A mtDNA analysis of spatiotemporal distribution of two sympatric larval populations of rainbow smelt (*Osmerus mordax*) in the St. Lawrence River estuary, Quebec, Canada

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Abstract: Ecological isolation through resource partitioning is invoked as a major factor for explaining the persistence of genetically distinct yet closely related sympatric populations. Two genetically distinct sympatric populations of anadromous rainbow smelt (*Osmerus mordax*) exist in the middle estuary of the St. Lawrence River. The persistence of these coexisting populations in sympatry is in conflict with current theoretical concepts predicting population richness. In the present study, we performed mtDNA PCR–RFLP analysis of 922 larvae from 33 sampling stations to test the hypothesis that the larvae belonging to the two sympatric smelt populations of the St. Lawrence middle estuary are spatially segregated and that such segregation may promote the persistence of the populations. Results clearly revealed spatial homogeneity in the relative distribution of larvae from the two populations. Consequently, they did not support our working hypothesis that larvae belonging to the two sympatric smelt populations are spatially segregated. Two alternative explanations may account for the lack of spatial partitioning observed here. Competition may not be important enough to promote resource partitioning at the larval stage. Alternatively, resource partitioning occurs, but not spatially. This study also demonstrated that the effect of historical events may have been as important as contemporary ecological settings in determining genetic population structure in smelt.

Résumé : Plusieurs auteurs suggèrent que le partage des ressources écologiques est le principal facteur permettant le maintien de populations en sympatrie. Des travaux récents ont démontré l'existence de deux populations sympatriques d'éperlans arc-en-ciel (*Osmerus mordax*) anadromes génétiquement distinctes mais ayant le potentiel de s'hybrider dans l'estuaire moyen du St-Laurent. La persistance de populations en sympatrie dans ce milieu est contraire aux prédictions théoriques de l'hypothèse du « member–vagrant ». Dans cette étude, nous avons effectué une analyse de PCR–RFLP sur l'ADNmt de 922 larves d'éperlans représentant 33 sites d'échantillonnage dans le but de tester l'hypothèse voulant que ces deux populations larvaires soient spatio-temporellement ségrégées et que cette ségrégation explique en partie leur persistance dans le milieu. Les résultats de ces analyses démontrent une homogénéité spatiale dans la distribution des larves appartenant aux deux populations. Conséquemment, ces résultats ne supportent pas l'hypothèse de départ. On peut expliquer ces résultats de deux façons différentes. Premièrement, le partage des ressources est peu important au niveau larvaire pour l'éperlan du St-Laurent. Alternativement, le partage des ressources est significatif, mais ne se fait pas spatialement. Ces résultats démontrent de plus que les effets des événements historiques sur la structure des populations peuvent, dans certaines circonstances, être plus importants que les événements écologiques contemporains.

Introduction

Many north-temperate fish species are characterized by the occurrence of sympatric ecotypes that remain genetically distinct despite their potential to hybridize (summarized in Bernatchez et al. 1996). Because of the close relationships and incomplete reproductive isolation, these sympatric ecotypes have been studied to better understand the early stages of speciation. Ecological resource partitioning may contribute to decreasing the intraspecific competition between sympatric populations and thus favours the coexistence of populations by disruptive or frequency-dependent selection (e.g., Schluter and McPhail 1993; Robinson and Wilson 1994; Skúlason and Smith 1995).

Resource partitioning generally occurs in sympatric freshwater fish populations by trophic-based adaptations that are manifested by morphological, behavioural, and life history characteristics (e.g., Bernatchez and Dodson 1990; Taylor and Bentzen 1993*a*, 1993*b*; McPhail 1994; Skúlason and Smith 1995). Despite intensive studies of sympatric populations found in freshwater, no study has yet documented resource partitioning between sympatric fish populations of the same species in estuarine or marine ecosystems. Resource partitioning studies in marine ecosystems have only involved sympatric and reproductively isolated fishes (e.g., Schmitt and Holbrook 1986) but never sympatric populations with the possibility of interbreeding. Furthermore, all studies related to resource partitioning among sympatric fish populations have generally focused on juvenile and adult life history stages, but have not considered the larval stage despite the presumed importance

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of this stage in contributing to population richness and abundance (Sinclair 1988).

The rainbow smelt (*Osmerus mordax* Mitchill) is an osmerid species found in salinity ranging from freshwater to 30‰ and in water temperatures from below 0°C to more than 20°C (Nellbring 1989). It also exhibits a broad life history diversity consisting of anadromous and landlocked populations as well as freshwater dwarf and normal ecotypes (Nellbring 1989; Taylor and Bentzen 1993*a*) respectively specialized for planktivorous and (or) macrophagous and (or) piscivorous modes of life (Taylor and Bentzen 1993*a*, 1993*b*). Anadromous populations of this species exhibit a similar life cycle: adult smelt ascend streams and rivers in early spring and spawn in freshwater. Hatching occurs 1–2 weeks later and larvae from different spawning sites are usually transported within short periods into estuarine, fjord, or marine coastal waters where they are retained (Ouellet and Dodson 1985; Laprise and Dodson 1989*a*, 1989*b*) until metamorphosis. In the St. Lawrence estuary, the larvae are transported from spawning sites to a nursery zone situated in the maximum-turbidity zone where they are retained by active vertical migration (Dodson et al. 1989; Laprise and Dodson 1989*a*, 1989*b*).

Recent molecular studies demonstrated the existence of two genetically distinct (F_{st} = 0.178) sympatric populations in the middle estuary of the St. Lawrence River associated with two phylogenetically distinct lineages separated by about 0.8% average pairwise sequence divergence (Baby et al. 1991; Bernatchez and Martin 1996). Smelt ascending tributaries along the south shore of the estuary to spawn are dominated by phylogenetic group A (80.9%) whereas those caught in the north channel between Beauport and the Saguenay fjord are dominated by phylogenetic group B (87.1%) (Bernatchez and Martin 1996). Hereafter, these two populations are referred to as the "south shore population" and the "north shore population." Bernatchez (1997) demonstrated that the allopatric origin of phylogenetic groups A and B was associated with distinct glacial refugia and that the middle estuary represents a zone of secondary contact between these races that have developed biological barriers to gene flow between them. The diversification of these two populations does not appear to involve the same mechanisms that have been observed in the lacustrine environment. No evidence of dwarf and normal ecotypes has been documented, and the geographical mixing of the adults belonging to the two populations has been demonstrated outside the spawning locations (Bernatchez et al. 1995). These observations suggest that the coexistence of the sympatric populations may be determined by differential resource partitioning at a younger life history stage, namely at the larval stage. Coexistence of two sympatric larval populations within the middle estuary also apparently contradicts theoretical predictions on the ecological causes of population structure in the marine environment. According to the member–vagrant hypothesis (Sinclair 1988), population richness and structure are a function of the number and locations of geographic settings permitting the retention of early life history stages. The well-mixed upstream part of the St. Lawrence middle estuary associated with the smelt nursery area represents a unique oceanographic system for larval retention (Dodson et al. 1989). Consequently, only one population should be found in the system according to the member–vagrant model. The presence of the two populations in one oceanographic entity suggests that the larvae might use this structure differently. It is plausible that behavioural mechanisms evolved differently for each population resulting in distinct spatiotemporal distributions permitting coexistence in the same estuarine system (Laprise and Dodson 1989*a*, 1989*b*).

In this study, we performed a mtDNA polymerase chain reaction – restriction fragment length polymorphism (PCR–RFLP) analysis to test the hypothesis that larvae belonging to the two sympatric smelt populations of the St. Lawrence middle estuary are spatially segregated and that such segregation may promote the coexistence of two populations in the system. As such, this study represents the first characterization of resource partitioning at the larval stage among sympatric fish populations and the first ecological assessment of sympatric fish populations in an estuarine environment.

Material and methods

Sample site

The St. Lawrence middle estuary is about 180 km long, receives an annual mean discharge of 10 000 m^3/s from the river, and comprises three main channels, north, middle, and south (e.g., Laprise and Dodson 1989*b*, 1993). The estuary is characterized by a well-mixed zone in the upstream section and a partially stratified zone downstream (El Sabh 1988). The salinity varies from 0‰ upstream of Île d'Orléans to over 30‰ downstream of Île aux Coudres (Fig 1). The estuary is highly energetic; tides vary between 3 and 5 m in height and current speed may reach 250 cm/s. Due to Coriolis force, tides, and the bottom topography of the estuary, important transverse currents are formed upstream and downstream of the central islands (Laprise and Dodson 1989*b*). Thus, this part of the St. Lawrence estuary between Île d'Orléans and Île aux Coudres is characterized by a cyclonic circulation that, in addition to the presence of a null zone (Dodson et al. 1989), contributes to the retention of suspended particulate matter inflowing from the river and salt marshes along the estuary forming a maximum-turbidity zone (Laprise and Dodson 1989*b*) (Fig. 1).

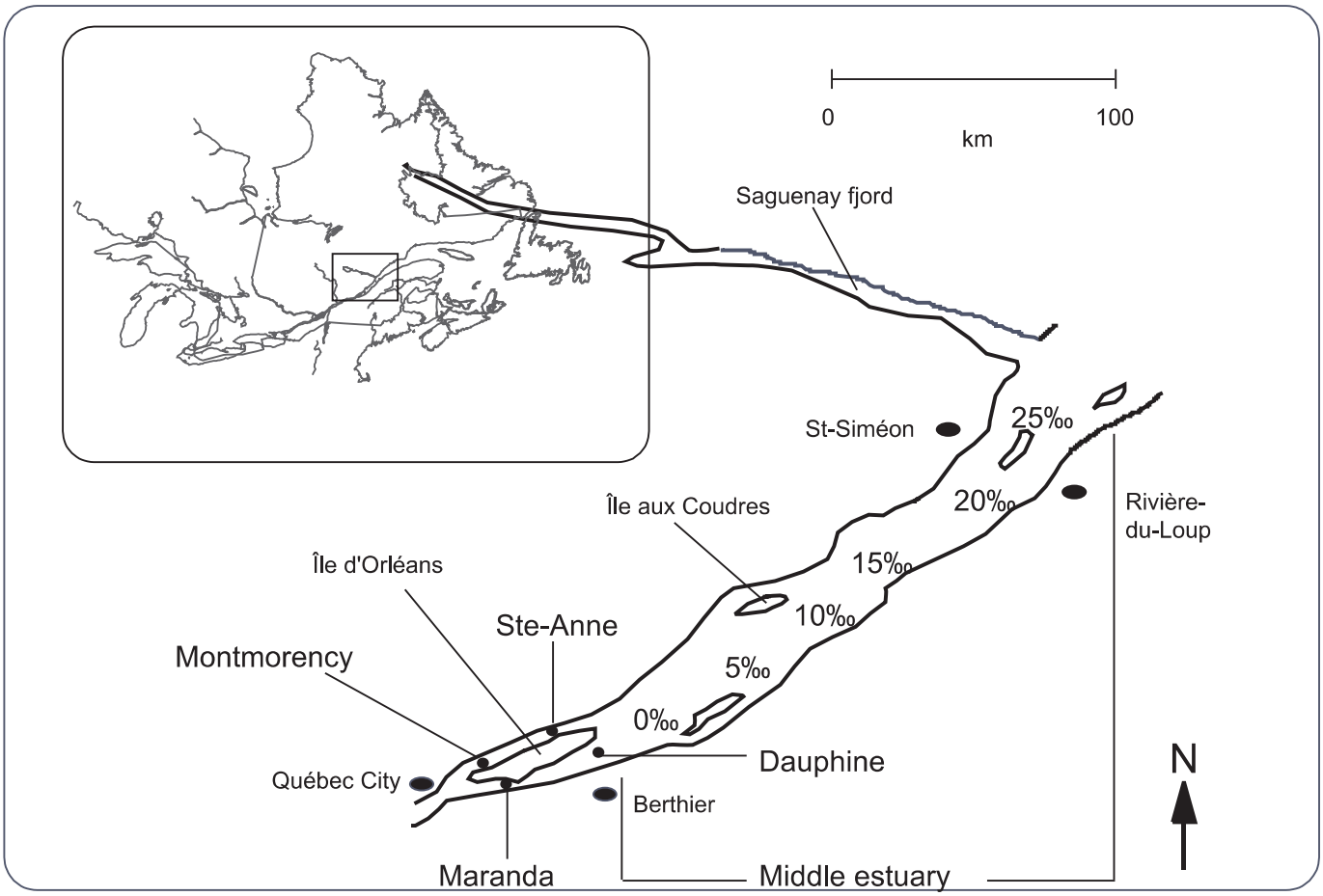
Sample collection

Larvae were sampled in the north and south channels over three consecutive months (Table 1). Pelagic trawling was carried out at five stations in May and six in June and July between Cap-Brulé and St-Siméon in the north channel and between St-Jean-Port-Joli and Cacouna in the south channel (Fig. 1). The stations were identified by the salinity of surface water masses. Larvae were also sampled twice in May at four stations around Île d'Orléans (Table 1; Fig. 1). Sampling consisted of step-oblique tows from the bottom to the surface using a 1-m plankton net equipped with a flowmeter and fitted with a 0.500-mm-mesh net for the sampling in May and around Île d'Orléans. Sampling in June and July was done in the same manner but with a 50-cm, 0.500-mm-mesh plankton net fitted to a Tucker trawl. The volume of water filtered was calculated from the distance towed and diameter of the net. Samples were preserved in 95% ethanol until genetic analysis.

Genetic analysis

Total DNA was extracted differently according to larval size. Small animals (ranging from 40 to 150 μg and from 5 to 13 mm in length) were soaked twice in distilled water for 15 min to remove ethanol. Water was discarded and each larva was put into 50 μL of extraction buffer (10 mM Tris–HCl (pH 8.0), 50 mM KCl, 0.5% Tween 20, 250 mg proteinase K/mL). Tubes were incubated for 3–4 h at 65°C and then heated at 95°C for 15 min. Digestion products were centrifuged for 10 min. For larger larvae, total DNA was purified

Fig. 1. Collection sites around Île d'Orléans and approximate locations of the six different water mass salinities (surface) that were sampled in the two channels of the middle estuary of the St. Lawrence River.



from the entire specimen following the procedures described in Bernatchez et al. (1992).

All larvae were allocated to mtDNA group A or B, defined previously by Bernatchez et al. (1995), by RFLP analysis performed with restriction endonucleases generating diagnostic sites on PCR-amplified segments. The analysis was initiated with the larger larvae. We performed PCR amplifications on a 2.4 kilobase pair (kb) segment encompassing the ND5 and ND6 subunits of NADH dehydrogenase using the primers developed by Cronin et al. (1993), which we named ND56R and ND56F. Each PCR reaction was composed of 1–5 μL of the total DNA extract (about 500 ng), 5 μL of 10× buffer (500 mM KCl, 100 mM Tris–HCl (pH9.0), 1% Triton X-100, 25 mM $MgCl_2$), 4 μL of dNTP mix (2.5 mM of each of dATP, dCTP, dGTP, and dTTP in sterile water), 2 μL of a 20 mM solution of the two primers, 2–4 units of *Taq* polymerase, and sterile water for a final volume of 50 μL per reaction. DNA amplification was performed in a programmable thermal cycler (Perkin-Elmer model 480) using the following profile: a preliminary denaturation at 95°C for 2 min followed by 40 cycles involving strand denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and primer extension at 72°C for 2 min and 30 s followed by a final elongation at 72°C for 10 min. The segments were subsequently digested with the hexameric enzyme *Apa* I used as recommended by the supplier (Pharmacia). This enzyme generated diagnostic fragment patterns for mtDNA groups A and B. mtDNA fragments were separated on 1.2 or 1.4% agarose gels at 90 V run for 5 h. Restriction fragments were revealed by ethidium bromide staining. Reliability was ensured by the analysis of 20 adult smelt characterized with RFLP analysis of the entire molecule and known to belong to mtDNA group A or B.

The small amount of DNA extracted from the small-size larvae in May was problematic for the amplification of the 2.4-kb segment. Development of primers for amplifying a shorter diagnostic segment was necessary to reduce the stringency of the PCR reactions. To do so, sequencing was performed on three potentially variable mitochondrial genes, the NADH subunits ND5 and ND6 and the ATPase subunit 6, to determine primer sets flanking diagnostic restriction sites between the two mtDNA groups (Fig. 2). PCR amplifications were performed on ND5 and ND6 subunit genes as described above and on the ATPase subunit 6 with the procedures described in Giuffra et al. (1994).

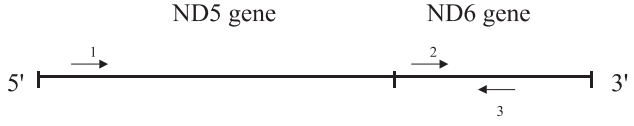
Amplified DNA was purified with the Quiagen DNA purification kit according to the supplier's recommendations. Double-stranded DNA sequencing was performed by applying the dideoxy chain-ending technique using the Sequenase kit (version 2.0, US Biochemical) as described in Bernatchez et al. (1992) with the following modifications. Sequence reactions were first performed with primers ND56R and ND56F for the ND5 and ND6 genes and H9208 and L8558 developed by Giuffra et al. (1994) for ATPase subunit 6. Internal primers developed in the present work were used for subsequent sequence reactions until the identification of diagnostic restriction sites between the two groups. Following this procedure, a 288 base pair (bp) segment of the ND6 gene localized between primers ND56R250 and ND56R450V was chosen to characterize the smallest larvae (Fig. 2). Diagnostic fragment patterns were generated

Table 1. Total number of caught larvae, density, samples sizes for genetic analysis, and relative frequency and absolute density of genetic groups A and B of rainbow smelt larvae for 42 stations in the St. Lawrence middle estuary for May 24–26, June 20–22, and July 15–16, 1994.

Month	Station	Total *n*	Density (*n*/100 m^3)	Genetic *n*	Relative frequency		Absolute density (*n*/100 m^3)	
					A	B	A	B
May 24–26	Dauphine (May 24)	2797	396.84	40	0.12	0.88	49.61	347.24
	Maranda (May 24)	135	20.11	43	0.05	0.95	1.01	19.10
	Montmorency (May 24)	210	36.85	24	0.00	1.00	0.00	36.85
	St-Anne (May 24)	130	32.63	37	0.03	0.97	0.88	31.75
	North (5‰)	766	179.20	40	0.05	0.95	8.96	170.24
	South (5‰)	50	9.22	36	0.03	0.97	0.28	8.94
	North (10‰)	75	9.60	33	0.15	0.85	1.44	8.16
	South (10‰)	396	65.18	40	0.05	0.95	3.26	61.92
	North (15‰)	0	—	—	—	—	—	—
	South (15‰)	0	—	—	—	—	—	—
	North (20‰)	3	0.43	2	0.00	1.00	0.00	0.86
	South (20‰)	0	—	—	—	—	—	—
	North (25‰)	0	—	—	—	—	—	—
	South (25‰)	14	1.83	10	0.00	1.00	0.00	1.83
	Dauphine (May 31)	237	35.46	38	0.05	0.95	1.88	33.58
	Maranda (May 31)	147	23.26	40	0.12	0.88	2.91	20.35
	Montmorency (May 31)	100	13.65	37	0.03	0.97	0.37	13.28
	St-Anne (May 31)	112	19.58	42	0.19	0.81	3.72	15.86
June 20–22	North (0‰)	154	11.46	41	0.10	0.90	1.12	10.34
	South (0‰)	6	0.49	5	0.2	0.8	0.10	0.39
	North (5‰)	1971	147.56	41	0.12	0.88	18.00	129.56
	South (5‰)	149	12.06	59	0.24	0.76	2.89	9.17
	North (10‰)	1172	77.79	43	0.12	0.88	9.02	68.77
	South (10‰)	15	1.18	12	0.17	0.83	0.20	0.98
	North (15‰)	16	1.12	7	0.00	1.00	0.00	1.12
	South (15‰)	0	—	—	—	—	—	—
	North (20‰)	24	1.62	14	0.00	1.00	0.00	1.62
	South (20‰)	0	—	—	—	—	—	—
	North (25‰)	5	0.42	5	0.00	1.00	0.00	0.42
	South (25‰)	241	17.29	21	0.00	1.00	0.00	17.29
July 15–16	North (0‰)	563	64.29	40	0.15	0.85	9.64	54.65
	South (0‰)	164	19.09	40	0.18	0.82	3.34	15.75
	North (5‰)	74	8.12	41	0.05	0.95	0.40	7.72
	South (5‰)	118	12.84	41	0.17	0.83	2.20	10.64
	South (10‰)	61	4.87	16	0.19	0.81	0.92	3.95
	North (15‰)	6	0.38	6	0.17	0.83	0.06	0.32
	South (15‰)	0	—	—	—	—	—	—
	North (20‰)	2	0.14	2	0.00	1.00	0.00	0.14
	South (20‰)	0	—	—	—	—	—	—
	North (25‰)	4	0.26	2	0.00	1.00	0.00	0.26
	South (25‰)	0	—	—	—	—	—	—

Note: Stations around Île d'Orléans are followed by the day of sampling and other stations are identified by channel and surface salinity.

Fig. 2. Primer locations on ND5 and ND6 genes in the rainbow smelt mtDNA molecule. ND56F300V (1) and ND56R450V (2) are located in the light strand, and ND56R250 (3) is located in the heavy strand. Primer 5′–3′ sequences are CCCGTTGCCCTTTACGTCACC for ND56F300V, GGACTACAAACAAAGTCAATAAG for ND56R450V, and ACTGGTCGTGTTTGTATAC for ND56R250.



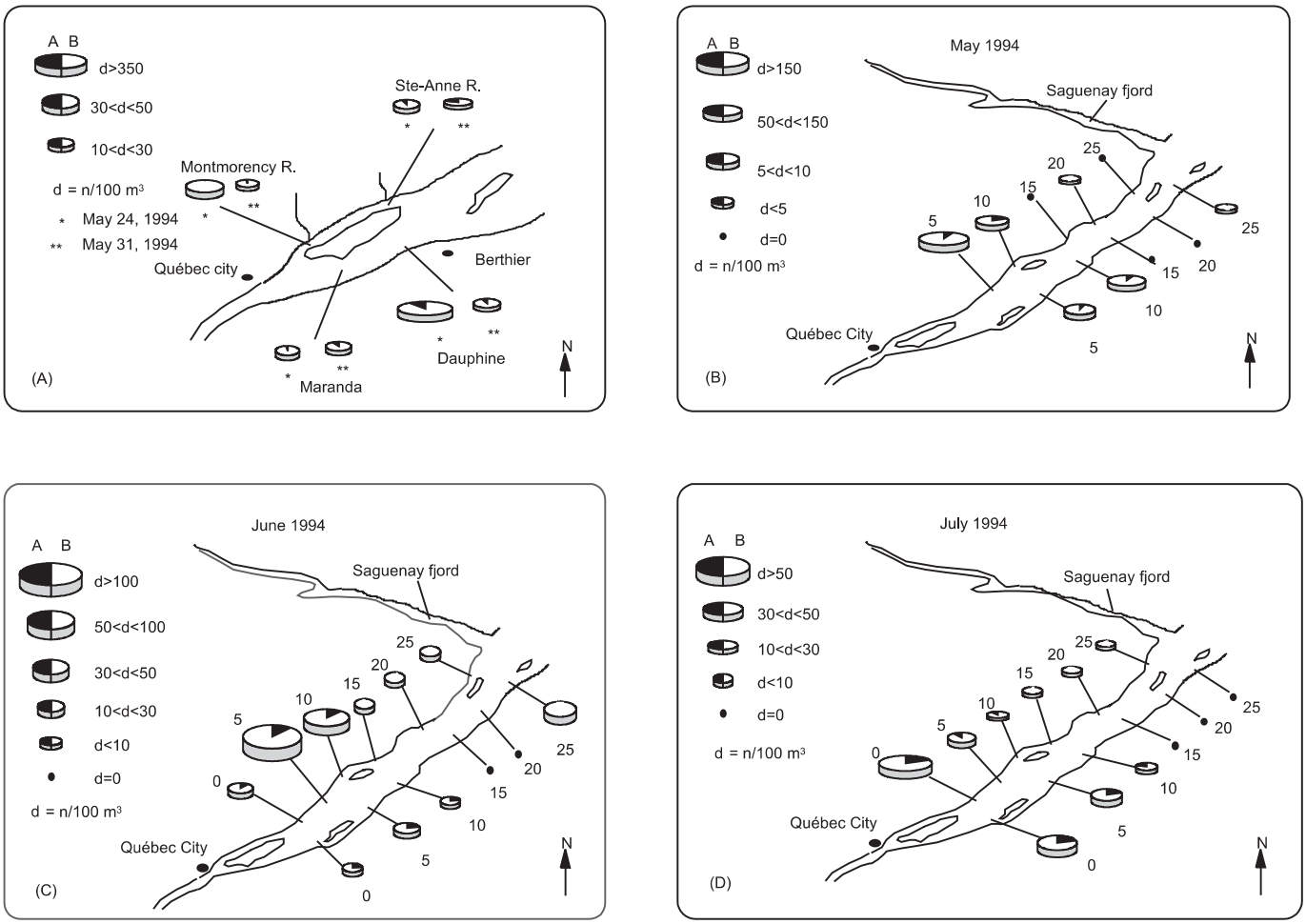
by *Dde* I on this segment. Procedures to genetically characterize larvae with this segment were done as described for the 2.4-kb segment, except that the elongation step was reduced to 45 s.

Data analysis

The density of larvae at each station (number of larvae per 100 m^3 of water filtered) was calculated at each station for each month and averaged. These estimates allowed us to quantitatively evaluate the number of larvae belonging to each genetic group, and therefore, each population, for the different stations or periods of sampling.

Heterogeneity in the distribution of larval populations among stations, groups of stations, and sampling periods was first evaluated by the analysis of frequency distribution of the two genetic groups for

Fig. 3. Frequency distribution and abundance (d = number of larvae/100 m^3 of filtered water) of larvae belonging to mtDNA groups A and B for (A) around Île d'Orléans on May 24 and 31, 1994, and (B–D) in the middle estuary for the two channels in May, June, and July, respectively. Names or numbers above pie charts identify sampling stations.



each station or group of stations (in cases where no distinction was detected among stations) using chi-square randomization tests (Roff and Bentzen 1989) with 1000 randomizations performed by the MONTE program of the REAP software package (McElroy et al. 1992). Significant heterogeneity was accepted when the probability (p) was <50/1000.

The relative contribution of the two breeding populations to larval samples was estimated for each station and group of stations for each period of sampling. This was calculated using the method of Lane et al. (1990) by first considering the frequency of genetic groups A and B in the two reproductive stocks characterized previously as baseline populations (Bernatchez et al. 1995). Final estimates, however, were calculated by using the distribution of the two mtDNA groups in May larvae as the baseline data for the north shore population. This substitution generated more plausible estimates (see Results section).

Results

Sequence analysis

Sequence analysis was performed until suitable primers flanking diagnostic restriction sites were identified. This resulted in a 5′–3′ sequence effort of 3042 bp of the light strand of mtDNA (Gene Bank accession Nos. AF034748–AF034752). Sequences were obtained for one representative of each mtDNA group: (*i*) 618 bp from the 68th nucleotide of the ATPase subunit 6 to 10 bp in the adjacent cytochrome oxidase subunit 3 (COIII) gene, (*ii*) the complete ND5 gene (1833 bp) and 32 bp of the adjacent leucine tRNA gene, and (*iii*) the complete ND6 gene (522 bp) and 28 bp of the adjacent glucine tRNA gene. Nine mutations were observed in these sequences and their spatial distribution was heterogeneous. The ATPase subunit 6 gene represented the least variable region, with no substitutions detected, compared with six in the ND5 gene and three in the ND6 gene discriminating the two mtDNA groups. The overall sequence divergence estimate between the groups was 0.30%. Seventy-eight percent (seven of nine) of these substitutions were transitions, three involving purine–purine differences (all in the ND5 gene) and four involving pyrimidine–pyrimidine differences (one in the ND5 gene and three in the ND6 gene).

Larval abundance and distribution

Zero to 2797 larvae were captured at each of the 42 stations in May, June, and July 1994 (Table 1; Fig. 3). Low densities of larvae were observed at the downstream sites of the middle estuary. Thus, at the 18 stations having a salinity between 15 and 25‰ and for the three sampling months, 17 had a larval

Table 2. Total number of larvae belonging to mtDNA groups A and B, their ratio, south shore contribution and variance estimates, abundance of larvae in the south and north shore populations in the St. Lawrence middle estuary, and their ratio for each period of sampling.

	Total *n*		A:B	Contribution	Variance	Abundance (*n*/100 m^3)		South:north
	A	B	ratio	(%)	(%)	South	North	ratio
May 24	65.44	686.89	1:10.5	0	0.086	0	730.2	—
May 31	8.88	83.07	1:9.4	—	—	—	—	—
June	31.33	222.37	1:7.1	5.0	0.109	12.7	241.0	1:19.0
July	16.92	95.22	1:5.6	8.7	0.173	9.7	102.4	1:10.6

Note: No contribution and variance estimates were calculated for May 31, since only Île d'Orléans was sampled on this date.

Table 3. Relative contribution and variance estimates of the south shore rainbow smelt population (dominated by genetic group A) to the larval samples around Île d'Orléans and from the St. Lawrence middle estuary.

South channel			North channel		
	Contribution	Variance		Contribution	Variance
May			May		
Dauphine (May 24, 1994)	0.0512	0.0033	Montmorency (May 24, 1994)	0.0000	0.0010
Maranda (May 24, 1994)	0.0000	0.0019	St-Anne (May 24, 1994)	0.0000	0.0016
5‰	0.0000	0.0017	5‰	0.0000	0.0020
10‰	0.0000	0.0020	10‰	0.0873	0.0043
25‰	0.0000	0.0010	20‰	0.0000	0.0010
Dauphine (May 31, 1994)	0.0000	0.0021	Montmorency (May 31, 1994)	0.0000	0.0016
Maranda (May 31, 1994)	0.0526	0.0033	St-Anne (May 31, 1994)	0.1427	0.0041
June			June		
0‰	0.1565	0.0313	0‰	0.0145	0.0028
5‰	0.2119	0.0035	5‰	0.0485	0.0032
10‰	0.1108	0.0117	10‰	0.0401	0.0030
25‰	0.0000	0.0010	15‰	0.0000	0.0010
			20‰	0.0000	0.0010
			25‰	0.0000	0.0010
July			July		
0‰	0.1219	0.0041	0‰	0.0873	0.0037
5‰	0.1163	0.0039	5‰	0.0000	0.0019
10‰	0.1399	0.0097	10‰	0.1108	0.0062
			15‰	0.1108	0.0228
			20‰	0.0000	0.0010
			25‰	0.0000	0.0010

Note: Date of sampling given in parentheses.

density <2/100 m^3 water (Table 1). Larval density was much higher in the upstream section of the middle estuary (≤ 10‰), with the maximum reached at pointe Dauphine in May where a density of 396.8 larvae/100 m^3 was observed.

Two to 59 larvae per station were genetically characterized (all larvae when $n < 40$ and random subsampling of 40–59 for the others) for a total of 922 larvae from 33 stations. No larvae were caught at the other stations. The frequency distribution of the larvae belonging to mtDNA groups A and B did not support the hypothesis of differential spatial segregation between the two larval populations. Thus, the distribution of larvae characterized by either mtDNA group A or B was very homogeneous among stations (Table 1). Larvae of mtDNA group B dominated each station at a frequency varying between 76 and 100% (Table 1; Fig. 3), and no difference in the frequency distribution of the two mtDNA groups was detected among individual samples for each month. Heterogeneity analysis was then performed each month among stations of the north and south channels individually, and no significant difference was observed except at Montmorency River and St-Anne River on May 31. Samples of each channel were then pooled for each month to compare the distribution of mtDNA groups between channels (May 31 observations excluded) and no heterogeneity was detected. Finally, samples were pooled by month to test for temporal heterogeneity of frequency distribution which indicated distinct distributions of the two mtDNA groups between May and June and May and July. The differences were explained by an increase in the number of larvae belonging to group A over time. This increase is illustrated by the ratio of absolute numbers of larvae characterized by mtDNA groups A and B for a given month (Table 2).

The distribution of mtDNA groups A and B among samples allowed the estimation of the relative contribution of the south shore breeding population to the number of larvae in each

station and for each month (Table 3). Initially calculated using the baseline populations of Bernatchez et al. (1995), contribution estimates of the south shore population were negative for May. Such results were due to the frequency of mtDNA group A (6%) observed in May, which was lower than the frequency reported by Bernatchez et al. (1995) for the north shore breeding population (13%). As the sample size for May 24–26 sampling was greater (n = 305 compared with 101) than that of Bernatchez et al. (1995), and always avoided negative contributions, we felt that the proportion of larvae belonging to groups A and B in this month was more representative of the north shore baseline population for estimating relative contributions.

The estimated contribution of the south shore population to larval samples was very small and homogeneous among stations for a given month (Table 3). Seventeen of the 33 contribution estimates were null and the average contribution of the 16 others was 10.0%. A temporal increase in the frequency of mtDNA group A was also reflected by the estimate of the south shore contribution to larval samples (pooled) for each month. Thus, the estimated contribution for this population increased from 0% in May to 5% in June and to 8.7% in July (Table 2).

The total larval density belonging to the south shore population in the system was calculated each month by multiplying the contribution estimate of the south shore population by the larval density for each month. The total abundance estimates of larvae belonging to the north shore population were estimated by the subtraction of the south shore larval density from the total larval density. No detectable contribution of the south shore population was observed in May. For the other two months, the larval south to north shore population density ratios were about 1:20 in June and 1:10 in July (Table 2). Therefore, the total larval density indicated an increase of the larvae belonging to the south shore population over time.

Discussion

Sequence analysis

Sequence analysis revealed heterogeneity in the mutational distribution between the two smelt mtDNA groups, suggesting differential mutation rates among genes, which corroborate previous studies (e.g., Meyer 1994; Zardoya et al. 1995). The divergence estimates between the two mtDNA groups were 0.0% (0/638) for ATPase subunit 6, 0.33% (6/1830) for the ND5 gene, and 0.57% (3/522) for the ND6 gene. However, an unexpected result was the overall low divergence (0.29%) between the two mtDNA groups obtained by sequencing compared with RFLP analysis performed over the entire mtDNA molecule (about 0.8%) (e.g., Bernatchez and Martin 1996). It has generally been reported that RFLP analysis detects less variation than sequencing because it is an indirect method of variation detection (e.g., Meyer 1994). The almost threefold difference in divergence estimates between the two methods indicates that the regions chosen for sequencing were less variable than averaged over the entire molecule. This was unexpected, since the chosen mtDNA regions were reported to be highly variable at the intraspecific level in other fishes, namely brown trout (*Salmo trutta*) (Giuffra et al. 1994; Bernatchez and Osinov 1995) and lake whitefish (*Coregonus clupeaformis*) (Pigeon et al. 1997). These results also suggested that it may not be possible to generally classify mtDNA genes as highly variable or not, since the extent of polymorphism in specific genes is apparently species dependent as stated above (also see Taylor and Dodson 1994). As mutation rate in mitochondrial protein-coding genes is mainly affected by selective functional constraints on the gene product (Meyer 1994), these results suggest that different mtDNA genes may be subject to differential selective constraints even among closely related species.

Analysis of smelt larvae

This study revealed four relevant results to the issue of the sympatric persistence of fish populations and to the biology of smelt in the estuary: the overall similarity of larval density distribution with previous studies, the lack of spatial segregation over time between the two larval populations, the increase of the south shore larvae relative to the north shore larvae with time and finally, the overall greater abundance of the north shore larval population.

Temporal stability of larval density distribution

Our results indicated the upstream displacement of larvae during the season, as maximum densities moved from water masses between 5 and 10‰ in May to waters of less than 5‰ in July. These results are identical to those obtained in detailed studies of smelt larval ecology in the St. Lawrence estuary. The low density of larvae in the downstream section of the middle estuary compared with the upstream section was previously described by Laprise and Dodson (1989*a*, 1989*b*). Larval dispersal seemed also to follow the same pattern described previously (e.g., Laprise and Dodson 1989*a*). Briefly, early after hatching, the larvae are transported into the estuarine nursery zone where they are retained by active vertical displacements in combination with the circulation system. The fact that older larvae use the tidal currents more efficiently by increasing the amplitude of their vertical migration explains the increase in density farther upstream in July (Laprise and Dodson 1989*b*). These results indicate that the adaptive mechanisms of vertical displacements exhibited by smelt larvae for their retention in a highly productive nursery area (Dauvin and Dodson 1990) are temporally stable.

Resource partitioning

Our results revealed spatial homogeneity in the relative distribution of the larvae from the south and north shore populations. Consequently, they did not support our working hypothesis that the larvae belonging to the two sympatric smelt populations in the St. Lawrence middle estuary are spatially segregated and that such segregation may promote in part the persistence of two populations in the system. Two alternative explanations may account for these observations.

First, the persistence of the two larval populations without apparent segregation may indicate that there is not sufficient competition to drive resource partitioning in the system at the larval stage. The maximum-turbidity zone of St. Lawrence middle estuary where the maximum density of smelt larvae is found represents a productive food web with high densities of both micro- and macro-zooplankton (e.g., Dodson et al. 1989). The densities of potential food for larvae, including copepods and *Neomysis americana*, are 10 and 100 times greater, respectively, within the turbidity zone than at its downstream limit (Dodson et al. 1989; Dauvin and Dodson 1990). This

abundance is presumably due to the common hydrodynamic control of suspended sediment and some pelagic components of the estuarine ecosystem (e.g., Laprise and Dodson 1989*a*, 1989*b*). The high content of food resources in this zone is also illustrated by a growth rate of smelt larvae of 0.33 mm/day within the turbidity zone as compared with growth rates varying from 0.10 to 0.24 mm/day downstream of the turbidity zone (Laprise and Dodson 1989*b*). In addition to the abundance of food resources, the existence of only one potential interspecific competitor, the Atlantic tomcod (*Microgadus tomcod*), renders plausible the persistence of coexisting smelt populations without spatial resource partitioning at the early larval stages. However, as suggested by the development of spatial segregation between tomcod and smelt later in the season (Laprise and Dodson 1989*b*), spatial segregation between the two smelt populations may also occur later in life in relation to an unknown resource gradient.

The second alternative explanation for these observations may be related to a temporal delay in the prey consumed by larvae of each population. The present results revealed a very low abundance, and possibly an absence, of larvae belonging to the south shore population in May and their gradual increase over time. This is indicative of a temporal delay in the entrance of the two larval populations to the estuary. These observations corroborate present knowledge of the breeding ecology of smelt. Briefly, although the north shore spawning locations are unknown, the capture of spent adults in early spring indicated that the breeding period takes place at the latest around the end of April (Bernatchez et al. 1995), possibly directly in the St. Lawrence River. In contrast, the south shore population breeding period has been well defined to occur 2–4 weeks later in the south shore tributaries (Ouellet and Dodson 1985). Assuming similar developmental rates, these observations imply that larvae of the south shore population are smaller than those of the north shore population at any given time. Many studies have demonstrated that the prey eaten varies with the length of the smelt larvae (e.g., Nellbring 1989; Dauvin and Dodson 1990). For the St. Lawrence middle estuary, Dauvin and Dodson (1990) observed that calanoid copepods were the major prey item for all length-classes, but mysids were proportionately more abundant among large larvae (>30 mm) and cladocerans were proportionately more abundant in the intermediate class (25–30 mm). These prey preferences may contribute to reducing diet overlap among larvae of different sizes. Globally, these observations are congruent with a possible resource partitioning by time lag between breeding periods of the two populations in the middle estuary. This hypothesis, however, remains to be tested by more detailed studies on the trophic ecology of smelt larvae belonging to each population.

Member–vagrant hypothesis versus historical factors

Whatever the explanation for the lack of spatial segregation of smelt larval populations in the middle estuary, their sympatric occurrence is in conflict with current theories predicting population richness in aquatic environments. Namely, the member–vagrant hypothesis (Sinclair 1988) predicts that the number of populations composing a given species will primarily be determined by the number of environmental settings (geographic, physical, oceanographic) to which (*i*) spawning adults are adapted to home and (*ii*) early life history is behaviourally adapted to permit retention. This implies that in situations where the distribution of the larvae is different from the distribution of the spawning sites, such as is the case with smelt in the middle estuary, the population structure should reflect the number of retention zones rather than the number of spawning sites (Baby et al. 1991). The middle estuary is characterized by a well-mixed zone in the upstream part and a partially stratified zone in the downstream part (El Sabh 1988). The well-mixed upstream part of the St. Lawrence middle estuary associated with the smelt nursery represents a unique oceanographic system (Dodson et al. 1989) within which smelt larvae are maintained by active and passive processes (Laprise and Dodson 1989*a*, 1989*b*). Furthermore, the general cyclonic circulation of the estuary permits the formation of strong transversal currents (Dodson et al. 1989) that promote the transport and mixing of the larvae of both populations across the estuary. As the mixing of the young life history stages reported in this study occurs in the middle estuary, the member–vagrant hypothesis would support the existence of a single population in that system.

The sympatric occurrence of both larval populations in a single retention zone therefore indicates that contemporary ecological settings may not be the most important mechanisms driving population divergence. It has been demonstrated that the two smelt populations of the middle estuary are derived from distinct glacial races that developed partial reproductive barriers (Bernatchez and Martin 1996; Bernatchez 1997). The fact that gene flow between them has been less than between any other anadromous ones studied in northeastern North America (e.g., Taylor and Bentzen 1993*a*; Bernatchez and Martin 1996) indicates that genetic divergence due to long-term geographic isolation may have been more important than contemporary ecological conditions in determining their divergence and persistence. As such, our results reemphasize the importance of considering not only ecological processes but also the evolutionary history to better understand the mechanisms determining a species' population structure.

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