Biogeography of resistance to paralytic shellfish toxins in softshell clam, *Mya arenaria* (L.), populations along the Atlantic coast of North America

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ABSTRACT

Blooms of *Alexandrium* spp., the causative agent of paralytic shellfish poisoning (PSP), recur with varying frequency and intensity on the Northwest Atlantic coast of North America, from New York, USA, to northern Canadian waters. Along this latitudinal range blooms co-occur with abundant, intertidal populations of softshell clams, *Mya arenaria*. Prior work identified a naturally-occurring genetic mutation in Domain II α-subunit of the clams' voltage-gated sodium channels (Na_V), which significantly reduces the binding affinity of the paralytic shellfish toxin,

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25 saxitoxin (STX). This mutation provides clams with resistance to the deleterious effects of STX,
26 allowing them to continue feeding during *Alexandrium* spp. blooms and attain very high tissue
27 toxicities. This study used genetic sequencing of the Na_V mutation locus in clams from four
28 coastal regions of the Bay of Fundy-Gulf of Maine and the mid-Atlantic to determine the
29 percentage of clams in each region that possess the resistant Na_V mutation. The genotype
30 composition was related to the occurrence and magnitude of PSP outbreaks based on shellfish
31 toxicity, primarily that of mussels, *Mytilus edulis*, used as a proxy for the prevalence and severity
32 of *Alexandrium* blooms in each region. As hypothesized, the proportion of clams bearing the
33 resistant mutation generally matched up well with the historical incidence and intensity of
34 *Alexandrium* spp. blooms. The highest percentage of homozygote resistant clams (RR = 70.0%),
35 and the lowest percentage of sensitive clams (SS = 4.5%) were found in eastern Gulf of Maine
36 populations. Exceptions at a few sites where anomalously high numbers of *M. arenaria* with the
37 resistant mutation were found despite the absence of blooms, may be attributable to larval gene
38 flow. There was no evidence that *Alexandrium* blooms occurring in Northport Harbor, Long
39 Island, have resulted in a shift in genotypic composition of the local clam population,
40 presumably due to their low cell toxicity. Seasonal mismatch of highly vulnerable *M. arenaria*
41 postset with toxic blooms at this latitude may also partly explain this result. This study provides
42 strong supporting evidence that *Alexandrium* blooms can select for resistance to PSP-toxins in
43 *M. arenaria* populations and proposes a mechanism for the persistence of the sensitive allele
44 throughout the region. Implications for clam aquaculture (seeding) efforts, as well as for shellfish
45 toxicity monitoring are discussed.
46

ADDITIONAL INDEX WORDS: paralytic shellfish poisoning; red tides; mutations; natural selection; *Mya arenaria*

1. Introduction

Harmful algal blooms (HABs) of dinoflagellate species, producers of paralytic shellfish toxins (PSTs), pose a threat to human health globally, primarily via consumption of bivalve mollusks that become contaminated via suspension-feeding. Such neurotoxins have played a critical role in the evolution of voltage-gated Na^+ channels (Na_V) essential for the function of excitable nerve and muscle cells across phyla (Anderson et al., 2005a; Catterall, 1992, 2000). The often annual recurrence of blooms of the *Alexandrium tamarense/fundyense* species complex and resulting paralytic shellfish poisoning (PSP) outbreaks have been well described along the Atlantic coast of North America (Anderson et al., 1994; Anderson, 1997; Bean et al., 2005; Thomas et al., 2010; Martin et al., 2014). Blooms vary in frequency and intensity (as measured by cell concentration and specific toxicity) along the coast; higher cell toxicities have been documented in northern *Alexandrium* isolates from Canada and Maine (ME), USA, waters than in those from southern New England and the mid-Atlantic (Maranda et al., 1985; Anderson et al., 1994; Anderson, 1997).

Toxicity in the main sentinel bivalve species, *Mytilus edulis*, provides a proxy for *Alexandrium* bloom occurrence and intensity, given that mussels accumulate PSTs very rapidly [achieve peak tissue toxicities within ~ 1 wk of peak *Alexandrium* densities (Bricelj and Shumway 1998)]. The timing of peak PSP toxicity coincides for *M. edulis* and *Mya arenaria*, although the magnitude is ~ 2 to 4x greater in the former. Shellfish toxicity usually peaks in early summer (June) in the western Gulf of Maine (GoM) and southern New England, in late summer (late July/early

August) in the eastern GoM and Bay of Fundy (BoF) (Anderson, 1997; Thomas et al., 2010), and in mid-late May on the north shore of Long Island, NY (Hattenrath et al., 2010). Populations of native Atlantic softshell clams, *Mya arenaria*, the target species in the present study, are ubiquitous component of the intertidal benthos throughout the geographic range of *Alexandrium* blooms, from Québec, Canada, to New York, USA, coastal waters, and the timing of *Alexandrium* blooms overlaps with that of softshell clam postlarvae/juveniles, the life history stage most vulnerable to PSTs (Bricelj et al., 2010). This overlap provides a unique opportunity to understand the adaptive responses of marine bivalve populations to neurotoxic HABs and the evolution of resistance to neurotoxins in a tractable and relatively simple invertebrate model system.

A genetic/molecular basis for the resistance of *M. arenaria* to PSTs was previously established. A single base pair substitution in the pore region of Domain II (DII) of the Na_V gene in resistant individuals of this species (as measured by a nerve *in vitro* bioassay) results in a single amino acid substitution in the encoded protein, where glutamic acid (E) is replaced with aspartic acid (D) (Table 1), causing a 1,000 fold decrease in the binding affinity for PSTs at this site (Bricelj et al., 2005; Connell et al., 2007). Six possible nucleotide combinations can occur at the Na_V mutation locus (Table 1): three that cause the E → D amino acid substitution and result in a STX-resistant genotype (RR), one wild type that results in a STX-sensitive genotype (SS), and two that produce a heterozygous genotype (RS). Both alleles were expressed in heterozygote individuals (Connell et al., 2007), which exhibited intermediate nerve resistance between that of RR and SS genotypes (Bricelj, MacQuarrie, and Connell, unpublished). These alleles are controlled at a single locus heritable through simple autosomal Mendelian inheritance patterns (Hamilton, 2009).

93 Resistance to PSTs in softshell clams was associated with enhanced fitness in PSP-affected
94 areas, as determined in laboratory studies in which clams were exposed to a simulated bloom of
95 a highly toxic *Alexandrium* isolate [Pr18b, ~60 to 98 pg saxitoxin equivalents (STXeq $cell^{-1}$
96 (MacQuarrie and Bricelj, 2008)] originally collected from the estuary of the Gulf of St.
97 Lawrence, Canada. Resistant juvenile clams exhibited increased burrowing capacity (and thus
98 presumably reduced risk of predation, desiccation, and advection in nature), increased feeding
99 (clearance rate) of toxic cells (and thus increased toxin accumulation), and ultimately higher
100 survival than sensitive individuals (MacQuarrie and Bricelj, 2008). Laboratory studies
101 demonstrated that post-metamorphic/juvenile clams represent the life history stage most
102 susceptible to the disabling effects of PST exposure (Bricelj et al., 2010), and are also known to
103 be the most vulnerable to predation in the field (Beal, 2006). Furthermore, when mixed
104 populations of resistant and sensitive *M. arenaria* postlarvae (~4-12 mm in shell length, SL)
105 were exposed under laboratory conditions to a single, one-week, simulated bloom of toxic
106 *Alexandrium*, the latter acted as a strong selective agent favoring resistant (RR) clams via
107 selective mortality of sensitive clams (Bricelj et al., 2010). Selection for PST resistance was also
108 demonstrated when juveniles of known SS or RR genotype, generated by controlled breeding,
109 were deployed together at several sites along the Atlantic coast varying in their history of PSP
110 (Connell, Bricelj, and Martin, unpublished data).
111 Selective pressure imposed by naturally occurring neurotoxins can have significant effects on
112 the genetic structure of populations of both terrestrial and aquatic organisms and affect their
113 abundance and geographic distribution. Adaptation to tetrodotoxin (TTX), another potent Na_V
114 blocker, is well characterized in the predator-prey system of garter snakes (*Thamnophis* spp.) and
115 poisonous TTX-producing newts (*Taricha* spp.) that overlap in their distribution in the Pacific

116 Northwest and California. Newts have evolved TTX resistance as a predator avoidance
117 mechanism, and garter snakes have co-evolved adaptive resistance to TTX that allows them to
118 prey on newts, an abundant and preferred food source (Brodie and Brodie, 1999; Geffeney et al.,
119 2005; Feldman et al., 2009, 2010, 2012). This has resulted in considerable variation in TTX
120 resistance among North American *Thamnophis* populations depending on their exposure to toxic
121 newts (Brodie et al., 2002). Tetrodotoxin resistance in garter snakes is the result of several amino
122 acid substitutions in DIII and DIV of the Na_V pore, which cause a variable, up to 40-fold
123 decrease in binding affinity of the TTX molecule to the channel (Geffeney et al., 2005; Feldman
124 et al., 2012). Differential resistance to PSTs in relation to historical toxin exposure was also
125 demonstrated in the copepod, *Acartia hudsonica*, from the Atlantic USA coast, such that higher
126 ingestion of toxic *Alexandrium* cells and higher fitness (egg production) were found in copepods
127 from a PST-affected ME site than for naïve populations (Colin and Dam, 2002, 2004). The
128 molecular basis for increased population resistance in this copepod species, however, remains
129 uncertain. Although a mutation in the Na_V of *A. hudsonica* was identified, it differs greatly from
130 that described in *Mya arenaria* and does not account for adaptation to PSTs in this copepod. It
131 was not found at the toxin binding site (Chen et al., 2015), there was no evidence that it led to
132 fitness advantages under toxic conditions or a cost under non-toxic conditions (Finiguerra et al.,
133 2015), and the expression of the mutant Nav isoform was not related to exposure to toxic
134 *Alexandrium* in the laboratory or to toxic blooms in the field (Finiguerra et al., 2014a and b).

135 Intraspecific differences in softshell clams' susceptibility to the effects of PSTs were first
136 characterized using a burrowing incapacitation assay under simulated HAB conditions in the
137 laboratory (Bricelj et al., 1996, 2002), as the genetic/molecular basis for the differential
138 sensitivity to PSTs had not yet been described at the time. The main objective of the present

study is to characterize the genotypic composition of toxin resistance of *Mya arenaria* populations in relation to their history of PSP along the northeastern and mid-Atlantic coasts of North America. Early characterization of resistance using the burrowing assay (conducted in 1996-2002) is compared to that determined by DNA sequencing of the Na_V mutation locus (2007-2013). It is hypothesized that *M. arenaria* populations in areas exposed to higher intensity HABs of toxic *Alexandrium* will be dominantly comprised of resistant individuals possessing the R allele (either RR or RS), whereas those where HABs are relatively rare or absent will be largely comprised of sensitive individuals with the SS genotype. Selection for resistance is further tested in a Long Island, NY, estuary where *Alexandrium* blooms are recurrent since 2006 but cell toxicities are typically an order of magnitude lower than in more northern latitudes. Persistence of the S allele in natural populations despite many decades of annual exposure to toxic blooms is discussed, as well as the significance of results for stock selection of aquaculture seeding operations. Finally, the stability (i.e., interannual variation) of the genotypic composition of *M. arenaria* populations at the identified locus, was determined at key sites along the US Atlantic coast representative of different PSP regimes.

1. Materials and methods

1.1. Phenotypic composition of *Mya arenaria* populations: burrowing behavior

The burrowing index measures, under standardized conditions (16^{o}C temperature, salinity = 30, coarse sand substrate), the ability of juvenile clams to re-burrow when exposed at the sediment surface after 24 h of exposure to high-toxicity cells of *Alexandrium tamarense* [strain PR18b isolated from the Gulf of St. Lawrence Estuary, 60-98 pg STX eq cell^{-1}, 100 cells ml^{-1}] (MacQuarrie and Bricelj, 2008). Briefly, clams of comparable size (mean ~ 35 mm in shell length, SL) were collected from natural populations at a time of the year when they contained no

detectable toxins and were acclimated to laboratory conditions for at least 3 weeks prior to toxification. Clams were then distributed among two aquaria per treatment: toxified vs. controls, fed a non-toxic diet of the diatom *Thalassiosira weissflogii*, (Actin strain, CCMP 1336 from the Bigelow National Center for Marine Algae and Microbiota, Maine). The percentage (%) of clams that were able to burrow (resistant phenotype) and that of clams that did not burrow (sensitive) in the toxified treatment was determined by the end of the trial (average calculated from duplicate aquaria; n = 60 to 100 clams per treatment). A correction factor was applied to account for the % of clams that did not burrow by the end of the trial in the control treatment due to incapacitation (e.g., poor condition) that could not be attributed to toxin exposure. Juvenile *M. arenaria* of a standardized size were used as burrowing capacity is size-dependent and declines in adults of this species. The burrowing index is non-destructive, allows characterization of resistance to PSP toxins of large sample sizes, and provides a relative measure of nerve resistance to STX as determined by the Twarog's in vitro nerve bioassay (Bricelj et al., 2002).

1.2. Genotypic composition

1.2.1. Sampling sites

Adult *M. arenaria* were collected using a hand rake from the upper intertidal zone of 26 sampling sites (Figure 1). The sample size for each site was based on the availability of clams found (Table 2). Repeated sampling over time was carried out at five sites with the time interval based on availability of field help, with the exception of Northport Harbor where samples was carried out to reflect the time needed between sampling for clams to become of legal size. These sites were clustered *a priori* into five different regions based on the major circulation patterns in the GoM and key hydrographic features (Pettigrew et al., 2005) (Figure 1 inset). These include the offshore veering of the Main Coastal Current (MCC) near Penobscot Bay (PB), and the

185 presence of Cape Cod, MA acting as a barrier that forces the predominant N-S flow offshore
186 (Anderson, 1997, Anderson et al., 2014).
187 Eastern Gulf of Maine (EGoM) was designated as the region from the Schoodic peninsula east
188 into southern New Brunswick, including Cobscook Bay (Townsend et al., 2001). Mount Desert
189 Island (MDI) extended between the Schoodic peninsula and the town of Brooklin, Maine (ME)
190 on the eastern side of PB. Penobscot Bay was designated as the region from Rockland, ME on
191 the western side of PB to Brooklin on the eastern side of the Bay, including the island of
192 Islesboro (IB). The Western Gulf of Maine (WGoM) region extended from PB to the border
193 between the states of ME and New Hampshire (NH). The southern New England and New York
194 (SNE-NY) region was defined as an area encompassing the southern shore of Cape Cod, and the
195 north and south shores of Long Island, NY. Clams were collected from eight sites in EGoM
196 (these included three populations from the BoF, Canada), five sites in MDI, four sites in PB, six
197 sites in WGoM, and three sites in SNE-NY. The number of *M. arenaria* collected at each site
198 that were successfully genotyped ranged from 19 to 386, with an average of 51 clams per site
199 (Table 2).
200
201 **1.2.2. DNA analysis**
202 Each individual *M. arenaria* was measured using calipers and the shell length was
203 recorded. Samples of DNA were obtained in a non-lethal manner using a 1 mL Monoject™
204 Tuberculin syringe (Covidien) to extract 0.3 mL of hemolymph from the anterior adductor
205 muscle. Following hemolymph extraction clams were returned to the flat. The hemolymph was
206 transferred to individually numbered sterile tubes and placed on ice for transport to the
207 University of Maine (UMO) where samples were processed within three hours of collection. The
208 hemolymph was processed as described by Hamilton et al. (2009). Samples from the Northport

209 Harbor site were frozen whole and shipped to UMO. A 5 mm x 5 mm piece of mantle tissue was
210 excised from each clam, and DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN)
211 following the manufacturer's protocol. All DNA concentrations were determined using
212 NanoDrop© ND-1000 Spectrophotometer (Thermo Scientific) and extracted DNA was stored at
213 -20°C. The shells of a subset of Northport Harbor clams were subsequently used for
214 determination of year class.

215 The sample DNA was used in PCR reactions designed to amplify a 172 bp segment of the Na^+
216 channel gene containing the mutation site. Amplification was carried out in 25 μL total volume
217 in Illustra PuReTaq Ready-To-Go PCR Bead tubes (GE Healthcare Life Sciences) containing 22
218 μL of HyClone™ HyPure Molecular Biology Grade Water (GE Healthcare Cell Culture), 0.5 μL
219 of each primer (oBTG-99F and oBTG-100R of 5 μM), and 2 μL of hemolymph (~50 ng of
220 DNA) or DNA extract. Using an MJ Research PTC-200 Thermal Cycler (GMI Laboratory
221 Instruments) PCR cycle conditions were as follows: (i) 95°C for 5 min, followed by 35 cycles of
222 (ii) 95°C for 30 s, (iii) 50°C for 30 s, and (iv) 72°C for 120 s, with final extension conditions of
223 72°C for 10 min.

224 The resulting PCR products were visualized on an $I.D._{N.A}$™ agarose gel to ensure proper
225 amplification. The PCR products were purified using the Wizard© SV Gel and PCR Clean-Up
226 System (Promega). The purified PCR products were sequenced using ABI BigDye™ Terminator
227 chemistry by the UMO DNA Sequencing Facility (Orono, ME). The raw data were analyzed
228 using Sequencher® 5.0.1 software (Gene Codes Corporation), and the genotype of each
229 individual was identified.

230 To determine if the percentage genotype composition of the populations changed at a specific
231 location, five of the original sites were resampled after a period of three to five years (Table 2).

232 The number of clams collected ranged from 18 to 120. At four of the five repeat sample sites
233 clam shell length was used as proxy for age to ensure that the same year classes were not being
234 resampled. At the Northport Harbor site a subset of the shells were thin sectioned and used to
235 calculate the age range of the sampled individuals following methods of Cerrato et al. (1991).
236 For age determination of the 2010 sample only the largest and smallest individuals (n = 15 out
237 of 100) were sectioned to obtain an age range of 1 to 7 years.

238 **1.3. Statistical analysis**

239 Homogeneity of the sampling sites within each of the five initially proposed regions was
240 tested using a replicated goodness-of-fit test based on the G-statistic (Sokal and Rohlf, 1981).
241 Significantly heterogeneous sites within each region were identified with unplanned tests of
242 homogeneity of replicates. These tests and all other multiple comparisons were Bonferroni
243 adjusted to maintain a family error rate of 0.05 (Miller, 1981). Individual genotype data were
244 pooled within each of the five regions, and statistical differences in the genotype distributions
245 between regions were conducted using pairwise G-tests of independence. For repeatedly
246 sampled sites, G-tests of independence were used to evaluate whether observed differences in
247 genotypic proportions at each site over time were statistically significant. The sample from the
248 Lawrencetown estuary, NS, Canada, was not included in the statistical analysis. All G-tests were
249 run in R (R Core Team, 2017) using the RVAideMemoire library (Herve, 2018).
250

251 **2. Results**

252 **2.1. Characterization of toxin resistance by burrowing**

253 Burrowing assays indicated that predominantly sensitive *Mya arenaria* populations (i.e., 71-
254 98% of the test population were unable to burrow following laboratory exposure to toxic

255 *Alexandrium*), occurred in Mt. Sinai Harbor (MS), central Long Island Sound, NY (Bricelj et al.,
256 1996), Lawrencetown Estuary (LE), SE Nova Scotia, and offshore in the Magdalen Islands (MI),
257 Québec (Figure 2), three regions with no known history of PSP prior to the time of clam
258 collection. In contrast, predominantly resistant populations prevailed in the Bay of Fundy (BoF)
259 and Gulf of Maine (GoM), regions historically affected by PSP. The clam population from the
260 PB so-called toxin-free "sandwich area", where shellfish toxicities are rare and below the
261 regulatory level or undetectable (Shumway et al., 1988), showed an intermediate genotype
262 composition, with about equal proportions of resistant and sensitive clams compared to other
263 Maine populations.

264 Overall, there was a clear relationship between the resistance of clam populations and their
265 prior history of PSP. It is noteworthy, however, that predominantly resistant populations
266 occurred as far south as Cape Cod, MA, where PSP outbreaks are more recent and less intense
267 (lower *M. edulis* peak toxicities) than in northeastern ME and the BoF (Anderson, 1997; Thomas
268 et al., 2010). The resolution of the burrowing assay in discriminating among these populations is
269 lower than that of the molecular analysis (Figure 3). An anomaly to the overall pattern was
270 observed in Oak Bay, where the clam population was predominantly resistant (Figure 2 inset),
271 although there are no records of PSP outbreaks in the upper reaches of Passamaquoddy Bay. This
272 is likely attributable to larval transport via the residual counterclockwise circulation gyre and
273 intense tidal mixing characteristic of the BoF (Arextabaleta et al., 2008; see discussion).

274 **2.2. Genotypic characterization of toxin resistance**

275 Testing for genetic homogeneity among the sample sites within each of the five regions
276 determined that each region was homogeneously grouped, with the exception of two sites. Based

277 on unplanned tests of homogeneity, the proportion of each genotype in the Morong Cove site,
278 EGoM region, and the Buttermilk Bay site in the SNE-NY region were significantly different
279 from the other sites in those regions (G-value = 13.853, df = 2, p = 0.001 and G-value = 12.946,
280 df = 2, p = 0.002, respectively). These two sites were left within the pooled groups for
281 subsequent analyses.
282 All three genotypes described with respect to STX resistance were found in the four GoM
283 regions. These regions showed a general trend of decreasing incidence of the RR genotype and
284 increasing incidence of the RS genotype from EGoM to WGoM, whereas the SS genotype was
285 most prevalent in PB (Figure 4). The EGoM region (n = 267) had the largest percentage of RR
286 individuals (70.0%) and had the lowest percentages of RS and SS individuals among GoM sites
287 (25.5% and 4.5%, respectively). The MDI region (n = 186) was comprised of 47.3% RS
288 individuals, a large percentage of RR individuals (34.9%), and 17.7% SS clams. The PB region
289 (n = 142) had an approximately even distribution of genotypes (RR = 34.5%; RS = 41.5%; SS =
290 23.9%). The WGoM region (n = 527) had the highest percentage of RS individuals of all the
291 regions, comprising over half of the clams sampled (53.5%), as well as the lowest percentage of
292 RR individuals in the GoM (27.7%). It also had a low percentage of SS individuals (18.8%).
293 In contrast to the GoM, the SNE–NY region (n = 220) had only two genotypes represented
294 and clams with the RR genotype were absent. This region was dominantly comprised of the SS
295 genotype (95.5%). A small number of clams in this region displayed the RS genotype (4.5%;
296 Figure 4).
297 Pairwise tests of independence performed among all five regions showed that genotype
298 proportions were significantly different between the EGoM region and all other regions, and
299 between the SNE-NY region and all other regions, and that genotype proportions among the

MDI, PB, and WGoM regions did not significantly differ from each other (Table 3). These results did not change when the two heterogeneous sample sites (Morong Cove and Buttermilk Bay) were excluded from the analysis. Overall, there was a highly significant positive linear relationship between the % of RR clams and latitude ($F = 37.29$, $df = [1,24]$, $p < 0.001$), and a highly significant negative relationship between % SS clams and latitude ($F = 147.22$, df [1,24], $p < 0.001$) (Figure 5A and B, respectively).

The temporal data showed that four of the five repeatedly sampled sites displayed no statistically significant shift in genotype proportions over the time interval considered. One repeatedly sampled site, Naskeag Harbor, did show a significant change in genotype proportions over a four-year period (2008 to 2012). Genotypic proportions differed significantly between the first and second samplings at this site (G-value = 7.7195, $df = 2$, $p = 0.021$). The number of individuals with the RR genotype decreased and the number of individuals with the RS genotype increased. There was no change in the number of SS individuals.

Analysis of the size and age data taken from clams at each of the repeated sites showed that the repeat samples did not belong to the same year classes as the initial samples. At four sites, the average shell length of individuals from the second sampling taken three to five years after the first sampling was either the same or smaller than the average shell length of individuals from the first sampling, indicating that a lack of temporal changes in genotype proportions was not due to resampling individuals from the same year class (Figure 6).

At the Northport Harbor, Long Island, site clams sampled in 2010 ranged from 38.2 to 83.6 mm SL, and in age from 1-7 years. This sample set included clams that experienced pre-bloom conditions at the time of recruitment, as well as clams recruited after the first PSP event in 2006. Individuals collected during the second sampling at this site in 2013 ranged from 38-42 mm in

323 SL and were 2-3 years old. All clam genotypes in 2013 recruited as postlarvae after *Alexandrium*
324 blooms appeared in this estuary.
325
326 **3. Discussion**
327 **3.1. Relationship between the resistance of *Mya arenaria* populations to PSTs and the**
328 **history of toxic algal blooms and PSP.**
329 The prediction that patterns of toxin resistance in softshell clam populations reflect the
330 selective pressure exerted by toxigenic *Alexandrium* was supported by the results of the present
331 study, both by the burrowing assay and genotypic analysis. The former, however, is unable to
332 discriminate an intermediate phenotype from the two sensitive and resistant end members, and
333 therefore provides reduced resolution relative to the molecular analysis.
334 High-toxicity PST blooms are a regular, annual phenomenon in the EGoM/BoF region, with
335 *Alexandrium* reaching cell concentrations as high as 2-3 x 10^6 L^{-1} (more typically 2-9 x 10^5), a
336 maximum *M. arenaria* tissue toxicity of 9,100 μg STX eq. 100 g^{-1} (Lepreau Basin, Martin et al.,
337 2014) and a peak mussel toxicity of 28,000 μg STX eq. 100 g^{-1} (reviewed by Bricelj and
338 Shumway, 1998) in the 1970s. This region had the highest percentage of the RR genotype
339 recorded in this study, with 90.6% of *M. arenaria* possessing at least one R allele, indicating
340 strong selection for PST resistance in this region. The fairly even distribution between RR and
341 SS genotypes in the MDI region suggests that HABs have a moderate impact on *M. arenaria*
342 populations in this area.
343 *Alexandrium* blooms are a regular occurrence in WGoM region since 1972 when PSP, which
344 had previously affected the EGoM and Canadian waters, extended southward due to transport of
345 *Alexandrium* cysts by hurricane activity. A high percentage (81.2%) of *M. arenaria* sampled in

346 the WGoM region possessed at least one R allele. Although most of those individuals were
347 heterozygotes (Figure 4), this still shows evidence that selection at the Na_V locus is taking place
348 in this region. There was no relationship between annual peak mussel toxicities, or the annual
349 frequency of blooms in which mussels exceeded the regulatory level, with latitude, during the
350 severe PSP outbreaks of 1985 and 2005 when only the ME coastline is considered (based on
351 DMR 1985-2005 records, plots not shown). Peak mussel toxicities, however, are higher in
352 WGoM than EGoM as illustrated during severe PSP outbreaks (e.g., 9587 and 4179 µg STXeq
353 100 g^{-1}, respectively, in 1986, and 4204 and 1047 µg STXeq 100 g^{-1}, respectively, in 2005), yet
354 the % of RR clams was higher in EGoM than WGoM (Figure 4). This may be attributable to the
355 more prolonged historic exposure to PSTs of *Mya* populations in the EGoM.
356 *Mya arenaria* from the SNE-NY region were almost entirely comprised of the SS genotype,
357 and the 4.5% of the population that possessed the R allele were all heterozygotes. Blooms of
358 *Alexandrium* spp. have only appeared and become a regular event in the Northport Harbor-
359 Huntington Bay estuary, Long Island, since 2006. Since peak spawning in the vicinity of the
360 study area occurs in June (Brousseau 1987) after the mid-late May peak in shellfish toxicity
361 (Hattenrath et al., 2010), softshell clam spat (at sizes when they are most vulnerable to PSTs)
362 may escape direct exposure to the bloom. A more likely explanation for the absence of selection
363 is the fact that these blooms are characterized by an order of magnitude lower cell toxicity [22
364 fmoles $cell^{-1}$ for field samples during the 2008 bloom (Hattenrath et al., 2010)] than in the GoM
365 due to the predominance of low-potency, N-sulfocarbamoyl toxins (Anderson et al., 1994).
366 These blooms, however, were characterized by high *Alexandrium* cell densities (maximum =
367 887,600 cells L^{-1} during the most severe 2008 bloom, resulting in peak mussel and softshell clam
368 toxicities of 1,400 and 600 µg STXeq 100 g^{-1}, respectively (Hattenrath et al., 2010). This finding

369 suggests that *Alexandrium* blooms of low cell toxicity (i.e., dominantly composed of N-
370 sulfocarbamoyl toxins) such as those experienced in the Northport estuary, despite their
371 relatively high densities, are incapable of selecting for resistance in *M. arenaria* populations.
372 Softshell clams exhibit very low capacity for the metabolic conversion of low-potency
373 sulfocarbamoyl toxins to more potent carbamate PSTs (Bricelj et al. 1996); thus the former may
374 result in lower toxicity once accumulated in tissues.

375 The PB clam population presents an anomaly in terms of the correlation between PSP
376 history and resistance to PSTs. *Alexandrium* blooms are rare in PB, and recorded toxicities in *M.*
377 *edulis* from PB rarely exceed the 80 μg STX eq 100 g^{-1} regulatory threshold (Thomas et al.,
378 2010). This is attributable to the fact that during the summer when *Alexandrium* typically
379 blooms, the convergent boundary between the EMCC and the WMCC near the mouth of PB
380 causes the EMCC waters to veer offshore, resulting in a net offshore transport of water,
381 effectively acting as a barrier that prevents *Alexandrium* spp. cells from entering the bay (Figure
382 1 inset; Pettigrew et al., 2005). Overall, the PB region contained an unexpectedly high
383 percentage of clams with the R allele (76%), and of RR clams (28%) (Figure 4). Clam Cove in
384 eastern PB, however, showed a lower prevalence of clams with the R allele (60%) in 2008,
385 which was more comparable to that found at Castine, also in eastern PB (~11 Km from the
386 Islesboro (IB) sampling site and thus closer to the head of the Bay), based on the burrowing
387 assay (49% resistant and 51% sensitive clams). It is possible that the R allele is being maintained
388 in PB *M. arenaria* populations by larval transport from an area where the R allele is dominant in
389 the population. Microgeographical differences in the proportion of resistant clams within PB
390 may also reflect the distance of a sampling site to the coastal source of *Alexandrium* cells.

391 Gene flow due to residual circulation patterns may also explain the anomalous presence of a
392 high proportion of resistant clams (burrowers) in Oak Bay, in the upper reaches of
393 Passamaquoddy Bay, Canada (Figure 2 inset), where PSP outbreaks have not been reported.
394 Shellfish toxicities have always occurred at low levels in Brandy Cove, a monitoring site in
395 southwestern Passamaquoddy Bay, and have been among the lowest detected anywhere in the
396 BoF, except for 2009, a year of very severe *Alexandrium* blooms, when *Mya arenaria* attained
397 4120 µg STXeq 100 g^{-1}at this site (McGillicuddy et al., 2014).
398 *Alexandrium* blooms expanded their distribution into southern New England waters (NH, and
399 MA waters) in 1972 and further intensified in this region following the massive PSP outbreak in
400 2005 (Anderson et al., 2005b), when mussel toxicities attained a maximum of 2357 µg
401 STXeq100 g^{-1} in Cape Cod ponds (data from the MA Division of Marine Fisheries, Shellfish
402 Sanitation and Management Program). A preliminary study, based on a very small sample size (n
403 = 11) indicated that a *M. arenaria* population from Essex, MA, was composed of 27% RR, 54%
404 RS, and 18% SS, clams (Connell et al., 2007), a relative composition comparable to that of
405 WGoM populations in the current study. Additional genotypic characterization of *M. arenaria*
406 populations from southern New England (MA) waters, including Massachusetts Bay and Cape
407 Cod, would be of interest to determine whether these populations have differentiated from those
408 in the WGoM. It is known that PSP outbreaks within Massachusetts Bay and Cape Cod are
409 much more infrequent than those in the WGoM region (Anderson et al., 2005b), likely exposing
410 *M. arenaria* populations to reduced selective pressure.
411 The present study also shows that differences in selective pressure may occur over relatively
412 short distances. Thus *M. arenaria* populations from the Gulf of S. Lawrence Estuary and the Bay
413 of Gaspé, Canada, were predominantly PSP-resistant, whereas those in the Magdalen Islands,

414 only ~260 km offshore, an area with no history of PSP (Blasco et al., 2003), were predominantly
415 sensitive based on the burrowing assay. *Alexandrium* cells occur almost every year in the
416 Magdalen Is. but remain very low in abundance, ≤100 cells L^{-1}. These findings correlate well
417 with the history of PSP in the region as maximum mussel toxicities occur in the lower Gulf of St.
418 Lawrence Estuary and northern coast of the Gaspé Peninsula, where up to 23,000 µg $100g^{-1}$ have
419 been documented (reviewed by Bricelj and Shumway, 1998). As demonstrated in the garter
420 snake (Feldman et al. 2009, 2010), toxin resistance may have evolved independently as a mosaic
421 in different *M. arenaria* populations, as resistant populations occur both in the GoM/BoF and
422 Gulf of St. Lawrence Estuary, two semi-enclosed bodies of water that show limited connectivity
423 (Holm and Bourget, 1994).
424 Since the *M. arenaria* population sampled on the south shore of Long Island was entirely
425 composed of individuals with the SS genotype, this appears to mark the southern limit of
426 distribution of the R allele. Based on the results of the present study, the northern distribution of
427 the Na_V mutation extends into the Canadian Maritime Provinces (Lawrencetown Estuary, NS,
428 and Bay of Fundy, NB), but a prior study found the R allele in softshell clam populations from
429 Havre-Aubert, north of Prince Edward Island, Canada (Connell et al., 2007). Analysis by
430 burrowing in the present study showed a predominance of resistant clams as far as the north
431 shore of the Gulf of St. Lawrence Estuary, Canada. It is very likely that the northern distribution
432 of the R allele extends as far as the northern limits of softshell clams' native range in the
433 northwest Atlantic, Newfoundland and the Labrador (Strasser, 1999). Given that the timing of
434 *Alexandrium* blooms varies seasonally along the Atlantic coast and that the susceptibility of *M.*
435 *arenaria* juveniles is inversely related to age/size (see Introduction), the selective pressure of

436 PSTs on clam populations along a latitudinal gradient may be modulated to some extent by the
437 size of the juvenile cohort when it is exposed to the toxic bloom.
438 Results of the statistical analysis comparing the genotype proportions of the five initially
439 designated regions suggest that overall, these could be redrawn into three, possibly four, discrete
440 sectors based on the distribution of genotypes. The first region encompasses eastern ME and
441 Cobscook Bay (EGoM in Fig. 2), and is characterized by a very high proportion of individuals
442 with the RR genotype. The second region encompasses MDI, PB, and western WGoM (Fig. 2),
443 and is characterized by a more even distribution of RR and SS individuals, and an increased
444 proportion of heterozygote clams. The third region encompasses the north and south shores of
445 Long Island, NY, and possibly Buzzards Bay and the south shore of Cape Cod, MA (SNE-NY),
446 and is characterized by dominance of SS individuals and absence of RR individuals (Figure 4).
447 A fourth region may include the coast of NH and MA north of Cape Cod, but genotyping of *Mya*
448 populations is not available to confirm this. Since statistical analysis within the SNE-NY region
449 indicated that the Buttermilk Bay site within Buzzards Bay was significantly heterogeneous,
450 additional sampling is needed to determine whether *M. arenaria* populations from southern New
451 England differ from those from New York waters. Buzzards Bay is connected via the Cape Canal
452 to Cape Cod Bay and coastal waters, and low mussel toxicities (~200 µg STXeq $100g^{-1}$) were
453 detected for the first time within the Bay during the massive 2005 *Alexandrium* bloom (Anderson
454 et al., 2005b). Given the historical southward expansion experienced by *Alexandrium* blooms
455 along the coast, conditions of toxin exposure may change in the future in this region and lead to
456 increased proportion of RR or RS in *M. arenaria* populations at this latitude. It is important to
457 note that the minimum *Alexandrium* bloom intensity that can result in toxin-induced selective
458 mortalities of sensitive *M. arenaria* remains to be established.

459 Strong genotypic spatial differentiation of marine bivalve populations, based on the allelic
460 frequency of specific functional genes as found in the present study, has been previously shown
461 in the response of *M. edulis* to natural selection by salinity, which combined with gene flow
462 resulted in a steep genotypic cline along Long Island Sound, NY, at the leucine aminopeptidase
463 (Lap) locus (Koehn et al., 1980a and b). Genotypic resistance to anthropogenic contaminants,
464 including pyrethroid pesticides that target Na_V channels in insects (e.g., Liu et al., 2002; Jones et
465 al., 2012), and heavy metals in benthic invertebrates, is well documented and occurs with or
466 without accompanying evidence of fitness costs. For example, rapid selection for resistance to
467 Cd, associated with a very steep spatial selection gradient and likely controlled by a single gene
468 of large effect, was shown in the aquatic oligochaete *Limnodrilus hoffmeisteri* (Klerks and
469 Levinton, 1989; Martinez and Levinton, 1996).
470 Stock enhancement efforts via transfer of juvenile of *M. arenaria* produced at a single regional
471 hatchery (Downeast Institute, Beals, ME) from local broodstock to more southern coastal waters
472 (southern New England) has been a common aquaculture practice over the years (Beal, 2004). It
473 is important to discuss the implications of the latitudinal differentiation in resistance of *M.*
474 *arenaria* populations to this activity. The high degree of genetic structure among softshell clams
475 with regard to the Na_V resistant mutation demonstrated in the present study indicates that seed
476 clams could potentially be planted into locations where their genetic makeup is poorly suited for
477 that habitat, hindering restoration efforts. Genetically tailoring seed clams to best fit the local
478 environment with respect to the history of PSP, given the strong selective pressure of PSTs, may
479 be a future consideration in the hatchery production of cultured seed. This approach has been
480 routinely implemented to breed disease-resistant bivalves (especially oysters) for deployment in
481 different regions along the Atlantic coast (e.g., Dégremont et al., 2015). Variable toxin

accumulation resulting from differential toxin resistance among *M. arenaria* populations is also expected to have important ecological impacts in terms of trophic transfer of PSTs to secondary consumers (multiple crab and fish species).

3.2. Persistence of the S allele and cost of STX resistance

It is noteworthy that the S allele has persisted along the portion of the Atlantic coast (BoF and EGoM) that has experienced recurrent and highly toxic *Alexandrium* blooms for more than 60 years based on PSP monitoring. There is increasing evidence suggesting that this may be explained by the fitness costs associated with Na_V mutations that may serve to constrain or delay the evolution of toxin resistance and maintain genetic variability. A tradeoff between TTX resistance and whole-organism performance was demonstrated in *Thamnophis* garter snakes. Their motility (maximum crawling speeds as determined using a locomotory performance bioassay), and thus presumably their ability to escape from predators and thermoregulate, are reduced in TTX-resistant individuals (Brodie and Brodie, 1999; Brodie et al., 2002; Geffeney et al., 2005). A biophysical cost of TTX resistance in Na_V function was also demonstrated: the Na^+ channels of TTX-sensitive snakes exhibit lower conductance, slower generation of the action potential and differences in gating properties, Na^+ ion selectivity and permeability relative to those of TTX-resistant snakes (Lee et al.,2011; Feldman et al., 2012).

Since the Na_V locus is highly conserved across taxa, we speculate that such neuromuscular costs may also be associated with the DII Na_v mutation in *M. arenaria*. This might occur via slowing down of burrowing and increased vulnerability to predation and tidal scour in the intertidal, and/or via reduced feeding by the gills, since coordination of the gill musculature is important to maintain normal feeding function in suspension-feeding bivalves (Medler and Silverman, 2001; Gainey et al., 2003). This would in turn lead to reduced growth and increased

duration of the juvenile period, when clams are known to be most vulnerable to predators (Beal 2006; Emerson et al., 1990), in areas that experience no detectable or low-intensity *Alexandrium* blooms. Field deployment of juvenile clams of known genotypes along the Atlantic coast showed that RR clams had a significantly reduced shell growth rate relative to SS clams at non-PSP affected sites, and RR clams also showed a significant reduction in growth rate than SS clams held under common laboratory conditions when fed the non-toxic diatom *Thalassiosira weissflogii* (Bricelj, Connell *et al*., unpublished data). This evidence in associating a fitness cost with PSP resistance is compelling, but may be confounded by the fact that RR and SS clams were obtained by controlled breeding of adults from two different *M. arenaria* populations from the BoF and NS. These results, however, support the hypothesis that, individuals with the R allele experience a fitness disadvantage in areas with limited or no PSP.

3.3. Interannual variability in clam population resistance to PSTs.

The genotypic composition with respect to PSP resistance remained relatively stable over a period of three to five years of exposure to *Alexandrium* blooms at three out of four sites sampled repeatedly in ME. This likely results because the latitudinal gradient in the severity of red tides is fairly predictable along the Atlantic coast. Characterization of PSP resistance of *M. arenaria* undertaken in this study over a maximum 8 year-period (2007-2015) are expected to be fairly robust in describing overall latitudinal patterns in PSP resistance. Longer-term, decadal changes, however, should be investigated based on the baseline data generated by this study.

At one site, Naskeag Harbor, however, there was a significant shift in the genotypic composition during the 4-year study period, with an overall reduction in the relative proportion of RR/RS individuals that shifted from 2.0 to 1.2 between 2008 and 2012. Considerable interannual variation in shellfish toxicities and *Alexandrium* along the NW Atlantic coast, has

528 been attributed to large-scale changes in the oceanographic regime and meterological conditions
529 (e.g., association between increased downwelling-favorable winds and higher nearshore shellfish
530 toxicities in Western ME and southern New England (Anderson et al., 2005b; Thomas et al.,
531 2010) as well as small-scale changes in local hydrographic features (Blasco et al., 2003). The
532 shift in genotypic composition at Naskeag Harbor may reflect these changes and/or be influenced
533 by the cost of resistance in years with low-intensity red tides at this site. It is noteworthy that
534 there was no detectable shift in the genotypic composition of softshell clams from the Northport-
535 Huntington Estuary associated with the recurrence of PSP starting in 2006. Thus, the
536 combination of high *Alexandrium* densities but low cell toxicities (in contrast with the relatively
537 low densities and high cell toxicities of *Alexandrium* in the GoM) did not select for increased RR
538 or RS allele proportion of the local *M. arenaria* population.
539 In conclusion, we suggest that the biogeographical distribution of RR, RS, and SS clams in the
540 Northwest Atlantic results from the interplay between the benefits of resistance to PSTs
541 conferred to clams possessing the R allele in highly toxic areas, and the potential neuromuscular
542 costs associated with this allele in areas relatively unaffected by PSP. This evolutionary trade-
543 off, superimposed on the environmental heterogeneity of the Northwest Atlantic in terms of the
544 prevalence and intensity of HABs, and local tidal and wind-driven hydrographic features that
545 affect larval flow, likely explain the genetic mosaic determined in the present study with respect
546 to the R allele.

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Figure 1. Coastal sites in the Bay of Fundy, Canada, Gulf of Maine, southern New England, and Long Island, New York (NY), USA, where *Mya arenaria* were collected for the present study (abbreviations shown in Table 2). Grey shading indicates five regions in which clam populations were hypothesized to differ in resistance to PSTs based on historical peak toxicities achieved (Figure 3): eastern Gulf of Maine (EGoM), Mount Desert Island (MDI), Penobscot Bay (PB), western Gulf of Maine (WGoM), and southern New England and Long Island, New York (SNE-NY) region. Lower-right inset shows a schematic of major residual circulation patterns for the upper 40 m in the GoM in summer (adapted from Pettigrew *et al.,* 2005). EMCC and WMCC = Eastern and Western Maine Coastal Currents, respectively. A GoM coastal current inshore of the EMCC that carries water from eastern ME across the mouth of the PB and into the western GoM (Anderson *et al*., 2005) is not shown.

Figure **2**. Percent of toxin resistant and sensitive clams (pie charts based on burrowing behavior) in Atlantic North American populations of *Mya arenaria* in relation to the history of exposure to PSP toxins (expanded from Bricelj et al. 2002). Site abbreviations and year of clam sampling are as follows from north to south: BC = Baie des Chevaux, ML = Mont Louis, RS = Rivière St. Jean Estuary, Baie de Gaspé, and MI = Magdalen Islands, Havre-Aux-Basques, in Québec (QC), Canada (2002) ; LE = Lawrencetown Estuary, NS, Canada (1998); SB = Sanborn Cove, Machias Bay, PB = Castine, Penobscot Bay, SC = Scarborough, in ME (2001); ES = Essex and OR = Orleans, in MA (2000), and MS = Mt. Sinai Harbor, Long Island Sound, NY (1996). Toxin susceptibility was determined by the burrowing assay, where sensitive clams are paralyzed and thus unable to burrow following toxin exposure (see text). Inset shows detail of sampling sites

784 and population resistance at sites in the Bay of Fundy, Canada: Lepreau Basin (1998), Crow
785 Harbour, Oak Bay and Stuart Town (1999).

786
787

788 **Figure 3.** Genotypic composition (% of each of three genotypes) with respect to PST resistance
789 of sampled *Mya arenaria* populations in each of the five study regions shown in Figure 1; RR
790 and SS indicate the homozygote resistant and sensitive genotypes, respectively, and RS the
791 heterozygous genotype. Data for sites sampled repeatedly represent the earlier year of sampling
792 (see Table 2). Red shading indicates areas of the coastline that have been affected by PSP, based
793 on toxicities exceeding the regulatory level for mussels, *Mytilus edulis*, used as the main sentinel
794 species for PSP (data sources: DMR toxicity records from 1985 to 2005 in ME (Thomas et al.
795 2010, Thomas pers. comm.), Anderson et al. 2005b, toxicity records for MA extending through
796 2017 (MA Division of Marine Fisheries, Shellfish Sanitation and Management Program), and for
797 NY through 2012 (Hattenrath 2014) (see Table 2 for site abbreviations).

798

799 **Figure 4.** Genotype frequencies with respect to STX resistance of sampled *Mya arenaria*
800 populations in each of the five study regions: eastern Gulf of Maine (EGoM), Mount Desert
801 Island (MDI), Penobscot Bay (PB), western Gulf of Maine (WGoM), and southern New
802 England-New York (SNE-NY). Genotypes RR, SS and RS as in Table 1.

803

804 **Figure 5**. Relationships between the percentage of clams with the RR genotype at various study
805 sites along the Atlantic coast of North America and latitude (A), and that of clams with the SS
806 genotype and latitude (^{o}N) (B) Equations indicate the fit of the linear relationship (solid line) to

807 the data and the coefficient of determination (R^2). (The Lawrencetown Estuary, Canada, was
808 excluded from the analysis as this site is found offshore rather than along the coastline).
809
810 **Figure 6.** Average shell length (in mm) of clams at sites where clams were sampled at least
811 twice over a 3 to 5-year period. From northeast to southwest, Mosquito Harbor was sampled in
812 2007 and 2012, Raccoon Cove in 2007 and 2012, Naskeag Harbor in 2008 and 2012, and
813 Poorhouse Cove in 2009 and 2012.
814
815
816
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LIST OF TABLES

Table 1. Six different nucleotide combinations observed at the Na^+ channel mutation locus, the encoded amino acid, and the resulting genotype exhibited in *M. arenaria:* SS, RR and RS indicate the STX-sensitive genotype, the STX-resistant genotype, and the heterozygous genotype, respectively. A = adenine, C = cytosine, T = thymidine; E = glutamic acid, D = aspartic acid.

Nucleotide	Amino Acid	Genotype
A/A	E/E	SS
A/C	E/D	RS
A/T	E/D	RS
C/C	D/D	RR
T/T	D/D	RR
C/T	D/D	RR

829 **Table 2.** *Mya arenaria* sample collection site, including the location (latitude in °N and
830 longitude in °W), year(s) sampled, and the number of clams that were sampled at each site (N).

831

Site Name	Abbreviation	Latitude, Longitude	Year(s) sampled	N
Lepreau Basin	LB	45.12852, -66.46151	2007	75
Beaver Cove	BC	45.07734, -66.73714	2009	29
Deadmans Harbour	DH	45.04906, -66.77008	2009	29
Prince Cove	PC	44.89589, -66.99306	2008	25
Gleason Cove	GC	44.97270, -67.05516	2008	28
Morong Cove	MC	44.85635, -67.10387	2010	41
Whiting Cove	TE	44.81096, -67.16156	2007	27
Duck Brook	DB	44.66274, -67.18790	2007	13
Mosquito Harbor	MH	44.37432, -68.07194	2007, 2012	22, 18
Youngs Shore	YS	44.48001, -68.11656	2007	22
Raccoon Cove	RC	44.47189, -68.28261	2007, 2012	22, 50
Ship Harbor	SH	44.23005, -68.32362	2007	30
Duck Cove	DC	44.25485, -68.37732	2007	22
Naskeag Harbor	NH	44.22959, -68.53542	2008, 2012	29, 27
Herrick Bay	HB	44.26508, -68.55417	2007	27
The Narrows	IB	44.31336, -68.90184	2015	29
Clam Cove	CC	44.13480, -69.09242	2015	30
Poorhouse Cove	PH	43.88363, -69.55011	2009- 2012	91, 66, 120, 109
Lowes Cove	LC	43.93675, -69.57684	2009	18
Dingley Island	DI	43.81715, -69.88579	2012	52
Biddeford Pool	BP	43.43741, -70.36946	2009	25

Webhannet River	WR	43.32035, -70.57177	2010	27
Kittery Point	KP	43.10706, -70.66622	2012	19
Buttermilk Bay	BB	41.75067, -70.62692	2007	29
Northport Harbor	NP	40.90103, -73.35386	2010, 2013	52, 40
Point Lookout	PL	40.59984, -73.56500	2009	35

832

833

834

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836 **Table 3.** G-tests of independence based on the distribution of genotype proportions among all
837 five regions. Comparisons that are significant based on a Bonferroni-adjusted p-value of 0.005
838 are shown in bold. EGoM = eastern Gulf of Maine, MDI = Mount Desert Island, PB =
839 Penobscot Bay, WGoM = western Gulf of Maine, SNE-NY = southern New England-New York.

840

	EGoM	MDI	PB	WGoM	SNE-NY
EGoM					
MDI	**1.0E-12**				
PB	**1.7E-12**	1.00			
WGoM	**<2.0E-16**	1.00	0.04		
SNE-NY	**<2.0E-16**	**<2.0E-16**	**<2.0E-16**	**<2.0E-16**	

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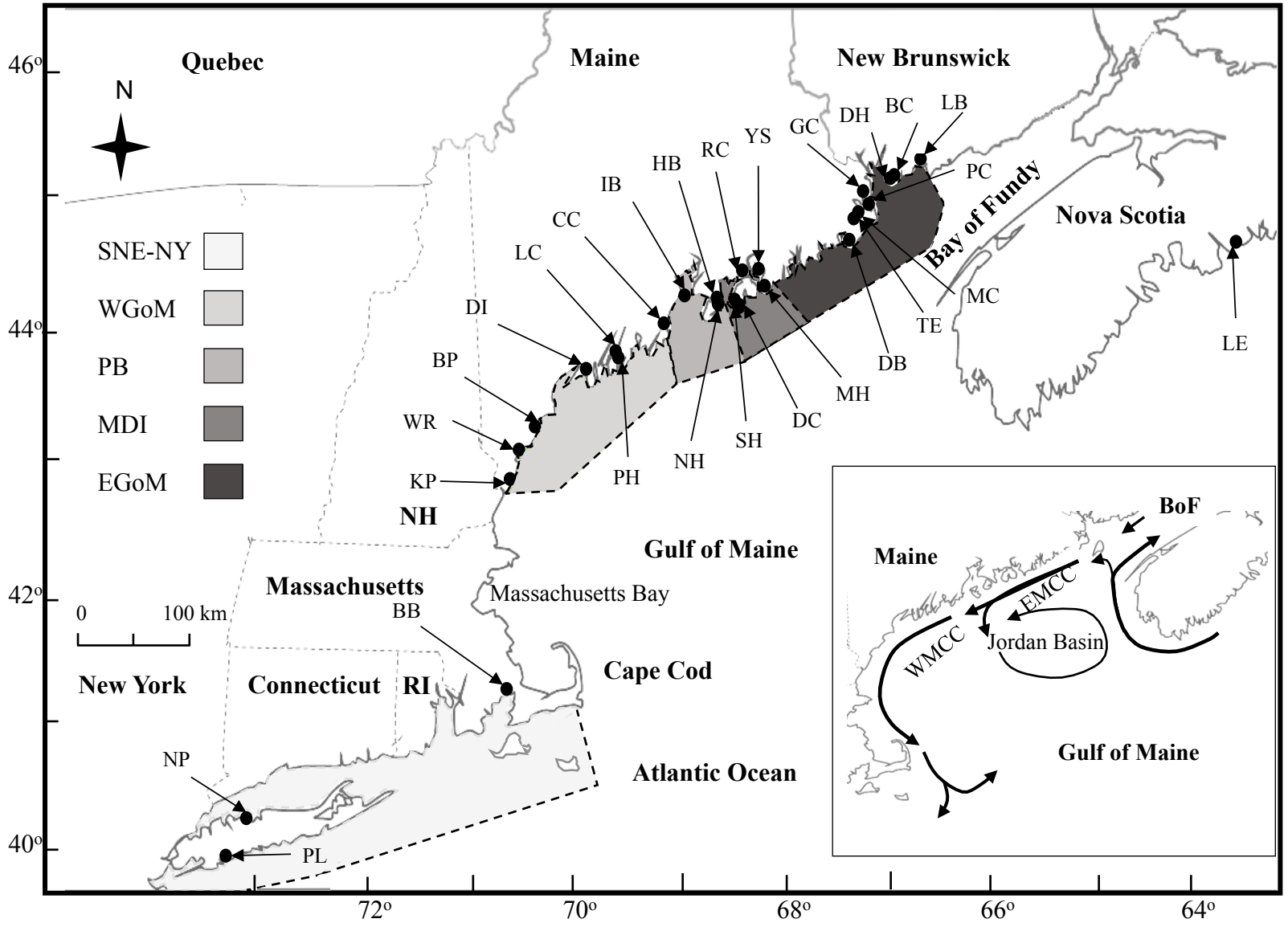
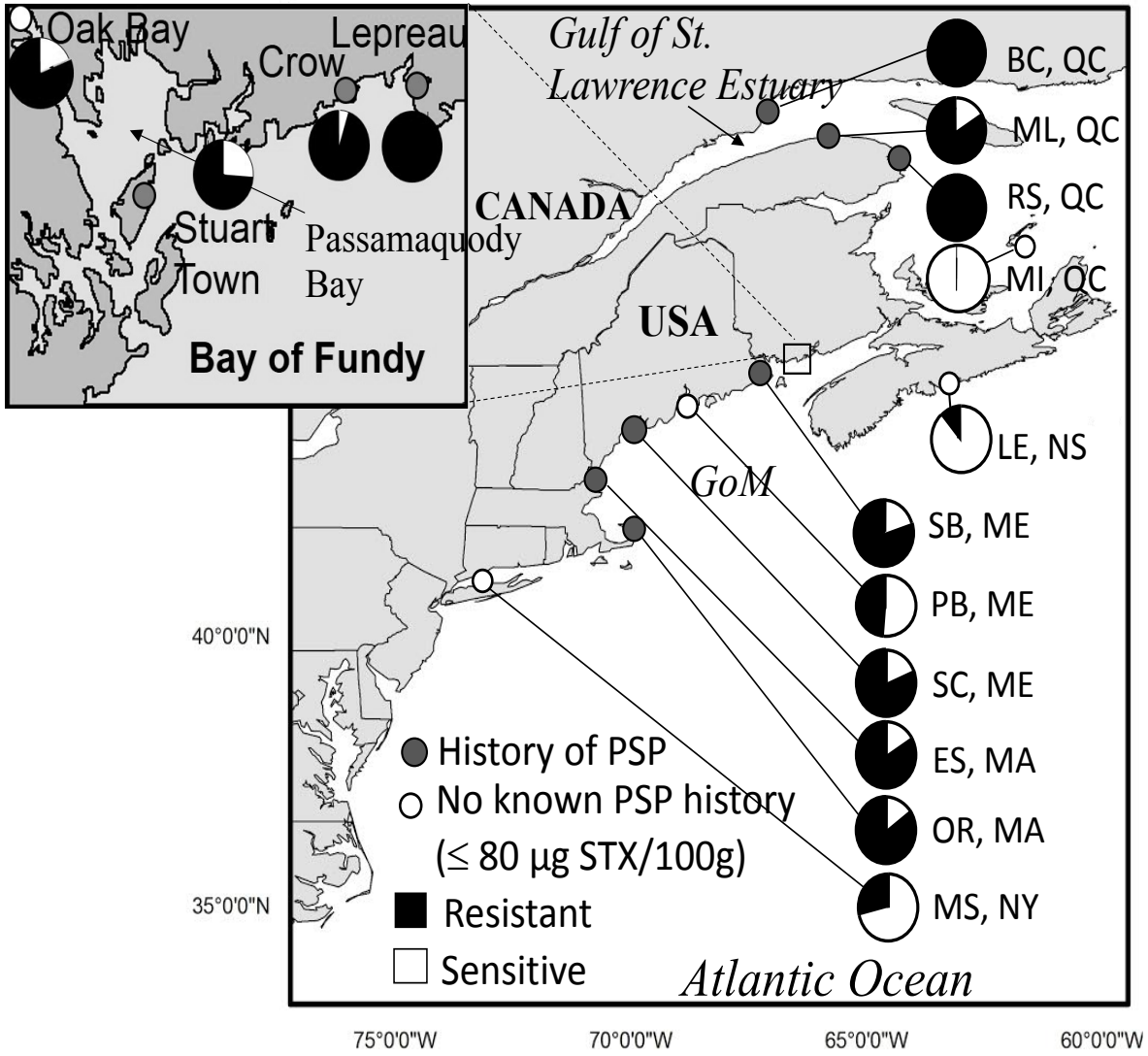
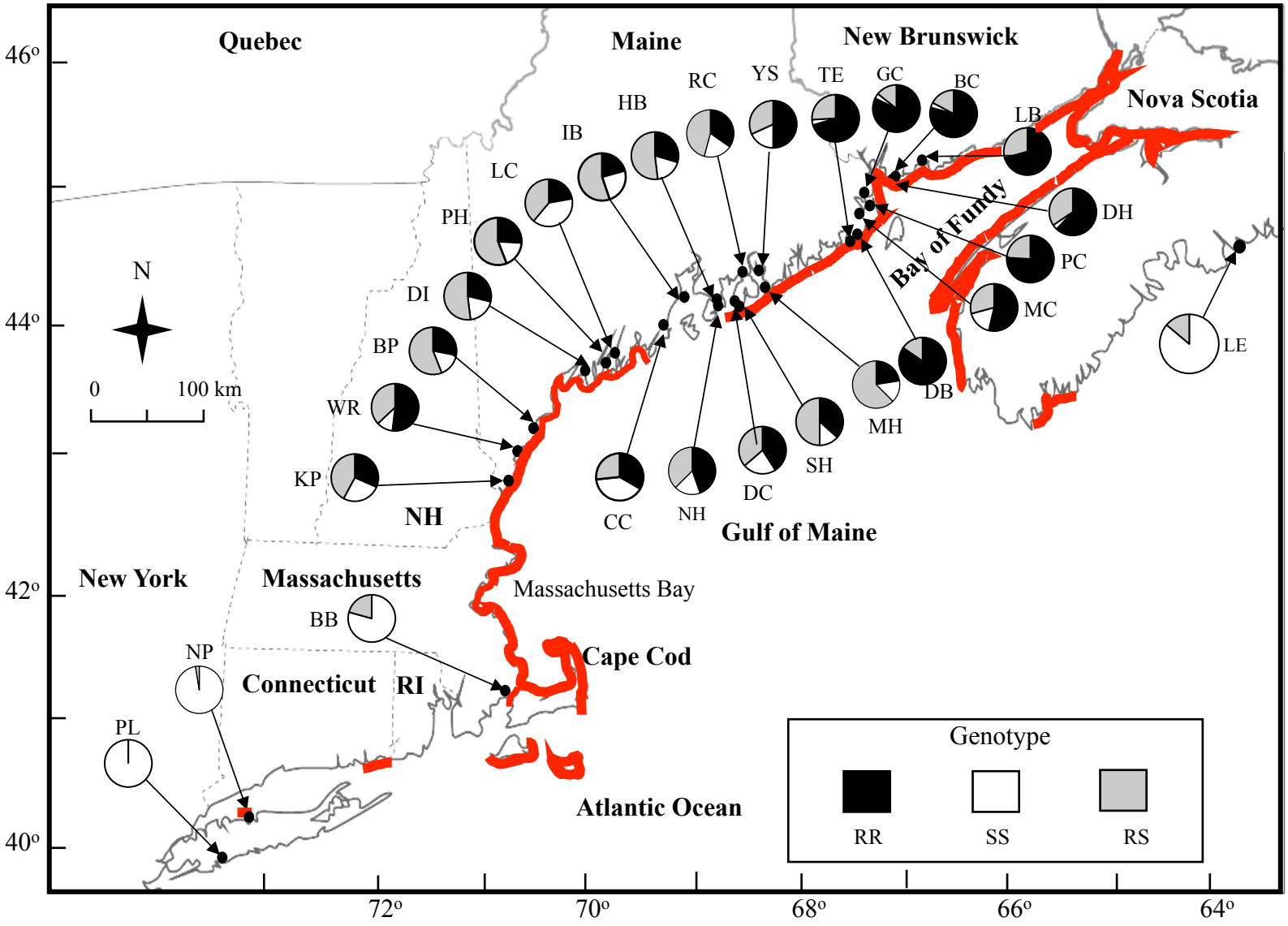
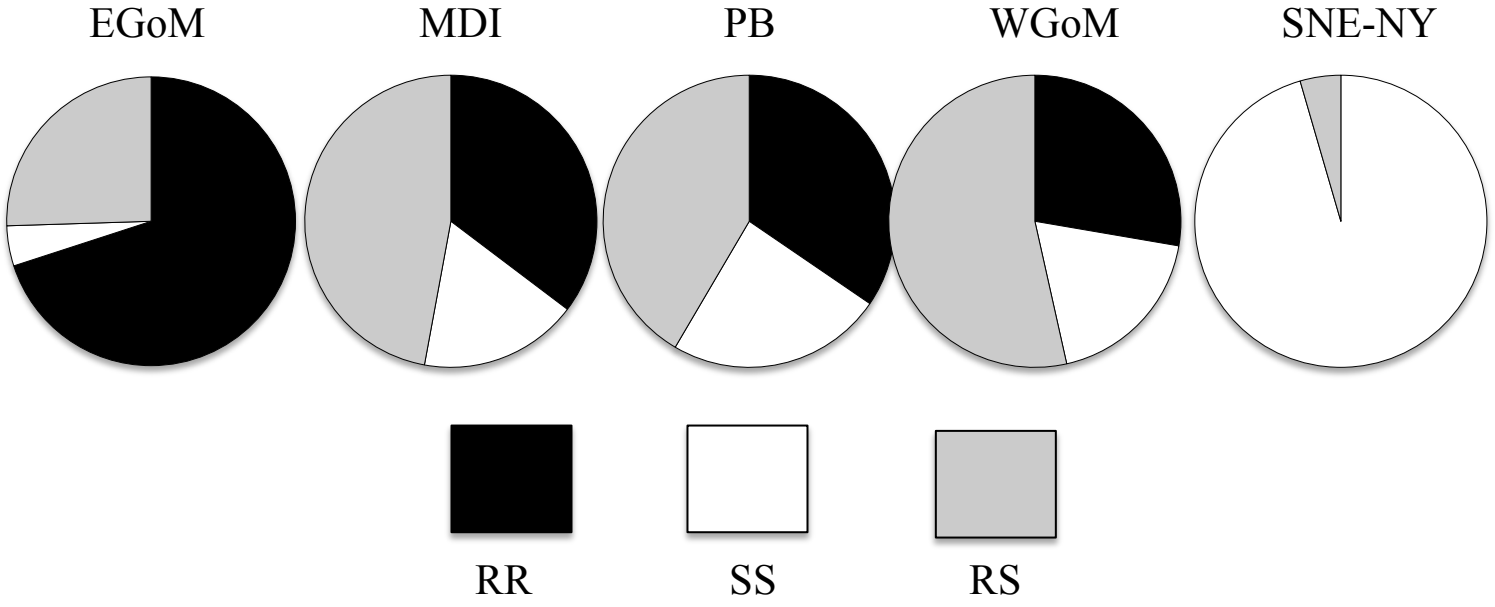
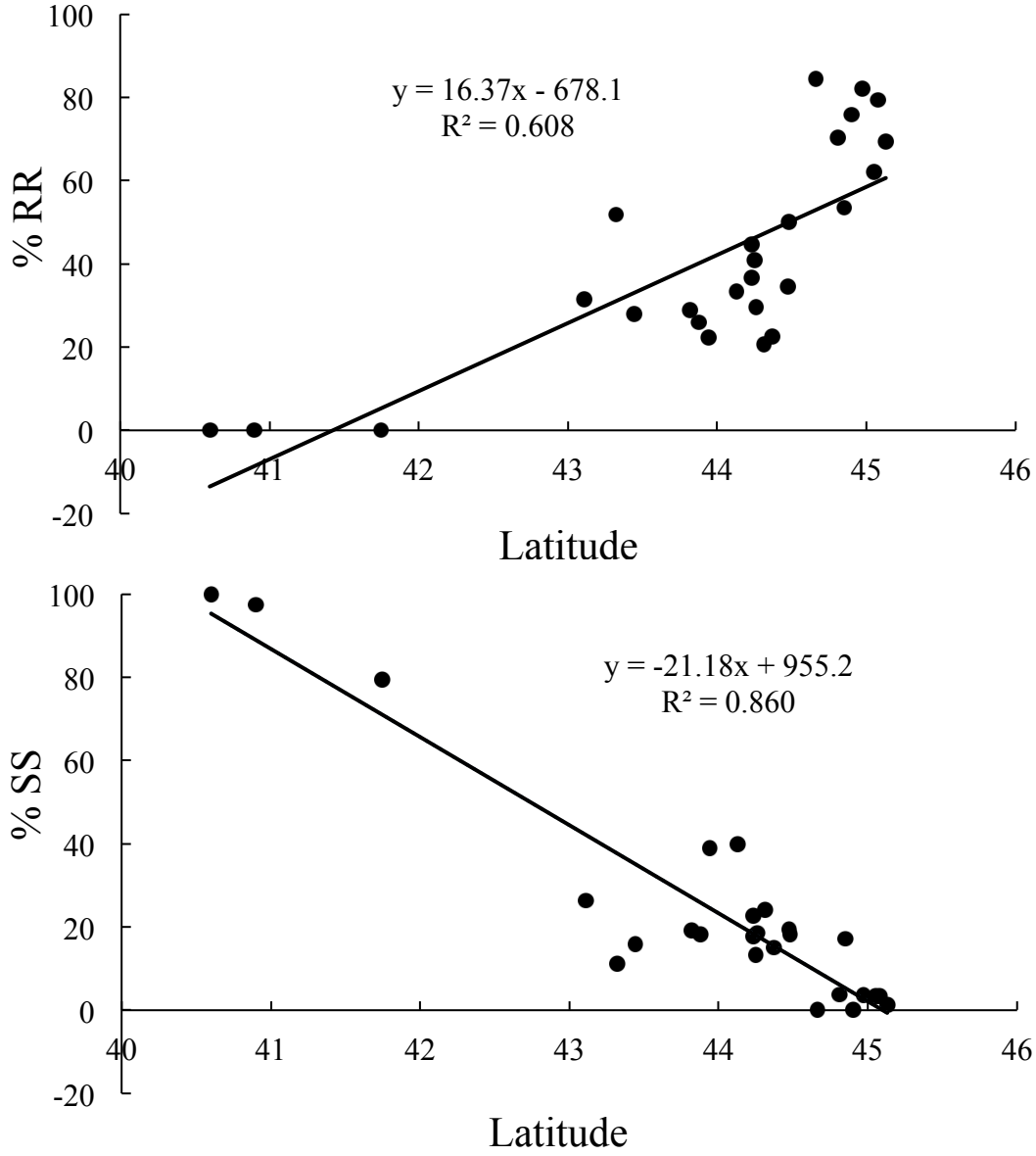
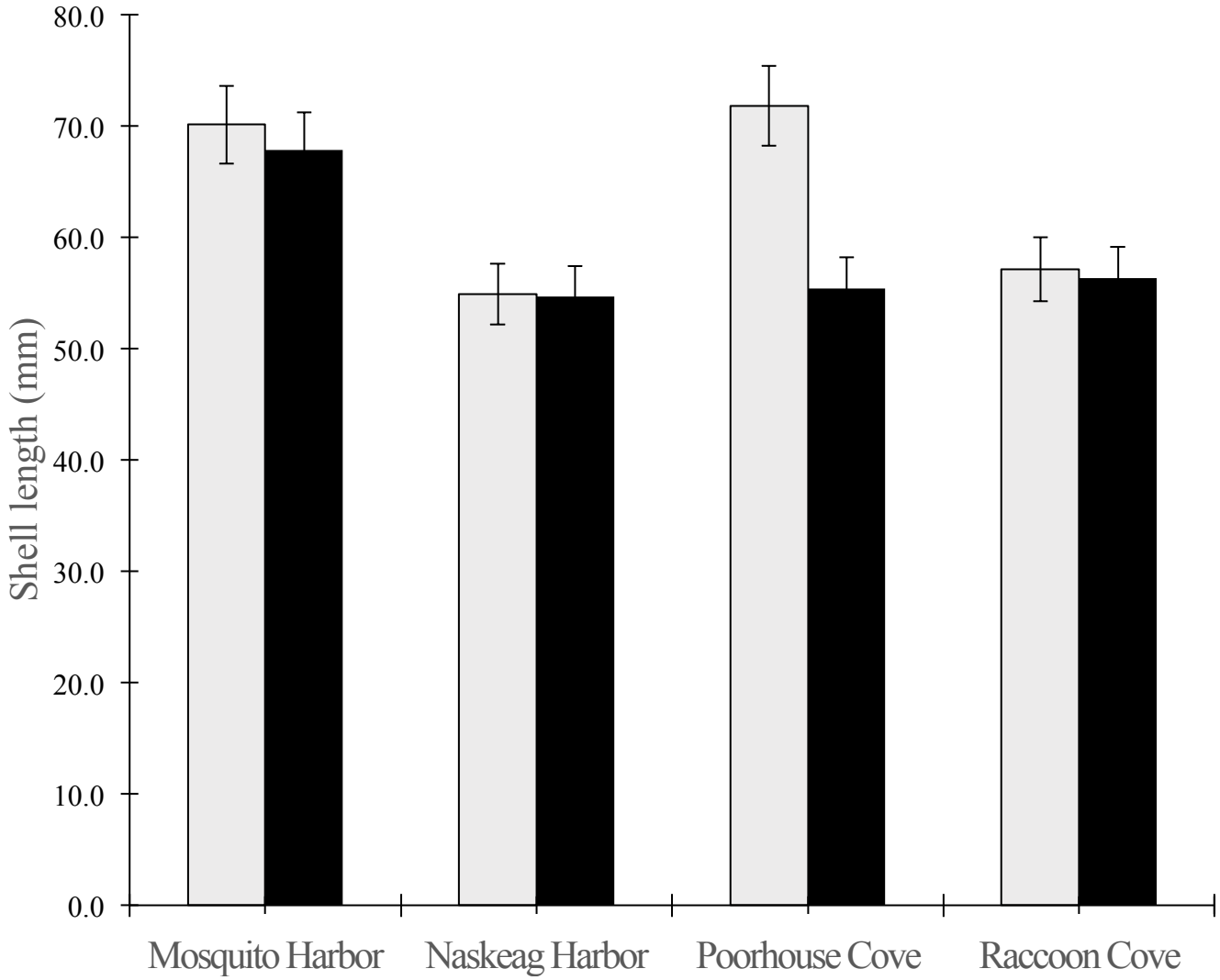

Quebec
Maine
New Brunswick
Nova Scotia
Bay of Fundy
N
SNE-NY
WGoM
PB
MDI
EGoM
46º
44º
42º
40º
72º
70º
68º
66º
64º
0
100 km
New York
Connecticut
RI
Massachusetts
NH
Massachusetts Bay
Gulf of Maine
Cape Cod
Atlantic Ocean
GC
DH
BC
LB
PC
YS
RC
HB
IB
CC
LC
DI
BP
WR
KP
PH
NH
SH
DC
MH
DB
TE
MC
LE
BB
NP
PL
BoF
Maine
EMCC
WMCC
Jordan Basin
Gulf of Maine


Oak Bay
Lepreau
Crow
Stuart Town
Passamaquody Bay
Bay of Fundy
Gulf of St. Lawrence Estuary
CANADA
USA
GoM
Atlantic Ocean
BC, QC
ML, QC
RS, QC
MI, QC
LE, NS
SB, ME
PB, ME
SC, ME
ES, MA
OR, MA
MS, NY
History of PSP
No known PSP history
(≤ 80 µg STX/100g)
Resistant
Sensitive
40°0'0"N
35°0'0"N
75°0'0"W
70°0'0"W
65°0'0"W
60°0'0"W


Quebec
Maine
New Brunswick
Nova Scotia
Bay of Fundy
NH
New York
Massachusetts
Massachusetts Bay
Cape Cod
Connecticut
RI
Gulf of Maine
Atlantic Ocean
N
0
100 km
46º
44º
42º
40º
72º
70º
68º
66º
64º
HB
RC
YS
TE
GC
BC
IB
LB
LC
PH
DH
DI
PC
BP
MC
LE
WR
DB
MH
KP
SH
DC
CC
NH
BB
NP
PL
Genotype
RR
SS
RS


EGoM
MDI
PB
WGoM
SNE-NY
RR
SS
RS


y = 16.37x - 678.1
R² = 0.608
% RR
Latitude
y = -21.18x + 955.2
R² = 0.860
% SS
Latitude


80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Shell length (mm)
Mosquito Harbor
Naskeag Harbor
Poorhouse Cove
Raccoon Cove