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# Population Structure of *Sebastes mentella* and *Sebastes fasciatus* in NAFO Divisions 3LNO Based on Microsatellite Genetic Markers and Geometric Morphometrics Data

by

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## Abstract

Redfish population structure was investigated in the NAFO Divisions 3LNO using microsatellite DNA markers and geometric morphometric analyses in order to assess the adequacy of existing management units Div. 3LN and Div. 3O. Genetic differentiation between pairs of samples was quantified by estimates of pairwise fixation indices (FST) based on variance in allelic frequencies. Comparisons were also made with outgroup samples from Subdivisions 3Ps and 3Pn. For *S. mentella*, analyses of both microsatellite and morphometric data show that redfish from Div. 3L are different from the outgroup samples from the Laurentian Channel confirming the microsatellite results of previous investigators. For *S. fasciatus*, there was a trend for genetic distances to match geographic distances with Div. 3LNO samples been closer to each other compared to outgroup samples. The results also suggest that *S. fasciatus* from Div. 3LNO and from the Subdivision 3PS area adjacent to Div. 3O form a population that exchanges individuals with the adjacent Unit 2 (Div. 3Ps4Vs4W$_{fgj}$+3Pn4Vn [Jun-Dec]). The exchange follows a gradient with Subdiv. 3PS and Div. 3O being more affected than Div. 3LN. Morphometric distances between Div. 3LNO samples for each species showed no clear geographic pattern that would suggest differences between these areas.

## Introduction

The Northwest Atlantic redfish consists of a complex of three species currently identified as *Sebastes mentella* and *S. fasciatus* which dominate the commercial fishery (Atkinson, 1987; Rubec *et al*., 1991) and *S. marinus*, which occurs at much lower abundance. Although recruitment of redfish is known to be sporadic in the Northwest Atlantic, there has been almost no important recruitment in the last 20 years. A consequence is that fishery is closed since 1995 in Unit 1 (Div. 4RST+3Pn4Vn [Jan-May]) and that quotas have been generally declining in other areas (DFO, 2000). In view of general low stock abundance, of absence or weak recruitment, and of the fact that fishery is not closed in all areas (e.g. Unit 2 - (Div. 3Ps4Vs4W$_{fgj}$+3Pn4Vn [Jun-Dec])) it is very important to understand redfish stock structure and their inter-relation. Such information is a prerequisite for the development of any sound management strategies.

From 1996-1998, a *Multidisciplinary Research Program on Redfish* was funded by the Canadian Department of Fisheries and Oceans (DFO) to basically create new knowledge on or address two fundamental issues: (1) How many species/stocks exist, (2) what are the basic biological characteristics of these species and subsequently how are they best managed. The project gave some important initial results with respect to unravelling the complex population structure that exists in the Northwest Atlantic for redfish species and gave additional insight into the basic biology of redfish (Gascon, 2003). In consideration of the project as a whole, the most compelling and encompassing tool employed to address the issues was the genetic research. The systematic application of molecular markers has allowed the clear discrimination between *S. fasciatus* and *S. mentella* in the Northwest Atlantic. These species also differ in their geographic distribution; *S. mentella* distribution ranges from the Gulf of St. Lawrence northward, *S. fasciatus* is distributed from the southern Grand Banks southward. *Sebastes fasciatus* is also found in Div. 2J+3KL and appears to reach its northernmost limit in Div. 2H. Although the distribution of both species overlaps mainly in Units 1 and 2, in NAFO Div. 3O, 3LN and 3M they hybridise and introgress essentially in Units 1 and 2 (Desrosiers *et al*., 1999; Roques *et al*., 2001). For both species, redfish from Unit 1 cannot be differentiated from those of Unit 2 based on their genetic characteristics (Roques *et al*., 2001), as well as on size distribution and on synchrony of recruitment pulses (Morin *et al*., 2004). They were only two exceptions to this general observation indicating that there may be weak structuring for *S. mentella* in these areas. Indeed, two *S. mentella* samples collected south of Newfoundland were genetically differentiated from the other samples collected in the same region (Roques *et al*. 2000; Roques *et al*., 2001), and parasite load was different between samples from Unit 1 and Unit 2 for *S. mentella* (Marcogliese *et al*., 2003). For *S. mentella*, there was also no genetic difference between redfish from Div. 3LN and 3O although sampling was limited (Roques *et al*., 2001). For *S. fasciatus*, genetic difference was detected between redfish from the Gulf of Maine and those of Unit 3 (Div. $4W_{dehkl}X$), but no information was available for Div. 3LNO (Roques *et al*., 2001).

Results from previous studies have significantly improved our understanding of the redfish biology and of stock structure; however some findings have created controversial issues whose consequences are still unclear and that must be addressed before these findings can be translated into management strategies. Furthermore some of these issues (e.g. Unit 3 - Gulf of Maine) may have international implications.

A three year research program focusing on the biological relevance of the boundary between management Units 1 and 2 has been undertaken in 2002. Sampling effort was mostly localized in these two units, but additional samples have also been gathered in other NAFO areas to allow for comparisons. This study was set to be in continuity with the 1996-1998 program, but with some interesting innovations. Sampling strategy has been revised in order to target large aggregations of redfish that are known to sustain the fishery. Besides, a multidisciplinary approach coupling molecular markers (microsatellites), geometric morphometrics and elemental otolith fingerprints was adopted. Great care was taken to gather the 3 sets of data on the same specimens, since data cross-validation is a way to generate more reliable results. For example, all specimens analyzed for microsatellites have served in the geometric morphometric part of the study.

Most fishery biologists are familiar with the use of molecular markers such as microsatellites, but geometric morphometrics may need some introduction. Geometric morphometrics is the most advanced approach for shape quantification (Rohlf and Marcus, 1993; Marcus and Corti, 1996; Adams *et al*., 2004). Traditional anatomical measures have been replaced by landmarks coded as 2D or 3D coordinates which capture the locations of anatomical characteristics. The coordinates are superimposed (making them invariant to scale, location and orientation) and transformed in shape variables that can be analysed with standard multivariate statistics. The relative spatial arrangement of landmarks is conserved throughout the analysis, which allows an easily interpretable visualization of morphological variability. Former redfish studies have confirmed the relevance of geometric morphometrics for species discrimination (Valentin *et al*., 2002), and shown its potential as a tool for population discrimination (Anon., 2004 and references therein).

The present paper focuses on the microsatellite and geometric morphometric analyses performed on samples from the Div. 3LNO area, in order to assess the relevance of managing Div. 3LN and 3O separately.

## Material and Methods

### *Samples*

For the whole study, more than 3000 redfish (representing 57 sets) were collected all over NAFO fishing areas in summer and autumn 2001 and 2002, with a bottom-trawl net. For the present study, 10 out of the 57 samples were considered, for a total of 463 specimens (Table 1 and Fig. 1). Eight samples have been caught in NAFO Div. 3LNO and represented aggregations dominated by *S. fasciatus* or *S. mentella*. Two sets, one for each species, came from the Laurentian Channel (close to Burgeo Bank) and were chosen as outgroups for comparison analyses.

### *Data collection*

For each set, up to 75 fishes were immediately frozen on board. In the laboratory, samples of liver and muscle tissues were taken on each thawing specimen. Liver samples were immediately frozen, pending MDH electrophoretic analyses according to Hebert and Beaton (1989). Muscle fragments were preserved in 100% ethanol pending DNA extraction with DNeasy® Tissue Kit (Quiagen). After complete thawing, geometric morphometric data consisting of 10 landmarks ($L_1$ to $L_{10}$) defining the body outline were captured (Fig. 2). The fish was laid on his right side on a Styrofoam board covered with a wax paper. The position of each landmark was determined by punching a hole in the paper with a needle. A support was used to maintain the needle perpendicular to the board. After landmarks collection, soft anal fin rays (AFC) were counted and the insertion pattern of the gasbladder muscle between ribs (EGM) was recorded along with sex, maturity and length. Finally, each wax paper was numerized. Landmark digitising was carried out using tpsDig (Rohlf, 2003a): *x*,*y* coordinates of the 10 landmarks were determined for each specimen, providing a total of 20 morphometric variables for each individual.

### *Genetic analyses*

Only six samples (3L29, 3L65, 3N23, 3O44, 3PN77, 3PS88b) were analyzed for variability at microsatellite loci. A total of 13 microsatellite loci were analyzed using fluorescent labelling method. Eight loci (SEB9, SEB25, SEB30, SEB31, SEB33, SEB37, SEB45, and SEB46) were originally characterized for the Atlantic redfish species (Roques *et al*., 1999a). The five additional loci used in this study were originally isolated from two Pacific redfish species and used for the first time on Atlantic species. They are SAL3 and SAL4 from *S. alutus* (Miller *et al*., 2000) and SPI4, SPI6, and SPI10 from *S. pinniger* (Gomez-Uchida *et al*., 2003).

The 13 microsatellite loci were amplified in three multiplex PCR reactions called hereafter MuxI (SEB9, SEB25, SEB31, SEB33), MuxII (SAL4, SEB30, SEB37, SEB46), and MuxIII (SEB45, SAL3, SPI4II, SPI6, SPI10II). Multiplex amplification demanded modification of three primers. Both forward (-F) and reverse (-R) primers for SPI4 and primer-F for SPI10 have been modified in order to yield longer PCR products. These loci are therefore called SPI4II and SPI10II in the present study. Moreover, the unlabelled SPI6 primer-R competed with the unlabelled SPI4II primer-R for amplification at locus SPI4II. After DNA sequence verification, it was confirmed that a site corresponding to primer-R SPI6 was present at locus SPI4II. Therefore, the unlabelled SPI6 primer-R was used to amplify both loci SPI4II and SPI6 and the primer-R SPI4II was not used further (Valentin, in prep.).

Each multiplex reaction was carried out in a 10 µl reaction volume using 2 µL of DNA template (20–50ng), 50µM dNTP, 0.245 U of Expand High Fidelity DNA polymerase (Roche), 1 × Expand High Fidelity buffer 2 with 1.5mM $MgCl_2$ (Roche, unknown composition), and 0.15–0.5 µM of each primer. For MuxI and MuxII, an initial denaturation time of 135s at 95°C was followed by 30 cycles of 30s denaturation at 94°C, 25s annealing at 55°C (MuxI) or 52°C (MuxII), 25s extension at 72°C, and a final 180s extension at 72°C. For MuxIII, initial denaturation was 180s at 94°C. It was followed by 30 cycles of 30s denaturation at 94°C, 30s annealing at 54°C, 30s extension at 72°C, and a final 180s extension at 72°C. PCR amplifications were performed with a Robocycler® Gradient 96 temperature cycler equipped with hot top (Stratagene®). For each sample, 1µL of the PCR product was mixed with 0.1µL GENESCAN® 400 HD ROX size standard (Applied Biosystems) and 15µL formamide before denaturation at 95°C for 3 min. Electrophoresis was conducted using an ABI 310 (Applied Biosystems) sequencer, with injection time and runtime set respectively to 5s and 30min. Data analysis and scoring were performed using Genescan and Genotyper software.

***Species assignment***

At this point, it is important to present how each individual was assigned to *S. fasciatus* or *S. mentella*. In the present study, the goal was to compare samples from Div. 3LNO to assess the relevance of management units 3LN and 3O. In this context, it was crucial to ensure that each sample was monospecific to avoid that species differences influence population comparisons within species. Species assignment is challenging for *S. fasciatus* and *S. mentella*, since assignment will vary according to the selected criterion (a recurrent problem with the three usual criteria MDH, AFC, EGM). Using a combination of highly polymorphic variables such as microsatellite loci allows for more accurate assignment (Roques *et al.*, 1999b).

Microsatellite data (representing samples 3L29, 3L65, 3N23, 3O44, 3PN77, and 3PS88b) were submitted to a factorial correspondence analysis (FCA), available in software Genetix (Belkhir *et al*., 1996-2004). FCA is an ordination method that projects individuals into a multidimensional space according to their allelic composition. Such analysis does not require setting *a priori* groups. Unsurprisingly, the first axis revealed two distinct clusters that were interpreted as representing *S. mentella* and *S. fasciatus*. The three usual criteria (MDH, EGM, AFC) were examined to decide which cluster corresponded to which species. Despite some discrepancies, it was clear that one cluster was dominated by individuals possessing *S. mentella* characteristics while the other comprised mostly individuals with *S. fasciatus* characteristics. So, the three usual criteria were used to identify the two clusters, but they were not used for individual assignment. Specimens were assigned to a given species according to the cluster they belonged to. According to microsatellites, the six samples were clearly dominated either by *S. mentella* (3L29, 3PN77) or by *S. fasciatus* (3L65, 3N23, 3O44, 3PS88b). Specimens not belonging to the dominant species of a given sample were discarded, leading to a total sample size of 177 individuals for microsatellite data (Table 1).

The challenge was then to assign individuals for which no microsatellite data were available (i.e. samples 3N24, 3N26, 3N27, and 3O63). The idea was to use geometric morphometric data. Like microsatellites, geometric morphometric data represent a combination of polymorphic variables. So, morphometric data for all specimens from Div. 3LNO and the two outgroups were submitted to a discriminant function analysis (DFA) after *generalized Procrustes analysis* and artefact correction (see below). The analysis was set to find the linear combination of morphometric variables that would maximize differences between the two species as determined by microsatellites (available only for a subset of the specimens). Every specimen without prior species information was assigned to one or the other species by the discriminant function. The four samples 3N24, 3N26, 3N27, and 3O63 were clearly dominated by *S. fasciatus*. As is was the case for microsatellite data, specimens not belonging to the dominant species of a given sample were discarded, leading to a total sample size of 463 individuals for morphometric data (Table 1).

***Usual criteria MDH, AFC, EGM***

MDH, AFC and EGM are routinely used for redfish species identification in the Northwest Atlantic. It was therefore interesting to analyse these data in the same way as in the 1996-1998 *Multidisciplinary Research Program on Redfish* to allow for comparison and to illustrate that species discrimination based on MDH does not necessarily match the one based on microsatellite data. The level of congruence between MDH, AFC, and EGM was used to define five groups within each monospecific sample as defined by microsatellites or morphometry. In the first two groups, the genotype at the *MDH-A** locus and the other two morphological characters (AFC and, EGM) were congruent. These two groups, in which all individuals were either homozygous for the allele *MDH-A*1* with AFC≥8 and EGM=2-3 or homozygous for the allele *MDH-A*2* with AFC≤7 and EGM≥3-4, were called typical *S. mentella* and typical *S. fasciatus* respectively. Two other groups were called introgressed *S. mentella* and introgressed *S. fasciatus*. In these groups, the specimens were homozygous at the *MDH-A** locus for either of the two alleles and at least one of the two morphological characters was not congruent with the genotype. A fifth group comprised all the fishes that were heterozygous at the *MDH-A** locus. The percentage of each group was calculated for each sample and represented graphically with pie charts (Fig. 1).

***Statistics on genetic data***

The number of alleles, number of private alleles, allelic richness, and observed and expected heterozygosities were calculated using software FSTAT (Goudet, 2001), Genetix (Belkhir *et al*., 1996-2004) and Arlequin (Schneider *et al*., 2000). Departure from Hardy-Weinberg equilibrium (HWE) was tested for each locus by the exact test of Guo

and Thompson (1992) available in Arlequin (Markov chain with 100 000 steps and 1 000 dememorization steps). Multilocus test by sample and multisample test by locus for heterozygote deficiency were performed in GENEPOP (Raymond and Rousset, 2003). All probability values were adjusted for multiple comparison tests using sequential Bonferroni adjustments (Rice, 1989).

Genetic differentiation between pairs of samples was quantified by estimates of pairwise fixation indices (FST) based on variance in allelic frequencies according to Weir and Cockerham (1984) available in Arlequin. Test of pairwise differences for FST values were computed using 100 000 permutations with probabilities values adjusted for multiple comparisons.

The extent of genetic divergence among pairs of samples was also quantified by Cavalli-Sforza and Edwards (1967) chord distance (DCE). The matrix of distance was submitted to a multidimensional scaling analysis or MDS (Kruskal, 1964a and b) using Systat (©2002, SYSTAT Software Inc.). MDS is an iterative ordination procedure based on monotone regression. It allows to represent distances between objects in a reduced space. This space has no dimension, since it is based on ranks, and it is chosen in a way to maximize the representation of true distances between objects. The level of concordance between the representation of the distances and the true distances is quantified by a measure of stress. Kruskal (1964a and b) suggested the following verbal evaluations for the goodness of fit associated with various levels of stress: poor (0.40), fair (0.20), good (0.10), excellent (0.05), and perfect (0.00). MDS analysis was performed with all 6 samples (2 *S. mentella* + 4 *S. fasciatus*) to illustrate the level of genetic differences between samples within species with respect to interspecific differences.

***Statistics on geometric morphometric data***

Morphometric data of the 463 specimens representing 10 samples have been submitted to a *generalized Procrustes analysis* (GPA) using software Relative Warps (Rohlf, 2003b). This procedure translated, rotated and scaled (to unit centroid size) the original configurations in order to achieve the best superimposition of all shapes. The new coordinates, called aligned coordinates, have been corrected for upward/downward arching artefact following Valentin *et al.* (2003). Mean configuration for each sample was computed separately for sex, because sexual dimorphism has been reported for North Atlantic redfish species (Valentin *et al.*, 2002 and references therein).

Pairwise Euclidian distances were computed between each sample. The corresponding distance matrix was introduced in a MDS analysis to illustrate morphometric differences between species, between samples within species and between sex within samples. Then, the analysis was performed separately for male and female *S. fasciatus* from Div. 3LNO to explore differences between samples at the species level.

## Results

***Usual criteria MDH, AFC, and EGM***

*Sebastes fasciatus* samples from areas 3N and 3O (3N23, 3N24, 3N26, 3N27, 3O44, 3O63) were homogenous regarding the percentage of typical, introgressed and heterozygote specimens (Fig. 1). Furthermore, some specimens within these samples showed lack of congruence between MDH and microsatellite or morphometry. Indeed, these samples were considered as monospecific on the basis of microsatellite and morphometry, but some specimens exhibited a *S. mentella* MDH pattern. The only sample from Div. 3L (3L65) was composed of typical *S. fasciatus* specimens, while the outgroup (3PS88b) was dominated by typical specimens with some introgressed ones. The two *S. mentella* samples were clearly different from each other with 3L29 comprising only few introgressed specimens while 3PN77 (the outgroup) exhibited almost 20% of heterozygote specimens and an equal percentage of typical and introgressed specimens.

***Microsatellite analysis***

A summary of the basic descriptive statistics for genetic data is presented in Table 2. The total number of alleles per locus varied between 6 (SAL3) and 43 (SEB30). With respectively 6 and 8 alleles, microsatellite loci originally isolated from *S. alutus* (SAL3, and SAL4) were less polymorphic than loci from North Atlantic redfish species or from *S. pinniger*. Allelic richness was higher for *S. mentella* (mean over samples = 162) than for *S. fasciatus* (mean

over samples = 137). Within *S. fasciatus*, allelic richness was higher for 3PS88b, the outgroup sample coming from the Laurentian Channel.

Private alleles were present in three samples, but at very low frequencies. However, these alleles were not exclusive to these samples. They were observed at other sites when the complete data set for samples from all over NAFO areas (N = 1 121) was considered. . In present case, interpretation of private alleles as a sign of population isolation is therefore rather limited (Valentin, in prep.).

Significant deviation from Hardy-Weinberg proportions was observed only in the *S. mentella* 3L29 sample after Bonferroni adjustment. In this sample, the deviation from Hardy-Weinberg was mostly caused by a heterozygote deficiency at the locus SEB37.

Pairwise FST values with associated probabilities are presented in Table 3. For *S. fasciatus* samples, FST pairwise values were small and ranged from -0.002 to 0.006. For the two *S. mentella* samples, pairwise FST was significant ($P$<0.0001), reaching a value of 0.018. Pairwise FST between species were higher and highly significant ($P$<0.00001), with values varying between 0.111 and 0.193.

Graphic representation of DCE genetic distances between samples after MDS analysis matched almost perfectly the real distances, considering the very small value of stress (0.003) associated to the configuration (Fig. 3). As observed for FST, DCE between species were larger than distances between samples within species. Interspecific genetic distances were smaller between the two outgroup samples from the Laurentian Channel, where introgressive hybridization is known to take place. For *S. fasciatus*, there was a trend for genetic distances to match geographic distances, with samples 3L65 and 3PS88b representing the extremities of an imaginary NE-SW axis on the MDS graph (Fig. 3). DCE between 3L65 and 3O44 was the largest distance between any two Div. 3LNO samples, and this distance was slightly inferior to DCE between 3O44 and the outgroup 3PS88b. The *S. mentella* sample from the 3LNO area (3L29) was clearly different from the outgroup (3PN77).

***Geometric morphometrics analysis***

Graphic representation of morphometric distances between samples was good with a stress value reaching 0.093 after MDS analysis (Fig. 4). Sexual dimorphism was confirmed by male and female samples not overlapping for each set, but the general pattern of morphometric distances between samples was identical for both sex. For each species, it was clear that the outgroup sample from the Laurentian Channel was different from the Div. 3LNO samples, indicating that redfish body shape was different between these two areas. Graphic representation produced by MDS analyses performed separately for sex on *S. fasciatus* distance data were excellent, with stress values reaching 0.040 for males and 0.023 for females. Morphometric distances between Div. 3LNO samples showed no clear geographic pattern that would suggest differences between these areas. In the whole, MDS analyses on morphometric and genetic data were convergent.

## Discussion

When results for both species are considered together, the three usual criteria give similar information as previously observed regarding species distribution and location of introgressive hybridization (Gascon, 2003). Although these results do not bring new information *per se*, it is important to present them. Indeed, the originality of these results resides in their comparison with results from the microsatellite analysis, a comparison that has never been done before. For the first time, it is possible to assess the accuracy of species assignment based on MDH. In the whole, MDH and microsatellites are rather congruent for species assignment (Fig. 1). It indicates that usual criteria are interesting to give an overview of the specific composition within samples. However, several *S. fasciatus* specimens according to microsatellites would be classified as *S. mentella* on the basis of MDH (see samples from Div. 3N and 3O). It suggests that microsatellites should be used when there is a need for species assignment at the individual level. This follows directly from the fact that genetic identity based on MDH has only three modes (corresponding to the three possible genotypes), but microsatellites has many modes that can describe a more complex genetic make-up.

For *S. mentella*, analyses of both microsatellite and morphometric data show that redfish from Div. 3L are different from the outgroup specimens caught in the Laurentian Channel. These observations are based only on one sample

from Div. 3L, but they confirm the microsatellite results of Roques *et al*. (2001) who showed significant differences between specimens from Newfoundland Grand Banks (Div. 3LN) and specimens form the Laurentian Channel (Unit 2). For *S. fasciatus*, morphometric differences between Div. 3LNO and Laurentian Channel have the same magnitude than for *S. mentella*, but genetic differences are lower with non significant FST pairwise values after Bonferroni adjustment. However, there is a trend for genetic distances to match geographic distances, with Div. 3LNO samples been closer to each other comparing to the outgroup. Analyzing these samples with additional *S. fasciatus* samples coming from the 3PS area adjacent to Div. 3O confirms the suggested trend (results not shown). It further suggests that *S. fasciatus* from Div. 3LNO and from the Subdiv. 3PS area adjacent to Div. 3O form a population that exchanges individuals with the adjacent Unit 2. The exchange follows a gradient with Subdiv. 3PS and Div. 3O being more affected than Div. 3LN. It has to be remembered that only one sample was available within each of the Div. 3LNO units. So, this interesting trend should be further investigated.

For *S. fasciatus*, the results are consistent with managing Div. 3L and 3N as a single stock. Besides, if the spatial and temporal stability of the observed population structure pattern is confirmed by further studies, it could be acceptable to manage Div. 3O together with Div. 3LN. For *S. mentella*, the present study can only confirm that Div. 3L is different from Unit 2, but the results of Roques *et al.* (2001) were consistent with managing Div. 3L and 3N together. In conclusion, for both species, additional information would be needed to address the validity of keeping Div. 3O as a separate unit. However, the present study (see discussion for *S. fasciatus*) suggests that Div. 3O is more prone to be influenced by migration events originating from or towards the Laurentian Channel. So keeping Div. 3O as a separate management unit could be in line with a precautionary approach, since Div. 3O may act as a buffer zone between the populations from Labrador and from the Laurentian Channel.

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**Table 1.** Description of the samples.

| Set | Species | Date | | NAFO | Longitude | Latitude | Depth (m) | N Microsat | N Morpho | (mal+fem) | N tot |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3L65 | FAS | autumn | 2001 | 3L | 4716.6 | 4651.8 | 404 | 24 | 24 | (17+7) | 24 |
| 3N23 | FAS | autumn | 2001 | 3N | 5022.2 | 4247.5 | 408 | 32 | 32 | (14+18) | 32 |
| 3N24 | FAS | autumn | 2001 | 3N | 5021.4 | 4248.1 | 294 | — | 74 | (23+51) | 74 |
| 3N26 | FAS | autumn | 2001 | 3N | 4955.4 | 4253.5 | 230 | — | 70 | (41+29) | 70 |
| 3N27 | FAS | autumn | 2001 | 3N | 4943.5 | 4259.1 | 292 | — | 68 | (51+17) | 68 |
| 3O44 | FAS | autumn | 2001 | 3O | 5256.0 | 4411.6 | 408 | 32 | 32 | (18+14) | 32 |
| 3O63 | FAS | autumn | 2001 | 3O | 5209.2 | 4342.5 | 320 | — | 74 | (30+44) | 74 |
| 3PS88b | FAS | autumn | 2002 | 3PS | 5727.3 | 4707.6 | 234 | 29 | 29 | (15+14) | 29 |
| 3L29 | MEN | autumn | 2001 | 3L | 4725.4 | 4803.1 | 506 | 28 | 28 | (10+18) | 28 |
| 3PN77 | MEN | autumn | 2002 | 3PN | 5824.0 | 4708.3 | 390 | 32 | 32 | (13+19) | 32 |
| | | | | | | | | 177 | 463 | (232+231) | 463 |

**Table 2.** Summary of descriptive statistics for microsatellite data showing sample size (n). number of alleles. number of private alleles. allelic richness. observed (Ho) and expected (He) heterozygosity. Probability values for deviation from Hardy-Weinberg proportions (HW) and significant *P*-values after Bonferroni adjustement at level 5% (*).

| | SEB25 | SEB31 | SEB33 | SEB9 | SAL4 | SEB30 | SEB37 | SEB46 | SAL3 | SEB45 | SPI10II | SPI4II | SPI6 | all loci |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3L65** | | | | | | | | | | | | | | |
| n | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| No. of alleles | 14 | 2 | 16 | 6 | 6 | 14 | 11 | 9 | 4 | 9 | 18 | 14 | 7 | 130 |
| No. of private all. | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| All. Richness (n=24) | 14.0 | 2.0 | 16.0 | 6.0 | 6.0 | 14.0 | 11.0 | 9.0 | 4.0 | 9.0 | 18.0 | 14.0 | 7.0 | 130.0 |
| Ho | 0.833 | 0.458 | 0.667 | 0.667 | 0.750 | 0.792 | 0.958 | 0.708 | 0.500 | 0.667 | 0.833 | 0.917 | 0.708 | 0.728 |
| He | 0.895 | 0.488 | 0.926 | 0.726 | 0.729 | 0.919 | 0.890 | 0.784 | 0.490 | 0.677 | 0.906 | 0.913 | 0.676 | 0.767 |
| HW | 0.273 | 1.000 | 0.004 | 0.951 | 0.443 | 0.244 | 0.799 | 0.547 | 1.000 | 0.516 | 0.307 | 0.652 | 0.605 | 0.000 |
| SD | 0.001 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 |
| **3N23** | | | | | | | | | | | | | | |
| n | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| No. of alleles | 13 | 2 | 15 | 6 | 8 | 19 | 12 | 11 | 3 | 7 | 18 | 17 | 8 | 139 |
| No. of private all. | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| All. Richness (n=24) | 11.8 | 2.0 | 14.3 | 5.7 | 7.2 | 16.2 | 11.9 | 10.1 | 2.9 | 6.7 | 16.2 | 15.5 | 7.4 | 127.8 |
| Ho | 0.844 | 0.281 | 0.844 | 0.688 | 0.719 | 0.875 | 0.875 | 0.750 | 0.438 | 0.688 | 0.813 | 0.906 | 0.813 | 0.733 |
| He | 0.874 | 0.424 | 0.916 | 0.669 | 0.659 | 0.902 | 0.900 | 0.815 | 0.449 | 0.663 | 0.896 | 0.898 | 0.743 | 0.749 |
| HW | 0.817 | 0.083 | 0.276 | 0.084 | 0.703 | 0.074 | 0.523 | 0.720 | 1.000 | 0.071 | 0.394 | 0.519 | 0.670 | 0.006 |
| SD | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 |
| **3O44** | | | | | | | | | | | | | | |
| n | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| No. of alleles | 14 | 2 | 16 | 10 | 7 | 23 | 12 | 10 | 3 | 9 | 18 | 16 | 10 | 150 |
| No. of private all. | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| All. Richness (n=24) | 12.4 | 2.0 | 14.3 | 8.6 | 6.7 | 20.4 | 11.4 | 9.5 | 2.8 | 7.7 | 16.6 | 14.7 | 9.2 | 136.3 |
| Ho | 0.844 | 0.219 | 0.844 | 0.656 | 0.813 | 0.813 | 0.938 | 0.719 | 0.375 | 0.406 | 0.938 | 0.781 | 0.719 | 0.697 |
| He | 0.837 | 0.479 | 0.826 | 0.687 | 0.725 | 0.941 | 0.900 | 0.807 | 0.378 | 0.630 | 0.928 | 0.899 | 0.756 | 0.745 |
| HW | 0.443 | 0.005 | 0.896 | 0.720 | 0.705 | 0.063 | 0.329 | 0.276 | 1.000 | 0.011 | 0.910 | 0.026 | 0.134 | 0.014 |
| SD | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 |

**Table 2.** continued

| | SEB25 | SEB31 | SEB33 | SEB9 | SAL4 | SEB30 | SEB37 | SEB46 | SAL3 | SEB45 | SPI10II | SPI4II | SPI6 | all loci |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3PS88b** | | | | | | | | | | | | | | |
| n | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 |
| No. of alleles | 18 | 6 | 21 | 9 | 6 | 19 | 13 | 11 | 3 | 11 | 23 | 16 | 9 | 165 |
| No. of private all. | - | - | 2 | - | - | 1 | - | - | - | - | - | 1 | - | 4 |
| All. Richness (n=24) | 16.8 | 5.5 | 19.2 | 8.5 | 5.7 | 17.8 | 12.5 | 10.3 | 3.0 | 10.1 | 20.9 | 14.7 | 8.6 | 153.3 |
| Ho | 0.862 | 0.517 | 0.931 | 0.690 | 0.655 | 0.862 | 0.966 | 0.793 | 0.414 | 0.690 | 0.897 | 0.828 | 0.724 | 0.756 |
| He | 0.873 | 0.596 | 0.930 | 0.722 | 0.744 | 0.921 | 0.901 | 0.801 | 0.492 | 0.732 | 0.939 | 0.884 | 0.776 | 0.786 |
| HW | 0.562 | 0.865 | 0.677 | 0.728 | 0.246 | 0.347 | 0.127 | 0.084 | 0.551 | 0.891 | 0.416 | 0.738 | 0.759 | 0.264 |
| SD | 0.000 | 0.001 | 0.000 | 0.001 | 0.002 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.029 |
| **3L29** | | | | | | | | | | | | | | |
| n | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 |
| No. of alleles | 15 | 12 | 28 | 8 | 3 | 18 | 18 | 10 | 6 | 18 | 18 | 10 | 8 | 172 |
| No. of private all. | - | - | - | - | - | 1 | 2 | - | - | 2 | - | - | - | 5 |
| All. Richness (n=24) | 14.2 | 11.8 | 26.1 | 7.7 | 3.0 | 16.5 | 17.1 | 9.1 | 5.8 | 16.9 | 17.3 | 9.6 | 8.0 | 163.2 |
| Ho | 0.893 | 0.786 | 0.964 | 0.714 | 0.179 | 0.786 | 0.679 | 0.357 | 0.393 | 0.893 | 0.893 | 0.821 | 0.714 | 0.698 |
| He | 0.888 | 0.845 | 0.974 | 0.723 | 0.262 | 0.904 | 0.934 | 0.594 | 0.456 | 0.924 | 0.914 | 0.871 | 0.855 | 0.771 |
| HW | 0.851 | 0.091 | 0.878 | 0.371 | 0.172 | 0.376 | 0.000 * | 0.004 | 0.195 | 0.838 | 0.428 | 0.856 | 0.037 | 0.000 * |
| SD | 0.000 | 0.000 | 0.000 | 0.002 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 |
| **3PN77** | | | | | | | | | | | | | | |
| n | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| No. of alleles | 15 | 13 | 27 | 9 | 4 | 21 | 20 | 13 | 5 | 12 | 15 | 13 | 11 | 178 |
| No. of private all. | - | - | - | - | - | - | - | 2 | - | - | - | - | - | 2 |
| All. Richness (n=24) | 13.8 | 11.9 | 23.8 | 8.4 | 3.9 | 18.5 | 17.6 | 11.7 | 5.0 | 10.4 | 13.1 | 12.1 | 10.2 | 160.5 |
| Ho | 0.938 | 0.750 | 0.938 | 0.656 | 0.594 | 0.938 | 0.844 | 0.813 | 0.719 | 0.906 | 0.781 | 0.875 | 0.875 | 0.817 |
| He | 0.885 | 0.789 | 0.961 | 0.705 | 0.494 | 0.930 | 0.903 | 0.832 | 0.680 | 0.836 | 0.807 | 0.888 | 0.886 | 0.811 |
| HW | 0.639 | 0.529 | 0.178 | 0.634 | 0.876 | 0.349 | 0.431 | 0.511 | 0.565 | 0.997 | 0.717 | 0.903 | 0.173 | 0.515 |
| SD | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.001 | 0.033 |
| **all** | | | | | | | | | | | | | | |
| n | 177 | 177 | 177 | 177 | 177 | 177 | 177 | 177 | 177 | 177 | 177 | 177 | 177 | 177 |
| No. of alleles | 20 | 15 | 39 | 14 | 8 | 43 | 30 | 18 | 6 | 25 | 36 | 26 | 13 | 293 |
| No. of private all. | 1 | 3 | 4 | - | - | 9 | 10 | 9 | 1 | 6 | 2 | 1 | 4 | 11 |
| All. Richness (n=24) | 15.4 | 9.6 | 21.4 | 8.6 | 5.7 | 22.7 | 16.1 | 11.3 | 4.8 | 11.8 | 18.5 | 15.3 | 8.8 | 170.0 |
| Ho | 0.870 | 0.497 | 0.870 | 0.678 | 0.621 | 0.847 | 0.876 | 0.695 | 0.475 | 0.706 | 0.859 | 0.853 | 0.762 | 0.739 |
| He | 0.897 | 0.742 | 0.945 | 0.800 | 0.715 | 0.954 | 0.923 | 0.852 | 0.637 | 0.772 | 0.911 | 0.903 | 0.816 | 0.835 |
| HW | 0.674 | 0.025 | 0.000 * | 0.243 | 0.705 | 0.000 * | 0.011 | 0.006 | 0.701 | 0.495 | 0.089 | 0.086 | 0.415 | 0.000 * |
| SE | 0.040 | 0.007 | | 0.026 | 0.018 | | 0.005 | 0.003 | 0.011 | 0.048 | 0.029 | 0.025 | 0.024 | 0.000 |

**Table 3.** Pairwise FST values (below diagonal) with associated *P*-values (above diagonal) between the 6 samples. Significant values after Bonferroni sequential adjustment are in bold. Shaded areas indicate interspecific comparisons.

| | 3L65 | 3N23 | 3O44 | 3PS88b | 3L29 | 3PN77 |
|---|---|---|---|---|---|---|
| **3L65** | — | 0.797 | 0.081 | 0.085 | **<0.00001** | **<0.00001** |
| **3N23** | -0.002 | — | 0.472 | 0.116 | **<0.00001** | **<0.00001** |
| **3O44** | 0.006 | 0.001 | — | 0.347 | **<0.00001** | **<0.00001** |
| **3PS88b** | 0.006 | 0.004 | 0.002 | □ | **<0.00001** | **<0.00001** |
| **3L29** | 0.179 | 0.193 | 0.187 | 0.167 | — | **<0.0001** |
| **3PN77** | 0.120 | 0.133 | 0.130 | 0.111 | 0.018 | — |

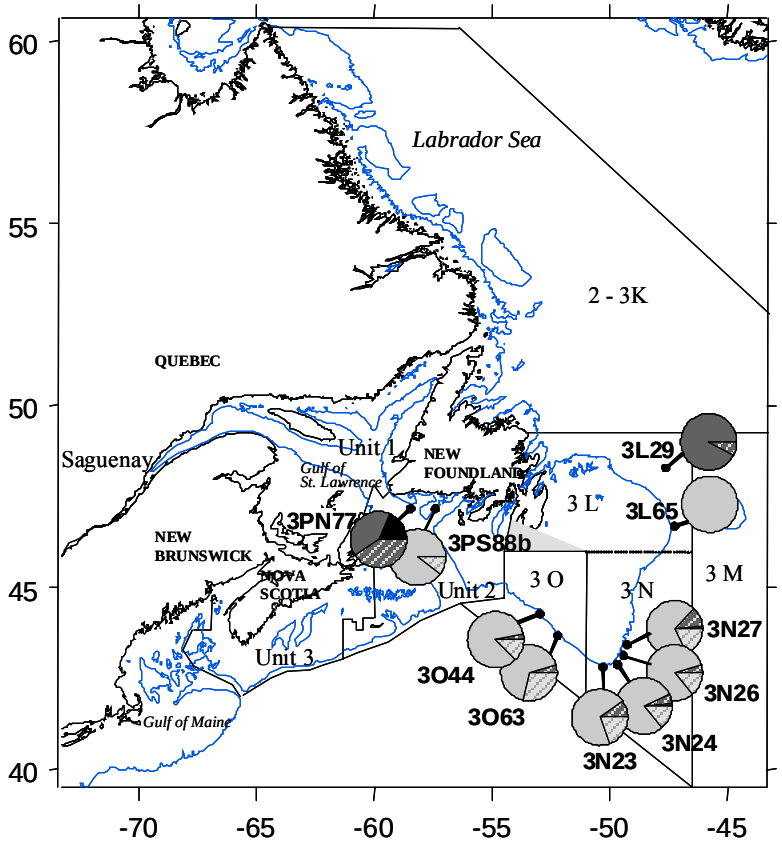


**Figure 1.** Map of the Northwest Atlantic showing location of the 10 samples. These samples are monospecific according to microsatellites or morphometry (*S. mentella*: 3L29, 3PN77; *S. fasciatus*: 3L65, 3N23, 3N24, 3N26, 3N27, 3O44, 3O63, 3PS88b). Pie charts represent the relative composition of each set for the following five groups based on the 3 usual criteria MDH, EGM, AFC: typical *S. mentella* ( ■ ), introgressed *S. mentella* ( ▩ ), typical *S. fasciatus* ( □ ), introgressed *S. fasciatus* ( ▨ ), and heterozygote specimens ( ■ ).

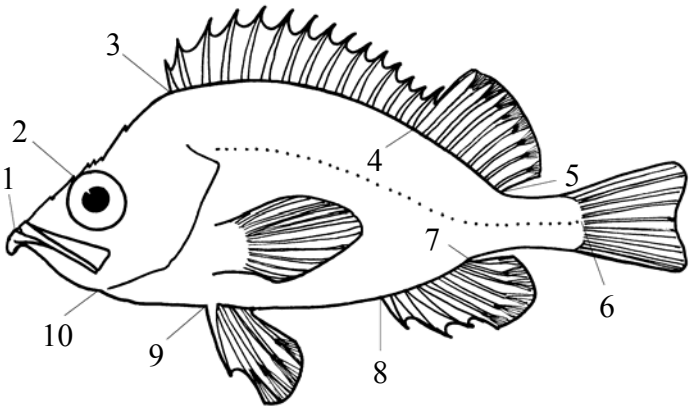


**Fig. 2.** Position of the 10 landmarks used to define body shape: (1) bottom of the teeth on the lower jaw; (2) preocular spine; (3) anterior insertion of the dorsal fin; (4) posterior base of the last hard ray on the dorsal fin; (5) posterior insertion of the dorsal fin; (6) posterior extremity of the lateral line; (7) posterior insertion of the anal fin; (8) anterior insertion of the anal fin; (9) anterior insertion of the pelvic fin; (10) posterior extremity of the lower jaw.

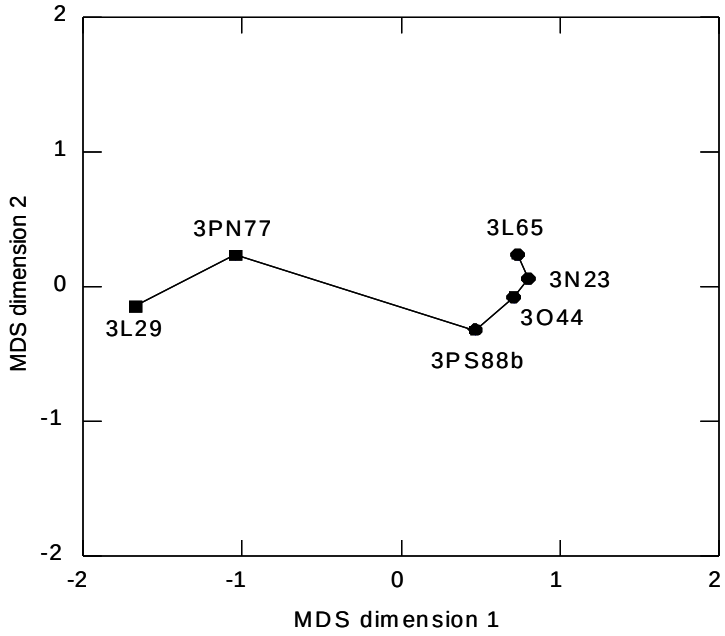


**Fig. 3.** Graphical representation of distances between samples after MDS analysis on Cavalli-Sforza and Edwards (1967) chord distance performed on both *S. mentella* (square) and *S. fasciatus* (dot) sex-pooled samples.

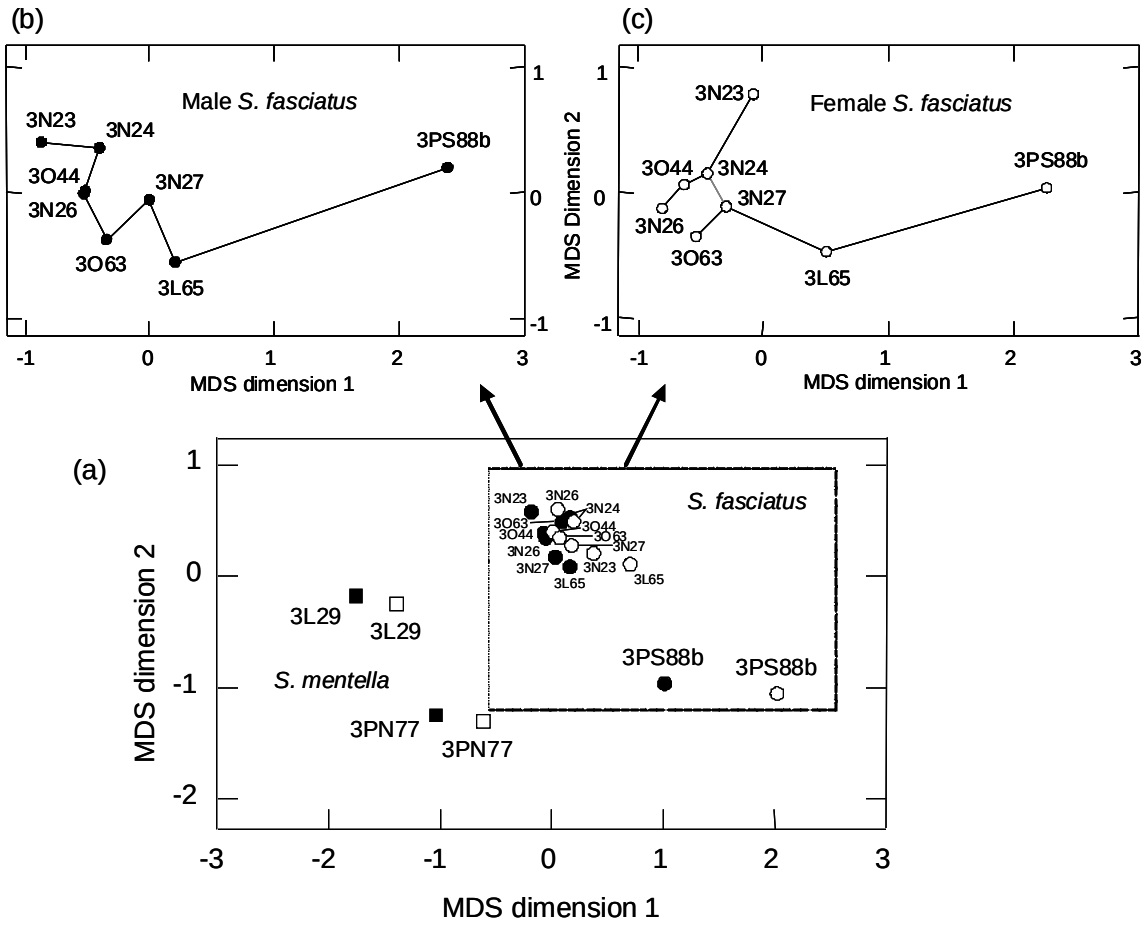


**Fig. 4.** Graphical representation of distances between samples after MDS analysis on geometric morphometric data performed (a) on both *S. mentella* (square) and *S. fasciatus* (dot) samples separated by sex (black for males and white for females), and on *S. fasciatus* separately for (b) males and (c) females.