybridization acting on a single stock. The new genetic information is presented in Appendix 1 (Artamonova *et al.*) and 2 (Makhrov *et al.*). Since most of the document deals with the critical review of the different genetic studies, the comments formulated are restricted to this aspect of the document.

The authors are using 5 criteria to evaluate the relevance of the results of the genetic studies in which *S. mentella* population structure has been described. However the use and applicability of these criteria can be questioned.

Firstly, the authors use those five criteria in such a stringent way that the end result is the rejection of all the studies that have described genetic differences between *S. mentella* stocks in the Irminger Sea and adjacent waters. Nevertheless, these studies still provide useful information on stock structure (e.g. studies using allozymes). An example of the stringent use of criterion 1 is the rejection of the study by Daníelsdóttir *et al.* (2008) based on the fact that the genetic analyses were carried out on morphotypes and not on samples separated by depth (i.e. above and below 500m). However, 1063 out of the 1212 deep-sea morphotypes were collected below 500m while all the 650 oceanic morphotypes were collected above 500m.

Secondly, although the application of the first 3 criteria could be rather straightforward, the application of the criteria 4 (selection) and 5 (hybridization) is more complicated as hybridization and selection are processes that have an evolutionary dimension. In this context, it is difficult to determine if the observed genetic characteristics are the results of historical or contemporary processes. For example, the presence of shared alleles among species does not necessarily indicate ongoing hybridization. In the same way invoking selection to explain difference in allelic frequencies among groups requires more rigorous demonstration. It is however on the basis of non compliance to these two criteria that the authors reject the results of all genetic studies that have shown genetic differentiation among *S. mentella* in the Irminger Sea and adjacent waters, mentioning that the authors did not presented nor discussed the study be Stefánsson *et al.* (2009b) that showed such differentiation.

Indeed, the authors could only reject the results of the studies by Stefánsson *et al.* (2009a) on the basis that selection is acting on microsatellites and that hybridization occurs between the different redfish species. More precisely, the authors of the present document mention that the study by Pampoulie and Daníelsdóttir (2008) contains evidence of selection acting at four of the nine microsatellite loci used. Actually, Pampoulie and Daníelsdóttir (2008) only suggest that homoplasy or selection may act at the four loci. Therefore, the study by Pampoulie and Daníelsdóttir (2008) does not constitute evidence that selection is acting on microsatellite loci as suggested by the authors. It is also worth mentioning that, in the recent and key study that is not referred to in the present document, Stefánsson *et al.* (2009b) carried out neutrality tests on the microsatellites that comprised all those used by Pampoulie and Daníelsdóttir

(2008) and all but 3 of the 12 used by Stefánsson *et al.* (2009a) and did not detect the influence of selection on any of the microsatellites used.

The multidisciplinary study carried out by Stefánsson *et al.* (2009b) is important because it reconciles the results obtained in other less sophisticated studies. Indeed, Stefánsson *et al.* (2009b) did confirm that the deep mesopelagic (below 550m) and shallow mesopelagic *S. mentella* (above 550m) are genetically differentiated. These genetic differences are temporally stable (1995–1996, 1999 to 2002). They also confirm that the deep mesopelagic and shallow mesopelagic *S. mentella* represent two morphotypes (based on traditional and geometric morphometrics and meristics). Although hybridization was observed between the two groups, Stefánsson *et al.* (2009b) found indication of restricted gene flow between them, which may be maintained by ecological isolation mechanisms. Stefánsson *et al.* (2009b) also hypothesize that redfish in the Irminger Sea are likely to represent a case of incipient speciation event based on historical imprints in the in the genetic data.

It is worth mentioning that the results obtained by Stefánsson *et al.* (2009b) are in line with the most recent scientific information regarding the importance of depth as a factor promoting *Sebastes* speciation. Indeed, a phylogenetic comparative study based on 66 *Sebastes* species from the northeast Pacific suggests that speciation is associated with divergence in habitat depth and depth associated morphology consistent with models of parapatric speciation along an environmental gradient (Ingram, 2010). Besides, speciation in *Sebastes* species occurs mostly within oceanic region (Hyde and Vetter, 2007).

In conclusion, the recent genetic information provided by Stefánsson *et al.* (2009a and b) supports the stock structure described in Cadrin et al. (2010). The present critical review cannot invalidate Stefánsson's studies that are based on a sound sampling design (spatial, temporal, bathymetric), a multidisciplinary approach (morphometrics and genetics), and powerful statistical analyses (on both individuals and samples). Stefánsson *et al.* (2009b) also test the neutrality of their microsatellites and address the question of hybridization.

There is compelling evidence for the presence of at least three *S. mentella* stocks in the Irminger Sea and adjacent waters. The fact that there is a consistent genetic signal despite overlapping in the distribution (geographic and depth) of the various developmental stages is also a strong evidence of stock structure.

References cited

Cadrin, S. X., Bernreuther, M., Daníelsdóttir, A. K., Hjörleifsson, E., Johansen, T., Kerr, L., Kristinsson, K., Mariani, S., Nedreaas, K., Pampoulie, C., Planque, B., Reinert, J., Saborido-Rey, F., Sigurðsson, T., and Stransky, C. 2010. Population structure of beaked redfish, *Sebastes mentella*: evidence of divergence associated with different habitats. – ICES Journal of Marine Science, 67: 1617–1630.

Daníelsdóttir, A. K., Gíslason, D., Kristinsson, K., Stefánsson, M., Johansen, T. and Pampoulie, C. 2008. Population structure of deep-sea and oceanic phenotypes of deepwater redfish in the Irminger Sea and Icelandic continental slope: are they cryptic species? Transactions of the American Fisheries Society, 137: 1723–1740.

Hyde, J.R., and Vetter, R. D. 2007. The origin, evolution, and diversification of rockfishes of the genus Sebastes (Cuvier). Molecular Phylogenetics and Evolution, 44: 790-811.

Ingram, T. 2010. Speciation along a depth gradient in a marine adaptive radiation. Proceedings of the Royal Society of London B, 278: 613-618.

Pampoulie, C., and Daníelsdóttir, A. 2008. Resolving species identification problems in the genus *Sebastes* using nuclear genetic markers. Fisheries Research, 93: 54-63.

Schmidt, C. 2005. Molecular genetic studies on species and population structure of North Atlantic redfish (genus *Sebastes*; Cuvier 1829). PhD thesis, University of Hamburg. 303 pp.

Stefánsson, M. O., Reinert, J., Sigurðsson, T., Kristinsson, K., Nedreaas, K., Pampoulie, C. 2009a. Depth as a potential driver of genetic structure of *Sebastes mentella* across the North Atlantic Ocean. ICES Journal of Marine Science, 66: 680–690.

Stefánsson, M. O., Sigurðsson, T., Pampoulie, C., Daníelsdóttir, A. K., Thorgilsson, B., Ragnarsdóttir, A., Gıslason, D., Coughlan, J., Cross, T. F., Bernatchez, L. 2009b. Pleistocene genetic legacy suggests incipient species of *Sebastes mentella* in the Irminger Sea. Heredity, 102: 514-524.

Appendix 1

*MEP-2*60* allele frequency correlation with invasion by copepod *Sphyrion lumpi* in beaked redfish (*Sebastes mentella*)
by Artamonova V.S., Bakay Y.I., Makhrov A.A., Popov V.I., Rolskiy A.Y.

The objective of the study described in this document is to demonstrate that the polymorphism observed at the *MEP-2** locus is influenced by selection. The authors suggest that the redfish possessing the allele *MEP-2**-60 are more sensitive to the infestation by the copepod *S. lumpi* and as a consequence suffer a higher mortality rate.

There are a number of limitations to this study. Table 1 shows that two groups of redfish were sampled. Redfish in samples 1, 2 and 3 are larger and were collected deeper than those of samples 4 to 7. In fact all but 4 specimens of the group collected deeper come from site 3. The results in Table 2 should be presented for each sample. It is actually impossible to determine if the difference in allelic frequencies is driven by depth. Is it possible that the group infested by 2 and more *S. lumpi* and exhibiting higher allelic frequency at *Mep2*-60* might correspond to samples 1, 2 and 3 that comprise larger specimens that were collected deeper?

Appendix 2

Redfish (genus *Sebastes*) hybridization in the Irminger Sea and its relevance for the assessment of population structure
by Makhrov A.A., Artamonova V.S., Popov V.I., Rolskiy A.Yu., Bakay Yu.I.

The objective of this study is to assess the importance of hybridization between *S. mentella*, as identified by external morphological characteristics, and other species in the determination of the genetic characteristics of *S. mentella* in the Irminger Sea.

The authors interpret the presence of some alleles at different allozyme loci as an indication that significant levels of hybridization take place between *S. mentella* and the other redfish species in the Irminger Sea. They mention that the difference in gene frequencies observed with depth is explained by hybridization except for the locus *MEP-2** that is influenced by selection. They conclude that existing allozyme data are not in agreement with the presence of two stocks of *S. mentella* in the Irminger Sea. However, this conclusion is very speculative and in contradiction with the results of recent genetic analyses based microsatellites that have indicated the presence of hy-

brids but still could identify the presence of two stocks of *S. mentella* (e.g. Stefánsson *et al.*, 2009).

The authors are not presenting the data on which they reach the conclusion. Indeed, the document is based on the analyses of allozymes carried out on *S. mentella* samples collected in 2007 (N=131; Appendix 1) and in 2010 (N=72). A sample of 30 individuals *Sebastes fasciatus* was also analysed. The information concerning the origin and characteristics of this sample is not provided. It seems that *Sebastes fasciatus* is used as a "proxy" to infer hybridization of *S. mentella* with *S. viviparus.* Furthermore, the genetic data collected on the specimens sampled in 2010 are not presented while those collected on the specimens sampled in 2007 are only partly (and not adequately) presented in the Appendix 1.

As presented, this document does not contribute to the assessment of the *S. mentella* population structure in the Irminger Sea.

References cited

Stefánsson, M. O., Sigurðsson, T., Pampoulie, C., Daníelsdóttir, A. K., Thorgilsson, B., Ragnarsdóttir, A., Gıslason, D., Coughlan, J., Cross, T. F., Bernatchez, L. 2009. Pleistocene genetic legacy suggests incipient species of *Sebastes mentella* in the Irminger Sea. Heredity, 102: 514-524.