J. Northw. Atl. Fish. Sci., Vol. 36: 65–80 Publication (Upload) date: 27 Jan 2006

Extensive Sampling and Concomitant Use of Meristic Characteristics and Variation at the *MDH-A** Locus Reveal New Information on Redfish Species Distribution and Spatial Pattern of Introgressive Hybridization in the Northwest Atlantic

Alexandra Valentin
Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski
310 Allée des Ursulines, Rimouski, Québec, Canada G5L 3A1

Jean-Marie Sévigny and Bernard Morin
Institut Maurice-Lamontagne, Pêches et Océans Canada
850 Route de la Mer, Mont-Joli, Québec, Canada G5H 3Z4

Don Power
Science, Oceans and Environment Branch, Fisheries and Oceans Canada
P. O. Box 5667, St. John's, Newfoundland, Canada A1C 5X1

Robert M. Branton
Marine Fish Division, Bedford Institute of Oceanography, Fisheries and Oceans Canada
P. O. Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2

Valentin, A., J-N, Sévigny, B. Morin, D. Power, and R. M. Branton. 2006. Extensive sampling and concomitant use of meristic characteristics and variation at the *MDH-A** locus reveal new information on redfish species distribution and spatial pattern of introgressive hybridization in the Northwest Atlantic. *J. Northw. Atl. Fish. Sci.*, **36**: 65–80. doi: 10.2960/J.v36.m573

Abstract

Variability at the *MDH-A** locus, of anal fin ray number and extrinsic gasbladder muscle pattern is used to describe the distribution of *Sebastes fasciatus* and *S. mentella* in the Northwest Atlantic and to define the geographical boundaries of the area of introgressive hybridization between the two species. *Sebastes mentella* distribution extends from the Gulf of St. Lawrence northward while *S. fasciatus* is distributed from the southern Grand Banks southward as described in earlier studies. *Sebastes fasciatus* is also found in the southern Labrador Sea where the species appears to reach its northernmost limit. The distribution of the two species overlaps mainly in the Gulf of St. Lawrence and the Laurentian Channel, around the Grand Banks and on Flemish Cap. This area of sympatry comprises a smaller area where heterozygous individuals at the *MDH-A** locus are observed and where introgressive hybridization occurs. This area is mostly restricted to the Gulf of St. Lawrence and the Laurentian Channel. The west-east gradient in the abundance of introgressed individuals suggests that the Gulf of St. Lawrence is the centre of introgressive hybridization and that larval dispersion or migration of juveniles and adults takes place from the Gulf of St. Lawrence towards south Newfoundland and the Grand Banks. In contrast, the absence of heterozygous individuals at the *MDH-A** locus outside the Gulf of St. Lawrence and the Laurentian Channel indicates that the dispersion of these individuals is limited for all stages of the life cycle.

Key words: distribution, hybridization, malate dehydrogenase, meristics, Northwest Atlantic, redfish, *Sebastes*, sympatry

Introduction

The management of several marine exploited species is based on the assumption that their distribution comprises different stocks and that these stocks represent populations with particular patterns of recruitment and mortality (Carvalho and Hauser, 1994; Waples, 1998; Ward, 2000). It is also assumed that the fishery will target unique stocks. Understanding the population structure is thus a prerequisite to the development and implementation of sound management and conservation measures.

The description of stock structure of redfish (*Sebastes* spp.) is complex since these stocks may comprise different sibling species, each of them being characterized by

different life history patterns. Three species are currently recognized in the Northwest Atlantic: *Sebastes mentella* (Travin, 1951), *S. fasciatus* (Storer, 1854) and *S. marinus* (Ascanius, 1772). They are traditionally managed as a single resource. For the fishery management, *S. fasciatus* (Acadian redfish) and *S. mentella* (deep-water redfish) are the two most important species in the Northwest Atlantic and show differential ecological preferences. *Sebastes fasciatus* typically occurs in shallower depths (150–300 m) whereas *S. mentella* is distributed at depths varying between 350 and 500 m. The distribution of the two species overlaps on Flemish Cap, in regions east and south of Newfoundland, and off the Scotian Shelf, but particularly in the Gulf of St. Lawrence, where they are both known to extrude their larvae (Sévigny *et al*., 2000). The northern (Labrador Sea) and southern regions (Gulf of Maine, Scotian Shelf) are respectively dominated by *S. mentella* and *S. fasciatus* (Atkinson, 1987; Gascon, 2003 for reviews).

Three characteristics are commonly used to discriminate *S. mentella* from *S. fasciatus* in the Northwest Atlantic. They are i) the number of soft rays in the anal fin (AFC) (≥8 for *S. mentella*, ≤7 for *S. fasciatus*) (Ni, 1981a; 1982), ii) the extrinsic gasbladder muscle passage patterns (EGM) (between ribs 2 and 3 for *S. mentella*, between ribs 3 and 4 or more for *S. fasciatus*) (Ni, 1981a; 1981b), iii) the genotype at the liver malate dehydrogenase locus (*MDH-A**). The *MDH-A** locus is polymorphic with two codominant alleles, *MDH-A*1* and *MDH-A*2*, that combine to form three possible genotypes. The genotype *MDH-A*11* characterizes *S. mentella*, while *MDH-A*22* is associated with *S. fasciatus* (Payne and Ni, 1982; McGlade *et al*., 1983). The significant number of heterozygous specimens (*MDH-A*12*) and a lack of concordance between the three classification criteria in the Gulf of St. Lawrence led Rubec *et al.* (1991) to hypothesize that introgressive hybridization takes place in this area. Other studies on ribosomal DNA (Desrosiers *et al.*, 1999) and body morphometry (Valentin, 1999; Valentin *et al.*, 2002) support this hypothesis. Recently, microsatellite DNA markers specific to redfish were developed (Roques *et al*., 1999a; 1999b) and used to assess the importance of introgressive hybridization between *S. fasciatus* and *S. mentella* in the Northwest Atlantic (Roques *et al*., 2001). This study shows that introgressive hybridization is mostly restricted to the Gulf of St. Lawrence and the Laurentian Channel and plays a key role in the genetic differentiation of the two species on the geographical scale.

Though currently used, AFC, EGM, and MDH have never been compared or even used concurrently in large scale sampling programs. This is mainly due to differences among studies in the methodology used to assess species identification. The present study remedies this situation in describing the AFC, EGM, and MDH characteristics of more than 7 500 individual redfish collected from 1994 to 1998 across the Northwest Atlantic. This large data base offers the first opportunity to document extensively the distribution and the concordance of the three usual criteria, with the objective to provide new insight about i) the geographic distribution of *S. fasciatus* and *S. mentella* in the Northwest Atlantic, ii) the localization and boundaries of areas of introgressive hybridization, iii) document the fine scale geographic variability of introgressive hybridization in the Gulf of St. Lawrence and the Laurentian Channel.

Material and Methods

Sampling Procedure

Sampling was carried out from 1994 to 1998 between January and December and covered the eight current redfish management areas in the Northwest Atlantic: NAFO Subarea 2+Division 3K, Div. 3LN, Div. 3M, Div. 3O, Unit 1 (4RST+3Pn4Vn[Jan–May]), Unit 2 (3Ps4Vs4Wfgj+3Pn4Vn[Jun–Dec]), Unit 3 (4X+4Wdehkl) and the Gulf of Maine. Some samples were also collected in the Saguenay Fjord (Fig. 1; Table 1). The important logistic involved in sampling such large areas across the Northwest Atlantic did not allow temporal and spatial homogeneity in the collection. Redfish samples were obtained from 545 bottom trawl tows using various gear types such as URI 81/114, Western 2A. Redfish from the Saguenay Fjord were caught using handlines during ice-fishing season. The length characteristics of the redfish collected are presented in Table 2.

All redfish were examined for three usual classification criteria. AFC and EGM were determined and recorded either at sea or in the laboratory. Some specimens presented patterns of EGM that were intermediary between those of *S. fasciatus* and *S. mentella*. These specimens were called "doubtful". The genotype at the *MDH-A** locus was determined from liver tissue samples, which were collected at sea on fresh fish and kept frozen pending the electrophoretic analyses, or were collected in the laboratory on frozen fishes just before the electrophoretic analysis.

Cellulose acetate electrophoretic analyses of the MDH and gel staining were carried out as described by Hebert and Beaton (1989). A heterozygous individual was used on every gel to assess the quality of the

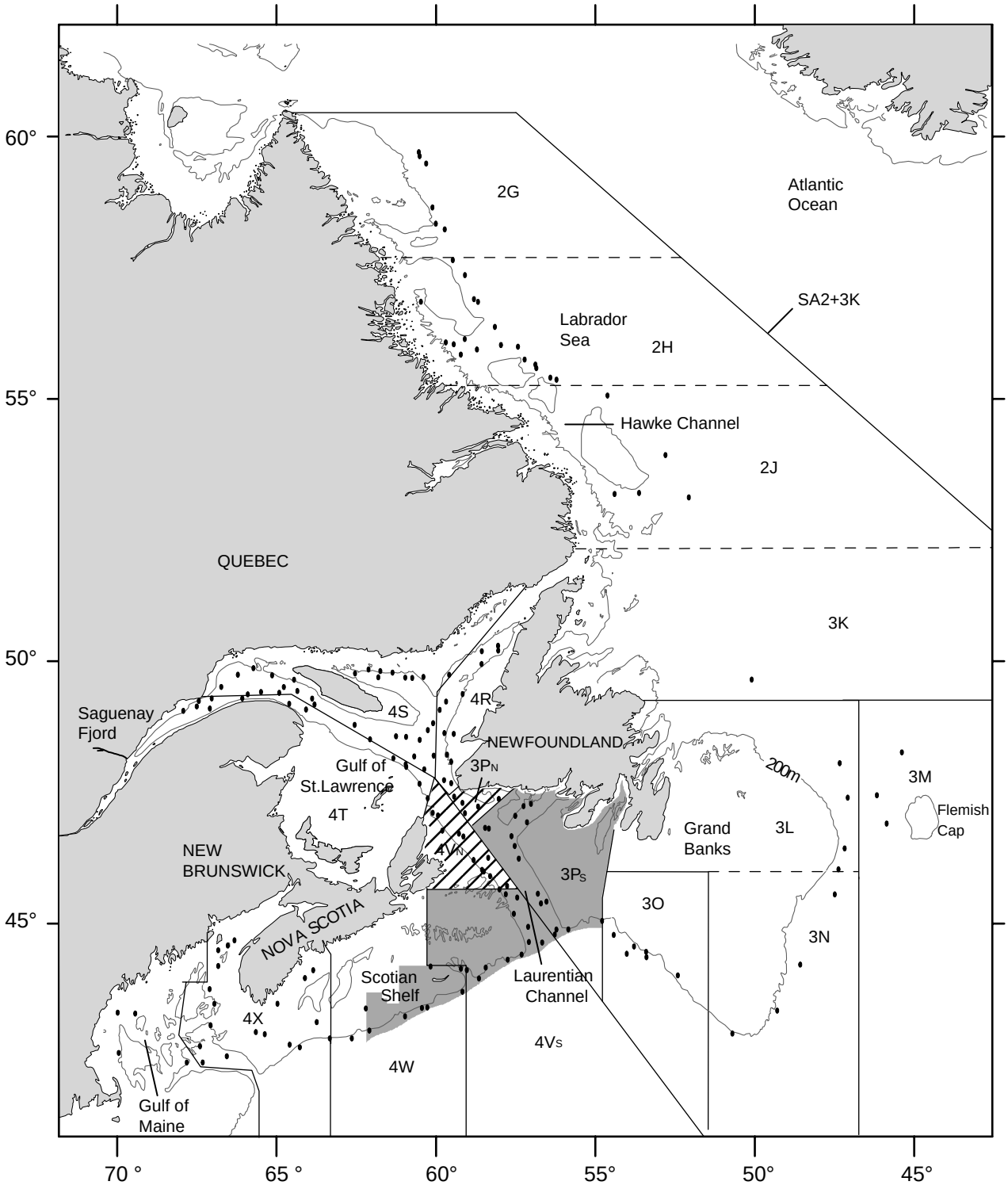

Fig. 1. Map of the Northwest Atlantic showing the location of the 184 sites representing the 545 bottom trawl tows carried out between 1994 and 1998. Each dot represents tows that were carried out within a square of 0.5 degree of latitude and were pooled. The boundaries of NAFO Divisions are illustrated. The hatched area corresponding to NAFO Subdiv. 3PN and 4VS indicates the region of seasonal overlap between redfish management Unit 1 (Gulf of St. Lawrence) and Unit 2 (Laurentian channel; grey area).

TABLE 1. Number of redfish collected in the current management areas of the Northwest Atlantic from 1994 to 1998. Northwest Atlantic Fisheries Organization (NAFO) Divisions are indicated. ? = precise date unknown. *N* = sample size.

	Redfish Current Management Area									
Sampling date	Labrador Shelf (SA2+3K)	Grand Bank (3LN)	Flemish Cap (3M)	Grand Bank (3O)	Saguenay Fjord	Gulf of Lawrence (Unit 1)	Laurentian Channel (Unit 2)	Scotian Shelf (Unit 3)	Gulf of Maine	Total *N*
1994	–	–	–	–	–	**602**	**685**	–	–	**1 287**
01–1994	–	–	–	–	–	310	–	–	–	310
03–1994	–	–	–	–	–	–	685	–	–	685
08–1994	–	–	–	–	–	292	–	–	–	292
1995	–	–	–	**61**	–	**388**	**877**	**376**	–	**1 702**
07–1995	–	–	–	61	–	–	754	376	–	1 191
08–1995	–	–	–	–	–	352	123	–	–	475
09–1995	–	–	–	–	–	36	–	–	–	36
1996	**28**	–	**23**	**128**	**69**	**618**	**1 203**	**247**	**89**	**2 405**
03–1996	–	–	–	–	–	–	102	–	–	102
04–1996	–	–	–	–	–	–	–	39	–	39
07–1996	–	–	–	128	–	–	934	–	–	1 062
08–1996	–	–	–	–	–	618	167	208	–	993
09–1996	–	–	5	–	–	–	–	–	12	17
10–1996	28	–	18	–	–	–	–	–	77	123
Winter 96	–	–	–	–	69	–	–	–	–	69
1997	**41**	**192**	–	**175**	–	**264**	**720**	–	–	**1 392**
05–1997	–	–	–	49	–	–	–	–	–	49
06–1997	–	102	–	–	–	–	–	–	–	102
07–1997	–	–	–	46	–	–	720	–	–	766
08–1997	–	–	–	–	–	264	–	–	–	264
10–1997	–	50	–	80	–	–	–	–	–	130
12–1997	–	40	–	–	–	–	–	–	–	40
? 1997	41	–	–	–	–	–	–	–	–	41
1998	**714**	–	–	–	–	**55**	**80**	–	–	**849**
06–1998	84	–	–	–	–	–	–	–	–	84
10–1998	616	–	–	–	–	–	–	–	–	616
11–1998	14	–	–	–	–	55	80	–	–	149
Total *N*	**783**	**192**	**23**	**364**	**69**	**1 927**	**3 565**	**623**	**89**	**7 635**

TABLE 2. Mean, minimum (Min), maximum (Max) and coefficient of variation (C.V.) of fish length (mm) collected in the redfish current management areas of the Northwest Atlantic from 1994 to 1998. North Atlantic Fisheries Organization (NAFO) Divisions are indicated. The sample size (*N*) does not correspond to that of Table 1 because length was not available for all individuals.

	Redfish Current Management Area									
	Labrador Shelf (2+3K)	Grand Bank (3LN)	Flemish Cap (3M)	Grand Bank (3O)	Saguenay Fjord	Gulf of Lawrence (Unit 1)	Laurentian Channel (Unit 2)	Scotian Shelf (Unit 3)	Gulf of Maine	Total *N*
N	782	186	23	363	69	1 927	3 565	623	89	7 627
Min.	93	146	90	80	220	7	60	80	150	7
Max.	505	440	380	430	330	515	530	460	280	530
Mean	222	261	198	256	265	235	288	252	198	261
C.V.	0.26	0.24	0.45	0.29	0.08	0.50	0.28	0.31	0.14	0.35

electrophoretic separation and to facilitate allele identification. Of the three criteria used, only the EGM pattern allows the discrimination between *S. mentella* and *S. marinus* (Power and Ni, 1982). In this study, *S. marinus* did not occur in the sampled redfish.

Data Analyses

Statistical analyses of the MDH variability were limited since the MDH data were used to provide a broad picture of the species distribution in the study area. Deviations from Hardy-Weinberg proportions were tested using a χ^2 test of goodness of fit for the management areas where genotypic variation was observed. Differences in genotypic frequencies between Units 1 and 2 were tested using heterogeneity χ^2 test.

The frequency distribution of AFC, EGM and MDH was calculated for each management area and represented as pie charts of relative frequencies.

The level of concordance between the three criteria was used to define five groups as in Valentin *et al*. (2002). In the first two groups, the genotype at the *MDH-A** locus and the other two morphological characters (AFC and EGM) were concordant. 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 concordant with the genotype. The fifth group called "heterozygous individuals" comprised all the fishes that were heterozygous at the *MDH-A** locus regardless of the values of the two morphological criteria.

The percentage of each group was calculated for each management area. Roques *et al*. (2001) have shown that *S. mentella* from the Labrador Sea and *S. fasciatus* from the Gulf of Maine and the Scotian Shelf may be considered as "pure species" based on the analyses of eight microsatellites markers. Considering this information, it was assumed that the lack of concordance between the three criteria observed in these regions reflected intraspecific natural variability of the AFC and EGM characters. Therefore, a correction was made to take this natural variability into account, keeping in mind that the determination of meristic characters is influenced by environmental conditions (Lindsey, 1988) which, in turn, may not be uniform across the species distribution range. The percentage of non-concordance was calculated for *S. mentella* (1.9%) in Div. 2G and 2H (Table 3A), and for *S. fasciatus* (11.2%) in Unit 3 and the Gulf of Maine (Table 3B). The value was higher for *S. fasciatus* mainly because AFC = 8 is not rare for this species. The percentages of non-concordance observed in Div. 2G–2H as well as in Unit 3 and the Gulf of Maine were attributed to natural variability of AFC and EGM instead of being a result of introgression. Therefore, in all areas and for each species, the corresponding percentage was subtracted from the percentage of introgression and added to the percentage of concordance. The percentages of concordance ("typical *S. mentella*" + "typical *S. fasciatus*"), of introgression ("introgressed *S. mentella*" + "introgressed *S. fasciatus*") and of "heterozygous individuals" were represented as pie charts of relative frequencies.

The spatial distribution of the five groups was also described on a smaller scale in the zone of hybridization (Gulf of St. Lawrence, Laurentian Channel) using 4 063 specimens caught during summer 1995, 1996 and 1997. For this analysis, all the individuals captured in tows that were carried out within a rectangle of 0.5 degree of latitude and longitude were pooled. This provided 54 sites comprising at least 30 specimens (mean = 78). The relative frequency of "typical *S. mentella*", "typical *S. fasciatus*", "introgressed *S. mentella*", "introgressed *S. fasciatus*" and "heterozygous individuals" was calculated for each sample and represented as shaded contour map using the mapping program available on the virtual data centre of the Maritimes DFO region.

Results

Species Distribution Based on MDH Variability

The distribution of the two redfish species differs significantly in the Northwest Atlantic (Fig. 2). Indeed, *S. fasciatus* is almost the only species represented in the southern part of the distribution, i.e. the Gulf of Maine and the Scotian Shelf. In these two regions, the frequencies of the allele *MDH-A*2*, which is characteristic of *S. fasciatus*, is equal to 1 and 0.97, respectively (Table 4). In these two areas, only 18 individuals out of 712 belong to *S. mentella*. In the northern part of the distribution (Labrador Sea: NAFO Subarea 2 and Div. 3K), *S. mentella* is the dominant species (85.7%) although *S. fasciatus* is also observed in small proportion (Fig. 2; Table 4). A detailed examination of the data reveals that the percentage of *S. mentella* reaches 98% in northern Labrador Sea (Div. 2G–2H). Indeed, 95 out of the 107 *S. fasciatus* specimens collected north of Newfoundland originate from the southern Labrador Sea (Div. 2J–3K) and 80 of these specimens were collected in Hawke

TABLE 3. Distribution of anal fin ray number (AFC) and of extrinsic gasbladder muscle passage patterns (EGM) for the genotype *MDH-A*11* in Div. 2G–2H (**A**) and for the genotype *MDH-A*22* on the Scotian Shelf (Unit 3) and the Gulf of Maine (**B**). Non typical patterns are in bold character. *N* = sample size.

EGM	AFC					
	6	7	8	9	10	N
			(**A**)			
2–3	–	**3**	231	321	17	572
2–3/3–4	–	–	**2**	**5**	–	7
3–4	–	**1**	–	–	–	1
N	–	4	233	326	17	580
			(**B**)			
2–3	–	**6**	**1**	–	–	7
2–3/3–4	**1**	**15**	**3**	**1**	–	20
3–4	10	572	**42**	–	–	624
3–4/4–5	–	27	**6**	–	–	33
4–5	–	7	**3**	–	–	10
N	11	627	55	1	–	694

Channel in a small number of tows. Both species are well represented in the Gulf of St. Lawrence (Unit 1), the Laurentian Channel (Unit 2), on the Grand Bank (Div. 3LN and 3O) and on Flemish Cap (Div. 3M). The change in the species distribution across the Northwest Atlantic is reflected in a north-south clinal change in allelic frequency. Indeed, allele *MDH-A*1* characteristic of *S. mentella* is the most frequent one in the Labrador Sea while allele *MDH-A*2* is almost fixed in the Gulf of Maine and on Scotian Shelf.

One of the most important features of the present study is the restricted distribution of heterozygous individuals (*MDH-A*12*) to the Gulf of St. Lawrence and the Laurentian Channel (Fig. 2; Table 4) although the genotypic frequencies between the two regions differ (χ^2 = 49.7; P <0.001). The apparent high level of heterozygosity observed on Flemish Cap is to be considered with some caution because of the small sample size (N = 23). However, unpublished data tend to confirm that heterozygotes represent about 5% (N = 137) of the redfish population on Flemish Cap (Johansen, pers. comm.).

The presence of the two redfish species in several areas of the Northwest Atlantic and the absence of heterozygotes translate not surprisingly into significant deviations from Hardy-Weinberg expectations; all of them being associated with deficits in heterozygotes (Table 4). The only exception is the Saguenay Fjord where *S. mentella* is the dominant species (Bourgeois, 1993).

AFC and EGM Variability

The geographic distribution of AFC and EGM provides the same broad picture as the MDH electrophoretic patterns regarding the distribution of the two species. Indeed, the characteristic modes for *S. fasciatus* (AFC ≤7 and EGM between ribs 3–4 or more) and for *S. mentella* (AFC ≥8 and EGM between ribs 2–3) are distributed in proportions (Fig. 3 and 4) that match those determined by the genotype at the *MDH-A** locus (Fig. 2).

Geographical Pattern of Concordance

The concordance between the three characters is high in the northern and southern areas (i.e., the areas strongly dominated by one or the other species), and weaker in the sympatric region. In the Labrador Sea, where *S. mentella* represent more than 85% of the specimens, the overall concordance reaches 93% whereas in the South, where *S. fasciatus* is almost the only species present, the concordance is close to 99% (Fig. 5; Table 5). The level of concordance is lower in the area of sympatry corresponding to the Gulf of St. Lawrence (Unit 1), the Laurentian Channel (Unit 2), the Grand Bank (Div. 3LN and 3O) and Flemish Cap (Div. 3M). Furthermore, the level of concordance is not homogenous across the whole area, as it follows a gradient with values diminishing from 85% in southern Grand Banks to 68%

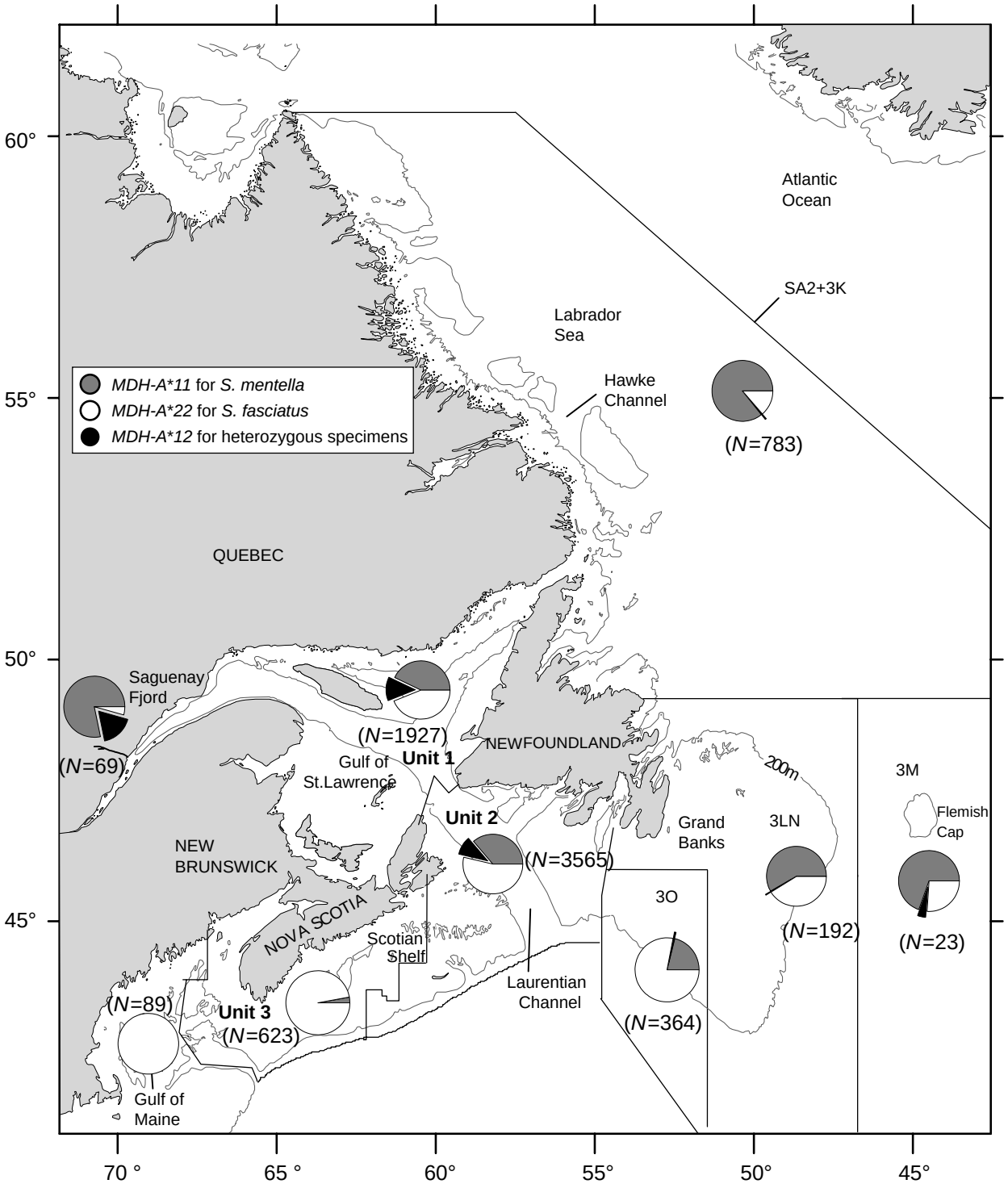

Fig. 2. Map of the Northwest Atlantic showing the geographic distribution of the three genotypes observed at the *MDH-A** locus in the current redfish management areas (NAFO Subarea 2+Division 3K, 3LN, 3M, 3O, Unit 1–3 and the Gulf of Maine).

TABLE 4. Allelic frequencies, relative genotypic frequencies, percentage of observed (H_o) and expected (H_e) heterozygosities at the *MDH-A** locus, and relative frequencies of anal fin ray number (AFC) and extrinsic gasbladder muscle passage pattern (EGM) classes for each current redfish management area. *N* = sample size; * indicates significant deficit of heterozygous specimens at $P \leq 0.05$.

Redfish Management Area	*N*	Allelic frequency		Heterozygosity		Genotype frequency		AFC		EGM		
		**1*	**2*	H_e	H_o	**11*	**22*	≤7	≥8	2–3	2–3/ 3–4	3–4 or +
Labrador Shelf (SA2+3K)	783	0.86	0.14	24.1	0.6*	85.7	13.7	9.1	90.9	84.2	3.3	12.5
Grand Bank (3LN)	192	0.59	0.41	48.6	0.5*	58.3	41.1	33.3	66.7	56.3	2.6	41.1
Flemish Cap (3M)	23	0.72	0.28	41.5	4.3*	69.6	26.1	17.4	82.6	56.5	4.4	39.1
Grand Bank (3O)	364	0.22	0.78	33.8	0.8*	21.2	78.0	61.8	38.2	18.4	6.0	75.6
Saguenay Fjord	69	0.87	0.13	22.8	17.4	78.3	4.3	8.7	91.3	65.2	24.6	10.2
Gulf of St. Lawrence (Unit 1)	1927	0.50	0.50	50.0	13.3*	42.9	43.8	35.9	64.1	35.1	21.3	43.6
Laurentian Channel (Unit 2)	3565	0.41	0.59	48.5	10.1*	36.2	53.7	44.2	55.8	28.7	16.5	54.8
Scotian Shelf (Unit 3)	623	0.03	0.97	5.6	0.0*	2.9	97.1	90.9	9.1	2.6	2.7	94.7
Gulf of Maine	89	0.00	1.00	0.0	0.0	0.0	100.0	89.9	10.1	0.0	3.4	96.6
All areas	7635	0.44	0.56	49.4	8.4*	40.1	51.5	43.0	57.0	34.2	14.3	51.5

TABLE 5. Relative frequencies of concordance, introgression and heterozygous specimens with relative frequencies by species for the current redfish management areas after correction for natural variability. *N* = sample size.

	Current Management Area								
	Labrador Shelf (2–3K)	Grand Bank (3LN)	Flemish Cap (3M)	Grand Bank (3O)	Saguenay Fjord	Gulf of St. Lawrence (Unit 1)	Laurentian Channel (Unit 2)	Scotian Shelf (Unit 3)	Gulf of Maine
Concordance	93.3	85.4	73.8	84.7	57.0	55.8	67.8	98.6	100.0
Typical *S. mentella*	84.5	53.2	53.5	17.7	55.1	24.1	22.1	1.5	0.0
Typical *S. fasciatus*	8.8	32.2	20.3	67.0	1.9	31.7	45.7	97.1	100.0
Introgression	6.1	14.1	21.9	14.5	25.6	30.9	22.1	1.4	0.0
Introgressed *S. mentella*	1.2	5.2	16.1	3.5	23.2	18.8	14.1	1.4	0.0
Introgressed *S. fasciatus*	4.9	8.9	5.8	11.0	2.4	12.1	8.0	0.0	0.0
Heterozygous specimens	0.6	0.5	4.3	0.8	17.4	13.3	10.1	0.0	0.0
N	783	192	23	364	69	1927	3565	623	89

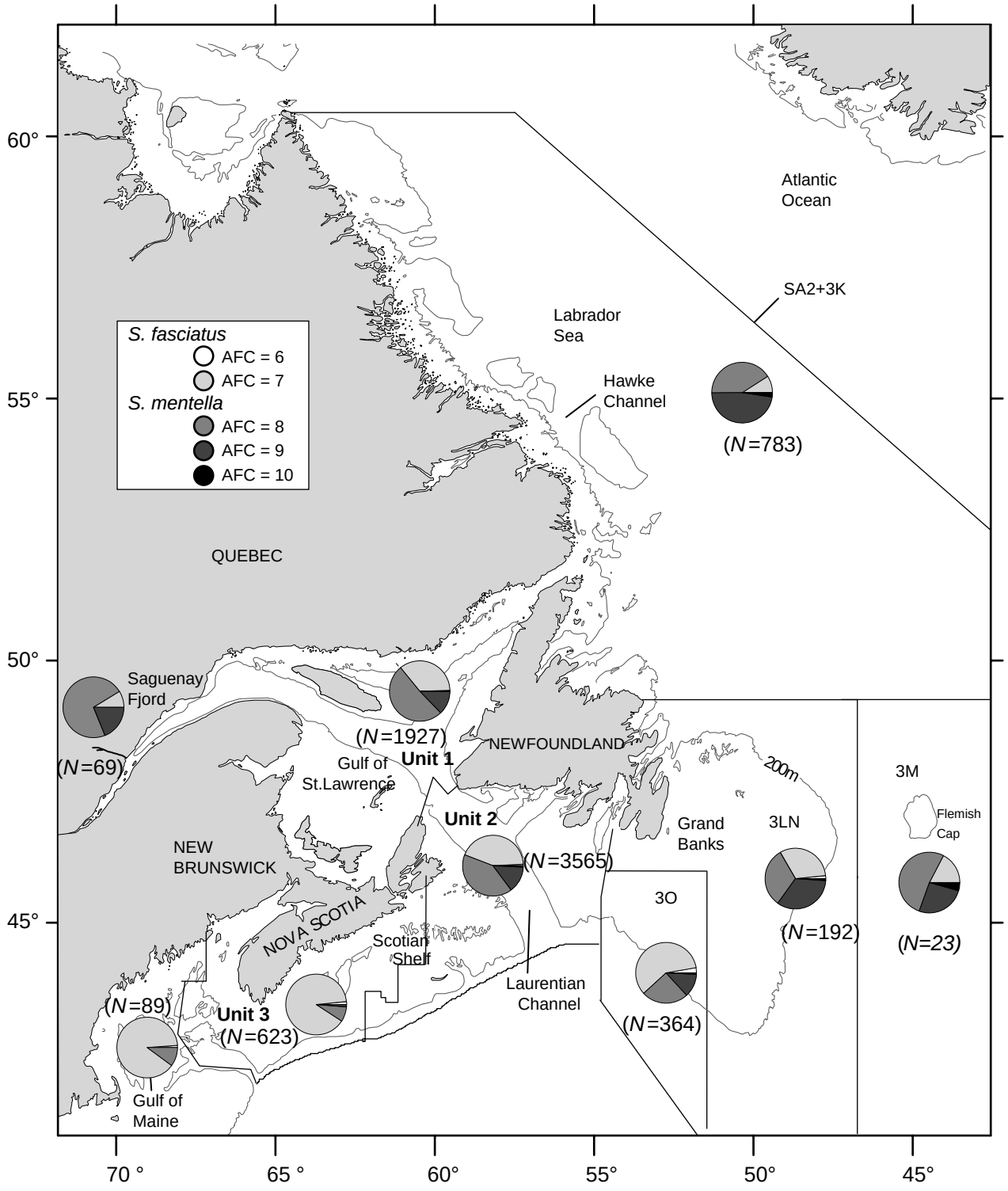

Fig. 3. Map of the Northwest Atlantic showing the relative frequency of the anal fin ray counts (AFC) in the current redfish management areas (NAFO Subarea 2+Div. 3K, 3LN, 3M, 3O, Unit 1–3 and the Gulf of Maine).

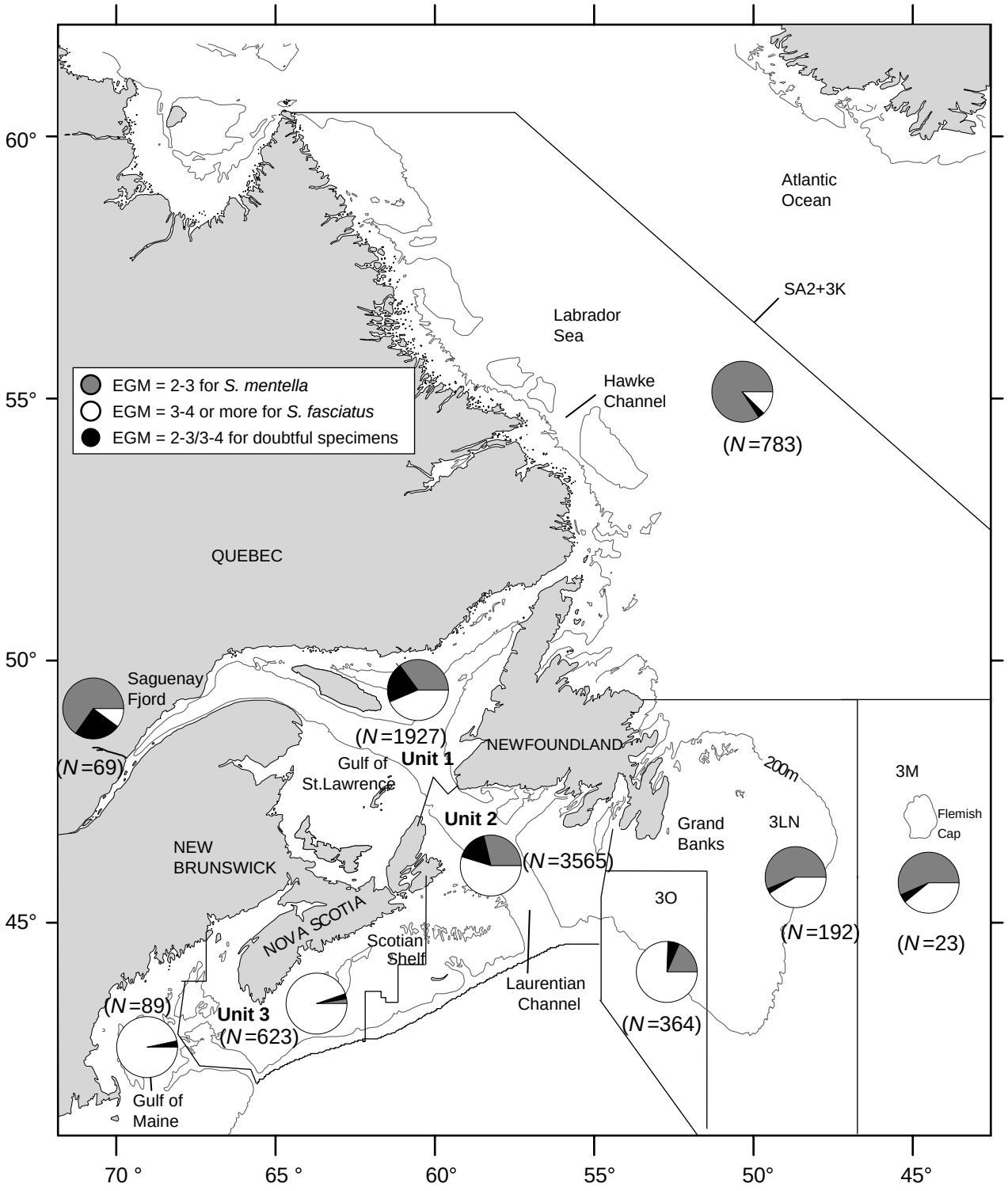

Fig. 4. Map of the Northwest Atlantic showing the relative frequency of the extrinsic gasbladder muscle passage patterns (EGM) in the current redfish management areas (NAFO Subarea 2+Div. 3K, 3LN, 3M, 3O, Unit 1–3 and the Gulf of Maine).

in Laurentian Channel and to 56% in the Gulf of St. Lawrence (Fig. 5; Table 5).

Spatial Distribution of Typical, Introgressed and Heterozygous Individuals in the Gulf of St. Lawrence, and the Laurentian Channel

The highest concentrations of *S. fasciatus* occur in Southern Grand Bank (Div. 3O) and Laurentian Channel (Unit 2), particularly in the regions of Banquereau, St. Pierre Bank, and Burgeo Bank. In these two management areas, "typical *S. fasciatus*" are strongly dominant and "introgressed *S. fasciatus*" are mostly distributed on the eastern slope of the Channel (Fig. 6 a, b). In the Gulf of St. Lawrence (Unit 1), high concentrations of *S. fasciatus* are present north of the Gaspé Peninsula, around Anticosti Island and in the area of Cape St. George. All these samples comprise a mixture of "typical *S. fasciatus*" and "introgressed *S. fasciatus*" specimens and are mostly dominated by "typical *S. fasciatus*".

Sebastes mentella is abundant in Honguedo Strait, in the Northeast Gulf of St. Lawrence, in Cabot Strait and, to a lesser extent, between St. Pierre Bank and Banquereau. Typical and introgressed specimens co-occur in these areas, but particularly high concentrations of "introgressed *S. mentella*" are present south of Anticosti Island and in Cabot Strait (Div. 3Pn). A concentration of *S. mentella*, mostly dominated by "typical *S. mentella*", is present south of Sable Island, but otherwise *S. mentella* is poorly represented along Banquereau and St. Pierre Bank outside the Laurentian Channel (Fig. 6 c, d).

The spatial variation in abundance of "heterozygous individuals" closely resembles that of "typical *S. mentella*" and "introgressed *S. mentella*" in the Gulf of St. Lawrence, the Laurentian Channel, and on southern Grand Banks (Fig. 6e).

Discussion

MDH, AFC and EGM are currently used for redfish species identification in the Northwest Atlantic. However, these three criteria were never recorded concurrently at the scale of the Northwest Atlantic. In the present study, the concomitant use of the three criteria at the scale of the Northwest Atlantic provides a better perspective on the species distribution and on the dynamic of this distribution. This study confirms and completes previous observations regarding redfish species distribution and reliability of the three usual criteria for species discrimination. Besides, this study is the first to describe the spatial distribution of typical, introgressed and heterozygous redfish at the scale of the Northwest Atlantic. It allows defining the boundaries of the area of introgressive hybridization and suggests original hypotheses regarding redfish dispersion and population structuring.

Distribution of *S. fasciatus* and *S. mentella*

The present results are in general agreement with those of previous studies showing that the northern Labrador Sea (Div. 2G–2H) and the Gulf of Maine and Scotian Shelf are strongly dominated by *S. mentella* and *S. fasciatus*, respectively (Barsukov and Zakharov, 1973; Ni, 1982, 1984; Atkinson, 1987; Gascon, 2003). In the present study, the MDH data shows that 98% of the specimens sampled in Northern Labrador Sea belong to *S. mentella* while more than 97% of those collected in the Scotian Shelf-Gulf of Maine area belong to *S. fasciatus*.

The north-south change in species distribution observed across the Northwest Atlantic translated into a north-south gradient of meristic characters that was also observed in earlier broadscale studies (Ni, 1982; Templeman and Pitt, 1961). Part of the gradient observed in AFC may be influenced by the north-south cline of the water temperature, since determination of meristic structures may be partly temperature-dependant (Lindsey, 1988; Templeman and Pitt, 1961).

It is worth mentioning that *S. fasciatus kellyi*, a presumptive subspecies of *S. fasciatus* in the Gulf of Maine (Litvinenko, 1979), does not seems to be an important factor in this study as there was no difference in genotypic distribution at the *MDH-A** locus, or in AFC and EGM distributions between the Gulf of Maine and Unit 3. Significant differences in allelic frequencies at microsatellite loci were detected between samples from these two areas (Roques *et al.*, 2001).

Reliability of the Three Usual Criteria to Discriminate Redfish Species

Each of the three characteristics (MDH, AFC and EGM) can be used to describe the distribution of the two species in the Northwest Atlantic (Fig. 2–4). These criteria are quite equivalent to discriminate the species in the areas strongly dominated by one or the other redfish species, as illustrated by the high level of concordance observed in northern Labrador Sea, on Scotian Shelf, and in the Gulf of Maine (Fig. 5; Table 5). However, it is worth mentioning that AFC alone tends to overestimate the importance of *S. mentella* in the southern part of redfish distribution, since AFC = 8 is not rare for *S. fasciatus*. As mentioned previously, higher temperature favours higher number of meristic characters such as AFC.

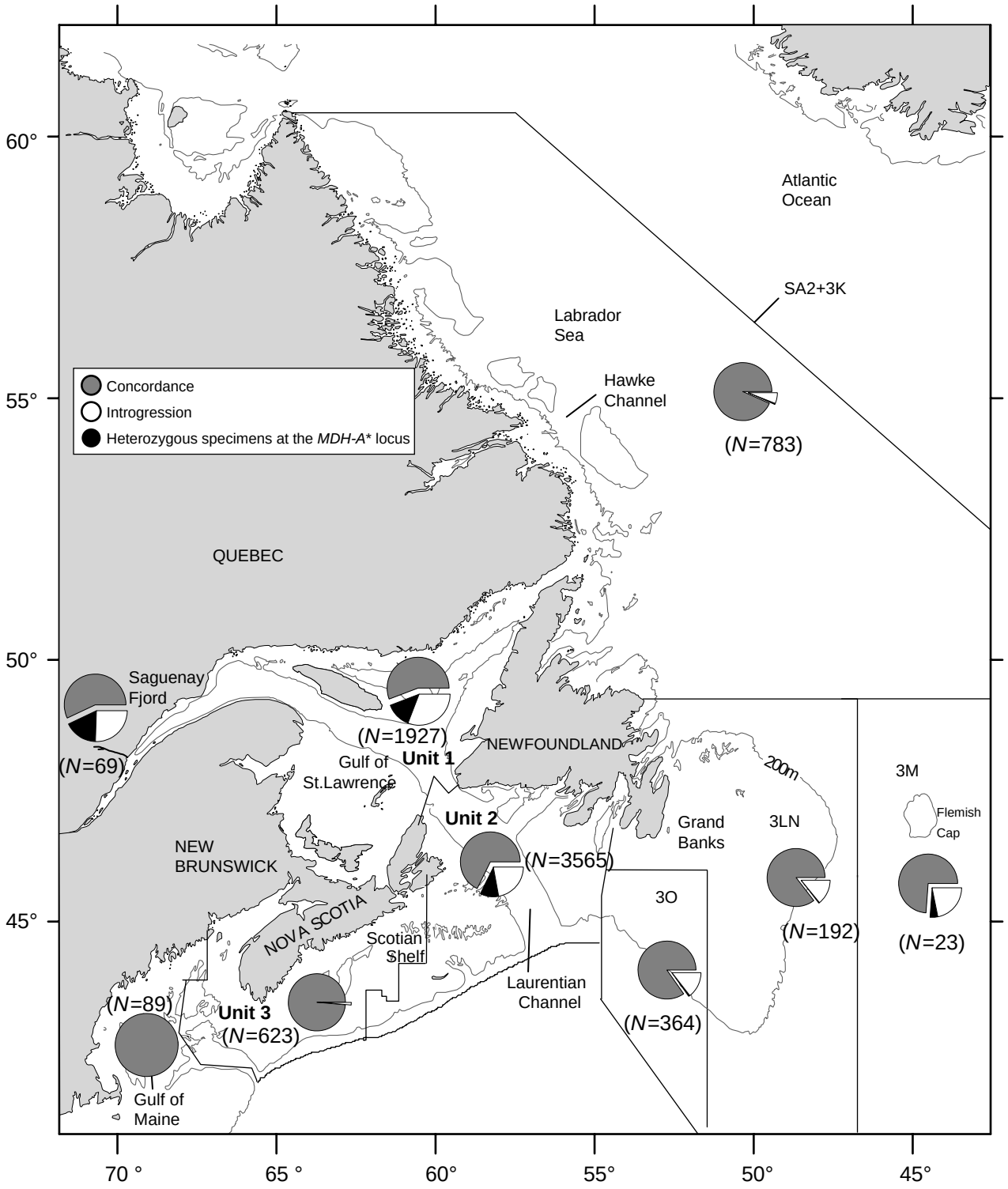

Fig. 5. Map of the Northwest Atlantic showing the relative frequency of individuals for which, the three characters are concordant (concordance), the three characters are not concordant (introgression), and of heterozygous specimens at the *MDH-A** locus in the current redfish management areas (NAFO Subarea 2+Div. 3K, 3LN, 3M, 3O, Unit 1–3 and the Gulf of Maine).

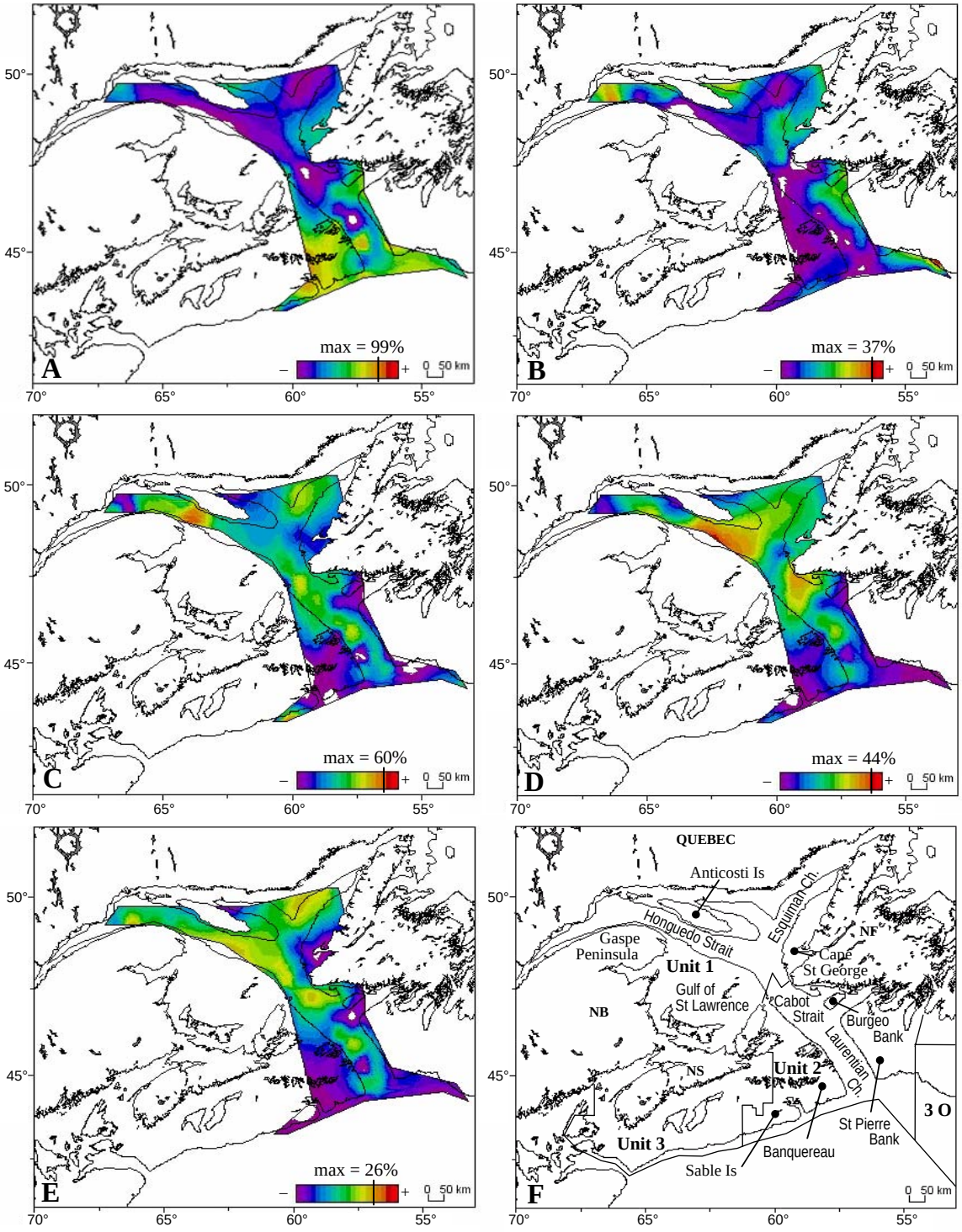

Fig. 6. Maps showing the spatial relative frequency of (**A**) "typical *S. fasciatus*", (**B**) "introgressed *S. fasciatus*", (**C**) "typical *S. mentella*", (**D**) "introgressed *S. mentella*", and (**E**) "heterozygous individuals" in the main zone of hybridization (Gulf of St. Lawrence and Laurentian Channel) using only the 4 063 specimens collected during the summer 1995–97. The location of main geographic sites is also provided (**F**). For each map, the colour scale was automatically calibrated according to the maximum percentage of the data (see value given on scale). Therefore, identical colour does not necessarily correspond to the same percentage in all maps. Name of geographic locations are provided in map **F**.

As expected, the reliability of the criteria is lower in the main area of sympatry (Units 1 and 2, and Div. 3LN, 3O and 3M), especially in the zone where heterozygote individuals are present in high proportion (Units 1 and 2). On Newfoundland's Grand Bank (Div. 3LN and 3O), the level of concordance is still high reaching 85%. This result is consistent with the studies of Ni (1981a; 1981b) who proposed that EGM and AFC are valid discriminating criteria on northeastern Grand Banks. On Flemish Cap (Div. 3M) the concordance is lower (74%) and the use of a single criterion would be less reliable. In the Gulf of St. Lawrence (Unit 1) and the Laurentian Channel (Unit 2), the level of concordance drops consistently reaching less than 56% in Unit 1. This is slightly superior but not significantly different ($\chi^2 = 1.41$; $P > 0.05$) from the value (50%) found by Valentin *et al*. (2002) on a much smaller sample ($N = 119$). Data from Rubec *et al*. (1991) provide information about the percentage of concordance between MDH and AFC (84%) and between MDH and EGM (73%). Our results support their observations with the concordance reaching 80% for AFC and 69% for EGM (data not shown). Overall, the low level of concordance observed between the three criteria in the Gulf of St. Lawrence and the Laurentian Channel questions the use of a single criterion for species identification in these regions, and reinforces the need for a multivariate approach.

Hypotheses Regarding Redfish Dispersion

The MDH data show that the Gulf of St. Lawrence, the Laurentian Channel, the Grand Banks, the southern Labrador Sea and the Flemish Cap form a large area of sympatry separating two areas largely dominated by one or the other species, as suggested by previous studies (Atkinson, 1987; Roques *et al*., 2001). The present study provides additional information thanks to the concomitant use of the three usual species identification criteria, which allowed the definition of five groups comprising "typical *S. mentella*", "typical *S. fasciatus*", "introgressed *S. mentella*", "introgressed *S. fasciatus*" and "heterozygous individuals". The distribution of these five groups at the scale of the Northwest Atlantic allows to propose hypotheses regarding the population connectivity.

Although "introgressed *S. mentella*" and "introgressed *S. fasciatus*" are present across the main area of sympatry, the importance of introgression varies within the zone. Introgression is the highest in the Gulf of St. Lawrence (30.9%), declines along a west-east gradient to reach the lowest value (14.1%) in the eastern half of the Grand Bank (Div. 3LN) and increases again in the Flemish Cap area (21.9%). This observation suggests that the Gulf of St. Lawrence and to a lesser extent Flemish Cap represent centres of introgressive hybridization. Dispersion of larvae or migration of juveniles and adults from these two centres may explain the presence of a substantial number of introgressed individuals in Unit 2, and in the Grand Bank area (Div. 3LN, 3O), although hybridization may also occurs in these areas. High gene flow between Unit 1 and 2 has been observed using microsatellite loci (Roques *et al*., 2001), supporting the hypothesis of dispersion-migration between the two units. In contrast, the same study showed significant genetic differences between the Gulf of St. Lawrence-Laurentian Channel area and the Grand Bank (for *S. mentella*), which suggested limited dispersion-migration between the two zones. Unfortunately, the study provided no information regarding the connectivity between the Grand Bank and Flemish Cap, so that no further inference can be made regarding the hypothesis of dispersion from the Flemish Cap towards the Grand Bank. To summarize, the results are consistent with the hypothesis of dispersion from centres of introgressive hybridization represented by the Gulf of St. Lawrence and Flemish Cap, and suggest that dispersion-migration is limited within the area of sympatry, especially between the Unit 1–Unit 2 area and the Grand Bank-Flemish Cap region. Such scenario is further supported by the distribution of the heterozygote specimens which are rarely found (<1%) in the Div. 3LNO units, unless this distribution is driven by different selection pressure on the heterozygotes.

The distribution of the heterozygotes, and of the introgressed specimens as well, suggests that dispersion-migration is limited not only within the main area of sympatry, but also towards the other areas of the redfish species distribution. In the southern part of the distribution, the very low rate of introgression, and the absence of heterozygotes on the Scotian Shelf (Table 5) suggest a strong barrier to dispersion-migration. This is surprising considering that larvae of the three genotypes at the *MDH-A** locus (*S. mentella*, *S. fasciatus* and heterozygotes) are present in the Gulf of St. Lawrence (Sévigny *et al*., 2000), and are likely to be transported out of the Gulf on the Scotian Shelf by the Gaspé current. Such limited dispersion would imply a strong mechanism of larval retention. There is now growing evidence that larval retention is more frequent than previously expected in the marine environment (e.g. Palumbi, 1999 and references therein), but such mechanism has not yet been identified for redfish from the Gulf of St. Lawrence. An alternative hypothesis would be that selection acts against the introgressed and heterozygote specimens drifted on the Scotian Shelf. Selection has already been proposed to explain the pattern of introgressive hybridization revealed by microsatellite loci (Roques *et al.*, 2001). The hypothesis assumed differential survival of certain hybrids (e.g. introgressed), relatively to either other hybrids (e.g. heterozygotes) or pure parental genotypes.

In the northern part of the redfish distribution, the rate of introgression observed in area SA2 + Div. 3K was about half the rate of the Grand Bank region, suggesting limited dispersion-migration towards the Labrador. This is contrasting with the microsatellite analyses that revealed no differences between the two areas (Roques *et al*., 2001). It is worth mentioning, however, that the study of Roques *et al*. (2001) was restricted to typical specimens only, an approach, which may have underestimated the level of genetic variability and missed information on genetic structure. Additional information is required to assess the interactions between the Grand Bank and the Labrador Sea.

Distribution of Species in the Main Area of Hybridization: Hypothesis Regarding Population Structuring

Our study clearly illustrates the bathymetric preferences of the two redfish species with *S. mentella* being associated with deeper parts of the Laurentian Channel while *S. fasciatus* prefers the slope of the Channel and the surroundings banks (Fig. 6). An important feature is that the distribution of heterozygotes closely resembles that of *S. mentella* specimens. Such a similarity was also observed for larvae (Sévigny *et al.*, 2000) and juveniles (Sévigny and de Lafontaine, 1992) and suggests that it is maintained throughout the life cycle.

The distribution of the "typical *S. mentella*", "typical *S. fasciatus*", "introgressed *S. mentella*", "introgressed *S. fasciatus*" and "heterozygous individuals" shows low level of patchiness (Fig. 6). The apparent discontinuity in the distribution of *S. mentella* and "heterozygous individuals" between the three areas consisting of the south of Anticosti Island, Cabot Strait and the Esquiman Channel could be explained by the scarcity of the data collected in this area (Fig. 6c, d, e).

The distribution patterns of *S. fasciatus* in the Laurentian Channel outside the Gulf of St. Lawrence is interesting. Indeed, "introgressed *S. fasciatus*" mostly occur in the eastern part (Fig. 6b), while "typical *S. fasciatus*" dominate the western part of the Channel (Fig. 6a), suggesting that the adults caught in summer on Banquereau Bank resemble specimens from Unit 3. Differences between the two slopes of the Channel have been observed across years for cod populations using otolith elemental fingerprint (Campana *et al*., 1999). Such consistent pattern is not clear for redfish, but the observed differences in the geographic distribution of "typical *S. fasciatus*" and "introgressed *S. fasciatus*" may be responsible for genetic differentiation on a small geographic scale since these individuals would have divergent genetic characteristics. Recent studies on microsatellite DNA proposed that introgressive hybridization played a significant role in the determination of genetic diversity, inter-specific difference and population structuring among redfish species in the Northwest Atlantic (Roques *et al*., 2001; Roques *et al*., 2002).

Acknowledgements

The authors wish to express sincere gratitude to Gerry Young, Brigitte Bernier, Brigitte Desrosiers and Wayne Burke for assistance in sampling and data collection. Particular and sincere thank to Éric Parent who participated in all aspects of this study at sea and in laboratory. Ralph Mayo provided the samples from the Gulf of Maine. This study was supported by the Department of Fisheries and Oceans, Canada.

Literature Cited

ATKINSON, D. B. 1987. The redfish resources off Canada's east coast. *In*: Proceedings of the International Rockfish Symposium. Lowell Wakefield Fisheries Symposium Series, Alaska Sea Grant College Program Report, **97-2**: 15–33.

BARSUKOV, V. V., and G. P. ZAKHAROV. 1973. Morphological and biological characteristics of the American redfish. *Fish. Res. Board Can. Transl. Ser*., No. **2488**, 66 p.

BOURGEOIS, A. 1993. Évaluation de l'exploitation du sébaste atlantique (*Sebastes mentella*) par la pêche sportive hivernale sur le Saguenay, saison 1990-91. Mémoire de Maîtrise, Université du Québec à Chicoutimi. 71 p.

CAMPANA, S. E., G. A. CHOUINARD, J. M. HANSON, and A. FRÉCHET. 1999. Mixing and migration of overwintering Atlantic cod (Gadus morhua) stocks near the mouth of the Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci*., **56**: 1873–1881.

CARVALHO, G. R., and L. HAUSER. 1994. Molecular genetics and the stock concept in fisheries. *Rev. Fish Biol. Fish*., **4**: 326–350.

DESROSIERS, B., J.-M. SÉVIGNY, and J.-P. CHANUT. 1999. Restriction fragment length polymorphism of rDNA in the redfishes *Sebastes fasciatus* and *S. mentella* (Scorpaenidae) from the Gulf of St. Lawrence. *Can. J. Zool*., **77**: 267–277.

GASCON, D. (ed.) 2003. Redfish Multidisciplinary Research Zonal Program (1995–1998): Final Report. C*an. Tech. Rep. Fish. Aquat. Sci*., **2462**: xiii + 155 p.

HEBERT, P. D. N., and M. J. BEATON. 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Education service of Helena Laboratories, Beaumont, TX, 31.

LINDSEY, C. C. 1988. Factors controlling meristic variation. *In*: Fish physiology, vol. XIB. W. S. Hoar and D. J. Randall (eds). Academic Press, Inc. San Diego, U.S.A., p. 197–274.

LITVINENKO, N. I. 1979. *Sebastes fasciatus kellyi* (Scorpaenidae) from coastal waters off Eastport Maine, USA. *J. Ichthyol*., **19**: 1–14.

McGLADE, J. M., M. C. ANNAND, and T. J. KENCHINGTON. 1983. Electrophoretic identification of *Sebastes* and *Helicolenus* in the northwestern Atlantic. *Can. J. Fish. Aquat. Sci*., **40**: 1861–1870.

NI, I.-H. 1981a. Separation of sharp-beaked redfish, *Sebastes fasciatus* and *S. mentella*, from Northeastern Grand Bank by morphology of extrinsic gasbladder musculature. *J. Northw. Atl. Fish. Sci*., **2**: 7–12.

1981b. Numerical classification of sharp-beaked redfishes, *Sebastes mentella* and *S. fasciatus*, from Northeastern Grand Bank. *Can. J. Fish. Aquat. Sci.*, **38**: 873–879.

1982. Meristic variation in beaked redfishes, *Sebastes mentella* and *S. fasciatus*, in the Northwest Atlantic. *Can. J. Fish. Aquat. Sci.*, **39**: 1664-1685.

1984. Meristic variation in golden redfish, *Sebastes marinus*, compared to beaked redfishes of the Northwest Atlantic. *J. Northw. Atl. Fish. Sci*., **5**: 65–70.

PALUMBI, S. R. 1999. The prodigal fish. *Nature*, **402**: 733–735.

PAYNE, R. H., and I.-H. NI. 1982. Biochemical population genetics of redfishes (*Sebastes*) off Newfoundland. *J. Northw. Atl. Fish. Sci*., **3**: 169–172.

POWER, D. J., and I.-H. NI. 1982. Morphology of the extrinsic gasbladder musculature in the golden redfish, *Sebastes marinus*. *J. Northw. Atl. Fish. Sci*., **3**: 165–168.

ROQUES, S., D. PALLOTTA, J.-M. SÉVIGNY, and L. BERNATCHEZ. 1999a. Isolation and characterization of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei: Scorpaenidae, genus *Sebastes*). *Mol. Ecol*., **8**: 685-686.

ROQUES, S., P. DUCHESNE, and L. BERNATCHEZ. 1999b. Potential of microsatellites for individual assignment: the North Atlantic redfish (genus *Sebastes*) species complex as a case study. *Mol. Ecol*., **8**: 1703–1717.

ROQUES, S., J.-M. SÉVIGNY, and L. BERNATCHEZ. 2001. Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the Northwest Atlantic: a rare marine example. *Mol. Ecol*., **10**: 149–165.

2002. Genetic structure of deep-water redfish, *Sebastes mentella*, populations across the North Atlantic. *Mar. Biol.*, **140**: 297–307.

RUBEC, P. J., J. M. McGLADE, B. L. TROTTIER, and A. FERRON. 1991. Evaluation of methods for separation of Gulf of St Lawrence beaked redfishes, *S. fasciatus* and *S. mentella*: malate deshydrogenase mobility patterns compared with extrinsic gasbladder muscle passages and anal fin ray counts. *Can. Fish. Aquat. Sci.*, **48**: 640–660.

SÉVIGNY, J.-M., and Y. DE LAFONTAINE. 1992. Identification of redfish juveniles in the Gulf of St. Lawrence using genotypic specific variations. *In*: Juvenile stages: the missing link in fisheries research. Y. de Lafontaine, T. Lambert, G. R. Lilly, W. D. McKone and R. J. Miller (eds). *Can. Tech. Rep. Fish. Aquat. Sci*., **1890**: 69–73.

SÉVIGNY, J.-M., P. GAGNÉ, Y. DE LAFONTAINE, and J. DODSON. 2000. Identification and distribution of larvae of redfish (*Sebastes fasciatus* and *S. mentella*: Scorpaenidae) in the Gulf of St. Lawrence. *Fish. Bull*., **98**: 375–388.

TEMPLEMAN, W., and T. K. PITT. 1961. Vertebral number of redfish, *Sebastes marinus* (L.), in the Northwest Atlantic. *ICNAF Spec. Publ*., **3**: 56–89.

VALENTIN, A. 1999. Application de la méthode d'analyse morphométrique en réseau à la différenciation des espèces *Sebastes fasciatus* et *Sebastes mentella* dans le golfe du Saint-Laurent. Mémoire de Maîtrise, Université du Québec à Rimouski, Rimouski, 137 p.

VALENTIN, A., J.-M. SÉVIGNY, and J.-P. CHANUT. 2002. Geometric morphometrics reveals body shape differences between sympatric redfish *Sebastes mentella*, *S. fasciatus* and their hybrids in the Gulf of St. Lawrence. *J. Fish Biol*., **60**: 857–875.

WAPLES, R.S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *J. Heredity*, **89**: 438-450.

WARD, R. D. 2000. Genetics in fisheries management. *Hydrobiologia*, **420**: 191–201.