Identification and distribution of larvae of redfish (*Sebastes fasciatus* and *S. mentella*: Scorpaenidae) in the Gulf of St. Lawrence

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Abstract.—Genetic variation at the liver *MDH** locus was used to describe the species composition and the geographic distribution of the larvae of the two redfish species, (*Sebastes mentella* and *S. fasciatus*) in the Gulf of St. Lawrence in 1991 and 1992. In both years, redfish larvae were more abundant at the junction of the Laurentian and the Esquiman Channels than in other areas surveyed. Electrophoretic analysis of 697 larvae in 1991 and 1041 in 1992 showed that larvae of both species were present in the Gulf during the two years of the study although in very different proportion. Larvae belonging to the genotype *MDH*A1A1* (*S. mentella*) represented at least 61% of the redfish larvae collected in the Gulf in 1991 and 77% in 1992. Strong spatial heterogeneity in the frequency of the two *MDH** alleles was observed; a higher proportion of *S. mentella* occurred in the central and deeper part of the channels and a higher proportion of *S. fasciatus* in shallower zones. Larvae of the genotype *MDH*A1A1* (*S. mentella*) were significantly larger than those of the genotype *MDH*A2A2* (*S. fasciatus*) for both years of the study, suggesting that the extrusion times differ between the two redfish species. The sizes and geographic distributions of the heterozygous larvae (*MDH*A1A2*) did not differ from those of *S. mentella* (*MDH*A1A1*).

The Gulf of St. Lawrence redfish consist of a complex of three putative species, currently identified as deepwater redfish, *Sebastes mentella,* and Acadian redfish, *S. fasciatus,* that dominate the commercial fishery (Atkinson, 1984; 1987; Rubec et al., 1991) and golden redfish, *S. norvegicus*, formerly named *S. marinus* (Fernholm and Wheeler, 1983) which occurs only sporadically and at very low abundance, namely at <1% of the catch (Ni and McKone, 1983; Rubec et al., 1991).

Although *S. fasciatus* differs morphologically from *S. mentella*, the overlap in meristic and morphometric characters is such that the two redfish species are difficult to separate and, frequently, individuals cannot be unequivocally identified (Ni, 1981; 1982; Kenchington, 1986; Rubec et al., 1991). Discrimination among redfish larvae is even more complex because the morphological characters used for adults and juveniles cannot be used for identifying species during the larval stages (Penney, 1987). The taxonomic confusion, as well as the absence of reliable morphological characters to identify the larvae, has limited our understanding of the ecology and population dynamics of redfish in the North Atlantic.

Electrophoretic analysis is an appropriate tool for identifing *Sebastes* species at all stages of their life cycle, including larvae (Payne and Ni, 1982; McGlade et al., 1983; Nedreaas and Naevdal, 1991; Rubec et al., 1991; Seeb and Kendall, 1991;

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Sévigny and de Lafontaine, 1992; Gagné, 1995; Seeb, 1998). In the Northwest Atlantic, differences in the electrophoretic mobility patterns of the dimeric protein malate dehydrogenase (MDH; EC 1.1.1.37) from liver tissue appear to distinguish *S. fasciatus* from *S. mentella* (Payne and Ni, 1982; McGlade et al., 1983; Rubec et al., 1991). The presence of two alleles (*MDH*A1* and *MDH*A2*) segregating at this locus results in three genotypes that can easily be identified by electrophoresis. A low-mobility banding pattern, corresponding to the genotype *MDH*A2A2* predominates in *S. fasciatus* whereas a high-mobility pattern, corresponding to the genotype *MDH*A1A1*, is more characteristic of *S. mentella*. However, there are two restrictions to using this approach. First and foremost, the heterozygous individuals sharing alleles of both *S. fasciatus* and *S. mentella* cannot be assigned unambiguously to one species. Second, electrophoretic mobility of MDH does not differ between *S. mentella* and *S. norvegicus* (McGlade et al., 1983). Despite these limitations and until a more reliable taxonomic tool becomes available, electrophoretic mobility patterns of liver MDH remain the best approach for identification of redfish species in the Gulf of St. Lawrence, especially at larval stages. However, one should remember that the taxonomic status of redfish is still under debate and although, for convenience, we use and refer to the species names *S. fasciatus* and *S. mentella* throughout the text, these may be putative species.

Redfish of the North Atlantic are ovoviviparous; their eggs are fertilized by sperm stored in the oviducts. Although the reproductive biology of the redfish of the Gulf of St. Lawrence is not well understood, mating (transfer of spermatozoa from male to female) probably takes place during late fall or early winter. Fertilization and embryogenesis take place in winter, and larvae hatch internally and are extruded during late spring and early summer (St-Pierre and de Lafontaine, 1995, and references therein). In consequence, the location of larval extrusion may differ significantly from the location where copulation has taken place. In order to avoid confusion, the term "larval extrusion," rather than "spawning," is used to distinguish between the hatching-extrusion phase and the mating phase of the redfish reproductive cycle. The importance of gene flow within and between species is established during the mating and fertilization phase of the reproductive cycle, whereas the location of larval hatching and extrusion will determine the geographic distribution of the species at the larval stage.

It is commonly assumed that the life cycle of the two species of redfish is completed inside the Gulf and that the redfish larval population represents a mixture of *S. fasciatus* and *S. mentella*. Although the presence of newly hatched redfish larvae in plankton samples from the Gulf did confirm that redfish extrude larvae in this area (de Lafontaine, 1990, and references therein; de Lafontaine et al., 1991), the species composition of these larvae has never been elucidated and their distribution in the Gulf has never been described. The objectives of the present study were 1) to describe the species composition of larval redfish in order to verify that the two species use the Gulf of St. Lawrence as an extrusion site, 2) to determine the spatial co-occurrence of *S. fasciatus* and *S. mentella* within the Gulf, and 3) to describe the larval size distribution of the two species as an indication of the variability in extrusion times.

Materials and methods

Sample collection

Sampling was conducted at 32 stations along three transects on the western and eastern side of Anticosti Island in the Gulf of St. Lawrence between 22 June and 4 July 1991 and at 74 stations, located mainly to the east of Anticosti Island, between 10 June and 20 June 1992 (Figs. 1 and 2). Numerous sampling stations were selected in order to obtain the largest spatial coverage for larval redfish distribution in the Gulf of St. Lawrence. Plankton samples were collected with a modified opening-closing 1-m^2 rigid "Tucker" trawl equipped with two, 333-m mesh nets and two G.O. model 2030 flowmeters. The gear was hauled in double-oblique tows from the ship's side at a cruising speed of 2.5–3.0 knots. Sampling was restricted to the 0–50 m upper layer where redfish larvae are concentrated (Kenchington, 1991; Runge and de Lafontaine, 1996). Maximum sampling depth was determined by a Vemco acoustic transducer attached to the gear frame and by calculation from the amount of wire length deployed and the wire angle. Tow duration varied between 6 and 10 min. Once back on board, nets were rapidly rinsed and samples were concentrated in codends and transferred to the laboratory on the vessel for larval sorting.

Upon collection, a visual examination of the samples was made. When the estimated number of larvae in one sample was greater than fifty, a subsample was taken for further sorting. All redfish larvae from the subsample were sorted individually and placed gently, while alive, in a drop of water on a plexiglass plate (95 mm diameter and 0.61 mm thickness) next to an inscribed number that served to identify individuals for the videotape recordings (see below)

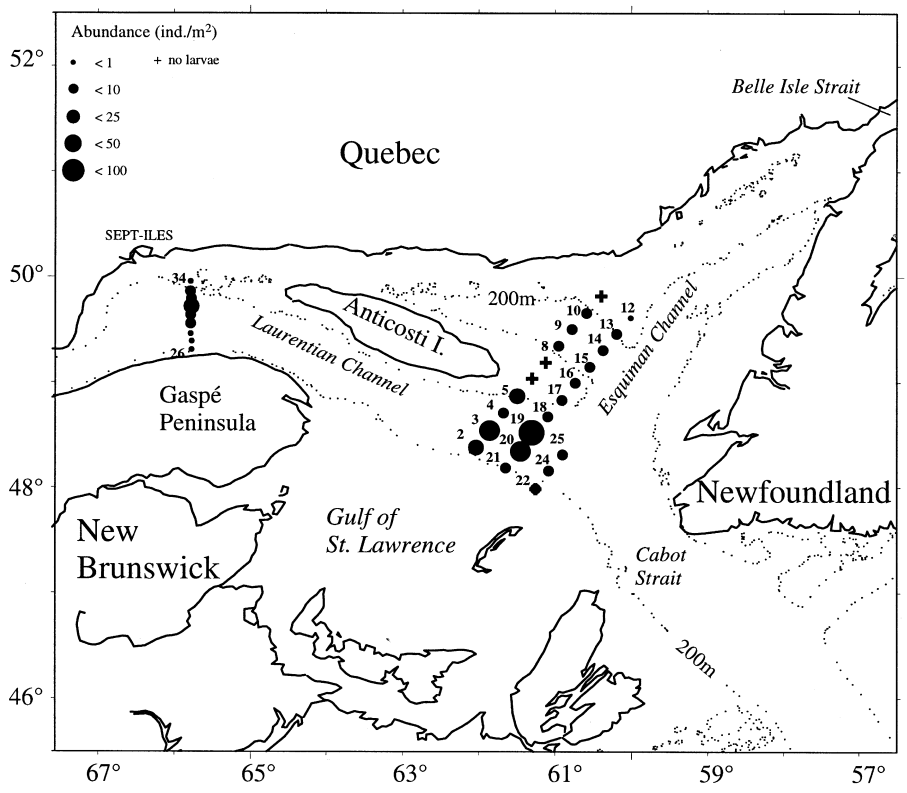

Figure 1

Geographic distribution and abundance of redfish larvae at stations sampled in June–July 1991 in the Gulf of St. Lawrence.

and for genotype determination. This procedure was adopted to minimize possible bias towards selection of larger larvae during sorting. A maximum of 10 larvae were placed on each plate. The total number of larvae so treated varied between stations owing to variable larval abundance at each station. The remaining sample with unsorted larvae and plankton was preserved in a 10% formalin seawater solution (4% formaldehyde) for subsequent laboratory sorting. Re-examination of the samples and subsamples in the laboratory showed that the proportion of larvae remaining in the samples was always less than 5% and that in most cases, larvae had been effectively sorted during the initial onboard sorting process.

The larvae placed on plates were individually video-recorded with a Hitachi black and white camera mounted on a stereomicroscope at 6× and 9× magnification and connected to a Sony Beta videotape recording machine. Periodic calibration of the magnification was made by filming a stage micrometer. The overall procedure (from sorting to filming) was completed in less than 5 min. and care was taken to ensure that larvae were alive during the video recording. The fish were sorted and video-taped on board while still alive so that morphometric measurements represented real values; these fish were later used for genetic analyses. This procedure eliminated the necessity of having one subsample for morphometric analyses and another one for biochemical analyses and did not introduce any measurement bias due to postmortem and preservation shrinkage (Magnússon[1]). Afterwards, each plate with larvae was immersed for a few seconds in liquid nitrogen, then placed in a petri dish and stored on board at –40°C. Samples were transferred to a –80°C freezer in the laboratory until electrophoresis analyses were carried out.

Laboratory analyses

Standard lengths of individual larvae was measured from the video recordings that were digitized with a Bioquant M8 image analyzing system. Repeated measurements of individual larvae indicated a measurement error of less than 3% (de Lafontaine, unpubl. data). Plankton samples were examined completely and the redfish larvae were sorted and enu-

[1] Magnússon, J. V. 1982. Shrinkage of dying redfish larvae. Int. Counc. Explor. Sea (ICES) Council Meeting 1982/G:23.

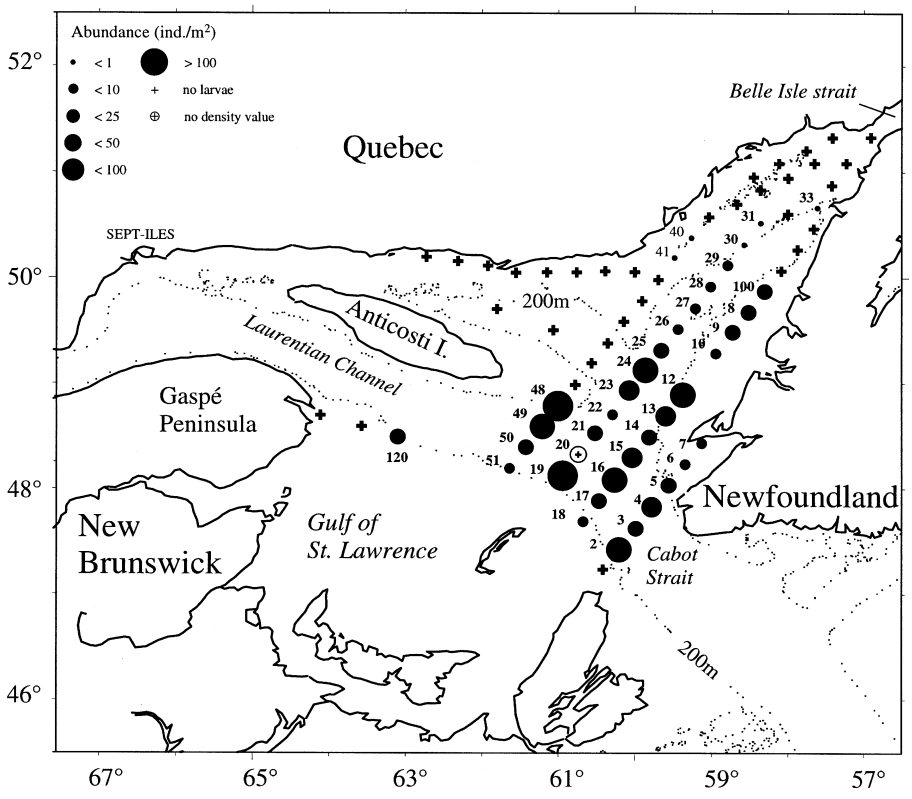

Figure 2

Geographic distribution and abundance of redfish larvae at stations sampled in June 1992 in the Gulf of St. Lawrence.

merated. The number of larvae frozen on board and those later sorted from the plankton samples were summed to provide estimates of the total abundance of redfish larvae (individuals/m^2) at each station.

Electrophoretic analyses

All electrophoretic analyses were performed within two to three months after specimen collection. The plexiglass plates supporting the larvae were always kept on ice during laboratory examination and analysis. The tissue in the liver area of individual larva was dissected under a stereomicroscope, placed directly into an individual sample well, and homogenized in an extraction solution containing 10 µL of a 0.01M Tris-HCl (pH 8.0) composed of 30% sucrose, 0.005M dithiothreitol (DTT), 0.5% polyvinylpyrrolidone (PVP) and 0.001M phenylmethylsulfonyl fluoride (PMSF). The tissue was homogenized with a fine glass rod and an aliquot was applied on the cellulose acetate gels. Cellulose acetate gel electrophoresis and gel staining were carried out as described by Hebert and Beaton (1989). A specimen of genotype *MDH*A1A2* was used as a standard on every gel to assess both the quality of the electrophoretic separation and to ensure allele identification. In 1991, a total of 735 larvae was frozen and analyzed for genotypic variation. Interpretable results were obtained for 697 specimens. In 1992, 1041 larvae were analyzed and scored.

Two alleles segregated at the *MDH** locus, resulting in the presence of three phenotypes (Rubec et al. 1991 and references therein). Homozygotes for the slow allele (*MDH*A2*) were assigned to *S. fasciatus,* and the homozygotes for the fast allele (*MDH*A1*) were classified as *S. mentella*. Heterozygotes at the *MDH** locus were left unclassified (see "Results" and "Discussion" sections).

Statistical analyses

The adequacy of genotypic proportions to Hardy-Weinberg expectations was tested for each sampling station where genotypic variation was observed by using the *G*-test of goodness-of-fit (Tables 1 and 2).

Standard length data were either not homoscedastic or not normally distributed. Therefore, comparisons of larval standard length between 1991 and 1992, between the different sampled areas of the Gulf, were carried out with a Mann Whitney *U*-test. Heterogeneity in the distribution of standard lengths

Table 1

Observed (Obs.) and expected (Exp.) number of larvae with each of the three genotypes and allelic frequencies in samples taken during June–July 1991. The mean standard length of the larvae and its coefficient of variation (CV, as a percentage) and the *G*-test values for deviation from Hardy-Weinberg expectations are given. * indicates that results were significant at $P<0.05$; ** indicates that results were significant at $P<0.001$.

Sampling station	Depth (m)	Larval density (no./m^2)	Genotype *A1A1* Obs.	*A1A1* Exp.	*A1A2* Obs.	*A1A2* Exp.	*A2A2* Obs.	*A2A2* Exp.	Allelic frequency *A1	Allelic frequency *A2	*n*	*G*-test value	Fish length Mean (mm)	Fish length CV (%)
2	340	19.9	20	20.0	9	9.0	1	1.0	0.817	0.183	30	0	9.63	10.12
3	437	28.5	23	22.7	12	12.5	2	1.7	0.784	0.216	37	0.274	9.28	8.58
4	380	7.2	10	9.8	5	5.5	1	0.8	0.781	0.219	16	-0.103	8.72	9.77
5	246	20.6	33	34.1	19	16.8	1	2.1	0.802	0.198	53	0.514	8.60	10.18
8	225	1.4	0	0	0	0	10	10.0	0	1	10	—	7.50	8.08
9	290	2.1	2	0.4	1	4.2	13	11.4	0.156	0.844	16	*6.982	6.99	9.28
10	267	1.6	0	0	0	0	9	9.0	0	1	9	—	6.98	11.78
13	216	1.4	6	3.2	1	6.5	6	3.2	0.500	0.500	13	*11.340	7.14	7.41
14	276	9.7	30	20.4	10	29.2	20	10.4	0.583	0.417	60	*27.865	8.17	14.29
15	244	4.5	1	0.2	1	2.7	13	12.2	0.100	0.900	15	2.884	7.29	8.55
16	146	1.5	7	6.4	2	3.2	1	0.4	0.800	0.200	10	1.207	8.80	15.92
17	174	5.5	24	24.0	14	14.0	2	2.0	0.775	0.225	40	0	9.46	11.06
18	375	5.9	11	10.2	8	9.5	3	2.2	0.682	0.318	22	0.772	9.64	13.33
19	416	62.6	30	27.8	5	9.4	3	0.8	0.855	0.145	38	*6.187	9.25	1.074
20	405	36.1	14	14.3	9	8.4	1	1.2	0.771	0.229	24	0.284	10.07	6.91
21	405	1.1	3	1.5	0	3.0	3	1.5	0.500	0.500	6	*8.318	8.91	13.64
22	64	1.4	6	6.0	0	0	0	0	1	0	6	—	7.16	31.04
24	369	3.8	13	12.4	7	8.2	2	1.4	0.750	0.250	22	0.440	9.80	16.52
25	475	7.0	33	32.4	6	7.2	1	0.4	0.900	0.100	40	0.856	10.36	11.99
26	222	0.4	0	0	0	0	4	4.0	0	1	4	—	6.47	11.89
27	350	0.6	0	0	0	0	7	7.0	0	1	7	—	6.97	8.19
28	346	0.5	0	0	0	0	4	4.0	0	1	4	---	6.83	4.83
29	330	4.0	1	0.5	5	6.0	19	18.4	0.140	0.860	25	0.782	7.69	15.13
30	329	7.5	38	33.6	10	18.8	7	2.6	0.782	0.218	55	*10.593	9.25	11.24
31	338	13.8	36	31.8	10	18.5	7	2.7	0.774	0.226	53	*9.965	8.96	14.35
32	335	3.6	13	7.5	4	15.0	13	7.5	0.5	0.5	30	*18.028	7.92	11.24
33	274	3.8	1	0.1	3	4.7	43	42.2	0.053	0.947	47	3.526	7.15	11.15
34	170	0.9	2	1.2	1	2.5	2	1.2	0.500	0.500	5	2.254	8.67	12.67
East sites			266	219.8	109	201.2	92	46.0	0.686	0.314	467	**95.410	8.93	15.79
West sites			91	50.2	33	114.5	106	65.3	0.467	0.533	230	*128.856	8.24	16.46
All sites			357	262.8	142	330.4	198	103.8	0.614	0.386	697	*234.634	8.70	16.42

among the three genotypes within and between sampled areas and between years was tested with a Kruskal-Wallis test. Nonparametric multiple comparisons (Dunn test) were used *a posteriori* to determine which genotypes had significantly different sizes of larvae. Differences between genotypic pairs were considered significant when $P<0.05$; the statistics Q is thus not provided in our text.

The relative proportion of *S. mentella* and of *S. fasciatus* in the sampled larval population was estimated by using two different approaches depending on the taxonomic status of the heterozygous individuals. With the first approach, the heterozygous individuals were not taken into account and the contribution of *S. mentella* and of *S. fasciatus* was based on the frequencies of the genotypes *MDH*A1A1* and *MDH*A2A2,* respectively. This approach yielded a conservative estimate. With the second approach, the heterozygous individuals were attributed to *S. mentella*. This procedure can be justified by the lack of difference in the size distribution of the *MDH*A1A1* and *MDH*A1A2* individuals and by the similar geographic distribution of these two genotypes (see "Results" and "Discussion" sections).

Table 2

Observed (Obs.) and expected (Exp.) number larvae with each of the three genotypes and allelic frequencies in samples taken during June 1992. The mean standard length of the larvae and its coefficient of variation (CV, as a percentage) and the *G*-test values for deviation from Hardy-Weinberg equilibrium are given. * indicates that results were significant at $P<0.05$; ** indicates that results were significant at $P<0.001$; nd = density value was not determined; therefore, this sampling station was not considered in the calculation of the contribution of each genotype to the larval population.

			Genotype						Allelic frequency				Fish length	
			A1A1		*A1A2*		*A2A2*							
Sampling station	Depth (m)	Larval density (mo./m^2)	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	**A1*	**A2*	*n*	*G*-test value	Mean (mm)	CV (%)
2	427	51.0	23	21.4	8	11.1	3	1.4	0.794	0.206	34	2.650	8.36	5.90
3	531	22.1	19	19.3	5	4.5	0	0.3	0.896	0.104	24	0.458	8.07	10.99
4	464	33.8	25	24.0	8	9.9	2	1.0	0.829	0.171	35	1.404	8.74	6.62
5	196	12.5	24	23.1	8	9.9	2	1.1	0.824	0.176	34	0.816	8.82	5.63
6	126	9.2	28	27.3	4	5.5	1	0.3	0.909	0.091	33	1.278	8.99	6.64
7	93	1.3	18	17.9	8	8.1	1	0.9	0.815	0.185	27	0.212	8.77	5.42
8	110	19.6	23	20.8	8	12.3	4	1.8	0.771	0.229	35	*4.130	9.00	4.83
9	128	16.0	18	15.6	10	14.9	6	3.6	0.676	0.324	34	*24.690	9.01	8.69
10	141	3.2	15	9.5	6	16.9	13	7.5	0.529	0.471	34	*15.577	8.30	12.01
12	115	51.2	26	25.5	6	7.0	1	0.5	0.879	0.121	33	0.546	8.58	8.23
13	158	33.4	28	26.5	4	7.1	2	0.5	0.882	0.118	34	*4.038	8.44	5.34
14	282	13.9	26	24.0	6	9.9	3	1.0	0.829	0.171	35	*4.745	8.35	7.18
15	418	46.1	26	26.4	7	6.3	0	0.4	0.894	0.106	33	0.681	8.66	5.10
16	490	87.6	27	27.5	8	7.1	0	0.5	0.886	0.114	35	0.919	8.53	7.06
17	451	14.1	24	23.4	5	6.2	1	0.4	0.883	0.117	30	0.897	7.11	7.91
18	69	2.3	30	29.1	1	2.9	1	0.1	0.953	0.047	32	*4.303	8.74	8.80
19	401	125.4	29	28.4	5	6.3	1	0.4	0.900	0.100	35	0.734	8.56	8.79
20	447	nd	30	30.2	5	4.6	0	0.2	0.929	0.071	35	0.435	8.70	8.24
21	393	20.2	29	28.3	4	5.5	1	0.3	0.912	0.088	34	1.277	7.91	7.31
22	317	1.8	3	0.8	2	6.3	14	11.8	0.211	0. 789	19	*8.128	6.93	14.99
23	321	39.9	29	27.5	4	7.1	2	0.5	0.886	0.114	35	*4.035	8.36	8.69
24	290	74.6	29	26.6	3	7.8	3	0.6	0.871	0.129	35	*8.934	8.21	9.42
25	271	22.4	23	21.4	8	11.1	3	1.4	0.794	0.206	34	2.650	8.65	8.87
26	246	4.6	22	15.6	2	14.9	10	3.6	0.676	0.324	34	**27.526	8.32	12.86
27	225	1.2	8	8.0	0	0	0	0	1	0	8	—	6.40	5.22
28	218	1.5	7	4.9	0	4.2	3	0.9	0.700	0.300	10	**12.217	7.20	12.35
29	263	4.1	7	1.4	0	11.1	27	21.4	0.206	0.794	34	**35.084	6.89	6.33
33	269	0.9	0	0	0	0	10	10.0	0	1	10	—	6.70	8.65
48	257	101.1	28	27.5	6	7.1	1	0.5	0.886	0.114	35	0.375	8.39	8.22
49	413	69.5	29	27.5	4	7.1	2	0.5	0.886	0.114	35	*4.035	8.44	8.81
50	415	14.3	26	24.9	7	9.3	2	0.9	0.843	0.157	35	1.464	8.53	12.35
51	179	4.6	25	24.5	6	7.0	1	0.5	0.875	0.125	32	0.547	8.10	10.26
100	123	11.9	19	15.1	8	15.8	8	4.1	0.657	0.343	35	*8.536	8.85	9.49
120	92	14.8	21	20.2	2	3.7	1	0.2	0.917	0.083	24	2.389	7.74	10.65
All sites			744	657.9	168	339.3	129	43.7	0.795	0.205	1041	**226.100	8.35	10.91

Larval abundance at each station was taken into account when calculating the contribution of each genotype. The contribution for the entire sampling area was estimated by the sum of the relative contribution of each genotype at each station calculated by the formula:

$$TC = S\left((N_{MDH^*} / n) \times larval\ abundance\right),$$

where TC = the total contribution of a genotype to the sampled larval population;

N_{MDH^*} = the number of individuals of a genotype at the *MDH** locus for a given station; and

n = the total number of individuals analyzed for MDH variation for a given station.

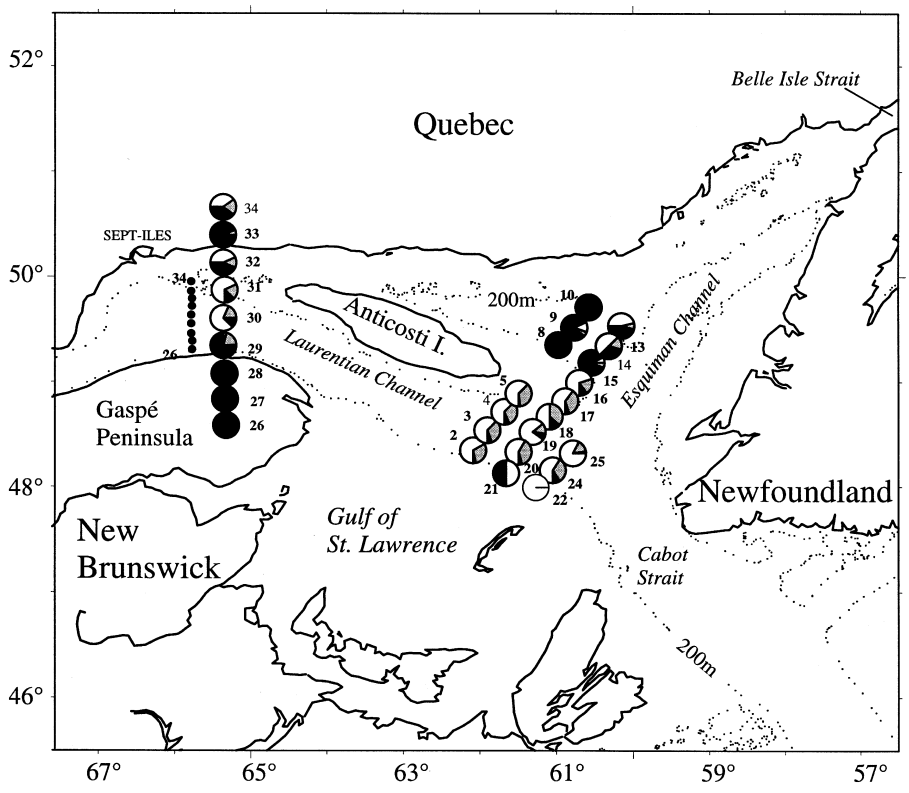

Figure 3

Geographic distribution of the larval genotypes *MDH*A1A1* characteristic of *S. mentella* (white), *MDH*A2A2* caracteristic of *S. fasciatus* (black) and *MDH*A1A2* (gray) in June–July 1991.

Results

Larval distribution, abundance, and size

In 1991, redfish larvae were caught at all but three sampling stations that were shallower than 200 m (Fig. 1). Larval abundance was highest (max.=62.6 individuals/m^2) over the central portion of the Laurentian Channel, on the southeastern side of Anticosti Island, and decreased northward along the Esquiman Channel. Only one larva was captured at the northeasternmost sampling station (12) (Fig. 1; Table 1). On the western side of Anticosti Island, larval abundance was highest (max.=13.8/m^2) at the central stations of the transect. Few larvae were caught at coastal stations within the Gaspé current (stations 26–28) or at the northernmost sampling station (<200 m deep) (Fig. 1; Table 1).

In 1992, larvae were collected at 34 of the 74 sampling stations (Fig. 2), and centers of abundance (max =125.4 larvae/m^2) were again located in the Laurentian Channel. Abundance gradually declined northwards along the Esquiman Channel. Larval redfish were virtually absent at the mouth of the Belle Isle Strait, along the Quebec north shore, at the shallow (<200 m) sampling stations on the north side of Anticosti Island or at the tip of the Gaspé peninsula (Fig. 2; Table 2).

The size of larvae ranged from 5.0 mm to 12.3 mm in 1991 and from 5.6 mm to 10.9 mm in 1992 (Tables 1 and 2). Larvae collected in 1991 were significantly larger than those collected in 1992 (Mann-Whitney *U*-test, $P<0.0001$; Table 1). Larvae from the eastern sector in 1991 were larger than those collected in the western sector (Mann-Whitney *U*-test, $P<0.0001$), and larvae collected in the eastern sector in 1991 were significantly larger than those collected in approximately the same region in 1992 (Mann-Whitney *U*-test, $P<0.0001$).

Spatial and temporal genotypic variations

The three genotypes were found in larval redfish and their relative proportion varied between sites and years (Figs. 3 and 4; Tables 1 and 2). In 1991, two groups of stations could be identified on the western side of Anticosti Island (stations 26–34) based on the frequencies of the genotypes observed. The first

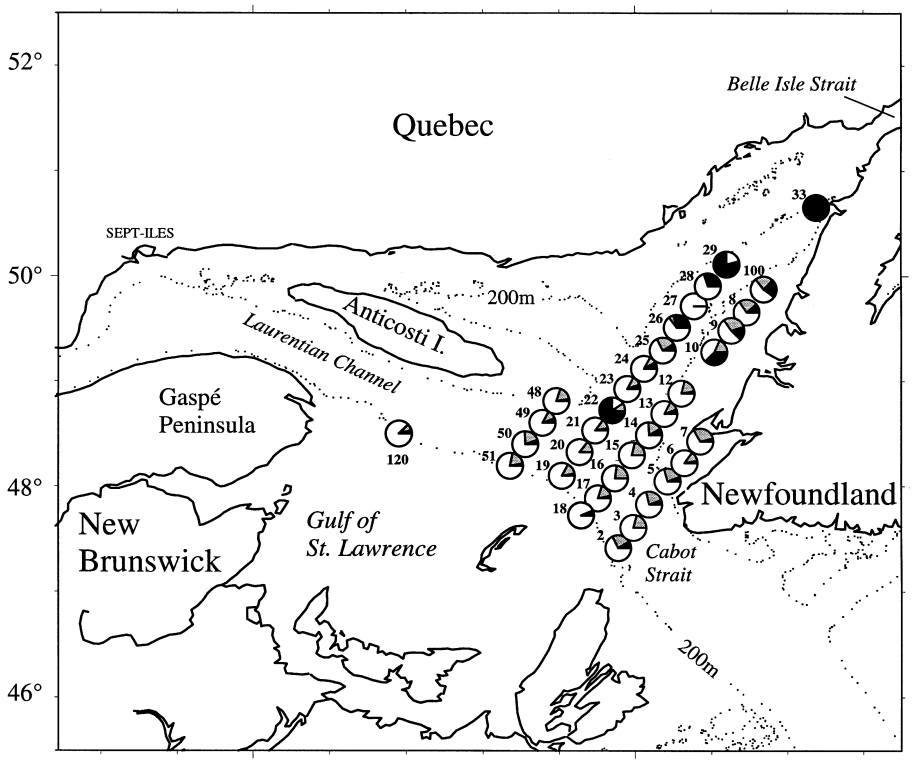

Figure 4

Geographic distribution of the larval genotypes *MDH*A1A1* characteristic of *S. mentella* (white), *MDH*A2A2* caracteristic of *S. fasciatus* (black) and *MDH*A1A2* (gray) in 1992. Sampling station numbering does not correspond with that for 1991.

group comprised stations 26–29 and 33–34 where the *MDH*A2A2* genotype is generally the most frequent. The frequency of allele *MDH*A2* at stations 26–29 within the Gaspé current system along the north shore of the Gaspé peninsula was very high, ranging from 0.860 to 1.0. At the northernmost station (33), the allele *MDH*A2* predominated and the frequency of both alleles was equal (0.500) at station 34. There was no significant deviation from Hardy-Weinberg expectations at the stations of this group. The second group comprised the central stations (30 31, 32). At theses stations, the three genotypes were represented but there was an abrupt inversion of the most frequent allele. At these stations, the frequency of allele *MDH*A1* ranged from 0.500 to 0.782. The frequency of this allele never equaled 1.0. Significant departure from Hardy-Weinberg expectations was also observed at these three central stations (Table 1).

On the eastern side of Anticosti Island, the genotype *MDH*A1A1* was most frequent in a group of stations located at the south-east end of the island near the junction of the Laurentian and Esquiman channels. The three genotypes were observed at each site except for station 22, where only *MDH*A1A1* was observed, and at station 21, where the heterozygous genotype was absent (Fig. 3; Table 1). Deviation from Hardy-Weinberg expectation was observed at stations 19 and 21 only (Table 1). The northern stations (8, 9, 10, 13, 14, 15) at the north-eastern end of Anticosti Island formed a distinct group where the genotype *MDH*A2A2* dominated. The frequency of allele *MDH*A2* varied from 0.417 to 1.0 (Table 1). The three genotypes were observed at stations 9, 13, 14, and 15 where significant deviation from Hardy-Weinberg was observed at all but sampling station 15 (Table 1).

In 1992, the genotype *MDH*A1A1* was also the most frequent genotype observed throughout the study area, except for stations 22, 29, and 33, where the genotype *MDH*A2A2* was dominant (Fig. 4). The allele *MDH*A1* was thus the most frequent except at these three sampling stations (Table 2). Stations 29 and 33 were the northernmost stations where larvae were found during our study. The frequency of the genotype *MDH*A2A2* increased towards the north within the sampling area although it was not the most frequent genotype. This trend was also observed in 1991 (Fig. 3). In general, the three genotypes were represented at all stations except at stations 3, 15, 16, 27, and 33, where only one or two genotypes

were observed. As noted in 1991, heterozygous individuals co-occurred with those of the *MDH*A1A1* genotype. The frequency of allele *MDH*A1* varied from 0.794 to 0.953 (Table 2). Allelic frequency of 1.0 was observed at stations 27 (*MDH*A1*) and 33 (*MDH*A2*) only. Deviations from Hardy-Weinberg expectations caused by a deficit in the number of heterozygous individuals were observed at 14 sampling stations (Table 2). When data for the entire study area were pooled, a significant departure from the Hardy-Weinberg equilibrium, also due to a deficit in heterozygotes, was noted for both years and for the eastern and western sectors of the Gulf in 1991 (Tables 1 and 2). Such deviations from Hardy-Weinberg equilibrium at any given station, within the different sectors of the Gulf or over the entire sampling area, indicate that redfish larval populations do not form a panmictic group in the Gulf of St. Lawrence but rather consist of a mixture of at least the two most frequent species, *S. mentella* and *S. fasciatus*.

A strong spatial heterogeneity in the distribution of genotypes was observed. The genotype *MDH*A2A2* dominated or was more frequent at stations located in the northern part of the Gulf whereas *MDH*A1A1* dominated in the southern part of the Gulf. The frequency distribution of the genotypes over the entire area in 1991 differed significantly between the eastern and the western Gulf of St. Lawrence (χ^2=53.0; P<0.0001), indicating important spatial variation on a large scale.

Genotype and larval-size distribution

In 1991, the size of the larvae varied significantly between the three genotypes (Kruskal-Wallis, P<0.0001); the *MDH*A2A2* larvae were smaller than those of the other two genotypes (Fig. 5). The size distribution of the larvae was not significantly different between the genotypes *MDH*A1A1* and *MDH*A1A2*. The size distribution of larvae of each genotype did not differ between the eastern and the western sectors of the Gulf. The obvious bimodal distribution of larval size (all genotypes confounded) observed on the west side of Anticosti Island can thus be explained by the difference in size of larvae of the different genotypes.

Similar results were obtained in 1992, where the size of the homozygous larvae *MDH*A2A2* was significantly smaller than those of the genotypes *MDH*A1A1* and *MDH*A1A2*. There was again no significant difference in the standard length between larvae of the *MDH*A1A1* and *MDH*A1A2* genotypes (Fig. 6).

A significant difference in the size of larvae between 1991 and 1992 (Kruskal-Wallis, P<0.0001; Fig. 6) was however noted and can be attributed to the smaller size of the *MDH*A1A1* and *MDH*A1A2* larvae in 1992 compared with those in 1991. The size distribution of the homozygous *MDH*A2A2* larvae was similar in both years.

In 1991, the genotype *MDH*A2A2* was more frequently represented in recently extruded larvae (<8.5 mm) and the genotype *MDH*A1A1* was most frequent for larvae >8.5 mm. In 1992, the genotype *MDH*A1A1* was the most frequent genotype in all size classes except for larvae <6.5 mm which were dominated by the genotype *MDH*A2A2*. In both years, the genotypic composition varied between the size classes of larval redfish, indicating that a larval cohort may be dominated by individuals belonging to a single species (Fig. 6). These differences are most likely associated with a difference in the extrusion time between *S. fasciatus* and *S. mentella*.

Relative proportion of each genotype in the overall larval population

Larvae of the *MDH*A1A1* genotype accounted for 61.8% and 77.6% of all collected larvae in 1991 and 1992 respectively. The contribution of the *MDH*A2A2* genotype was 14.4% in 1991 but dropped to 5.6% in 1992. The heterozygote genotype accounted for 23.8% in 1991 and 16.8% in 1992. The combined contributions of the genotypes *MDH*A1A1* and *MDH*A1A2* thus represented 85.6% and 94.4% of the larval population in 1991 and 1992, respectively.

Discussion

Our results clearly show that the larval redfish population in the Gulf of St. Lawrence is not genetically homogeneous but rather is multispecific and composed of at least two genetically distinct species currently identified as *S. mentella* and *S. fasciatus*. As previously indicated in the introduction, *S. norvegicus* may also occur in the Gulf but at very low abundance and cannot be distinguished from *S. mentella* at the *MDH** locus. The co-existence and the larval extrusion of the two species of redfish, identified by the number of anal-fin rays, have been inferred for Gulf species in previous studies based on the presence of gravid adults (Ni and Sandeman, 1984) and on the presence of sexually mature females in spawning and postspawning condition for both species (St-Pierre and de Lafontaine, 1995). We report here the first evidence that the two species are clearly identified in the larval population.

The taxonomic status of the heterozygous individuals is however problematic. Rubec et al. (1991) suggested that the presence of heterozygotes was best

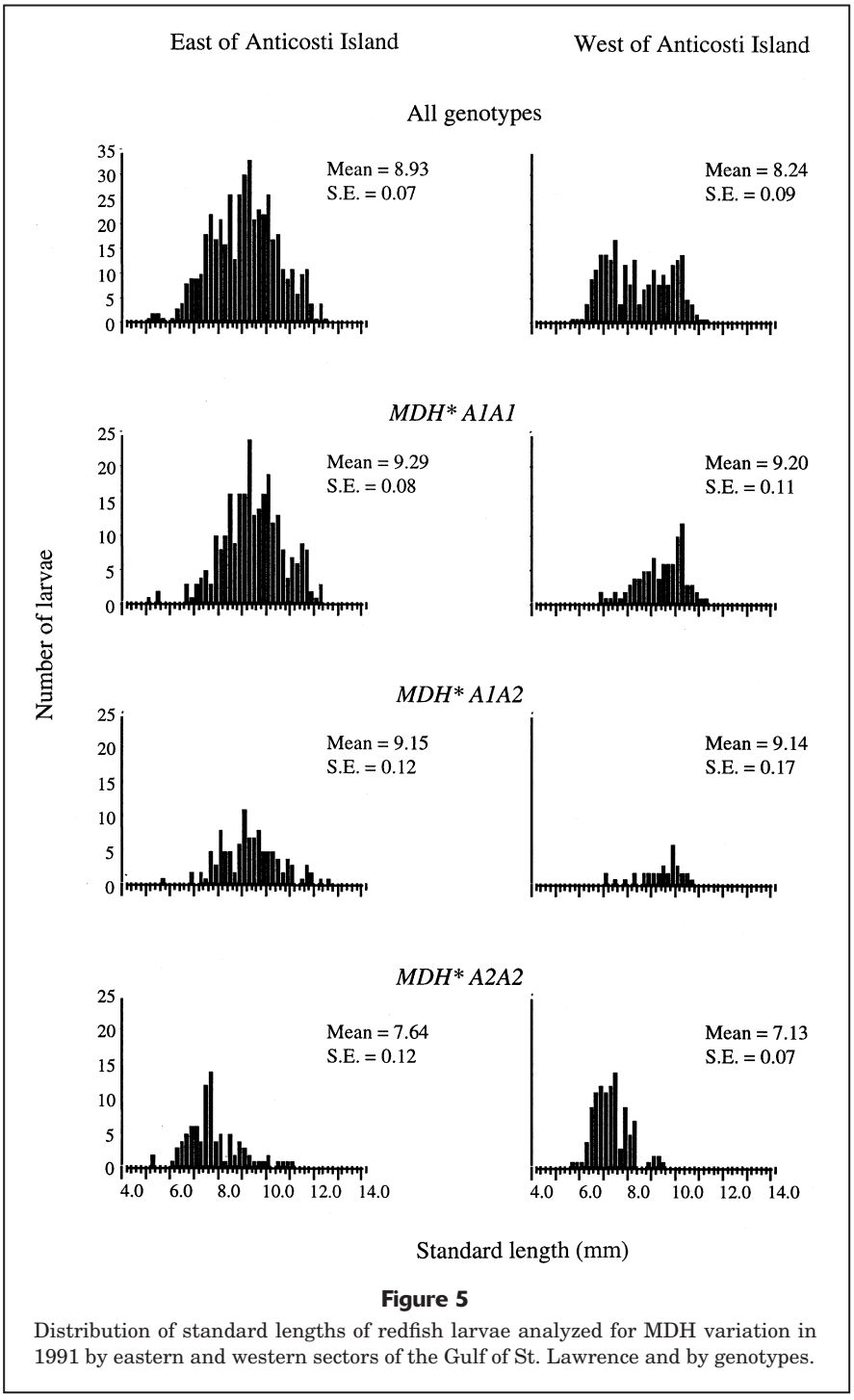

Figure 5

Distribution of standard lengths of redfish larvae analyzed for MDH variation in 1991 by eastern and western sectors of the Gulf of St. Lawrence and by genotypes.

explained by introgressive hybridization between *S. fasciatus* and *S. mentella* in the Gulf. Our results do not rule out this possibility. Hybridization between *S. fasciatus* and *S. mentella* was also suggested as one possibility to explain the RFLP patterns of rDNA observed in adult redfish from the Gulf of St. Lawrence (Desrosiers et al., 1999), where individuals with the rDNA hybrid type had more affinities with *S. mentella* than with *S. fasciatus*. In fact, hybridization between *Sebastes* species may not be an uncommon phenomenon because introgression was recently observed between three rockfish species in Puget Sound (Seeb, 1998). In our study, the similar size distribution of larvae of the *MDH*A1A1* and *MDH*A1A2* genotypes suggests that heterozygous individuals have more affinity with the *S. mentella*

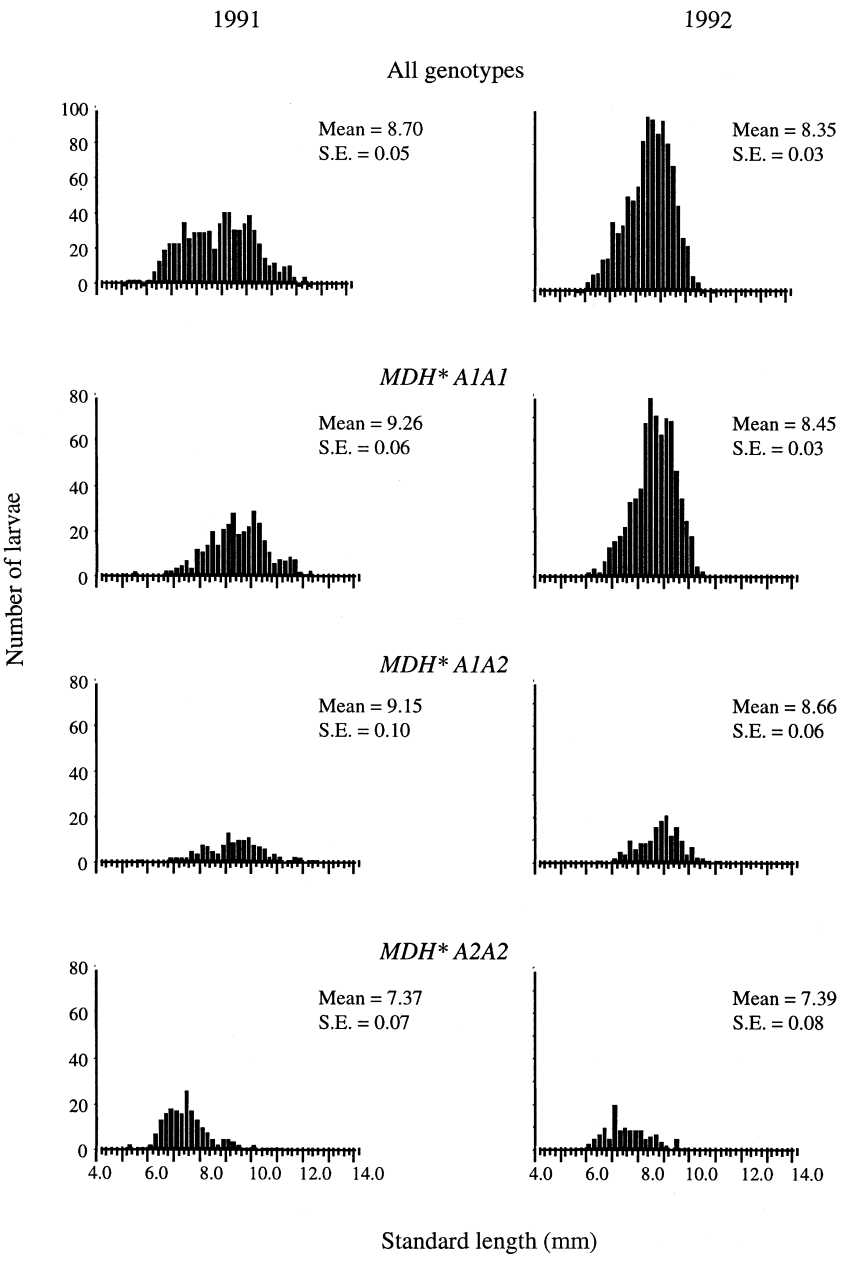

Figure 6

Distribution of standard lengths of all redfish larvae analyzed for MDH variation in 1991 and 1992 by year and genotypes.

than with *S. fasciatus*. The geographic distribution of the heterozygote larvae also matched closely that of the genotype *MDH*A1A1*.

Overall, clear geographic patterns in the abundance and the geographic distribution of redfish larval genotypes were observed in the Gulf of St. Lawrence. In both sampling years redfish larvae were mainly concentrated in offshore areas associated with the deep (>200 m) waters of the Laurentian and Esquiman Channels, as previously reported (Kenchington, 1991; de Lafontaine et al., 1991; Runge and de Lafontaine, 1996). The high abundance of redfish larvae on the southeastern side of Anticosti Island is consistent with the observed occurrence of gravid redfish during May and June in the same area (St-Pierre and de Lafontaine, 1995). High abundance of larval redfish east of Anticosti Island has been frequently observed from mid-May to mid-June

and would correspond to the seasonal peak in larval extrusion as indicated by the maturity cycle of adults (Ni and Templeman, 1985; St-Pierre and de Lafontaine, 1995). On the western side of Anticosti Island, the highest larval abundance is associated with a cyclonic residual circulation (El-Sabh, 1976) as found in previous studies (de Lafontaine, 1990; de Lafontaine et al., 1991). Our data suggest however, that in 1991 and 1992 the contribution of the western sector to the entire redfish larval production of the Gulf of St. Lawrence was small and less important than that of the eastern sector. The western sector contributed approximately up to 10.2% of the *S. mentella* (*MDH*A1A1 +MDH*A1A2*) and up to 34.4% of the *S. fasciatus* larvae in the 1991 samples. This observation is in agreement with the estimated lower biomass of adult redfish in the western Gulf of St. Lawrence in relation to the eastern sector (Atkinson, 1984; Morin and Bernier[2]). The proportionally higher contribution of *S. fasciatus* in the western sector would be indicative of slightly higher abundance of *S. fasciatus* periodically observed in that part of the Gulf (Morin[3]).

The different geographic distributions of the various larval genotypes strongly suggest differences in the preferred extrusion sites of *S. fasciatus* and *S. mentella* (Figs. 3 and 4). *Sebastes mentella* seems to prefer zones located in the central and deeper parts of the channels whereas *S. fasciatus* preferably uses shallower zones near the shelf break and along the sides of the channels. This observation is consistent with the reported summer distribution patterns of adult redfish in the Gulf of St. Lawrence (Rubec et al. 1991; St-Pierre and de Lafontaine, 1995). The apparent low proportion of *S. fasciatus* in the southern part of the Gulf may be due to the relative lack of shallower stations. Attempts to infer more precisely the locations of extrusion sites from the observed larval distributions would, however, require that the size frequency of sampled larvae be considered. In this case, size at extrusion would therefore be the best basis from which to infer the vicinity of the extrusion sites. The size at extrusion for redfish larvae in the Gulf of St. Lawrence is unknown. Penney and Evans (1985) indicated that newly extruded redfish (presumably *S. mentella*—see Penney, 1987) larvae from Flemish Cap (east of Newfoundland Grand banks) ranged between 6.2 and 8.9 mm. From the age-length relationships, these authors estimated that the mean size of newly extruded larvae was 7.68 and 8.23 mm in two consecutive years. Magnusson and Magnusson[4] also reported considerable variation in the size at extrusion (5.5–7.2 mm) of redfish larvae from the Northeast Atlantic waters. Penney (1985) indicated that the mean size of pre-extruded larvae in gravid females of *S. fasciatus* and *S. mentella* collected in southern Newfoundland waters was 7.34 and 7.89 mm, respectively. The size range of larvae in our study was 5.0 to 12.3 mm in 1991 and 5.6 to 10.9 mm in 1992. The proportion of larvae <8.5 mm was 46% and 54% in 1991 and 1992, respectively, suggesting that a large number of sampled larvae were recently extruded. Assuming an average size at extrusion of 7.5 mm and given the estimated growth rates (0.1 to 0.15 mm/day) reported in the literature (Penney and Evans, 1985; Herra, 1989), the maximum length of larvae in our samples would correspond to larvae of approximately 22 to 45 days old. The majority of larvae being <10 mm long, the sampled population was certainly less than 1 month old. Although the period of time between larval extrusion and time of collection may allow for some horizontal drift of larvae by the surface currents, the relatively small size and the presumably young age of the larvae would tend to indicate that these larvae did not disperse much (in relation to the large area sampled in our study) and would have been collected close to their extrusion sites. Consequently, the differences in the relative distribution of the genotypes of larvae indicate that the extrusion sites of the two species do not overlap to a large extent within the Gulf of St. Lawrence.

The mean and the range of the size of larvae varied between genotypes where larvae of the *MDH*A2A2* were significantly smaller (by 1.5 to 2.0 mm) than those of the two other genotypes (Fig. 6). This finding is consistent with other observations, suggesting that the length of *S. fasciatus* at extrusion is smaller than that of *S. mentella*, although considerable variability exists (Penney, 1985; 1987; Penney and Evans, 1985). The similar results obtained in the two sampling years of our study would tend to reveal a species-specific characteristic. In addition, the difference between the mean (and modal) size of each homozygote genotype was larger than that for the reported size at extrusion between the two species (0.5–0.6 mm; see Penney, 1985; Penney and Evans,

[2] Morin, B., and B. Bernier. 1997. The status of redfish in Unit 1 (Gulf of St. Lawrence). Can. Stock Assess. Sec. Res. Doc. 97/112, 23 p. Sciences Branch, Department of Fisheries and Oceans, Maurice Lamontagne Institute, 850 Route de la Mer, Mont-Joli, Québec, Canada G5H 3Z4.

[3] Morin, B. 1998. Unpubl. results. Sciences Branch, Department of Fisheries and Oceans, Maurice Lamontagne Institute, 850 Route de la Mer, Mont-Joli, Québec, Canada G5H 3Z4.

[4] Magnússon, J. V., and J. Magnússon. 1977. On the distinction between larvae of *S. marinus* and *S. mentella*: preliminary report. Int. Counc. Explor. Sea (ICES) Council Meeting 1977/F:48.

1985). This finding suggests that the larvae of *S. fasciatus* were extruded slightly later than those of *S. mentella* in the Gulf of St. Lawrence. Once again assuming that larval growth rates can vary between 0.10 mm and 0.15 mm/day, one can conlude that the peak in larval extrusion as determined by the modal size groups of each homozygote genotype (7.5 mm for *MDH*A2A2* and 10.2 mm for *MDH*A1A1* in 1991) would be approximately 15–25 days apart. From age estimates of larvae from otolith microstructure, Penney (1987) concluded that extrusion time of *S. mentella* was earlier than that of *S. fasciatus* in Flemish Cap area. Previous larval collections made over three consecutive summers at a fixed site along the Gaspé coast in the Gulf of St. Lawrence also showed that larvae extruded earlier tend to be larger than those extruded later in the season (Jean, 1955). Such a sampling strategy combined with genetic identification of the larvae (as presented our paper) would permit a more precise description of the seasonal variability in the extrusion of the two redfish species in the Gulf of St. Lawrence. The significantly smaller larvae collected in 1992, compared with 1991, is probably due to the earlier sampling time in 1992.

Sebastes mentella dominated the redfish larval population in the Gulf of St. Lawrence, contributing 61.8% and 77.6% of all larvae collected in 1991 and 1992, respectively. If we assume that the heterozygous individuals belong to *S. mentella*, the proportion of *S. mentella* increases to 85.6% and 94.4% for these years, respectively. Furthermore, if the heterozygous individuals belong to *S. mentella*, it might be expected that a small proportion of homozygous *MDH*A2A2* individuals were actually *S. mentella,* although misclassified as *S. fasciatus*. In which case, we would have slightly underestimated the abundance of *S. mentella*. The presence of these individuals in the *MDH*A2A2* group might correspond to the largest individuals of this group (Figs. 5 and 6). In any case, the values we obtained for larval stages are very close to those (90.3%) for adult *S. mentella* from samples in the Gulf of St. Lawrence in 1989 and 1990 (St-Pierre and de Lafontaine, 1995).

These results contrast with those of Sévigny and de Lafontaine (1992) who showed that *S. fasciatus* dominated the population of juveniles sampled in the northeastern sector of the Gulf in summer 1990 and 1991. Those juveniles mostly belonged to the 1988 cohort whose origin is not known, and Sévigny and de Lafontaine (1992) did not rule out the possibility that these fish may have originated from outside the Gulf. This rather distinct variation in the genotypic frequency among the various life stages of redfish in the Gulf of St. Lawrence remains unexplained in the light of present knowledge of the ecology of redfish over the entire distribution area. Change in the relative proportion and the geographic distribution of these two species (both at the larval and juvenile stage) may result from variable annual recruitment or from different larval drift and retention patterns among species. The mechanisms responsible for these recruitment patterns are not known but are consistent with the view that the Gulf of St. Lawrence does not form a homogeneous environmental unit but rather may consist of distinctly different pelagic ecosystems in the eastern and western sectors (de Lafontaine et al., 1991).

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