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Distribution and frequency of mitochondrial DNA polymorphisms in *Mytilus edulis* populations of southwestern Nova Scotia (Canada)

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Abstract: The Atlantic blue mussel, *Mytilus edulis* Linnaeus, 1758, exhibits doubly uniparental inheritance of mitochondrial (mt) DNA. Females are usually homoplasmic for a female-transmitted mt genome (the F-type), and males are heteroplasmic for an F-type and a male-transmitted mt genome (the M-type). F-types can undergo “role-reversal” events, resulting in new male-transmitted mtDNA genomes known as recently-masculinized (RM) types that co-occur in populations with evolutionarily older standard-male (SM) types. Phylogenetic analyses have shown that RM-types periodically replace SM types. It has also been shown that sperm with RM mtDNA have greater swimming velocity and more efficient components of the electron transport chain compared to sperm with SM mtDNA, thus leading to the hypothesis that RM sperm may have a selective advantage over SM sperm. The present study examines the distribution of RM and SM mitotypes in male *M. edulis* ($n = 225$) from thirteen localities in southwestern Nova Scotia (Canada). The SM-type was more common in all populations, with the proportion of RM-types ranging from 0–24.1%. The highest proportion of RM-types was observed in an aquaculture operation. Analyses of additional populations are required to evaluate the selective pressures affecting the geographic distribution of RM and SM mitotypes in *M. edulis*.

Keywords: masculinization, blue mussels, DUI, paternally inherited mtDNA, blue mussel, *Mytilus edulis*

Introduction

Animals typically inherit mitochondrial DNA (mtDNA) through the maternal line, a form of uniparental inheritance termed strict maternal inheritance or SMI (Breton and Stewart 2015). However, several lineages of bivalves in the orders Mytiloida, Unionoida, Veneroida and Nuculanoida demonstrate an unusual system of mtDNA transmission referred to as “doubly uniparental inheritance” or DUI (reviewed in Gusman et al. 2016). The blue mussel, *Mytilus edulis* Linnaeus, 1758, (Mytilidae: Bivalvia) is one species that exhibits DUI (reviewed in Breton et al. 2007; Zouros 2013). Rather than transmitting mtDNA exclusively through the maternal line as in SMI, *Mytilus* spp. are characterized by the presence of two distinct sex-associated mtDNA lineages inherited either maternally through eggs or paternally through sperm. Mitochondrial (mt) genomes that are inherited through the maternal line are referred to as female-transmitted or F-types and mt genomes inherited through the paternal line are referred to as the male-transmitted or M-types (Stewart et al. 2009). In general, female mussels are homoplasmic for the female-transmitted genome (Garrido-Ramos et al. 1998). However, traces of M-type mtDNA have been reported in female somatic tissues (Obata et al. 2007). In contrast, male mussels are heteroplasmic in terms of their mtDNA, with varying ratios of the maternal and paternal mtDNA in all tissues with the exception of spermatozoa, which contain the M-type exclusively (Venetis et al. 2006).

Occasionally, an M-type and an F-type recombine and undergo a “role-reversal event” in which an F-type mt genome becomes “masculinized” and invades the male route of transmission/inheritance (Burzyński and Śmietanka 2009; Ladoukakis and Zouros 2001; Robicheau et al. 2017a; 2017b). These recombinant genomes are referred to as “recently masculinized” male-transmitted genomes or RM-types (Breton et al. 2007; Stewart et al. 2009)

because they are transmitted from fathers to sons via sperm (Zouros 2013). Evolutionarily older M-types within the same population are referred to as standard male-transmitted or SM-types (Stewart et al. 2009). Sequencing of RM mtDNAs has revealed that these genomes are primarily composed of an F-type genome's protein-coding genes, ribosomal RNA genes and transfer RNA genes with the addition of an SM genome's control region (e.g. Zbawicka et al. 2007). In addition to the M-type control region, RM genomes may also contain the control region of the F-type genome; however, this residual F-type control region appears to have degenerated, or be in the process of degenerating, in some RM genomes (Mizi et al. 2005; Stewart et al. 2009). When comparing the genomes of the RM and SM types in *Mytilus* mussels, the protein-coding genes and RNA genes exhibit approximately 20–30% divergence in nucleotide sequence depending upon the specific genes and genomes examined and the nucleotide substitution model used (Hoeh et al. 1997; Breton et al. 2006). Everett et al. (2004) first studied potential functional differences in *M. edulis* sperm that contain mitochondria housing either the SM-type mt genome or the highly divergent RM-type. Everett et al. (2004) predicted that the high level of amino acid substitution between the two mitotypes could impact electron transport chain and ATP synthase efficiency and consequently sperm motility. In this initial study, Everett et al. (2004) reported no significant difference in sperm motility parameters associated with either of these two mitotypes. Jha et al. (2008) conducted a more sophisticated examination of this question for *M. edulis* mussels from an aquaculture operation near Halifax, Nova Scotia, using computer assisted sperm analysis (CASA) and examined much larger sample sizes (both in total number of individuals and in numbers of sperm per individual). Jha et al. (2008) found that sperm of RM males exhibited faster average curvilinear and average path velocities than the sperm of SM males. In a complementary analysis using the same mussel samples, more efficient mitochondrially-encoded

enzyme activity for the cytochrome *c* oxidase complex IV was reported in RM-type mussels than in SM-type mussels (Breton et al. 2009). Differences in the efficiency of the oxidative phosphorylation system may be associated with differences in adenosine triphosphate (ATP) production, which is the core function of mitochondria. Thus, observed differences in swimming velocities may be explained by differences in the efficiencies of mitochondrial enzyme complexes of RM versus SM-type mussels (Breton et al. 2009). In summary, the sperm of RM-type mussels are associated with higher enzymatic activity and faster sperm motility in comparison to the sperm of SM type mussels.

In addition to studies comparing mtDNA mitotypes, effects of sperm interactions and egg homogenate on sperm velocity in *M. edulis* has been studied. Stewart et al. (2012) determined that neither the presence of eggs, nor sperm from another male, had a significant effect on sperm velocity. In other words, the velocity of individual sperm was not responsive to further environmental cues after spawning. Based on these results, Stewart et al. (2012) hypothesized that selection favored a fixed strategy in males, in which the velocity of a male's sperm does not change after spawning.

Because *M. edulis* demonstrated a fixed velocity spawning strategy, Stewart et al. (2012) proposed that spawning velocities of populations may reflect local conditions rather than environmental cues post spawning. Levitan (2004) conducted a comparative study on the effects of variable population density and sperm velocities among species of sea urchins and proposed a trade-off between sperm velocity and longevity. Species with relatively high population densities and close to 100% fertilization success were observed to have relatively fast but short-lived sperm. In contrast, species with lower population densities and 50% fertilization success had

125 slower, long-lived sperm (Levitan 2004). Similar to sea urchins, *M. edulis* are also broadcast
126 spawners and may demonstrate comparable behavior.

127 Based on phylogenetic analyses of M and F-types in various bivalve lineages, it has been
128 hypothesized that RM mtDNA genomes replace SM mtDNA genomes over time, not only in the
129 Mytilidae, but in other bivalve lineages as well (e.g., Hoeh et al. 1997; Passamonti and Scali
130 2001,; Stewart et al. 2009). As noted by Zouros (2013), the presumed complexity of doubly
131 uniparental inheritance coupled with the observation that both mytilids and venerids exhibit
132 female-dependent sex-biased progeny ratios and that sperm-derived mitochondria either cluster
133 together in embryos destined to become males or diffuse and gradually disappear in embryos
134 destined to become female in both these taxa, is strong, albeit indirect, evidence that DUI
135 evolved once in an ancestral bivalve. (Note: these patterns have not yet been explored for
136 Unionids.) However, an alternate hypothesis has been proposed (e.g., Milani et al. 2016); DUI
137 may have originated several times independently as a consequence of the repeated
138 endogenization of “selfish” viral sequences that influence offspring sex ratios. Given the
139 challenges of establishing homology of between the unique mtORFan genes in the mitochondrial
140 DNA genomes of DUI species (e.g., Breton et al. 2011) and known viral nucleotide motifs, this
141 argument also relies on indirect evidence. Establishing the origins and evolutionary dynamics of
142 DUI clearly continues to be an active area of investigation.

143 Earlier studies that first noted the frequency of RM versus SM types in *M. edulis* mussels
144 found a higher proportion of SM- than RM-type mussels in Nova Scotia (Stewart et al. 1995; Jha
145 et al. 2008). If environmental factors play a role in determining the frequency of the RM versus
146 SM mitotypes in populations of *Mytilus edulis* mussels in Nova Scotia, we predict that there will
147 be differences in the frequencies of these haplotypes among beds of mussels sampled across

diverse habitats in Nova Scotia that differ in their environmental characteristics. For example, the southern coast of Nova Scotia is bordered by the Atlantic Ocean which differs in factors such as temperature, salinity, and tidal height compared to the Bay of Fundy, which has the highest tides in the world (Department of Fisheries and Oceans 2002; Hasegawa et al. 2011). Indeed, the tidal range of 15 m in the Bay of Fundy is five times the range observed along the Atlantic Coast. This study reports the proportion of RM and SM-types in mussels obtained from thirteen localities in southwestern Nova Scotia, and discusses the implications of these results for studying the evolutionary dynamics of male-transmitted mitotypes in bivalves with DUI.

Materials and Methods

Sample collection of Mytilus edulis in western Nova Scotia

Sampling of *M. edulis* was completed over three years from 13 different sites in southwestern Nova Scotia (Figure 1; Table 1), either by scuba diving, by collection at exposed mussel beds during low tides (intertidal zone), or by purchasing specimens from aquaculture operations. Live specimens were stored in aerated, running salt water supplemented with algae (i.e., Spat Formula, Aquaculture Innovation Products LTD) as a food source, and maintained at 6°C in the Weston Animal Care Facility at Acadia University (Wolfville, NS) until processed.

Sex determination, species identification and mitotype identification

To determine specimens' sex, a 1 cm² piece of gonad tissue was dabbed on a microscope slide and examined under a cover slip at 400× magnification to detect motile sperm or large sessile eggs. (Anecdotally, the proportion of males and females at each site was roughly equal, but these data were not retained in all years.) To determine species and score the SM versus RM mitotype of each male mussel sample, DNA was isolated from an additional 1 cm² piece of

gonad tissue using a saturated NaCl DNA isolation protocol (Miller et al. 1988; Sutherland et al. 1998). DNA concentration and absorbance ratio (A₂₆₀/A₂₈₀) of samples were measured using a nanospectrophotometer (Implen) and diluted to 25 ng/μl for further use as template in downstream polymerase chain reactions (PCRs). Diluted DNA samples were stored at -20°C in 1× Tris-EDTA (TE) buffer.

Species determination

Because *Mytilus edulis* and *Mytilus trossulus* are morphologically similar (Inoue et al. 1995), PCRs and a subsequent *HhaI* restriction enzyme digest were performed to confirm species identity (Heath et al. 1995). The target gene in PCRs was the internal transcribed region (*ITS*) located between the 18S and 28S nuclear rRNA coding regions (Heath et al. 1995; Dalziel and Stewart 2002). The primers used were ITS-F 5'-GTTTCCGTAGGTGAACCTG-3' and ITS-R 5'-CTCGTCTGATCTGAGGTCG-3' (Heath et al. 1995). Each 25 μl PCR reaction mixture consisted of 21 μL of Invitrogen Platinum Blue Super Mix (Life Technologies Inc.), 0.4 μM of each primer (ITS-F and ITS-R), and 1 μl additional MgCl₂ solution (25 mM). The reactions were performed in a thermo-cycler for 40 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 60 seconds. The initial denaturation period was 94°C for 4 minutes and the final extension period was 72°C for 5 minutes (Dalziel and Stewart 2002). Following amplification, *ITS* PCR amplicons were digested using the restriction enzyme *HhaI* (Promega Corp.) by combining 10 μL PCR product, 2 μL 10× restriction enzyme buffer, 7.5 μL ultrapure water, 0.3 μL *HhaI* (10 U/μL), and 0.2 μL BSA (10 μg/μL). The mixture was incubated at 37°C for ≥ 2 hours. Following incubation, the restriction digest reaction was visualized under UV light by running cut DNA on a 1% agarose gel (w/v) stained with EtBr in 1× Tris-acetate-EDTA (TAE) buffer. Fragment sizes were determined using a 100bp Low Scale DNA ladder (Fisher

Scientific). The presence of two bands at 180bp and 450bp indicated an *M. edulis* individual. The presence of a band at 180bp and a band at 280bp indicated an *M. trossulus* individual. Both *M. edulis* and *M. trossulus* samples had additional bands smaller than 180bp (for examples of patterns, see figure 1 in Heath et al. 1995).

Determination of RM and SM mitotypes

Two approaches were used to determine the male mitotype of each sample. First, the primers M-ed1-COIII-F 5'-TGGAGTCGCTTTATTTATTTTATCTGA-3' and M-ed1-COIII-R 5'-ATACTACAAACCACAGCCTCACTCATA-3' were used to identify samples that contained the SM mitotype (see for example Garrido-Ramos et al. 1998; Sutherland et al. 1998; Dalziel and Stewart 2002). These primers amplify a 530 base pair region of the SM cytochrome c oxidase subunit III (*cox3*) gene in the mtDNA of Nova Scotia *M. edulis* but not of the RM-type mtDNA (Sutherland et al. 1998; Garrido-Ramos et al. 1998). Each 25 µL PCR mixture for the "SM-specific" primers consisted of 22 µL of Invitrogen Platinum Blue 1.1× Taq DNA polymerase PCR Supermix, 0.4 mM of each primer (Med1-COIII-F primer and M-ed1-COIII-R primer), 1 µL additional MgCl₂ solution (25 mM) and 1 µL of DNA template. Reactions were performed in a thermo-cycler for 40 cycles of 94°C for 30 seconds, 63°C for 90 seconds, and 72°C for 30 seconds. The initial denaturation period and final extension period were both 4 minutes. Positive amplification was assessed via visualization of PCRs (as described above) under UV light and a 1% agarose gel.

Samples that did not amplify with the SM-specific primers were analyzed further using the modified *cox1* primers HCO2198 short 5'-TAAACTTCAGGGTGACCAAAAAAT-3' and LCO1490 short 5'-GGTCAACAAATCATAAAGAT-3' (Folmer et al. 1994). These PCR mixtures consisted of 42 µL of Invitrogen Platinum Blue PCR Supermix, 0.4 mM each primer, 2

μl additional MgCl₂ solution (25 mM) and 2 μL DNA template. Reactions were performed in a thermo-cycler for 40 cycles of 94°C for 30 seconds, 40°C for 45 seconds, and 72°C for 60 seconds. The initial denaturation period at 94°C was for 3 minutes and the final extension period was at 72°C for 4 minutes. Unpurified PCR samples were sent to the McGill University and Génome Québec Innovation Centre (Montréal, QC, Canada) for Sanger sequencing.

Phylogenetic similarity of the newly sequenced samples was used to assign the mitotypes to the SM or RM category as per Jha et al. (2008). Specifically, RM mitotypes were identified using Clustal Omega as those identical or nearly identical to sequences with one of the following GenBank accession numbers: EU018152, EU018181, EU018166, EU018178, EU018196, or EU018202. Similarly, any male samples for which the SM-specific *cox3* primers did not produce a product and for which *cox1* sequence was obtained were identical to sequences with one of the following GenBank accession numbers: EU018148- EU018151, EU018153- EU018165, EU018167- EU018177, EU018179- EU018180, EU018182- EU018195, EU018197- EU018201, or EU018203- EU018204. Statistical testing of differences in RM and SM haplotype frequencies among populations (Fisher's exact test for count data) was completed in R (R Core Team 2017). We also made use of the rcompanion package for R (Mangiafico 2017).

Results and discussion

The proportion of *Mytilus edulis* at each site as determined by the *ITS* PCR-Restriction fragment profile analysis ranged from 65–100%; thus *M. edulis* mussels predominated in the localities/zones sampled. Only male *M. edulis* were characterized further. For all samples examined from 2012–2014, a total of 225 individuals were identified as male *M. edulis*. The proportion of RM types ranged from 0–20% with an overall mean of 8.0% RM mitotypes (Table 1; Figure 1). A Fisher's exact test for differences in proportions of RM and SM types among

populations was not significant ($p = 0.06714$), although the fact that it was approaching the $p = 0.05$ value suggests that there was a trend towards differences in haplotype frequencies in some populations.

Samples collected from both the Atlantic Ocean side of Nova Scotia, as well as the Bay of Fundy side had some locations with no RM-types detected. “Natural” (i.e., non-aquaculture) intertidal populations from both coasts had locations where the percentage of RM-types reached highs of 6.3% (Chester Basin and Shelburne Harbour, Atlantic side of Nova Scotia) to 13.3% (Scots Bay, Bay of Fundy side of Nova Scotia). Curiously, the frequency of RM-types were at their highest values for the two aquaculture operations in Mahone Bay (20.0%) and Ship Harbour (24.1%), both on the Atlantic Ocean side of the province. Aquaculture farms are subtidal and are, of course, managed to obtain maximal density to increase commercial yield (Cubillo et al. 2012). While a higher frequency of RM-types in high-density aquaculture farms is consistent with our hypothesis that faster swimming speed is advantageous in high-density environments, more detailed analyses of these patterns and broader geographic coverage will be necessary to fully evaluate this hypothesis. Although we did not conduct any direct functional analyses on the specific males analyzed in this study (e.g., swimming speed or enzyme efficiencies of sperm), we assume that these functions for the RM and SM-types are consistent with the patterns described by Jha et al. (2008) and Breton et al. (2009). In Nova Scotia, and likely in other jurisdictions as well, aquaculture operations artificially introduce spat from one area to another (P. Darnell, Indian Point Marine Farms, Ltd. personal communication), which can alter local mitotype ratios. For example, At Indian Point Marine Farms, spat was collected from Cape Breton and from additional unspecified locations in the Gulf of St. Lawrence to reduce the number of *M. trossulus* mussels and increase production of *M. edulis* (P. Darnell,

personal communication). For various reasons, including overall size and shell characteristics, *M. edulis* are more commercially advantageous for mussel aquaculture in this region (Mallet and Carver 1995). No data are currently available on the frequency of RM and SM-types from the source populations used to collect the spat, but this information would clearly be useful moving forward. The introduction of spat from elsewhere may also impact local RM/SM ratios in natural populations of *M. edulis* situated near aquaculture operations.

Although speculative, another factor that could affect the ratio of RM to SM-types in a population may be the likelihood of embryos fertilized by sperm containing an RM versus SM mitotype, respectively, developing into a male. Although Zouros (2013) has proposed a model in which the sex of fertilized eggs is determined by the genotype of the female parent (and accordingly, not influenced by the mitotype of the male parent), Yusa et al. (2013) reanalyzed the published results of crossing experiments and sex ratio variation in *Mytilus* spp. and proposed that sex in this genus is indeed controlled by a pair of nuclear sex ratio alleles expressed in the mother, but also by minor sex-determining genes inherited from the father (potentially mitochondrial) and also possibly from the mother. If there are molecular signals contained within these male-transmitted mt genomes that do influence sexual development (as has been proposed for freshwater mussels; see Breton et al. 2011), then it is possible that SM mt genomes may be more efficient at influencing male development and consequently more SM containing sperm would be present in the population.

Finally, the manner in which RM vs. SM types interact with nuclear encoded proteins (as per Kyriakou et al. 2015) may have developmental consequences that influence the relative fitness of RM versus SM individuals. The process of mitochondrial recombination creates chimeric RM genomes of various lengths that may contain a mixture of F-type and SM-type

control regions (e.g., Cao et al. 2009). There is also a lack of information on the general growth patterns and, for example, age of sexual maturation of RM versus SM containing males. The somatic tissues of male mussels are heteroplasmic for the F- and M-type (Garrido-Ramos et al. 1998) and the M-type is expressed in somatic tissues (Dalziel and Stewart 2002). Differences in the functional efficiency of RM versus SM mitotypes could affect the overall metabolic efficiency, and hence growth rates, *et cetera*, of male mussels. Differences in the rate of growth, size of gonads, and age of sexual maturation between RM and SM containing males could be important fitness factors affecting the evolutionary dynamics of these mitotypes. Indeed, Pozzi et al. (2017) have recently hypothesized that mitochondrially-encoded micro RNAs (“SmithRNAs”) could influence gonad development and patterns of nuclear gene expression in DUI bivalves. If the RM vs. SM M-type genomes have different expression patterns of SmithRNAs, this too could affect the relative fitnesses associated with these two categories of mitochondrial genomes.

Implications for the evolutionary dynamics of RM and SM types in bivalves

An intriguing difference among bivalve taxa that exhibit DUI is the frequency with which role-reversal events occur (Gusman et al. 2016). For example, the M and F lineages of species in the freshwater mussel family Unionidae may be >50% divergent in amino acid sequence (Doucet-Beaupré et al. 2010). The M and F lineages present in the family Unionidae likely diverged over 200 million ago (Hoeh et al. 2002). It has been hypothesized that perhaps some unique characteristics, such as the unique *COX2* extension (Curole and Kocher 2002), that is only found in the M-type genome, or the role played by novel open reading frames (i.e., *orfan* genes (Breton et al. 2011; Guerra et al. 2017) may limit the opportunity for successful recombination events to occur (Stewart et al. 2009). In contrast, the most common M and F

mitotypes in many species in the families Mytilidae and Veneridae are in the range of ~20% divergent (Breton et al. 2007), although recently it has been shown that the M and F types present in the horse mussel, *Modiolus modiolus* (Family Mytilidae), are also extremely divergent with an M/F-type p-distance of 38% (Robicheau et al. 2017). As mentioned above, Jha et al. (2008) and Breton et al. (2009) demonstrated statistically significant differences in sperm swimming speed and in aspects of the efficiency of the electron transport chain between RM and SM containing sperm, with RM sperm exhibiting faster swimming speed and higher *COXI* activity on average. Stewart et al. (2009) therefore suggested a primarily deterministic model for the periodic replacement of SM types by RM types in bivalve taxa experiencing masculinization events. As mentioned above, whether an RM-type will replace an SM-type at all, and how quickly that will happen is, however, likely to be dependent on a complex interplay of various factors. The strength of selection acting on the RM versus SM types will clearly be important. While increased ATP synthesis efficiency and corresponding sperm swimming speed are likely to be important, an individual male's optimal sperm swimming speed will reflect the demographic characteristics of the population. For example, optimal sperm swimming speed experiences a trade-off with longevity with respect to population density (e.g., various species of sea urchins; Levitan 2000; 2004) or male competitive advantage and volume of sperm (e.g., parental versus sneaker bluegill sunfish; Burness et al. 2004) to provide but two examples. Clearly additional *in vivo* and *in situ* studies are required to further characterize the various factors affecting the evolutionary dynamics of the RM and SM types in marine mussels.

Competing interests: We declare no competing or financial interests.

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Table 1. Number and proportions of *Mytilus edulis* recently-masculinized (RM) and standard-male (SM) mitotypes at locations sampled in southwestern Nova Scotia. For sampling methods: Intertidal Zone = IZ; scuba diving = SD. Locality codes refer to pie chart numbering in Fig 1.

Locality	Locality Code	Coordinates	Description (Method)	Sampling Date	<i>n</i>	Mitotype Count		% RM
						RM	SM	
Ship Harbour, Halifax Co.	A	44.812832, -62.844454	Aquaculture	July 2012	29	7	22	24.1%
Cow Bay	B	44.653990, -63.424080	Mixed sand/gravel (IZ)	May 2013	15	0	15	0.0%
Chester Basin, Mahone Bay	C	44.561426, -64.301949	Subtidal (SD)	July 2013	16	1	15	6.3%
Gold River, Mahone Bay	D	44.530719, -64.310188	Rocky (IZ)	June 2013	16	0	16	0.0%
Indian Point, Mahone Bay	E	44.456973, -64.318085	Aquaculture	June 2013	15	3	12	20.0%
Lunenburg, Lunenburg Bay	F	44.361706, -64.335058	Submerged boat mooring line (SD)	June 2013	11	0	11	0.0%
Shelburne, Shelburne Harbour	G	43.716775, -65.359898	Sand and gravel (IZ)	July 2013	16	1	15	6.3%
Argyle, Argyle River	H	43.713953, -65.832739	Sand and gravel (IZ)	August 2013	10	0	10	0.0%
Sandy Cove, St. Mary's Bay	I	44.486868, -66.084640	Wharf pilings, (IZ)	June 2012	10	0	10	0.0%
Sandy Cove, Bay of Fundy	J	44.486868, -66.084640	Rocky (IZ)	July 2012	10	0	10	0.0%
Joggin Bridge, Annapolis Basin	K	44.617065, -65.691032	Sand and gravel (IZ)	August 2013	11	1	10	9.1%
Scots Bay, Minas Basin	L	45.314555, -64.424343	Rocky (IZ)	May 2013	15	2	13	13.3%
Scots Bay, Minas Basin	M	45.314555, -64.424343	Rocky (IZ)	May 2014	51	3	48	5.9%
TOTAL					225	18	207	8.0%

Figure Captions

Figure 1. Map showing the blue mussel, *Mytilus edulis*, sampling locations in southwestern Nova Scotia, (see Table 1 for location codes and other sampling site details) and pie charts indicating the relative proportions of the RM (black) and SM (gray) haplotypes recovered at each location.

Figure 1.