

Assessment of Biodiversity of American Oyster (*Crassostrea virginica*) populations of Cape Breton, N.S. and the Maritimes

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by

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ABSTRACT

Vercaemer B., P. St-Onge, K. Spence, S. Gould and A. McIsaac, 2010. Assessment of biodiversity of American oyster (*Crassostrea virginica*) populations of Cape Breton, N.S. and the Maritimes. Can. Tech. Rep. Fish. Aquat. Sci. 2872: vi + 32 p.

Rejuvenation of depleted stocks through seeding and cultivation programs has been proposed for the Bras d'Or lake's oysters (*Crassostrea virginica*) by several stakeholders including DFO and First Nations. Maintenance of genetic diversity in natural or re-seeded beds is critical to the long-term sustainability of the oyster industry, as genetic diversity is essential in providing the Bras d'Or lake's populations the resilience to adapt to both environmental changes, including diseases outbreaks, and fishing pressure. By using molecular tools, such as microsatellite DNA markers, to construct a database of genetic profiles of populations, it is possible to make informed management decisions, which will maximize the potential for sustained genetic diversity over time for both wild and aquaculture populations. The objective of this work was to assess biodiversity and genetic profiles of 25 oyster populations in Atlantic Canada with emphasis on Bras d'Or lake's populations in Cape Breton where 10 sites were assessed as well for temporal variation. Oysters originating from the Bras d'Or lake are genetically different from all other oysters originating from Atlantic Canada.

RÉSUMÉ

Vercaemer B., P. St-Onge, K. Spence, S. Gould and A. McIsaac, 2010. Assessment of biodiversity of American oyster (*Crassostrea virginica*) populations of Cape Breton, N.S. and the Maritimes. Can. Tech. Rep. Fish. Aquat. Sci. 2872: vi + 32 p.

Le rajeunissement des stocks par des programmes d'ensemencement et de culture a été proposé pour les huîtres (*Crassostrea virginica*) du lac Bras d'Or par plusieurs parties prenantes, y compris le MPO et les Premières Nations. Le maintien de la diversité génétique des stocks naturels ou réensemencés est essentiel à la viabilité à long terme de l'industrie de l'huître. La diversité génétique est essentielle pour donner aux populations du lac Bras d'Or la résilience de s'adapter à la fois aux changements de l'environnement, y compris aux maladies, et à la pression de pêche. En utilisant des outils moléculaires, tels que les marqueurs microsatellites, pour construire une base de données des profils génétiques des populations, il est possible de prendre des décisions de gestion éclairées, ce qui permettra de maximiser le potentiel de la diversité génétique au fil du temps pour les populations sauvages et aquacoles. L'objectif de ce travail était d'évaluer la biodiversité et les profils génétiques de 25 populations d'huîtres au Canada atlantique en mettant l'accent sur les populations du lac Bras d'Or au Cap-Breton, où 10 sites ont été aussi évalués pour la variation temporelle. Les huîtres en provenance du lac Bras d'Or sont génétiquement différentes de toutes les autres huîtres en provenance du Canada atlantique.

NIKANATUEK

Vercaemer B., P. St-Onge, K. Spence, S. Gould and A. McIsaac, 2010. Assessment of biodiversity of American oyster (*Crassostrea virginica*) populations of Cape Breton, N.S. and the Maritimes. Can. Tech. Rep. Fish. Aquat. Sci. 2872: vi + 32 p.

Alsusultijik we’kaw DFO aqq L’nu’k kisutmi’tij ta’n tli-ila’laten mn’tmu’k ewe’wmumkl teli-sika’ta’tij aqq ta’n teli-kwenuj mn’tmu’k (*Crassostrea virginica*) Pitu’poq. Keknue’k ta’n teli-istueyuj mn’tmu’k wjit mn’tmukemk elmi’knik, mita ta’n teli-istuo’ltijik keknu’e’k wjit ta’n teli-qamutmi’tij pilua’si’k Pitu’poq wsitqamuey we’kaw ksnukwaqnn ika’q kisna awsami-ktanujik . Ewe’wasikl apje’jkl lukwaqne’l, staqa nike’ microsatellite’l DNA keknu’a’tekek, eltumk maw-wikasik ta’n wetapeksulti’tij mn’tmu’k kulaman menaqaj kisutten ta’n ketu’tla’tekemk, kulaman elmi’knik kisi-we’wten ula kina’masuti wjit etlikwenikemk samqwaniktuk aqq elt ta’n tl-maliamatan seyo’ltijik mn’tmu’k. Ula lukwaqn iloqaptmi’tij te’sijik mn’tmu’k Pitu’poq etek Unama’kik ta’n tett newtiska’ql keknu’e’kl mn’tmu’k etlikuti’tij wjit tel-pilu’a’sik te’sipunqekl. Mn’tmu’k tle’k Pitu’poq piluamuksultijik jel mu Apaqtukewaq mn’tmu’k.

INTRODUCTION

The American oyster, *Crassostrea virginica* (Gmelin 1791), an ecologically and economically important species in Atlantic Canada, has experienced major declines in natural populations in Cape Breton, NS, for a variety of reasons including overfishing, illegal harvesting, loss/degradation of habitat and more recently diseases (e.g. MSX in 2002, Malpeque disease in 2007). Some oyster beds have been almost completely depleted while others show poor recruitment. Also, historical factors contribute to the problems such as small lease sizes and a seasonal market.

Rejuvenation of the depleted private leases or public beds through seeding and cultivation programs has been proposed for the Bras d'Or lake, a land-locked estuarine system in Cape Breton, by several stakeholders including DFO and First Nations, historically the main fishers. Mi'kmaw fishers have fished the oyster for food and ceremonial purposes for centuries and are currently taking part in the commercial fisheries and aquaculture operations. Maintenance of genetic diversity in natural or re-seeded beds is critical to the long-term sustainability of the oyster industry, as genetic diversity is essential in providing the Bras d'Or populations the resilience to adapt to both environmental changes and fishing pressure. By using molecular tools to construct a database of genetic profiles of populations, it is possible to make informed management decisions, which will maximize the potential for sustained genetic diversity over time. Oyster beds along the shores of the Bras d'Or lake are discontinuous in nature and mostly occur in small bays and coves where larval retention could be high.

The Bras d'Or lake is a unique brackish water body where importation of oysters from outside the Bras d'Or lake have been prohibited since the onset of Malpeque disease in the 1950s. In addition, closure of shellfish beds around the Bras d'Or lake has continued in recent years despite public and municipal awareness about sewage contamination. Environment Canada under the Shellfish Harvesting Area Water Classification Program as part of the Canadian Shellfish Sanitation Program, temporarily or permanently closes certain areas because of bacterial contamination. Closed areas could also be created for conservation reasons such as insufficient spawning stock biomass. Illegal harvesting of oysters in closed areas is not uncommon and thefts from aquaculture operations or private leases have been reported as well. The ability to identify the origin of illegally-harvested oysters using a database of genetic profiles could be an important tool for the successful prosecution of poachers. Microsatellite DNA markers profiling has been used intensively and has proven proven to be a useful tool not only for

determining conservation strategies for endangered and/or commercially exploited species but also for fisheries forensics. These molecular markers provide a way to uniquely assign individuals to populations for management and enforcement purposes.

The overall objective of this work was to assess biodiversity and genetic profiles of oyster populations in Atlantic Canada with emphasis on Cape Breton populations.

MATERIALS AND METHODS

Samples collection

Between 2003 and 2005, 41 samples of American oysters (*Crassostrea virginica*), at least one year old, were collected at 25 sites across Atlantic Canada: 5 in New Brunswick, 3 in Prince Edward Island and 17 in Nova Scotia (Figure 1; Table 1). Oysters were collected from water depths between 0.5 -2.5 m by hand or rake depending on the depth. Of the 17 Nova Scotia sites, 10 were situated within the Bras d'Or lake, 3 on the periphery of Cape Breton Island and 4 others on mainland Nova Scotia. In order to assess temporal variation, 6 sites of the Bras d'Or lake (BHI, GC, WB, NB, CC and CI) were sampled every year between 2003 and 2005 while 4 others, 2 from the Bras d'Or and 2 from New Brunswick (WT, BB, RB and SS) were sampled twice during those years (Table 1). Morphological and physiological information of collected oysters within the Bras d'Or lake can be found in Gould (2004). All remaining sites were only sampled once, either in 2003, 2004 or 2005. Sample size ranged between 35 and 100 individuals (Table 1). Upon arrival at the laboratory, oysters were shucked and 0.5 g of mantle and/or adductor muscle was preserved in 100% ethanol until DNA extraction.

DNA extraction and Genotyping

Genomic DNA was extracted from approximately 25 mg of mantle and gill tissue using a Qiagen DNeasy tissue kit (Qiagen Inc., Santa Clara, CA). A total of 12 published microsatellite loci were evaluated and 9 were utilized: 2 dinucleotides (Cvi-1g8 and Cvi-2g14) (Reece et al., 2004), 2 trinucleotides (Cvi-6, Cvi-8, Cvi-9) (Brown et al., 2000) and 4 tetranucleotides (Cvi-12, Cvi-2i4, Cvi-2j24 and Cvi-2i23) (Reece et al., 2004). Trinucleotides and tetranucleotides are preferable to minimize scoring errors. 2 µl of DNA from each individual were used for PCR amplification (PTC-200 thermal cycler, MJ Research Inc., Watertown, MA) and fragment analysis was performed using a MJ BaseStation platform (MJ Research Inc., Watertown, MA). Microsatellite characteristics and PCR conditions were optimized from Brown et al. (2000) and Reece et al. (2004) (Table 2).

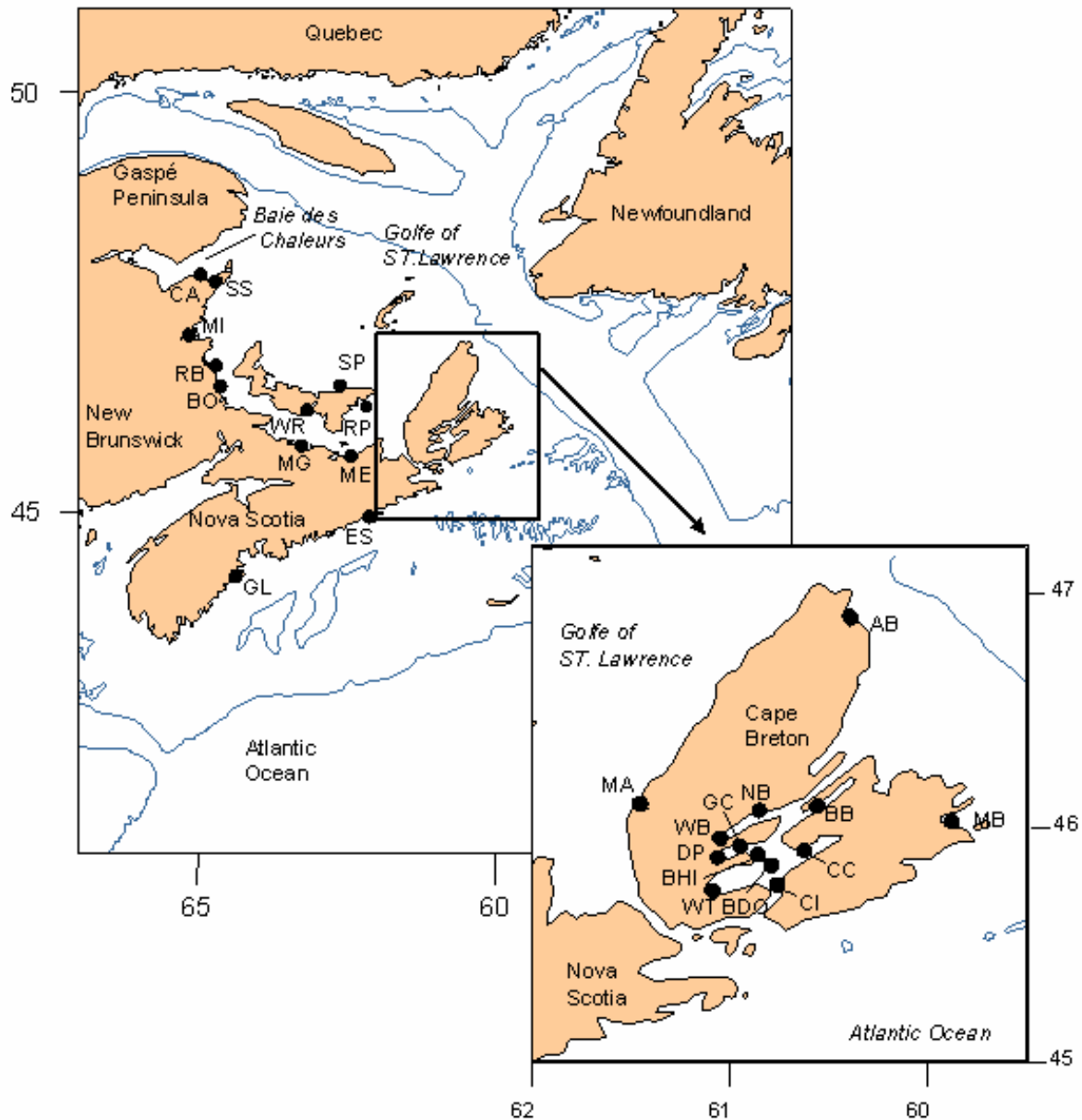


Figure 1. Geographical map of Atlantic Canada and Gulf of St. Lawrence showing all 25 sampled sites. Nova Scotia sites: BDO: Bras d'Or Lake, WT: West Bay, BHI: Big Harbour Island, GC: Gillis Cove, DP: Denys Pond, WB: Whycocomagh Bay, NB: Nyanza Bay, BB: Barachois Harbour, CC: Crane Cove, CI: Chapel Island, MB: Mira Bay, AB: Aspy Bay, MA: Mabou, GL: Goat Lake, ES: Eastern shore of NS, ME: Merigomish, MG: Malagash; PEI sites: RP: Red Point, WR: West River, SP: St-Peters; New Brunswick sites: BO: Bouctouche, RB: Richibucto Bay, MI: Miramichi, SS: St-Simon, CA: Caraquet.

Table 1. Sampling locations and respective letter codes along with numbers of *C. virginica* individuals sampled during each year of the study. The different color shades indicate the origin of the different sampled localities (Cape Breton, NS, rest of Nova Scotia, PEI and NB).

Population	Code	Number of individuals sampled per year (<i>N</i>)			
		2003	2004	2005	Total
1. Bras d'Or Lake, NS	BDO	35	---	---	35
2. West Bay, NS	WT	---	96	98	194
3. Big Harbour Island, NS	BHI	90	98	99	287
4. Gillis Cove, NS	GC	88	100	88	276
5. Denys Pond, NS	DP	61	---	---	61
6. Whycocomagh Bay, NS	WB	96	100	98	294
7. Nyanza Bay, NS	NB	96	100	100	296
8. Barachois Harbour, NS	BB	---	100	95	195
9. Crane Cove, NS	CC	94	98	100	292
10. Chapel Island, NS	CI	95	100	100	295
11. Mira Bay, NS	MB	---	---	60	60
12. Aspy Bay, NS	AB	---	---	59	59
13. Mabou, NS	MA	---	---	60	60
14. Goat Lake, NS	GL	---	---	85	85
15. Eastern shore of NS	ES	---	---	55	55
16. Merigomish, NS	ME	---	100	---	100
17. Malagash, NS	MG	---	100	---	100
18. Red Point, PEI	RP	---	99	---	99
19. West River, PEI	WR	---	---	92	92
20. St-Peters, PEI	SP	56	---	---	56
21. Bouctouche, NB	BO	---	---	58	58
22. Richibucto Bay, NB	RB	48	---	99	147
23. Miramichi, NB	MI	---	---	57	57
24. St-Simon, NB	SS	---	100	60	160
25. Caraquet, NB	CA	---	---	56	56
Total		759	1191	1519	3469

Table 2. Characteristics of the 9 microsatellite markers used to detect genetic variation and population structure of *Crassostrea virginica* populations originating from Atlantic Canada.

Name of locus	Type of locus	Repeat motif	Annealing temp. (°C)	Source	A (n) ¹
Cvi-6	Trinucleotide	(GAT) ₁₇	50	Brown et al. (2000)	15 (44)
Cvi-8	Trinucleotide complex	[(CAAA) ₂ (CAA)] ₂	55	Brown et al. (2000)	14 (40)
Cvi-9	Trinucleotide	(CAT) ₁₄	55	Brown et al. (2000)	14 (40)
Cvi-12	Tetranucleotide complex	(CAAA) ₆ (CAGAAAAA) (CAAA) ₄	55	Brown et al. (2000)	10 (40)
Cvi-1g8	Dinucleotide	(CT) ₁₆	46	Reece et al. (2004)	14 (60)
Cvi-2i4	Tetranucleotide	(GATT) ₂₄	46	Reece et al. (2004)	11 (60)
Cvi-2g14	Dinucleotide complex	(TC) ₄ (TT)(TC) ₂₀	52	Reese et al. (2004)	6 (60)
Cvi-2j24	Tetranucleotide	(CAAT) ₉	53	Reece et al. (2004)	4 (60)
Cvi-2i23	Tetranucleotide	(GTTT) ₇	51.5	Reece et al. (2004)	10 (60)

¹ A represents number of alleles observed in 40-44 individuals from native populations in Virginia (n=40) and Connecticut (n=44) (Brown et al. 2000) and 60 individuals from 10 hatchery-produced reference families (Reece et al. 2004)

Analysis of genomic DNA was performed with CARTOGRAPHER (MJ Research Inc., Watertown, MA) to assess raw scores of alleles. If genotypes were not properly expressed for certain loci, they were accordingly re-amplified. At the end of each year, a systematic re-amplification of approximately 10 to 20% of individuals was carried out to validate original raw scores. Raw scores were then thoroughly scrutinized to eliminate allele-scoring errors, as it is often the case with platform automation (Amos et al., 2006). This was done for all 9 loci datasets by randomly selecting 20 individual genotypes of each population and processing them in the FLEXIBIN macro (Amos et al., 2006). Final allele identity for all genotypes was based on resulting allele intervals generated by FLEXIBIN. Once alleles were accordingly scored, the whole database was then processed with MICRO-CHECKER (van Oosterhout et al., 2004) to determine whether allele identities corresponded to the known periodicity of markers and to assess the probability of null (non-amplifying) alleles and stuttering patterns for each combination of loci and populations.

Statistical analyses

Descriptive statistics and Hardy-Weinberg equilibrium

The number of detected alleles and allelic richness for each combination of loci and populations were assessed with FSTAT v.2.9.3 (Goudet, 2001). The number of private alleles for each combination of loci and populations were determined from the summary output file generated by CONVERT v.1.31 (Glaubitz, 2004). Observed heterozygosity and unbiased expected heterozygosity (Nei, 1978) were determined with GENETIX v.4.05 (Belkhir et al., 2001). Inbreeding coefficients (F_{IS}) and associated P values were assessed using GENEPOP v.4.0 (Raymond and Rousset, 1995) while the analysis of linkage disequilibrium between pairs of loci was performed with FSTAT v.2.9.3 (Goudet, 2001) with a significance level of 5% and 0.1389% after Bonferroni correction and 7020 permutations.

Temporal genetic variation

Genetic structure can be evaluated by testing the F_{ST} estimate (Weir and Cockerham, 1984), which measures the reduction in the average proportion of heterozygous genotypes among samples. A first pair-wise F_{ST} analysis with all 41 samples was performed with FSTAT v. 2.9.3 (Goudet, 2001). Interpretation of temporal genetic differences was only completed for pairs of samples originating from the 10 sites which were sampled more than once over several years (WT, BHI, GC, WB, NB, BB, CC, CI, RB and CA) (Table 3). Significance level between samples was set at a nominal level of 5% and Bonferroni-adjusted to 0.0061% after 16,400 permutations. Samples that yielded significant differences were all kept for further analyses of geographical genetic variation. In the absence of significant differences, only the most recent samples from each population were kept for further analyses.

Geographical population structure

Upon completion of analyses pertaining to temporal variations, 27 samples out of the original 41 were kept for testing geographical population structure (Table 3). The population structure of *C. virginica* in Atlantic Canada was mainly described with 4 series of analyses. A pairwise F_{ST} analysis with all remaining 27 samples was firstly performed with FSTAT v.2.9.3 (Goudet, 2001). Significance level between samples was

set at a nominal level of 5% and Bonferroni-adjusted to 0.0142% after 7020 permutations. Secondly, genic differentiation for each population pair was analyzed with GENEPOP v.4.0 (Raymond and Rousset, 1995) using a Fisher's exact G test with Markov chain parameters set at 10,000 de-memorization steps and 100 batches with 5,000 iterations per batch. Significance of *P* values was once again set at a nominal level of 5% with a Bonferroni correction of 0.0142%. Thirdly, the Bayesian clustering method (Pritchard et al., 2000; Falush et al., 2003) was used to presume population structure by probabilistically assigning all 2,118 individuals to an inferred number of populations (*K*) using a model-based clustering method. This software works under the assumption that loci within populations are at Hardy-Weinberg and linkage equilibrium. Parameters for analysis allowed admixture of individuals and correlations between genotypes with a burn-in period of 10,000 steps followed by 10,000 Markov chain Monte Carlo repetitions (MCMC). Ten simulations were carried out for each *K* ranging between 2 and 20. The most probable number of clusters was determined by computing the ad hoc statistic ΔK (Evanno et al., 2005) with the estimated log probabilities of data obtained from STRUCTURE. Probabilities of individual and population membership to each inferred cluster were consequently taken from a randomly selected STRUCTURE run carried out with the most probable value of *K*. Finally, genotypes were analyzed with PHYLIP v.3.67 (Felsenstein, 2005) to create a neighbor-joining tree based on pairwise Cavalli-Sforza and Edwards (1967) genetic chord distances (DCE) with degree of support for each branch assessed with a bootstrap analysis of 1,000 re-samplings over loci.

Table 3. Results of a preliminary pairwise F_{ST} analysis of temporal genetic variation for 10 of the 25 *C. virginica* populations that were sampled over either 2 or 3 years (total of 41 samples). Significance (*) of P -values denoting temporal genetic variation was set at a nominal level of 5% with a Bonferroni-adjusted level of 0.000061 for multiple comparisons after 16400 permutations. Samples that were kept for further geographical analysis of genetic variation are listed with specified letter coding in brackets.

Population	Code	Number of individuals sampled per year (N)			Significant (*) or non-significant (NS) temporal variation			Samples kept for further geographical analysis
		2003	2004	2005	2003 & 2004	2003 & 2005	2004 & 2005	
1. Bras d'Or Lake, NS	BDO	35	---	---	---	---	---	2003
2. West Bay, NS	WT	---	96	98	---	---	NS	2005
3. Big Harbour Island, NS	BHI	90	98	99	NS	NS	NS	2005
4. Gillis Cove, NS	GC	88	100	88	NS	NS	NS	2005
5. Denys Pond, NS	DP	61	---	---	---	---	---	2003
6. Whycocomagh Bay, NS	WB	96	100	98	NS	NS	NS	2005
7. Nyanza Bay, NS	NB	96	100	100	NS	NS	NS	2005
8. Barachois Harbour, NS	BB	---	100	95	---	---	NS	2005
9. Crane Cove, NS	CC	94	98	100	NS	NS	NS	2005
10. Chapel Island, NS	CI	95	100	100	NS	*	*	2004 (CI4) 2005 (CI5)
11. Mira Bay, NS	MB	---	---	60	---	---	---	2005
12. Aspy Bay, NS	AB	---	---	59	---	---	---	2005
13. Mabou, NS	MA	---	---	60	---	---	---	2005
14. Goat Lake, NS	GL	---	---	85	---	---	---	2005
15. Eastern shore of NS	ES	---	---	55	---	---	---	2005
16. Merigomish, NS	ME	---	100	---	---	---	---	2004
17. Malagash, NS	MG	---	100	---	---	---	---	2004
18. Red Point, PEI	RP	---	99	---	---	---	---	2004
19. West River, PEI	WR	---	---	92	---	---	---	2005
20. St-Peters, PEI	SP	56	---	---	---	---	---	2003
21. Bouctouche, NB	BO	---	---	58	---	---	---	2005
22. Richibucto Bay, NB	RB	48	---	99	---	*	---	2003 (RB3) 2005 (RB5)
23. Miramichi, NB	MI	---	---	57	---	---	---	2005
24. St-Simon, NB	SS	---	100	60	---	---	NS	2005
25. Caraquet, NB	CA	---	---	56	---	---	---	2005

RESULTS AND DISCUSSION

Genotyping, descriptive statistics and Hardy-Weinberg equilibrium

Once the final allele scoring with the FLEXIBIN macro was completed, an analysis of data with MICRO-CHECKER showed that there were no significant departures in allele identities as they all matched the known repeat motifs and periodicities of selected loci (Brown et al., 2000, Reese et al., 2004). However, the program showed probable evidence of null alleles, as a general excess of homozygotes was found in several combinations of loci and populations. The lowest and highest amounts of homozygote excess were respectively detected in loci Cvi-12 (4 out of 41 samples) and Cvi-1g8 (100% of samples). Cvi-12, along with Cvi-9, was also a locus which conformed approximately to Hardy-Weinberg equilibrium (HWE) in Brown et al. (2000). Null allele detection pertaining to populations ranged between 3 (BDO, GL, MI and SS) and 8 (CI5 and RB5) of all 9 loci. The program also indicated that some stuttering may have resulted in scoring errors, mostly for loci Cvi-8 (35 of 41 samples), Cvi-1g8 (25 samples), Cvi-6 (18 samples) and Cvi-2i4 (16 samples). All remaining loci showed stuttering in less than 9 populations with Cvi-9 showing none. However, no evidence of large allele dropout was found at any locus.

Correction of genotype data for null alleles could have been attempted (Chapuis and Estoup, 2007) but is nonetheless either questionable or does not change the values of estimates of genetic variation (Galindo-Sanchez, 2008). Alternatively, in a recent study, after three re-amplification steps with modified primer sets, all microsatellite loci were corrected for null alleles in a pearl oyster species (Lemer et al., 2011) and, once corrected, all populations appeared at HWE, demonstrating that null alleles were responsible for the initial disequilibrium of the populations. However, this corrective step-by-step method involves expensive and time consuming re-amplification of homozygotes and null individuals with redesigned primer pair combinations, which is unwarranted in large scale population studies, such as the present one. In this context, the final allele scores obtained using the software FLEXIBIN were deemed acceptable. In addition, no significant linkage disequilibrium was detected. Therefore, it was assumed that the nine loci were independent genetic markers.

Descriptive statistics of all 25 localities are summarized in Appendix 1. The number of alleles (allelic richness) in a population is a fundamental measure of genetic variation. The total number of alleles per locus ranged between 11 (Cvi-8) and 43 (Cvi-

2i23) while the mean number of alleles per population ranged between 6.11 (Cvi-8) and 23.07 (Cvi-2g14) (Appendix 1). Interestingly, two samples (WT: West Bay, NS and SS: St-Simon, NB) had maximal numbers of alleles at 3 different loci. However, allelic richness is biased by the effect of sample size: large samples are expected to have more alleles. Interpretation of population differences are therefore more accurate if based upon allelic richness corrected for differences in sample size with the rarefaction method (Leberg, 2002 and references therein). Average allelic richness per locus (corrected for sample size $N=30$) ranged more than 3-fold between loci showing the lowest and highest richness values (respectively Cvi-8 and Cvi-2g14) (Appendix 1). SS (St-Simon, NB, Gulf of St. Lawrence) was found to be the most diverse sample (14.3) while GL was observed to be the least diverse sample (10.1), with a difference of more than 4 alleles between them. The GL population was actually collected from Goat lake, a deep tidal lake-like estuary, isolated on the Atlantic shore of NS and considered to be a refuge (Spares and Dadswell, 2001). Private alleles are alleles found in only one of several populations in a larger collection. In the present study, there were several instances of private alleles across samples and loci and most private alleles were found in the more diverse populations from the Gulf (Appendix 1). The two populations with the highest amount of private alleles were ME and MG (Merigomish and Malagash, northern shore NS, southern Gulf), each comprising 17.39% of all observed private alleles. The Bras d'Or populations had no private alleles, indicative of intense gene flow with little outside contact.

Total observed within-sample heterozygosity H_O for all loci averaged 0.680, ranging from 0.366 (Cvi-8) to 0.870 (Cvi-2g14) and between 0.640 (SP) and 0.762 (SS) (Appendix 1). Total expected and unbiased within-sample heterozygosity H_E (Nei, 1978) averaged a value of 0.822, ranging from 0.596 (Cvi-8) to 0.917 (Cvi-1g8) and between 0.796 (MB) and 0.843 (SS), a range showing in fact similar levels of heterozygosity between localities. The observed heterozygosity H_O was substantially lower than the expected values H_E for most loci in nearly all samples, which is suggestive of the presence of null alleles and is a typical observation in bivalves (McGoldrick et al. 2000; Hedgecock et al., 2004). However the values reported here are within the ranges reported for other natural populations of *C. virginica* ($H_E \approx 0.80$, Brown et al., 2000; $H_E=0.69 - 0.97$, Carlsson and Reece 2007; $H_E=0.800-0.845$, Galindo-Sanchez, 2008).

Not surprisingly, the F_{IS} values were positive, high and significant for several loci, including 25 of all 27 samples for Cvi-1g8, 24 for Cvi-6, 21 for Cvi-2i4 and 18 for Cvi-8. This indicates significant departures from HWE because of heterozygote deficits.

All remaining loci only had between 0 and 2 out of 27 sampled populations yielding significant F_{IS} values. The SP (St-Peters, PEI) locality, which is a sample taken from an aquaculture site, had six loci out of nine that yielded significant P values for F_{IS} while the BDO (Bras d'Or lake, NS), a truly natural population sample, showed none. For the great majority of population-locus combinations, positive F_{IS} values were encountered which are usually attributed to sampling from across subdivided populations (e.g. Wahlund effect), inbreeding, natural selection and/or the presence of null alleles. In this study, it seems unlikely that there was genetic patchiness or inbreeding within locations. In the present context of heavy mortalities due to diseases, natural selection may be at play, although it would have to be acting across all unlinked loci (Rose et al. 2006). It seems then that these large heterozygote deficits relative to Hardy-Weinberg expectations are best explained by the presence of null alleles.

These results seem to concur with other genetic studies of wild bivalve populations, including oysters such as *C. virginica* and *Crassostrea gigas*, which are known to be generally deficient in heterozygotes (McGoldrick et al. 2000, Reese et al. 2004, Rose et al. 2006, Carlsson and Reece 2007). In a recent population study of *C. virginica* from the state of Veracruz, Gulf of Mexico, Galino-Sanchez et al. (2008) found significant deviations from HWE at all 5 microsatellite loci in all 6 populations sampled and these were attributed to the presence of null alleles. Nonetheless, it has been argued that allelic richness may reflect more effectively a population's potential to respond to selection pressures over the long-term than would heterozygosity (Allendorf, 1986; Spencer et al., 2000).

Temporal genetic variation

Out of 25 sampled populations, 10 were sampled over several years and thus eligible to be screened for temporal genetic variation. The pair-wise F_{ST} analyses only confirmed the presence of significant temporal variation at the Chapel Island (CI) and Richibucto Bay (RB) locations. The CI sample collected in 2005 was significantly different from both CI samples collected in 2003 and 2004 (Table 3) but the collections from 2003 and 2004 were not different. The 2 most recent samples of CI (2004 and 2005) were thus kept for further geographical analyses of population structure and were respectively labelled CI4 and CI5. The RB sample collected in 2003 was also significantly different from the one collected in 2005 (Table 3). For this location, no collection was done in 2004. Both samples (respectively labelled RB3 and RB5) were thus kept for further analyses. As aforementioned, it is in the CI5 and RB5 samples that

homozygote excesses were mostly observed. The 8 other eligible populations did not show any temporal significant difference between samples.

Geographical population structure

Pair-wise F_{ST} P -values are presented in Appendix 2. It was shown that 234 sample pairs had significant P -values out of a possible 351 (66.67%). In total, 12 Cape Breton samples out of a possible 14 were shown to be significantly similar with each other on a genetic level, except for Mira Bay (MB) having occasional significant P - values, i.e. with Gillis Cove (GC) and Nyanza Bay (NB). The remaining 2 samples were restricted to Chapel Island collection 2005 (CI5) and Mabou (MA) which were nearly all significantly different from all other Cape Breton samples. Mabou is located on the western side of Cape Breton Island within the waters of the southern Gulf of St. Lawrence and Chapel Island is located on south-eastern shore of the Bras d'Or lake, furthest away from the two main northern channels that connects it with oceanic waters, but very close to the St. Peter's Canal, which offers intermittent passage between the Bras d'Or lake and the Atlantic through a system of locks.

A great majority of the 14 Cape Breton samples yielded significant P -values of F_{ST} when paired with the 4 remaining Nova Scotia samples (10 pairs with significant P values out of a possible 16), with the 3 samples from Prince Edward Island (27 pairs out of a possible 30) and with all 6 samples from New Brunswick (83 pairs out of possible 90). The 2 samples originating from the Atlantic shore of mainland Nova Scotia, Eastern Shore (ES) and Goat lake (GL) were shown to be genetically different from each other and from all 8 samples originating from Prince Edward Island and New Brunswick. However, sampled oysters from the northern part of Nova Scotia (ME Merigomish and MG Malagash) only genetically differed with these 8 aforementioned samples at three different occasions out of a possible 16 pairs. Since ME and MG are directly associated with the Northumberland Strait, these results are suggestive of a close genetic link between oysters originating from these locations and those coming from Prince Edward Island and New Brunswick. It is also important to note that nearly all oysters sampled from these two latter provinces were genetically similar with each other, except for the RB5 sample that yielded significant P values with all the others.

As opposed to the pairwise F_{ST} analysis that is useful for testing whether differences in heterozygosities are significantly different from one population to another, the Fisher's exact G test rather discriminates whether populations have significantly

different allelic diversities from each other. The P -values that originated from this latter test are also presented in Appendix 2. It was shown that 273 sample pairs out of a possible 351 (77.78%) significantly differed from each other. Three samples originating from Cape Breton (CI5: Chapel Island 2005, MB: Mira Bay and MA: Mabou) showed extensive numbers of significant P values with all the other Cape Breton samples (e.g. Bras d'Or lake localities and Aspy Bay), which were similar to each other, with the exception of a few significant P values. All remaining sampled oysters originating from other parts of Nova Scotia, Prince Edward Island and New Brunswick had significantly different allelic diversities than oysters sampled in Cape Breton. Furthermore, only the RB5 and MI (Richibucto Bay 2005 and Miramichi, NB) samples yielded a majority of significantly different allelic diversities when paired with the remaining samples of the Gulf of St. Lawrence, which were all significantly similar to each other.

Several iterations of different values of K with the STRUCTURE program were necessary to detect the most probable number of clusters (K) that best explained the membership of each of the 2118 sampled oysters to an inferred number of clusters assuming Hardy-Weinberg and linkage equilibriums. This type of analysis was determined with the help of the ΔK graphical method of Evanno et al. (2005) since raw results from STRUCTURE were inconclusive because of high variability between iterations. It was shown that assignment of individual membership was best explained with 4 clusters since plotting of ΔK in relation to $K=4$ showed the highest value (Figure 2).

A random iteration with $K= 4$ was taken from STRUCTURE and is graphically represented in Figure 3. All samples from Cape Breton except Mabou (MA) seem to be linked with similar patterns of color, i.e. green and yellow. Different color patterns (i.e. blue and red) were detected for samples coming from the other parts of Nova Scotia, including those from Prince Edward Island and New Brunswick, except for oysters from RB5, which were predominantly assigned to the blue cluster. More information pertaining to membership probabilities of populations to each 4 inferred clusters are presented in Table 4. This confirms how oysters originating from Cape Breton are genetically different from all other oysters originating from Atlantic Canada. It also detected that the Chapel Island collection of 2005 is somewhat different from the Bras d'Or ensemble and is raising some question regarding this particular sampling.

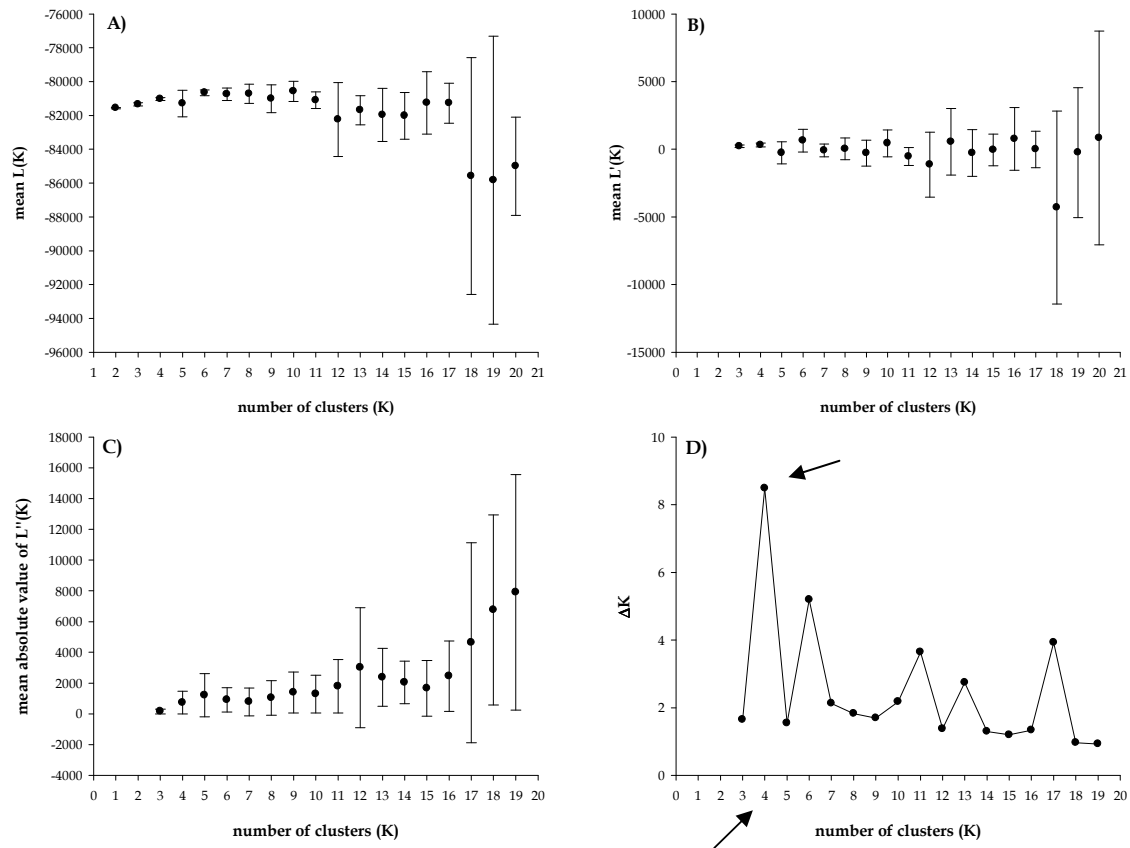


Figure 2. Assessment of the most probable number of clusters (K) explaining the genetic structure of 2118 individuals from 27 *C. virginica* populations sampled across the Gulf of St. Lawrence and analyzed with 9 microsatellite loci using the ΔK graphical method developed by Evanno et al. (2005). A) Mean (\pm SD) posterior probability of data (also known as LnP(D) or Pr(X | K)) for given values of K as assessed by the STRUCTURE software (Pritchard et al., 2000, Falush et al., 2003) after a burn-in period of 10,000 steps followed by 10,000 Markov chain Monte Carlo (MCMC) repetitions under an admixture and correlated model. B) Mean rate (\pm SD) of change of the likelihood distribution (also known as $L'(K)$) for given K values. C) Mean (\pm SD) absolute values of the second order rate of change of the likelihood distribution (also known as $L''(K)$) for given K values. D) ΔK for given K values. Arrows point to the greatest value of ΔK and the most probable number of clusters (K = 4) explaining the population structure of all 2118 sampled individuals under Hardy-Weinberg and linkage equilibrium.

The population structure detected in the previous analyses was further validated with the analysis of Cavalli-Sforza and Edwards (1967) genetic chord distances measured between samples. These distance measures were used in building a neighbour-joining tree which is presented in Figure 4.

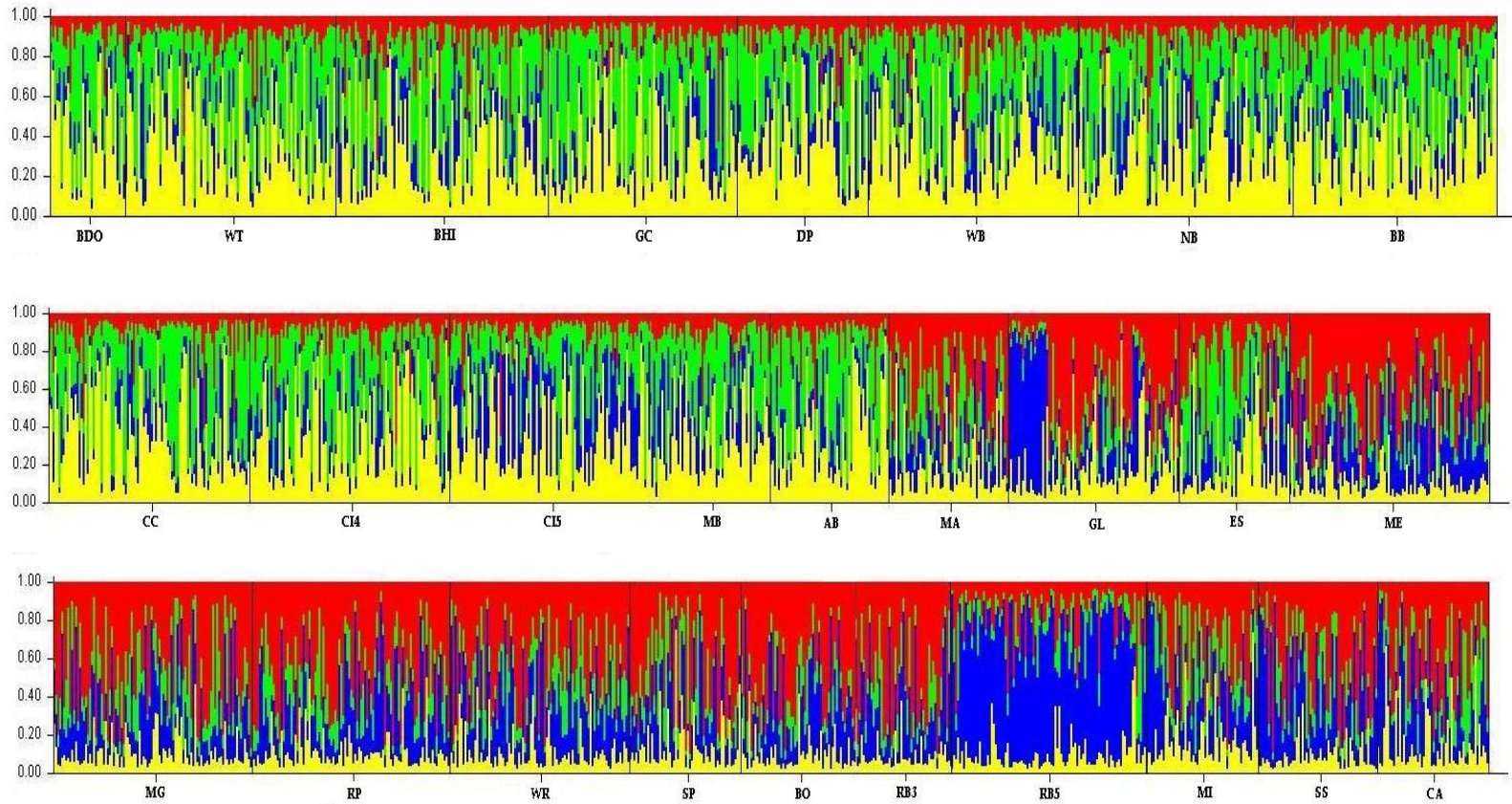


Figure 3. Summary plot of the 2118 *C. virginica* individual coefficients of membership to the most probable number of groups ($K = 4$) estimated with STRUCTURE software and the ΔK graphical method of Evanno et al. (2005) analyzed with 9 microsatellite markers for 27 populations sampled across the Gulf of St. Lawrence. Nova Scotia sites: BDO: Bras d'Or Lake, WT: West Bay, BHI: Big Harbour Island, GC: Gillis Cove, DP: Denys Pond, WB: Whycocomagh Bay, NB: Nyanza Bay, BB: Barachois Harbour, CC: Crane Cove, CI: Chapel Island, MB: Mira Bay, AB: Aspy Bay, MA: Mabou, GL: Goat Lake, ES: Eastern shore of NS, ME: Merigomish, MG: Malagash; PEI sites: RP: Red Point, WR: West River, SP: St-Peters; New Brunswick sites: BO: Bouctouche, RB: Richibucto Bay, MI: Miramichi, SS: St-Simon, CA: Caraquet.

Table 4. Probabilities of membership to each of the 4 detected clusters (K = 4) are finally presented with the two most probable clusters for each population highlighted in bold font. Probabilities were computed with STRUCTURE.

Population	Code	Probabilities of membership to each inferred cluster			
		1	2	3	4
1. Bras d'Or Lake, NS	BDO	0.101	0.348	0.127	0.424
2. West Bay, NS	WT	0.108	0.351	0.135	0.406
3. Big Harbour Island, NS	BHI	0.138	0.338	0.168	0.356
4. Gillis Cove, NS	GC	0.108	0.372	0.150	0.370
5. Denys Pond, NS	DP	0.118	0.313	0.160	0.409
6. Whycocomagh Bay, NS	WB	0.124	0.322	0.166	0.387
7. Nyanza Bay, NS	NB	0.121	0.330	0.172	0.378
8. Barachois Harbour, NS	BB	0.115	0.343	0.142	0.400
9. Crane Cove, NS	CC	0.113	0.356	0.154	0.376
10. Chapel Island, NS	CI 2004	0.125	0.348	0.146	0.381
	CI 2005	0.103	0.228	0.287	0.382
11. Mira Bay, NS	MB	0.114	0.319	0.208	0.358
12. Aspy Bay, NS	AB	0.127	0.363	0.151	0.359
13. Mabou, NS	MA	0.403	0.212	0.190	0.194
14. Goat Lake, NS	GL	0.393	0.106	0.305	0.196
15. Eastern shore of NS	ES	0.237	0.323	0.203	0.237
16. Merigomish, NS	ME	0.479	0.146	0.256	0.119
17. Malagash, NS	MG	0.468	0.177	0.242	0.114
18. Red Point, PEI	RP	0.476	0.152	0.259	0.113
19. West River, PEI	WR	0.434	0.161	0.288	0.118
20. St-Peters, PEI	SP	0.465	0.162	0.224	0.148
21. Bouctouche, NB	BO	0.515	0.118	0.264	0.103
22. Richibucto Bay, NB	RB 2003	0.509	0.138	0.258	0.095
	RI 2005	0.168	0.107	0.622	0.103
23. Miramichi, NB	MI	0.320	0.189	0.318	0.173
24. St-Simon, NB	SS	0.428	0.136	0.342	0.094
25. Caraquet, NB	CA	0.389	0.173	0.280	0.157

After a bootstrap analysis of 1000 re-samplings over loci, all samples originating from the Cape Breton except Mabou (MA) were clustered together 95.2% of the time and the Eastern Shore (ES) sample was included in the same cluster 85.6% of the time. Other clusters consist of the pairing of the Miramichi (MI) and Richibucto 2005 (RB5) collection samples in 69.7% of re-samplings and of the grouping of the rest of the southern Gulf of St. Lawrence (MG, SP, RP, ME, RB3, BO, WR and SS) in 77.6% of re-samplings over loci. Surprisingly, the sample from Goat Lake (GL) clusters with 2 samples from the southern Gulf of St. Lawrence, Caraquet (CA) and Mabou (MA). All remaining bootstrap values were below 50.0% and were not included in the final neighbour-joining tree. These results support fairly well the results observed with the pair-wise F_{ST} and Fisher's exact G test analyses.

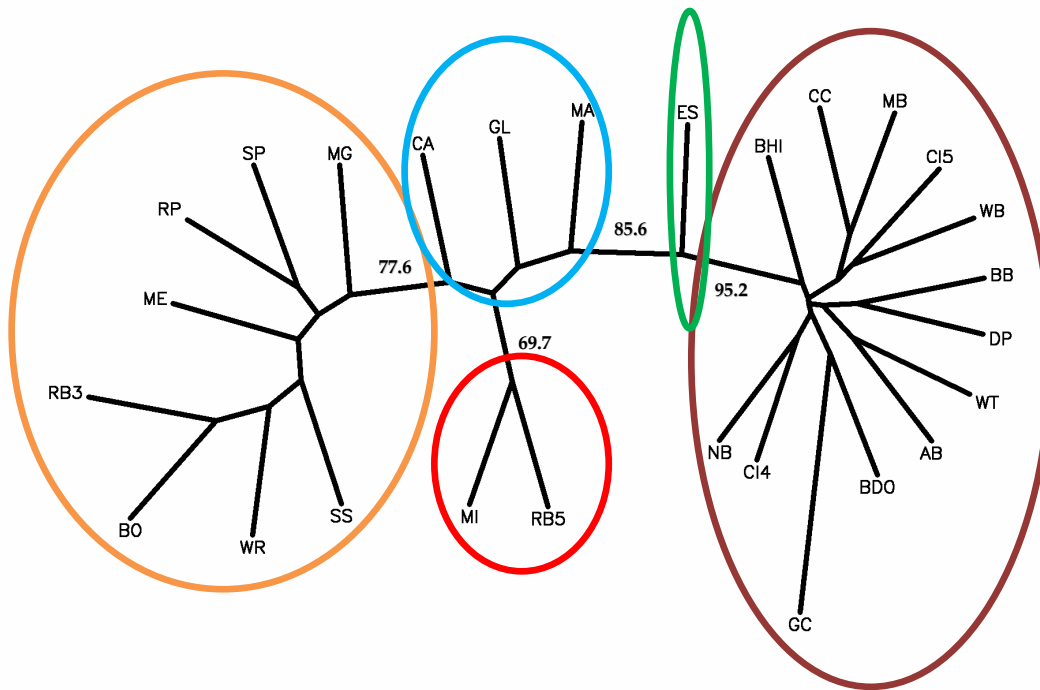


Figure 4. Neighbor-joining tree based on pairwise Cavalli-Sforza and Edwards (1967) genetic chord distances. Bootstrap values (in %) indicate degree of support for each branch after 1000 re-samplings over loci. Nova Scotia sites: BDO: Bras d’Or Lake, WT: West Bay, BHI: Big Harbour Island, GC: Gillis Cove, DP: Denys Pond, WB: Whycocomagh Bay, NB: Nyanza Bay, BB: Barachois Harbour, CC: Crane Cove, CI: Chapel Island, MB: Mira Bay, AB: Aspy Bay, MA: Mabou, GL: Goat Lake, ES: Eastern shore of NS, ME: Merigomish, MG: Malagash; PEI sites: RP: Red Point, WR: West River, SP: St-Peters; New Brunswick sites: BO: Bouctouche, RB: Richibucto Bay, MI: Miramichi, SS: St-Simon, CA: Caraquet.

The four series of analysis above reasonably agree to form a spatial structure of Atlantic Canada oyster populations. The Bras d’Or populations sampled in this study, along with Aspy Bay and to some degree the Mira Bay samples, were genetically very similar and different from the rest of the Atlantic shore of Nova Scotia and the Southern Gulf of Saint Lawrence, including Northern shore of Nova Scotia and Western Cape Breton (Mabou). The Eastern shore and Goat lake localities are also somewhat distinguishable from the others and this could be explained by their origin: the Eastern shore collection was probably from the remnant population of a past aquaculture operation and Goat lake in fact acts as an estuarine refugee for *C. virginica* on the South shore of Nova Scotia. However, the analysis showed that questions needed to be raised

regarding some collections such as the Chapel Island or Richibucto Bay, both in 2005. While anthropogenic factors (e.g. sampling biases) could be invoked, other life characteristics could explain this observed temporal variation. High fecundity and stochastic larval survival have been suggested by Hedgecock (1994) to potentially create genetic heterogeneity among cohorts. This can result in higher genetic heterogeneity over time in one location than what is observed spatially among populations. However, there was no difference in allelic richness, a very sensitive indicator, between years in Chapel Island, although it might have been the case for Richibucto Bay (see Appendix 1).

The planktonic phase of *C. virginica* lasts ~3 weeks suggesting that larvae could potentially travel hundreds of kilometres before settlement. This life history characteristic is conducive to high or long-range gene flow and thus provides demographic connections between different areas. The Bras d'Or lake presents restricted entrances and convoluted shores delimitating numerous bays and coves which would act as traps with respect to oyster recruitment if flushing rates were low. Nonetheless, the connectivity of the various bays within the lake was demonstrated by flushing times and estimates of particle distribution by Petrie and Bugden (1992) and the associated time scales varied from days to a few months. The shallow portions where the oyster beds occur are closer to the lower end of this time range and retentive local circulation in small bays is only partial.

Gene flow within the Bras d'Or lake and within the Gulf of St. Lawrence can be attributed not only to natural processes but also by human interference (past or current restocking effort and transfer for aquaculture purposes). In particular, to accelerate recovery of oysters stocks that would succumb to the Malpeque disease, mass transplantations of infected but disease tolerant oysters from PEI (Drinnan, 1967) have probably depopulated original naïve stocks in NS and NB and genetically homogenized populations of the southern Gulf of St. Lawrence. Also, oyster stocks were transferred repetitively from the Bras d'Or lake to Aspy Bay, which probably explains the inclusion of that location to the Bras d'Or lake cluster.

Although technical difficulties were encountered in this analysis of genetic diversity (i.e. null alleles, typical of bivalves populations studies), this study successfully characterized the genetic diversity of *C. virginica* in Eastern Canada. With four different but complementary analysis of genetic biodiversity, the observed pattern of genetic diversity can be attributed to differentiation at a large regional scale overlaying genetic panmixia over small regional scales.

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Appendix 1. Descriptive statistics for each of 27 *Crassostrea virginica* samples according to 9 microsatellite loci and all loci together. Statistics include sample size (N), number of detected alleles, number of private alleles, allelic richness as calculated from a sub-sample of 30 diploid individuals, Nei's observed heterozygosity (H_O), Nei's expected heterozygosity (H_E), inbreeding coefficient (F_{IS}) and probability of heterozygote deficiency (P). Global statistics at the end of the table are given as within sample mean values. Significant F_{IS} values after sequential Bonferroni correction are noted in bold.

	Microsatellite Loci									
	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
1. Bras d'Or Lake, NS, 2003 (BDO)										
Number of individuals (<i>N</i>)	34	35	34	35	30	34	35	33	34	33.78
Number of alleles	8	7	10	7	16	11	19	7	15	11.11
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	7.752	6.695	9.857	6.571	16.000	10.645	18.061	6.895	14.385	10.762
Observed heterozygosity (<i>H_O</i>)	0.588	0.514	0.706	0.800	0.667	0.618	0.886	0.636	0.824	0.693
Expected heterozygosity (<i>H_E</i>)	0.800	0.602	0.832	0.740	0.890	0.865	0.900	0.711	0.856	0.799
Inbreeding coefficient (<i>F_{IS}</i>)	0.2675	0.1470	0.1534	-0.0824	0.2540	0.2889	0.0163	0.1064	0.0380	0.1350
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0019	0.1169	0.0358	0.8623	0.0006	0.0004	0.4726	0.1953	0.3578	0.0002
2. West Bay, NS, 2005 (WT)										
Number of individuals (<i>N</i>)	97	95	98	98	87	98	98	97	98	96.22
Number of alleles	10	8	12	8	21	13	22	11	17	13.56
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	7.619	6.367	9.283	6.257	16.439	10.847	17.629	6.846	13.964	10.583
Observed heterozygosity (<i>H_O</i>)	0.608	0.358	0.847	0.755	0.540	0.480	0.878	0.650	0.776	0.655
Expected heterozygosity (<i>H_E</i>)	0.789	0.667	0.853	0.772	0.908	0.852	0.912	0.722	0.888	0.818
Inbreeding coefficient (<i>F_{IS}</i>)	0.2303	0.4647	0.0075	0.0221	0.4061	0.4387	0.0374	0.1005	0.1272	0.2010
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.4796	0.3708	0.0002	0.0002	0.1391	0.0504	0.0010	0.0002
3. Big Harbour Island, NS, 2005 (BHI)										
Number of individuals (<i>N</i>)	97	98	99	99	88	95	99	99	99	97.00
Number of alleles	14	6	12	9	23	15	22	11	17	14.33
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	9.659	5.908	9.643	6.999	16.441	11.493	16.442	7.700	13.940	10.914
Observed heterozygosity (<i>H_O</i>)	0.557	0.276	0.889	0.828	0.534	0.600	0.869	0.707	0.788	0.672
Expected heterozygosity (<i>H_E</i>)	0.815	0.690	0.844	0.792	0.901	0.846	0.898	0.746	0.859	0.821
Inbreeding coefficient (<i>F_{IS}</i>)	0.3182	0.6020	-0.0538	-.0468	0.4088	0.2920	0.0311	0.0527	0.0829	0.1830
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.9374	0.8582	0.0002	0.0002	0.1914	0.1988	0.0233	0.0002

Appendix 1. (continued)

	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
4. Gillis Cove, NS, 2005 (GC)										
Number of individuals (N)	87	85	87	88	80	86	88	87	88	86.22
Number of alleles	9	6	12	8	19	14	22	12	18	13.33
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness ($N = 30$)	7.496	5.341	9.467	6.709	15.478	11.835	16.892	7.512	13.448	10.464
Observed heterozygosity (H_O)	0.540	0.388	0.805	0.807	0.650	0.477	0.830	0.644	0.750	0.654
Expected heterozygosity (H_E)	0.804	0.720	0.842	0.792	0.903	0.868	0.899	0.753	0.835	0.824
Inbreeding coefficient (F_{IS})	0.3296	0.4622	0.0477	-0.0185	0.2813	0.4521	0.0781	0.1461	0.1025	0.2070
Probability of F_{IS} (P)	0.0002	0.0002	0.1885	0.6385	0.0002	0.0002	0.0220	0.0095	0.0175	0.0002
5. Denys Pond, NS, 2003 (DP)										
Number of individuals (N)	61	61	61	61	59	57	58	59	60	59.67
Number of alleles	11	5	13	6	20	12	21	9	18	12.78
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness ($N = 30$)	8.905	4.984	11.187	5.905	15.459	10.705	17.556	7.260	14.869	10.759
Observed heterozygosity (H_O)	0.557	0.410	0.885	0.738	0.441	0.544	0.845	0.576	0.817	0.646
Expected heterozygosity (H_E)	0.805	0.617	0.865	0.750	0.897	0.873	0.915	0.738	0.878	0.815
Inbreeding coefficient (F_{IS})	0.3096	0.3376	-0.0237	0.0159	0.5106	0.3791	0.0722	0.2206	0.0703	0.2090
Probability of F_{IS} (P)	0.0002	0.0002	0.7469	0.4640	0.0002	0.0002	0.0484	0.0021	0.0928	0.0002
6. Whycocomagh Bay, NS, 2005 (WB)										
Number of individuals (N)	97	96	98	98	90	93	98	97	97	96.00
Number of alleles	12	8	12	8	26	13	26	12	19	15.11
Number of private alleles	1	---	---	---	---	---	---	---	1	2
Allelic richness ($N = 30$)	8.977	6.107	9.882	7.338	18.314	10.742	20.293	7.548	14.023	11.469
Observed heterozygosity (H_O)	0.619	0.469	0.898	0.776	0.567	0.570	0.939	0.701	0.794	0.704
Expected heterozygosity (H_E)	0.797	0.629	0.858	0.774	0.923	0.858	0.924	0.750	0.867	0.820
Inbreeding coefficient (F_{IS})	0.2243	0.2556	-0.0468	-0.0022	0.3874	0.3366	-0.0157	0.0660	0.0844	0.1430
Probability of F_{IS} (P)	0.0002	0.0002	0.9035	0.5556	0.0002	0.0002	0.7712	0.1512	0.0220	0.0002
7. Nyanza Bay, NS, 2005 (NB)										
Number of individuals (N)	99	99	100	100	91	99	100	100	100	98.67
Number of alleles	9	6	12	9	22	14	24	10	19	13.89
Number of private alleles	---	---	---	---	---	1	---	---	---	1
Allelic richness ($N = 30$)	8.232	5.493	10.397	6.978	17.675	11.169	17.401	7.555	13.950	10.983
Observed heterozygosity (H_O)	0.626	0.273	0.810	0.700	0.560	0.586	0.890	0.630	0.800	0.653
Expected heterozygosity (H_E)	0.802	0.603	0.873	0.783	0.926	0.851	0.886	0.753	0.879	0.818
Inbreeding coefficient (F_{IS})	0.2201	0.3488	0.0735	0.1070	0.3963	0.3127	-0.0051	0.1643	0.0906	0.2020
Probability of F_{IS} (P)	0.0002	0.0002	0.0364	0.0286	0.0002	0.0002	0.6216	0.0016	0.0117	0.0002

Appendix 1. (continued)

	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
8. Barachois Harbour, NS, 2005 (BB)										
Number of individuals (<i>N</i>)	94	92	93	86	87	92	95	93	95	91.89
Number of alleles	11	6	11	10	20	15	23	9	19	13.78
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	9.209	5.789	10.349	7.480	16.539	11.151	17.191	6.671	12.978	10.817
Observed heterozygosity (<i>H_O</i>)	0.543	0.435	0.871	0.744	0.552	0.663	0.863	0.656	0.768	0.677
Expected heterozygosity (<i>H_E</i>)	0.782	0.684	0.861	0.767	0.902	0.871	0.900	0.731	0.853	0.817
Inbreeding coefficient (<i>F_{IS}</i>)	0.3077	0.3658	-0.0118	0.0305	0.3897	0.2401	0.0414	0.1032	0.0996	0.1720
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.6613	0.3428	0.0002	0.0002	0.1354	0.0521	0.0105	0.0002
9. Crane Cove, NS, 2005 (CC)										
Number of individuals (<i>N</i>)	97	99	100	100	93	96	100	100	100	98.33
Number of alleles	9	7	11	7	19	13	23	10	17	12.89
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	7.064	6.055	9.418	6.014	14.693	11.428	18.895	7.431	13.064	10.451
Observed heterozygosity (<i>H_O</i>)	0.557	0.404	0.760	0.690	0.688	0.375	0.830	0.700	0.780	0.643
Expected heterozygosity (<i>H_E</i>)	0.786	0.667	0.861	0.777	0.895	0.855	0.910	0.732	0.864	0.816
Inbreeding coefficient (<i>F_{IS}</i>)	0.2924	0.3958	0.1173	0.1123	0.2321	0.5629	0.0886	0.0437	0.0974	0.2140
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.0031	0.0235	0.0002	0.0002	0.0041	0.2383	0.0097	0.0002
10. Chapel Island, NS, 2004 (CI4)										
Number of individuals (<i>N</i>)	99	97	100	99	95	90	98	97	98	97.00
Number of alleles	12	6	12	10	22	13	25	11	20	14.56
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	8.946	5.256	9.651	7.410	16.962	9.862	19.098	7.948	14.864	11.111
Observed heterozygosity (<i>H_O</i>)	0.636	0.454	0.844	0.818	0.621	0.322	0.908	0.650	0.714	0.663
Expected heterozygosity (<i>H_E</i>)	0.796	0.657	0.820	0.804	0.906	0.848	0.922	0.778	0.874	0.825
Inbreeding coefficient (<i>F_{IS}</i>)	0.2017	0.3104	0.0281	-0.0174	0.3158	0.6212	0.0149	0.1656	0.1833	0.2010
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.2872	0.6689	0.0002	0.0002	0.3463	0.0016	0.0002	0.0002
11. Chapel Island, NS, 2005 (CI5)										
Number of individuals (<i>N</i>)	98	96	100	100	91	96	100	99	100	97.78
Number of alleles	12	6	11	11	22	14	25	12	19	14.67
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	9.706	5.203	9.960	7.826	16.891	11.302	18.214	8.237	14.179	11.280
Observed heterozygosity (<i>H_O</i>)	0.653	0.302	0.790	0.740	0.703	0.521	0.820	0.707	0.760	0.666
Expected heterozygosity (<i>H_E</i>)	0.834	0.490	0.864	0.794	0.914	0.872	0.900	0.824	0.866	0.817
Inbreeding coefficient (<i>F_{IS}</i>)	0.2178	0.3842	0.0859	0.0678	0.2312	0.4040	0.0896	0.1427	0.1227	0.1860
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.0249	0.1008	0.0002	0.0002	0.0045	0.0029	0.0021	0.0002

Appendix 1. (continued)

	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
12. Mira Bay, NS, 2005 (MB)										
Number of individuals (<i>N</i>)	59	60	60	60	55	60	60	60	60	59.33
Number of alleles	10	6	12	9	19	14	21	8	23	13.56
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	7.918	5.364	10.818	7.717	16.378	12.127	18.051	6.851	17.060	11.365
Observed heterozygosity (<i>H_O</i>)	0.627	0.367	0.883	0.783	0.509	0.650	0.850	0.667	0.850	0.687
Expected heterozygosity (<i>H_E</i>)	0.795	0.496	0.873	0.770	0.902	0.860	0.896	0.738	0.832	0.796
Inbreeding coefficient (<i>F_{IS}</i>)	0.2129	0.2617	-0.0115	-0.0172	0.4376	0.2453	0.0517	0.0975	-0.0219	0.1370
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0025	0.6665	0.6679	0.0002	0.0002	0.1395	0.1072	0.7331	0.0002
13. Aspy Bay, NS, 2005 (AB)										
Number of individuals (<i>N</i>)	59	58	59	59	57	58	59	59	59	58.56
Number of alleles	10	6	11	7	21	10	21	9	17	12.44
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	8.775	5.920	10.306	6.268	17.630	8.965	16.669	7.046	13.531	10.568
Observed heterozygosity (<i>H_O</i>)	0.661	0.293	0.864	0.729	0.597	0.517	0.780	0.695	0.797	0.659
Expected heterozygosity (<i>H_E</i>)	0.799	0.669	0.850	0.786	0.911	0.817	0.888	0.729	0.850	0.659
Inbreeding coefficient (<i>F_{IS}</i>)	0.1738	0.5638	-0.0174	0.0730	0.3473	0.3690	0.1229	0.0471	0.0634	0.1890
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0056	0.0002	0.6864	0.1726	0.0002	0.0002	0.0068	0.3115	0.1360	0.0002
14. Mabou, NS, 2005 (MA)										
Number of individuals (<i>N</i>)	60	58	59	60	56	59	60	60	60	59.11
Number of alleles	15	5	12	13	18	15	23	13	18	14.67
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	11.728	4.466	10.946	11.126	15.588	10.957	19.444	10.525	14.504	12.143
Observed heterozygosity (<i>H_O</i>)	0.517	0.345	0.898	0.783	0.518	0.441	0.850	0.783	0.817	0.661
Expected heterozygosity (<i>H_E</i>)	0.761	0.492	0.895	0.852	0.907	0.733	0.883	0.785	0.878	0.798
Inbreeding coefficient (<i>F_{IS}</i>)	0.3228	0.3004	-0.0038	0.0813	0.4313	0.4006	0.0380	0.0023	0.0698	0.1730
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0008	0.5973	0.0852	0.0002	0.0002	0.2313	0.5502	0.1056	0.0002
15. Goat Lake, NS, 2005 (GL)										
Number of individuals (<i>N</i>)	85	84	85	85	77	83	85	84	85	83.67
Number of alleles	10	5	11	10	16	15	19	10	16	12.44
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	8.564	4.586	8.692	9.461	12.873	11.536	15.132	7.859	12.215	10.102
Observed heterozygosity (<i>H_O</i>)	0.765	0.226	0.800	0.882	0.688	0.590	0.894	0.738	0.871	0.717
Expected heterozygosity (<i>H_E</i>)	0.836	0.573	0.825	0.855	0.882	0.855	0.874	0.762	0.848	0.812
Inbreeding coefficient (<i>F_{IS}</i>)	0.0855	0.6066	0.0303	-0.0326	0.2205	0.3107	-0.0230	0.0309	-0.0274	0.1170
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0578	0.0002	0.3095	0.8243	0.0002	0.0002	0.7912	0.3296	0.7846	0.0002

Appendix 1. (continued)

	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
16. Eastern Shore of NS, 2005 (ES)										
Number of individuals (<i>N</i>)	53	52	55	55	52	51	55	53	55	53.44
Number of alleles	9	6	10	11	18	13	22	7	15	12.33
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	8.268	5.575	9.942	9.136	16.182	11.476	19.368	6.375	11.917	10.915
Observed heterozygosity (<i>H_O</i>)	0.547	0.289	0.818	0.782	0.769	0.451	0.946	0.717	0.782	0.678
Expected heterozygosity (<i>H_E</i>)	0.777	0.698	0.887	0.825	0.920	0.832	0.933	0.770	0.825	0.678
Inbreeding coefficient (<i>F_{IS}</i>)	0.2980	0.5890	0.0778	0.0524	0.1653	0.4603	-0.0139	0.0695	0.0532	0.1840
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.0807	0.2453	0.0010	0.0002	0.7368	0.2113	0.2204	0.0002
17. Merigomish, NS, 2004 (ME)										
Number of individuals (<i>N</i>)	91	97	100	100	94	99	98	95	100	97.11
Number of alleles	17	5	14	14	24	16	26	15	30	17.89
Number of private alleles	---	---	---	---	---	---	---	2	2	4
Allelic richness (<i>N</i> = 30)	11.923	4.290	11.768	11.674	19.086	13.294	21.542	11.689	19.565	13.870
Observed heterozygosity (<i>H_O</i>)	0.517	0.392	0.860	0.890	0.660	0.546	0.959	0.747	0.820	0.710
Expected heterozygosity (<i>H_E</i>)	0.809	0.533	0.899	0.874	0.939	0.803	0.946	0.816	0.890	0.834
Inbreeding coefficient (<i>F_{IS}</i>)	0.3630	0.2653	0.0430	-0.0185	0.2989	0.3214	-0.0144	0.0848	0.0791	0.1500
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0010	0.1403	0.7418	0.0002	0.0002	0.7988	0.0403	0.0101	0.0002
18. Malagash, NS, 2004 (MG)										
Number of individuals (<i>N</i>)	98	94	100	100	90	98	99	98	99	97.33
Number of alleles	14	7	13	18	25	17	25	12	21	16.89
Number of private alleles	1	---	---	---	2	---	---	---	1	4
Allelic richness (<i>N</i> = 30)	10.996	5.101	11.153	12.394	18.652	12.932	20.493	8.878	14.557	12.795
Observed heterozygosity (<i>H_O</i>)	0.561	0.372	0.790	0.840	0.589	0.429	0.859	0.714	0.859	0.668
Expected heterozygosity (<i>H_E</i>)	0.820	0.542	0.891	0.835	0.928	0.759	0.941	0.781	0.891	0.821
Inbreeding coefficient (<i>F_{IS}</i>)	0.3166	0.3135	0.1135	-0.0061	0.3664	0.4365	0.0875	0.0853	0.0370	0.1870
Probability of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0006	0.0027	0.6208	0.0002	0.0002	0.0021	0.0630	0.1681	0.0002
19. Red Point, NS, 2004 (RP)										
Number of individuals (<i>N</i>)	91	96	78	98	75	97	93	92	95	90.56
Number of alleles	12	6	15	15	22	17	24	13	23	16.33
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	9.325	4.516	12.661	11.548	18.408	14.674	19.497	10.575	16.625	13.092
Observed heterozygosity (<i>H_O</i>)	0.539	0.333	0.859	0.888	0.627	0.732	0.946	0.717	0.832	0.719
Expected heterozygosity (<i>H_E</i>)	0.761	0.562	0.892	0.869	0.932	0.860	0.933	0.815	0.897	0.836
Inbreeding coefficient (<i>F_{IS}</i>)	0.2939	0.4079	0.0375	-0.0221	0.3289	0.1498	-0.0148	0.1199	0.0734	0.1400
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.2111	0.7718	0.0002	0.0002	0.7636	0.0078	0.0230	0.0002

Appendix 1. (continued)

	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
20. West River, PEI, 2005 (WR)										
Number of individuals (N)	90	92	92	92	78	92	92	92	92	90.22
Number of alleles	18	7	15	17	24	17	28	12	24	18.00
Number of private alleles	---	---	---	1	1	---	---	---	---	2
Allelic richness ($N = 30$)	13.217	4.989	12.699	12.597	18.711	12.368	20.545	9.440	16.003	13.403
Observed heterozygosity (H_O)	0.500	0.261	0.826	0.804	0.372	0.565	0.935	0.717	0.870	0.650
Expected heterozygosity (H_E)	0.825	0.464	0.893	0.893	0.928	0.780	0.930	0.809	0.883	0.823
Inbreeding coefficient (F_{IS})	0.3954	0.4395	0.0752	0.0994	0.6007	0.2761	-0.0048	0.1136	0.0152	0.2110
Probability of F_{IS} (P)	0.0002	0.0002	0.0329	0.0091	0.0002	0.0002	0.6237	0.0136	0.3802	0.0002
21. St-Peters, PEI, 2003 (SP)										
Number of individuals (N)	54	53	51	54	45	53	48	53	48	51
Number of alleles	12	7	14	15	19	16	23	12	21	15.44
Number of private alleles	1	---	---	---	---	---	1	---	---	2
Allelic richness ($N = 30$)	10.116	5.867	12.932	13.007	17.150	14.438	20.199	10.419	18.111	13.582
Observed heterozygosity (H_O)	0.500	0.245	0.667	0.907	0.622	0.679	0.896	0.642	0.604	0.640
Expected heterozygosity (H_E)	0.774	0.569	0.890	0.881	0.927	0.895	0.930	0.813	0.896	0.842
Inbreeding coefficient (F_{IS})	0.3560	0.5712	0.2526	-0.0304	0.3310	0.2426	0.0372	0.2126	0.3281	0.2410
Probability of F_{IS} (P)	0.0002	0.0002	0.0002	0.8033	0.0002	0.0002	0.2325	0.0010	0.0002	0.0002
22. Bouctouche, NB, 2005 (BO)										
Number of individuals (N)	56	58	56	58	48	58	57	57	58	56.22
Number of alleles	17	5	13	12	21	16	23	12	26	16.11
Number of private alleles	---	---	---	---	---	---	1	---	2	3
Allelic richness ($N = 30$)	12.443	4.408	12.115	11.182	18.324	14.165	20.355	10.274	18.665	13.548
Observed heterozygosity (H_O)	0.446	0.448	0.893	0.862	0.708	0.776	0.912	0.597	0.810	0.717
Expected heterozygosity (H_E)	0.775	0.568	0.890	0.866	0.935	0.800	0.944	0.754	0.885	0.824
Inbreeding coefficient (F_{IS})	0.4262	0.2125	-0.0031	0.0051	0.2446	0.0299	0.0340	0.2098	0.0849	0.1310
Probability value of F_{IS} (P)	0.0002	0.0259	0.6142	0.5235	0.0002	0.3506	0.2101	0.0014	0.0395	0.0002
23. Richibucto Bay, NB, 2003 (RB3)										
Number of individuals (N)	46	48	48	48	40	46	48	48	48	46.67
Number of alleles	13	5	14	12	19	12	22	11	21	14.33
Number of private alleles	---	---	---	---	---	---	---	---	1	1
Allelic richness ($N = 30$)	11.757	4.250	13.005	11.750	17.888	11.335	19.440	9.718	17.543	12.965
Observed heterozygosity (H_O)	0.522	0.417	0.938	0.813	0.575	0.696	0.813	0.667	0.792	0.693
Expected heterozygosity (H_E)	0.767	0.568	0.910	0.876	0.927	0.828	0.926	0.796	0.868	0.829
Inbreeding coefficient (F_{IS})	0.3210	0.2691	-0.0305	0.0728	0.3827	0.1613	0.1236	0.1637	0.0883	0.1670
Probability value of F_{IS} (P)	0.0002	0.0088	0.8259	0.1263	0.0002	0.0093	0.0060	0.0163	0.0677	0.0002

Appendix 1. (continued)

	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
24. Richibucto Bay, NB, 2005 (RB5)										
Number of individuals (<i>N</i>)	96	99	99	99	89	98	99	99	99	97.44
Number of alleles	16	7	12	13	26	16	26	13	21	16.67
Number of private alleles	---	---	---	---	1	---	---	---	---	1
Allelic richness (<i>N</i> = 30)	11.988	5.347	11.015	10.651	19.166	12.908	20.560	8.631	16.076	12.928
Observed heterozygosity (<i>H_O</i>)	0.583	0.384	0.818	0.778	0.573	0.571	0.748	0.667	0.859	0.665
Expected heterozygosity (<i>H_E</i>)	0.823	0.641	0.887	0.861	0.921	0.818	0.946	0.765	0.878	0.838
Inbreeding coefficient (<i>F_{IS}</i>)	0.2925	0.4024	0.0782	0.0967	0.3788	0.3021	0.2108	0.1296	0.0226	0.2080
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.0305	0.0142	0.0002	0.0002	0.0002	0.0105	0.2969	0.0002
25. Miramichi Bay, NB, 2005 (MI)										
Number of individuals (<i>N</i>)	55	56	56	56	50	57	54	50	54	54.22
Number of alleles	17	5	13	13	19	13	23	11	18	14.78
Number of private alleles	---	---	---	---	---	---	---	---	---	---
Allelic richness (<i>N</i> = 30)	13.723	4.533	11.921	11.601	17.093	11.460	19.293	9.508	14.704	12.648
Observed heterozygosity (<i>H_O</i>)	0.618	0.393	0.911	0.839	0.600	0.772	0.870	0.640	0.796	0.715
Expected heterozygosity (<i>H_E</i>)	0.822	0.476	0.882	0.861	0.907	0.862	0.911	0.789	0.881	0.821
Inbreeding coefficient (<i>F_{IS}</i>)	0.2500	0.1760	-0.0328	0.0258	0.3407	0.1048	0.0450	0.1903	0.0969	0.1300
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0416	0.8264	0.3669	0.0002	0.0374	0.1868	0.0082	0.0389	0.0002
26. St-Simon, NB, 2005 (SS)										
Number of individuals (<i>N</i>)	56	60	60	60	54	60	60	59	60	58.78
Number of alleles	16	6	15	13	21	18	24	15	25	17.00
Number of private alleles	1	---	---	---	---	---	---	---	---	1
Allelic richness (<i>N</i> = 30)	12.753	5.232	13.201	11.221	19.126	14.597	20.705	12.397	19.206	14.271
Observed heterozygosity (<i>H_O</i>)	0.536	0.500	0.817	0.933	0.759	0.783	0.850	0.814	0.867	0.762
Expected heterozygosity (<i>H_E</i>)	0.845	0.588	0.897	0.861	0.942	0.797	0.943	0.828	0.888	0.843
Inbreeding coefficient (<i>F_{IS}</i>)	0.3679	0.1501	0.0903	-0.0847	0.1955	0.0167	0.0996	0.0176	0.0237	0.0970
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0689	0.0377	0.9829	0.0002	0.4416	0.0068	0.4453	0.3467	0.0002
27. Caraquet, NB, 2005 (CA)										
Number of individuals (<i>N</i>)	52	53	55	55	46	52	55	54	56	53.11
Number of alleles	13	6	10	14	25	14	21	12	19	14.89
Number of private alleles	---	---	---	---	1	---	---	---	1	2
Allelic richness (<i>N</i> = 30)	11.101	5.449	9.555	11.898	20.502	12.570	18.189	10.328	15.907	12.833
Observed heterozygosity (<i>H_O</i>)	0.539	0.340	0.764	0.782	0.587	0.654	0.836	0.778	0.875	0.684
Expected heterozygosity (<i>H_E</i>)	0.822	0.589	0.861	0.825	0.914	0.813	0.931	0.804	0.862	0.825
Inbreeding coefficient (<i>F_{IS}</i>)	0.3471	0.4252	0.1144	0.0532	0.3605	0.1972	0.1021	0.0326	-0.0151	0.1720
Probability value of <i>F_{IS}</i> (<i>P</i>)	0.0002	0.0002	0.0290	0.2119	0.0002	0.0010	0.0109	0.3636	0.6889	0.0002

Appendix 1. (continued)

	Cvi6	Cvi8	Cvi9	Cvi12	Cvi1g8	Cvi2i4	Cvi2g14	Cvi2j24	Cvi2i23	All loci
All 27 sampled populations										
Total number of individuals (N_{TOT})	2061	2071	2083	2103	1897	2057	2091	2074	2097	2059.33
Total number of alleles	30	11	19	21	36	22	30	17	43	25.44
Mean number of alleles per population	12.44	6.11	12.30	11.07	21.00	14.30	23.07	11.07	19.85	14.58
Total number of private alleles	4	---	---	---	5	2	2	2	8	23
Mean allelic richness ($N = 30$)	10.854	5.767	11.132	10.393	17.966	12.636	19.363	9.115	16.039	12.590
Obs. within-sample heterozygosity (H_O)	0.573	0.366	0.833	0.803	0.603	0.578	0.870	0.687	0.803	0.680
Exp. within-sample heterozygosity (H_E)	0.803	0.596	0.871	0.820	0.917	0.838	0.916	0.771	0.870	0.822
Inbreeding coefficient within-sample (F_{IS})	0.2820	0.3960	0.0420	0.0220	0.3450	0.3240	0.0480	0.1070	0.0780	0.1760
Probability value of F_{IS} (P)	< 0.001	< 0.001	< 0.001	0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Appendix 2. Geographical analysis of genetic variation carried out with 27 *C. virginica* populations. Significance (in bold) of *P*-values from pairwise F_{ST} analyses after 7020 permutations (above grey cells) and from Fisher's exact test of allelic diversity (below grey cells) were set at a nominal level of 0.05 with Bonferoni-adjusted level of 0.000142.

	BDO	WT	BHI	GC	DP	WB	NB	BB	CC	CI4	CI5	MB	AB
Bras d'Or Lake, NS (BDO)		0.73889	0.81296	0.65883	0.39274	0.48932	0.37664	0.12906	0.15883	0.63632	0.00128	0.08191	0.13789
West Bay, NS (WT)	0.52995		0.51567	0.45670	0.06652	0.43490	0.32664	0.31410	0.57393	0.31111	0.00014	0.00057	0.61823
Big Harbour Island, NS (BHI)	0.42877	0.09296		0.81168	0.47066	0.10142	0.41097	0.05584	0.44801	0.74217	0.00014	0.00057	0.34715
Gillis Cove, NS (GC)	0.261176	0.08292	0.36940		0.18476	0.20057	0.35684	0.08063	0.77593	0.62863	0.00014	0.00014	0.07792
Denys Pond, NS (DP)	0.09232	<0.00001	0.05448	0.00554		0.03348	0.18504	0.05684	0.00285	0.04274	0.00014	0.00228	0.00242
Whycocomagh Bay, NS (WB)	0.26207	0.16951	0.00041	0.00278	0.00015		0.41339	0.05456	0.29245	0.18960	0.00014	0.02621	0.03661
Nyanza Bay, NS (NB)	0.11901	0.05951	0.05619	0.03194	0.04806	0.11572		0.00328	0.02051	0.30242	0.00014	0.00014	0.00442
Barachois Harbour, NS (BB)	0.01441	0.05906	0.00073	0.00074	0.00019	0.00080	<0.00001		0.00912	0.07108	0.00014	0.00028	0.13205
Crane Cove, NS (CC)	0.03047	0.12665	0.03457	0.24196	0.00006	0.04646	0.00065	0.00008		0.09658	0.00014	0.00527	0.05413
Chapel Island, NS, '04 (CI4)	0.38706	0.01638	0.04664	0.15578	0.00050	0.00543	0.01568	0.00090	0.00634		0.00014	0.00997	0.00826
Chapel Island, NS, '05 (CI5)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001		0.00014	0.00014
Mira Bay, NS (MB)	0.00333	<0.00001	<0.00001	<0.00001	0.00007	0.00055	<0.00001	<0.00001	0.00004	0.00014	<0.00001		0.00043
Aspy Bay, NS (AB)	0.00935	0.11154	0.03057	0.00057	<0.00001	0.00101	<0.00001	0.00434	0.00075	0.00001	<0.00001	<0.00001	
Mabou, NS (MA)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Goat Lake, NS (GL)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Eastern Shore of NS (ES)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Merigomish, NS (ME)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Malagash, NS (MG)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Red Point, PEI (RP)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
West River, PEI (WR)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
St-Peters, PEI (SP)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Bouctouche, NB (BO)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Richibucto Bay, NB, '03 (RB3)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Richibucto Bay, NB, '05 (RB5)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Miramichi Bay, NB (MI)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
St-Simon, NB (SS)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Caraquet, NB (CA)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	0.00002	<0.00001	<0.00001

Appendix 2. (continued)

	MA	GL	ES	ME	MG	RP	WR	SP	BO	RB3	RB5	MI	SS	CA
Bras d'Or Lake, NS (BDO)	0.00014	0.00014	0.00043	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
West Bay, NS (WT)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Big Harbour Island, NS (BHI)	0.00014	0.00014	0.00043	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Gillis Cove, NS (GC)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Denys Pond, NS (DP)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Whycocomagh Bay, NS (WB)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Nyanza Bay, NS (NB)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Barachois Harbour, NS (BB)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Crane Cove, NS (CC)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Chapel Island, NS, '04 (CI4)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Chapel Island, NS, '05 (CI5)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Mira Bay, NS (MB)	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Aspy Bay, NS (AB)	0.00014	0.00014	0.00057	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Mabou, NS (MA)		0.00014	0.00014	0.00014	0.00755	0.00014	0.00014	0.00399	0.00014	0.00043	0.00014	0.00199	0.00014	0.00028
Goat Lake, NS (GL)	<0.00001		0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Eastern Shore of NS (ES)	<0.00001	<0.00001		0.00014	0.00028	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014	0.00014
Merigomish, NS (ME)	<0.00001	<0.00001	<0.00001		0.00499	0.00071	0.00256	0.09957	0.27806	0.08091	0.00014	0.00014	0.21154	0.08903
Malagash, NS (MG)	0.00002	<0.00001	<0.00001	0.00019		0.00114	0.00271	0.39373	0.17593	0.10157	0.00014	0.00684	0.12236	0.01766
Red Point, PEI (RP)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001		0.00057	0.18048	0.03063	0.00214	0.00014	0.00014	0.00413	0.00071
West River, PEI (WR)	<0.00001	<0.00001	<0.00001	0.00001	<0.00001	<0.00001		0.17293	0.64587	0.38177	0.00014	0.10171	0.13960	0.00969
St-Peters, PEI (SP)	<0.00001	<0.00001	<0.00001	0.00669	0.00362	<0.00001	0.00319		0.37749	0.07308	0.00014	0.26524	0.40997	0.20171
Boucoute, NB (BO)	<0.00001	<0.00001	<0.00001	0.10297	0.00252	<0.00001	0.20567	0.06476		0.55328	0.00014	0.00043	0.61211	0.03476
Richibucto Bay, NB, '03 (RB3)	<0.00001	<0.00001	<0.00001	0.00337	0.00103	<0.00001	0.02572	0.00024	0.15655		0.00014	0.01353	0.22051	0.00456
Richibucto Bay, NB, '05 (RB5)	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001		0.00014	0.00014	0.00014
Miramichi Bay, NB (MI)	<0.00001	<0.00001	<0.00001	<0.00001	0.00001	<0.00001	0.01256	0.00358	0.00002	0.00011	<0.00001		0.01311	0.00271
St-Simon, NB (SS)	<0.00001	<0.00001	<0.00001	0.08403	0.00371	<0.00001	0.00925	0.02561	0.16960	0.02484	<0.00001	0.00056		0.40285
Caraquet, NB (CA)	<0.00001	<0.00001	<0.00001	0.00668	0.00009	0.00002	<0.00001	0.01411	0.00605	0.00002	<0.00001	0.00003	0.15688	