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Species composition and genetic diversity of farmed mussels in British Columbia, Canada

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ABSTRACT

The common blue mussel (*Mytilus edulis*) was introduced to British Columbia, Canada, in the 1980s as an aquaculture alternative to native mussel species. Since then, the mussel industry in Pacific Canada has expanded and includes operations utilizing traditional methods of broodstock selection based on visual qualitative and quantitative traits. The impacts of hatchery propagation on genetic diversity and implications for animal fitness have been previously studied for other aquatic species, and this study further examines the effect of hatchery production on three *M. edulis* aquaculture populations in relation to a wild originator population. Prior to microsatellite genetic analysis, animals were identified to species using nuclear markers and were found to contain varying proportions of pure *M. edulis* as well as other pure species and hybrids from the '*Mytilus edulis* complex'. Subsequently seven microsatellite markers were used to genotype 166 pure adult *M. edulis* individuals, all of which exhibited high levels of polymorphism. Allele frequencies at multiple loci did not conform to Hardy–Weinberg expectations and substantially less genetic diversity and very low effective population size estimates (N_e, calculated from linkage disequilibrium) were observed in farmed populations compared to the wild reference population. All populations were found to be genetically distinct based on F_{ST} estimates. Mean allelic richness was approximately three times higher in the wild reference population than the three farmed populations (21 compared to 7.51, 7.91 in the two populations selecting for size and 8.24 in the population selecting for a colour morph). Observed heterozygosity was not significantly decreased in the cultured colour morph population, but was significantly different in the two other culture populations in comparison to the wild group. Reduced genetic diversity of the aquaculture populations is likely at least partially due to small effective breeding groups during hatchery propagation, creating genetic drift over successive generations. Speculations about the influence of broodstock selection practices are tentative and should be addressed in further temporal studies. These results indicate the need for the effective management of hatchery operations, the importance of rigorous site inventory, genetic broodstock characterization, and that ideally pedigree programs should be developed to help maintain healthy and productive shellfish culture populations with adaptive fitness capacity.

Statement of relevance: Hatchery methods impact species purity and genetic diversity.

1. Introduction

On the Pacific coast of North America, in British Columbia (BC) Canada, there are four *Mytilus* species of marine mussels; the native *Mytilus trossulus* (or bay mussel) and *M. californianus* (California mussel), as well as two non-native cultured species *M. edulis* (blue mussel) and *M. galloprovincialis* (Mediterranean mussel). *Mytilus trossulus*, *M. galloprovincialis* and *M. edulis* are considered together as the '*Mytilus edulis* species complex'; they are phenotypically similar and are known to hybridize readily (Shields et al., 2008), hence genetic methodologies are needed to reliably distinguish individual species or hybrids (Heath et al., 1995). Whilst there have been introductions of the non-native species for aquaculture purposes in BC since an initial transfer in 1987, Heath et al. (1995) speculated that other sources of entry may have occurred through ballast water or hull-fouling, or that these species may have been present in BC for a considerable time period and were not previously accurately identified (N. Bourne, Fisheries and Oceans Canada, *pers. comm.*).

Intentional introductions of *M. edulis* occurred due to difficulties culturing the native *M. trossulus* in the 1980s, including post-spawning mortalities (Bower, 1989; Emmett et al., 1987) and shell breakages in market processing. Likewise, culture of *M. californianus* was not pursued

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due to very strong byssus attachments and tough meats, making it an undesirable product from a culture standpoint (B. Kingzett, Vancouver Island University, *pers. comm.*). Fisheries and Oceans Canada developed a mussel aquaculture program in 1987, when *M. edulis* broodstock were intentionally introduced to BC from Prince Edward Island (PEI) (Bill Heath, BC Ministry of Forests, Lands and Natural Resource Operations, *pers. comm.*). This initial introduction is believed to have been the base broodstock population for a number of aquaculture operations in BC, including those sampled in this study. Since that time, aquaculture operations have undertaken traditional selective *M. edulis* breeding programs for different visual qualitative and quantitative traits, such as colour morphs and larger size, but no pedigree-assisted broodstock programs are currently being employed.

Artificial selection in domesticated aquatic and terrestrial animals is an established practice to increase desirable traits in offspring such as growth rate, disease resistance, and marketability, but the majority of aquaculture operations do not genetically manage their populations to maintain diversity, which could have long-term adaptive benefits (FAO, 2008). Hatchery propagation methods can create small effective population sizes (Beaumont et al., 2010; Hedgecock and Sly, 1990) due to the high variation in the number of effective breeders contributing to progeny, and has been observed in shellfish populations (Boudry et al., 2002; Gaffney et al., 1993). This will limit the genetic variation available for the offspring (Hedgecock et al., 2007) and will increase the effects of random genetic drift. Domestication of aquatic stocks over generations will also alter a population gene pool (Aho et al., 2006) by selecting for genetic variants able to exploit hatchery culture conditions. Over time, this can create a markedly different population to the wild originator (FAO, 2008) and may cause deleterious phenotypic effects, such as reduced performance and viability in non-hatchery settings (Beaumont and Hoare, 2003). In bivalve aquaculture the broodstock are either collected from the wild and are therefore genetically 'replenished' annually, or from a cultured population where a broodstock program may be employed, such as may be the case for non-native aquaculture species. Selective breeding programs can maintain genetic diversity and have proven successful in increasing yield and growth rates in many species of bivalves including the Pacific oyster *Crassostrea gigas* (Langdon et al., 2003), hard shell clam *Mercenaria mercenaria* (Hadley et al., 1991), Catarina scallop *Argopecten circularis* (Ibarra et al., 1995; Ibarra et al., 1999) and bay scallop *Argopecten irradians irradians* (Zheng et al., 2012; Zheng et al., 2006; Zheng et al., 2008; Zheng et al., 2004).

The aims of this study are to compare neutral genetic diversity at DNA microsatellite markers in *M. edulis* from three aquaculture populations in BC and one wild reference population from PEI, to gain insight into if and how hatchery propagation methods may have altered patterns of genetic diversity within farmed populations. Genetic diversity in farmed populations may be affected by random genetic drift caused by hatchery methods, trait selection, and/or a past population bottleneck. While we requested only pure *M. edulis* individuals from mussel hatcheries for our analysis, we tested to confirm species and discovered the presence of hybrid and non-*M. edulis* individuals in these farmed stocks. Thus an additional aim of our study became to determine the species composition of the farmed populations and provide possible explanations for the occurrence of hybrids. Genetic diversity analysis was only performed on genetically confirmed pure *M. edulis* individuals. Results are interpreted in the context of their implications for subsequent mussel aquaculture in BC.

2. Materials and methods

2.1. Sample collection and DNA extraction

Marine mussels were collected from the licensed field tenures of three different aquaculture populations in British Columbia (BC); Blue Frontier Adventures Inc. (BFA) (50°09′30.68″N, 125°11′28.37″W), Island Sea Farms (ISF) (50°03′01.21″N, 124°59′26.64″W) and Taylor Shellfish Canada (TSC) (49°59′57.64″N, 124°42′51.44″W). At the time of sampling, the hatchery propagation strategies of the three farmed operations were as follows: BFA were breeding the F_{13} generation of a blond colour morph of *M. edulis*; ISF were culturing *M. edulis* and *M. galloprovincialis*, as well as producing *M. edulis* – *M. galloprovincialis* hybrids and use these as broodstock lines for subsequent seed generation; and TSC were collecting *M. edulis* broodstock from operations in Okeover Inlet BC, generating seed in a hatchery with seed juveniles on-grown in BC. Each of these aquaculture on-growing operations is located in nearshore coastal zones and all animals are cultured on ropes suspended from rafts or longlines. All samples provided for analysis were phenotypically identified by aquaculture operators as pure *M. edulis* individuals, regardless of other broodstock operations, such as hybrid development, that may be occurring by the same operator. Samples provided were also from the same hatchery cohorts, according to the author's knowledge. Reference wild populations were collected from two locations in Prince Edward Island; North London (46°28′10.52″N, 63°30′49.48″W) and Murray River (46°00′29.39″N, 62°31′23.48″W). These two reference populations are derived from wild seed inputs and combined to form one genetically diverse wild reference group, whereas all the above aquaculture populations are derived from hatchery-generated individuals. Laboratory processing of samples began with excision of a piece of mantle tissue and preservation in 96% undenatured ethanol for subsequent DNA extraction, which was done using Qiagen DNeasy® preparation kits.

2.2. Species identification

Morphologically based species identification is not possible for the *M. edulis* species complex so previously developed molecular methods were used for species identifications. Known *M. edulis*, *M. galloprovincialis* and *M. trossulus* standards were obtained from the Pacific Biological Station, Fisheries and Oceans Canada in Nanaimo, BC (courtesy R. Withler) and were used as positive controls. DNA fragments were PCR-amplified, and individuals identified to species level using the following four co-dominant marker loci. All PCR reactions were 25 μL in volume and contained 1.5 μL DNA, 10 pmol each forward and reverse primer, 200 μM dNTPs, 0.65 U HotStar™ Taq polymerase (QIAGEN) in 1 × reaction buffer. A PCR-RFLP method based on the internal transcribed spacer region of ribosomal DNA (*ITS*) developed by (Heath et al., 1995) was used to discriminate *M. trossulus* from *M. edulis*/*M. galloprovincialis* using the following cycling conditions: initial denaturation at 95 °C for 15 min, followed by 35 cycles 94 °C for 1 min (denature), 50 °C for 1 min (annealing), 70 °C for 2 min (extension), with a final elongation step of 70 °C for 5 min. PCR products were digested with *HhaI* (New England Biolabs). *Glu-3′* and *Glu-5′* markers amplify different segments of the polyphenolic adhesive byssal thread protein and can identify all three *Mytilus* species (Rawson et al., 1996). *Glu-3′* was amplified using primers JH-4 and PR-8 (Rawson et al., 1996), but modified so that JH-4 was labelled with a HEX tag and PR-8 with a poly-A tail, using the following thermal cycling conditions: initial denaturation of 95 °C for 15 min, followed by 30 cycles of 94 °C for 30 s (denaturation), 52 °C for 30 s (annealing), 72 °C for 30 s (extension), with a final elongation step of 70 °C for 5 min. The same cycling conditions were used to amplify *Glu-5′* using primers JH-5 and JH-54 (Rawson et al., 1996), modified by the attachment of a 6-FAM fluorescent tag to the 5′ end of JH-5 and the addition of a poly-A tail to the 5′ end of JH-54. The fourth marker, *Mytilus* Anonymous Locus-1 (*Mal-1*) can discriminate *M. trossulus* from *M. edulis*/*M. galloprovincialis* (Rawson et al., 1996), and was amplified with a cycling protocol of: initial denaturation of 95 °C for 15 min, followed by 35 cycles of 94 °C for 30s (denaturation), 54 °C for 1 min (annealing), 70 °C for 1 min (extension), with a final elongation step of 70 °C for 5 min. PCR products were digested with *SpeI* (New England Biolabs).

Depending on the marker, digested or undigested PCR products were visualized using DNA 7500 Agilent chips (Cat. No. 5067-1506), analysed using the 2100 Agilent Bioanalyzer, and identified to species or hybrid complex based on fragment lengths compared to known standards and according to published methods. Additional TSC samples were required to produce sufficient *M. edulis* for later genotyping and only those individuals confirmed to be pure *M. edulis* from all populations were used in subsequent genotyping studies. The type of species and species composition between the groups was compared using Chi-squared analysis (χ^2) in Excel and post-hoc Fisher Exact pairwise analysis was used to determine statistically significant groupings using Microsoft Office 2007.

2.3. Microsatellite genotyping of M. edulis

Samples were genotyped at seven previously isolated microsatellite loci (Lallias et al., 2009). All loci were amplified using a single-reaction nested PCR method using forward primers with fluorescently labelled M13 tail attached to their 5′ ends (Schuelke, 2000). Reverse primers were 'PIG-tailed' to improve scoring (Brownstein et al., 1996). All PCR reactions were 10 μL in volume and contained 5–50 ng genomic DNA, 200 μM dNTP, 2 pmol each of fluorescently labelled M13 primer and reverse primer, 0.5 pmol of M13-labelled forward primer, and 0.5 U Platinum Taq (Invitrogen) in 1× PCR amplification buffer. Reactions for loci Med362, Med740, and Med747 contained 1.5 mM $MgCl_2$, whereas for Med367, Med379, Med733, and Med737, 1× PCRx Enhancer Solution (Invitrogen) and 2.0 mM $MgSO_4$ were used instead. All loci except two specified below were amplified using the following 'touchdown' after a 3 min initial denaturation step at 95 °C: 30 s at 95 °C, 30 s at 62 °C to 54 °C (dropping 2 °C every two cycles), 30 s at 72 °C; followed by 23 cycles of 30 s at 95 °C, 30 s at 54 °C, 30 s at 72 °C, and one final cycle of 10 min at 72 °C. Med367 was amplified using a similar touchdown, except that only 5 touchdown cycles were used (from 65 °C to 53 °C dropping 3 °C every cycle) and 30 cycles of annealing at 54 °C were used. Med737 was amplified using 10 cycles of 30 s at 95 °C, 1 min at 63 °C, 1 min 72 °C; followed by 25 more cycles with the annealing temperature dropped to 53 °C, and ending with one final cycle of 10 min at 72 °C. PCR products were capillary electrophoresed using an ABI 3130*xl* Genetic Analyzer. Data were analysed using GeneMapper v4.0 (Applied Biosystems).

2.4. Microsatellite data analysis

MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004) was used to check for null alleles and scoring errors due to stuttering or large allele dropout at each microsatellite locus in each population. Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were performed using GENEPOP (Raymond and Rousset, 1995), with the dememorization number set at 1000 and the number of batches at 300 batches (2000 iterations/batch) for each test. Weir and Cockerham's (Weir and Cockerham, 1984) inbreeding coefficient (f) for each locus in each population was also calculated in GENEPOP with significance values interpreted after Bonferroni correction for multiple tests (Rice, 1989). At the population level, the inbreeding coefficient F_{IS} was generated using an analysis of molecular variance (AMOVA) (Excoffier et al., 1992) in Arlequin v3.5.1.2 (Schneider et al., 2000) with significance testing by 1023 permutations. Descriptive diversity measures for each locus including allele number and observed and expected heterozygosities were generated using GenAlEx v6.3 (Peakall and Smouse, 2006). Tests for population genetic structure on a global and pairwise population basis were done using F_{ST} derived from an AMOVA, again using Arlequin software and 1023 data permutations. To ensure that results were not confounded by possible null alleles, F_{ST} estimates were generated using all loci and excluding the four loci that deviated from HWE in the wild population, for which some of the conditions required for HWE are more plausible (e.g. large population size, random mating, and panmixia). For a visual representation of genetic patterns, a principal co-ordinates (PCO) analysis of pairwise population F_{ST} values was performed (using GenAlEx).

Genetic diversity in wild and farmed mussels was examined in the context of the hypothesis that farmed mussels may have lost diversity through a population bottleneck associated with a founder event when originally introduced to the West coast of Canada (from the East coast), and/or through inbreeding effects associated with broodstock selection. Either of these is expected to cause a significant reduction in effective population size, which in turn is expected to cause a population to lose substantial genetic variation, corresponding to a reduction in allele number and heterozygosity at polymorphic loci (Chakraborty and Nei, 1977; Nei et al., 1975). We tested for heterozygosity differences among all populations across all loci, with non-proportional heterozygote and homozygote data, using a Cochran–Mantel-Haenszel (χ^2_{MH}) contingency test. This was followed by a generalized linear effects model using the binomial family applied to observed non-proportional heterozygote data to determine population differences, using R (version 2.11.1; R Development Core Team 2010). We used the method of Cornuet and Luikart (1996) for detecting recent population bottlenecks based on the expectation that bottlenecked populations experience a transient phase whereby the observed heterozygosity is higher than the expected heterozygosity at mutation-drift equilibrium (Piry et al., 1999). A one-tailed Wilcoxon signed rank test implemented in the program BOTTLENECK (Piry et al., 1999) was used to test for heterozygosity excess under the two-phase mutation model with the proportion of single step mutations set to 95% (as recommended by Piry et al. (1999) for microsatellite data) and using 1000 data permutations. Tests were performed separately on each population and after pooling the farmed populations. As an alternative to using heterozygosity as the basis for detecting low population sizes, evidence of a restricted number of breeders in the hatchery propagated populations was assessed by estimating effective populations sizes (N_e) in all four sampled populations using the linkage disequilibrium method in NeEstimator v2 (Do et al., 2014). This method is based on the principle that N_e decreases as genetic drift with few parents generates non-random associations among alleles at unlinked loci (Luikart et al., 2010). N_e estimates were generated excluding alleles with frequencies smaller than 0.02 to reduce bias related to rare alleles (Waples and Do, 2008) and 95% confidence intervals (CI) are reported.

3. Results

3.1. Species composition of sampled populations

All mussel samples were phenotypically identified by operators as pure *Mytilus edulis* individuals at time of provision for analysis. It was therefore assumed that all "*M. edulis*" farmed populations would contain pure *M. edulis* individuals, but the sampled populations unexpectedly contained other pure species and hybrids from the '*Mytilus edulis* complex' in varying proportions, and therefore all individuals were genetically identified to species. The four genetic markers used for molecular species identification revealed that the only pure *Mytilus edulis* population was the wild reference population from Prince Edward Island. In addition to some pure *M. edulis* individuals, the aquaculture populations also contained pure *M. galloprovincialis, M. trossulus* and hybrids (Table 1). The degree of hybridization and species observed depended upon the population sampled. The BFA population contained predominantly pure *M. edulis* individuals (98%) and a small percentage of *M. trossulus* x *M. edulis* hybrids (2%). The ISF and TSC populations contained 37% and 53% *M. edulis* x *M. galloprovincialis* hybrids, respectively. Fisher's Exact pairwise analysis (Fisher, 1922) revealed highly significant differences in the species composition observed between the wild and BC aquaculture populations ($p < 0.001$). Specifically, the PEI and BFA populations were significantly different from the ISF and

Table 1
Mytilus species composition based on genotype data (%) for the wild reference population (PEI) and three B.C. aquaculture populations.

Population	N	Pure species			Hybrid species	
		M.e	*M.g*	*M.t*	*M.e:M.g*	*M.e.:M.t*
PEI[a]	100	100	0	0	0	0
BFA[a]	100	98	0	0	0	2
ISF[b]	100	58	5	0	37	0
TSC[c]	100	32	15	0	53	0

(*M.e* = *M. edulis*, *M.g* = *M. galloprovincialis*, *M.t* = *M. trossulus*, PEI = Prince Edward Island, BFA = Blue Frontier Adventures Inc., ISF = Island Sea Farms and TSC = Taylor Shellfish Canada). Letters denote statistically significant groupings following Fisher exact pairwise analysis tests.

TSC populations (Table 1). In addition, there were significant differences in population species composition between the ISF and TSC populations.

3.2. Microsatellite analyses of M. edulis

The seven microsatellite loci analysed here exhibited high levels of polymorphism; the number of alleles per locus across all populations varied from 18 to 54, with allelic diversity within populations ranging from 4 to 38 alleles per locus (Table 2). Several locus/population comparisons showed significant departures from HWE. Before Bonferroni correction for multiple tests, 21 of 28 tests were statistically significant; after the correction, 14 remained significant (Table 2). These large deviations from HWE did indeed correspond with highly significant F_{IS} estimates at the population level, as follows: PEI ($F_{IS} = 0.20$, $p < 0.0001$), ISF ($F_{IS} = 0.20$, $p < 0.0001$), BFA ($F_{IS} = 0.10$, $p < 0.01$), and TSC ($F_{IS} = 0.26$, $p < 0.0001$).

Heterozygosity analysis using a Cochran–Mantel-Haenszel test found significant differences among populations (χ2MH = 36.526, df = 3, $p < 0.0001$). Further analysis using a generalized linear mixed effect model (binomial family) found that the BSF population (co-efficient = −0.2761, $p = 0.1338$) did not significantly differ in levels of heterozygosity to the wild population, whereas both ISF (co-efficient = −0.7367, $p < 0.001$) and TSC (co-efficient = −1.0473, $p < 0.001$) did differ significantly. Plot analysis and Table 2 show that TSC showed by far the lowest levels of heterozygotes at loci Me362, Me740 and Me747 whereas ISF showed the lowest levels at loci Me367 and Me733. There was no evidence of heterozygosity excess (as evaluated by Wilcoxon signed ranks tests in BOTTLENECK) when farmed populations were considered separately or together.

Results of MICRO-CHECKER indicated possible null alleles for 16 of 28 locus/population comparisons and cited an excess of homozygotes as the reason for suspicion in all cases. All 14 of the locus/population comparisons that yielded highly significant p values (i.e. $p < 0.001$) in the HWE exact tests (see Table 2) were among those suspected as having null alleles. MICRO-CHECKER also returned the TSC population at Med367 and the BFA population at Med362 as possibly having null alleles. Scoring errors and large allele dropout were not implicated except for in two of the 28 locus/population comparisons, which suggested stuttering causing a scoring problem at locus Med733 in TSC population and locus Med362 in the PEI population. However, we considered it unlikely that a scoring error would manifest in only one of four populations genotyped.

Tests for linkage disequilibrium were statistically significant for nine out of 84 locus/population tests before Bonferroni correction (Rice, 1989). Five tests remained significant after correction, all of which were in farmed populations: Med367/Med379 in all three farmed populations and Med747/Med737 and Med733/Med362 in BFA. No evidence of linkage disequilibrium was found for any of the locus pairs in the wild PEI population here (or in a wild population from the Irish Sea tested by Lallias et al. (2009)). N_e estimates indicated very small contemporary effective population sizes in all three farmed populations (ISF: $N_e = 49$, CI 31–95; BFA: $N_e = 33$, CI 24–49; TSC: $N_e = 47$, CI 27–115) whereas there was no evidence of a limited population size in the wild PEI population ($N_e = \infty$).

Table 2
Estimates of the number of alleles per locus (N_A), allelic richness (A_R) and the expected (H_E) and observed (H_O) heterozygosities for 7 microsatellite loci in 4 populations of genetically confirmed *Mytilus edulis* mussels in British Columbia (N = sample size at each locus).

Population		Locus							
		Med362	Med367	Med379	Med733	Med740	Med747	Med737	Average
PEI	N	43	47	44	46	46	45	34	43.6
	N_A	21	20	38	13	20	42	27	25.9
	A_R	17.64	15.91	29.3	11.46	16.02	32.03	24.75	21.02
	H_E	0.91	0.89	0.96	0.85	0.86	0.92	0.94	0.9
	H_O	0.51	0.81	0.93	0.28	0.8	0.73	0.71	0.68
	f	0.45	0.1	0.04	0.67	0.07	0.25	0.27	
	p	<0.001*	0.15	0.26	<0.001*	0.33	<0.001*	<0.001*	
ISF	N	45	43	41	43	47	41	39	42.7
	N_A	6	6	15	4	8	6	10	7.9
	A_R	6.00	5.92	13.61	3.96	7.80	5.68	9.63	7.51
	H_E	0.82	0.68	0.82	0.39	0.79	0.8	0.84	0.73
	H_O	0.8	0.35	0.81	0.12	0.68	0.37	0.64	0.54
	f	0.03	0.49	0.03	0.71	0.15	0.55	0.25	
	p	<0.05	<0.001*	<0.05	<0.001*	<0.05	<0.001*	<0.001*	
BFA	N	45	46	45	44	46	44	39	44.1
	N_A	9	6	13	5	7	12	11	9
	A_R	8.85	5.22	11.24	4.51	6.55	10.80	10.54	8.24
	H_E	0.84	0.61	0.85	0.66	0.79	0.83	0.86	0.78
	H_O	0.67	0.52	0.84	0.21	0.89	0.52	0.8	0.64
	f	0.21	0.16	0.02	0.7	0	0.38	0.09	
	p	<0.01	<0.01	0.2	<0.001*	0.31	<0.001*	0.19	
TSC	N	41	37	37	36	36	30	28	35
	N_A	8	6	15	7	8	6	8	8.3
	A_R	7.76	5.50	14.09	6.46	7.64	5.93	8.00	7.91
	H_E	0.77	0.65	0.9	0.64	0.7	0.77	0.83	0.76
	H_O	0.32	0.49	0.89	0.36	0.33	0.23	0.71	0.48
	f	0.6	0.27	0.02	0.44	0.54	0.71	0.15	
	p	<0.001*	0.2	<0.05	<0.001*	<0.001*	<0.001*	<0.05	

Population names are abbreviated as follows: Prince Edward Island (PEI), Island Sea Farms (ISF), Blue Frontier Adventures Inc. (BFA), Taylor Shellfish Canada (TSC). (f = Weir and Cockerham's (1984) inbreeding coefficient, p values are from Hardy–Weinberg exact tests, * denotes significant p values after Bonferroni correction).

Table 3
Patterns of allele presence of genetically confirmed Mytilus edulis samples, as indicated by a symbol, or absence, as indicated by a blank space, at seven microsatellite loci in three farmed and one wild (PEI) mussel population.

Allele No.	Med362				Med367				Med379				Med733				Med740				Med747				Med737			
	PEI	ISF	BFA	TSC	PEI	ISF	BFA	TSC	PEI	ISF	BFA	TSC	PEI	ISF	BFA	TSC	PEI	ISF	BFA	TSC	PEI	ISF	BFA	TSC	PEI	ISF	BFA	TSC
1	x				x	○	●	Δ	x	○	●	Δ	x				x				x				x			
2	x		●	Δ	x						●		x				x						●		x	○	●	Δ
3	x				x				x	○	●	Δ		○			x				x					○		Δ
4	x	○	●	Δ	x	○	●	Δ	x				x				x	○	●	Δ	x	○	●	Δ	x			
5	x				x	○	●	Δ	x				x				x	○		Δ	x				x	○	●	Δ
6	x	○	●	Δ	x		●		x				x			Δ	x				x					○	●	Δ
7	x				x				x			Δ	x	○	●	Δ	x	○	●	Δ	x				x	○	●	
8	x				x	○	●	Δ	x				x			Δ	x	○	●	Δ		○	●	Δ	x	○	●	Δ
9	x	○	●	Δ	x	○	●	Δ	x	○	●	Δ		○	●	Δ	x	○	●	Δ	x				x	○		
10	x				x				x	○	●		x				x		●		x				x		●	
11	x	○	●	Δ	x				x				x	○	●	Δ	x		●		x				x		●	
12	x	○	●	Δ	x				x				x				x				x				x			
13				Δ	x	○		Δ	x				x					○		Δ	x	○	●	Δ	x		●	
14	x				x				x				x				x				x		●		x			
15	x				x				x						●	Δ	x				x				x	○	●	
16	x				x				x				x					○		Δ	x				x			
17	x		●		x				x		●	Δ			●			○	●	Δ	x				x			
18	x				x				x							Δ	x				x				x			
19		○	●	Δ	x				x								x				x				x			
20	x				x				x								x				x				x			
21			●						x			Δ					x				x				x	○	●	Δ
22	x								x	○		Δ					x				x				x			
23	x								x								x				x		●		x	○	●	Δ
24	x								x		●										x				x			
25									x												x				x			
26										○	●										x	○	●	Δ	x			
27											●										x				x			
28									x												x				x			
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Allelic diversity patterns in the three farmed populations in British Columbia were similar to one another, but differed markedly from those in the wild PEI population (Table 3), so for descriptive measures of genetic diversity the three farmed populations are considered together. Across all loci, the total number of alleles and the number of private (i.e. population-specific) alleles were both much higher in the wild PEI population than the three farmed populations combined, despite average sample size per locus being much lower in the former (44) than in the latter (122) (Table 3). The allelic richness per locus in the wild PEI population ranged from 11.46 to 32.03, whereas that in the farmed populations ranged from 3.96 to 14.09 (Table 2); indeed, the mean allelic richness per locus was 21.02 for the wild population and 7.51 for ISF, 8.24 for BFA and 7.91 for TSC.

Higher allelic diversity at microsatellite loci in wild vs. farmed mussels is consistent with expectations associated with a founder event, inbreeding, and / or random genetic drift, all of which predict a loss of rare alleles. Across all loci (Table 2), a total of 181 alleles were detected in the PEI population, 125 (69%) of which were private to this population, compared to 56 alleles sampled from the three farmed populations combined, 27 (48%) of which were private. We compared the frequencies of alleles private to the wild population with the frequencies of those that were shared with the farmed populations for evidence that founder effects or random genetic drift may have eliminated low frequency alleles in the latter. The mean frequency of alleles in wild mussels from PEI that were also found in the farmed mussels in British Columbia (0.062, $n = 56$) was higher than the mean frequency of alleles unique to the wild population (0.028, $n = 125$), and this difference was highly significant ($p < 0.001$). Patterns of allele presence / absence shown in Table 3 depict a much more continuous distribution of sampled alleles at each locus in the wild compared to the farmed mussels.

F_{ST} estimates generated using only the three loci that were in HWE in the wild population were remarkably similar to those generated using all seven loci (i.e. including the other four loci that were not in HWE in the wild population). The global F_{ST} across all loci and populations was 0.068 and 0.076 for the three-locus and seven-locus data sets, respectively, both of which were highly statistically significant ($p < 0.0001$). All pairwise population F_{ST} estimates generated from both the three-locus and seven-locus data sets depicted highly statistically significant levels of genetic differentiation ($p = 0.001$; Table 4, Fig. 1).

4. Discussion

This study sought to examine the impact of hatchery propagation strategies on the genetic diversity of three aquaculture populations of marine mussels, in comparison to a wild reference population. We expected all sampled populations to contain either totally pure or very high proportions of pure *Mytilus edulis*; however, high proportions of *M. edulis* × *M. galloprovincialis* hybrids were discovered. There may be a number of factors influencing the species composition of these populations. Intuitively, it seems likely that the phenotypic similarity among species in the *M. edulis* complex may have resulted in other species being included as hatchery broodstock for subsequent juvenile

Table 4
F_{ST} estimates based on 3 microsatellite loci that were in HWE in the wild population (above diagonal) and those based on all 7 loci (below diagonal). Each of the twelve pairwise population comparisons gave highly significant p values (<0.001) based on 1023 permutations. All animals screened were confirmed as pure *Mytilus edulis* individuals. (PEI = Prince Edward Island, ISF = Island Sea Farms, BFA = Blue Frontier Adventures Inc., TSC = Taylor Shellfish Canada).

	PEI	ISF	BFA	TSC
PEI	–	0.079	0.074	0.081
ISF	0.089	–	0.033	0.056
BFA	0.075	0.075	–	0.083
TSC	0.078	0.057	0.089	–

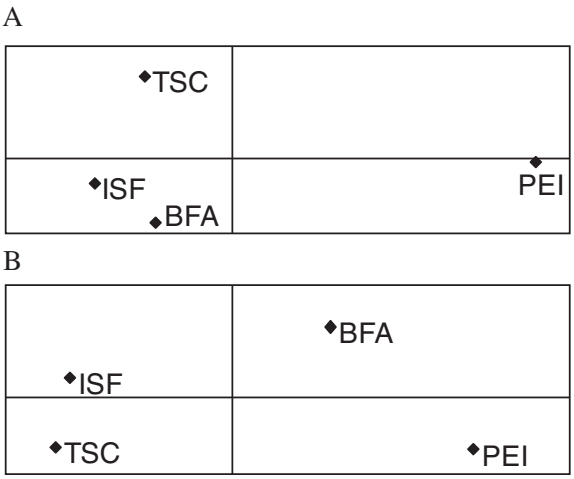

Fig. 1. Principal coordinates analysis plot of pairwise population F_{ST} values for three farmed populations (TSC = Taylor Shellfish Canada, ISF = Island Sea Farms, BFA = Blue Frontier Adventures Inc.) and one wild population (PEI = Prince Edward Island) of genetically confirmed *Mytilus edulis* based on: (A) 3 microsatellite loci, all of which were in HWE in the wild population; and (B) 7 microsatellite loci, four of which were not in HWE in the wild population. See Table 2 for results of HWE tests per locus and Table 4 for F_{ST} values and significance testing.

production. Other potential factors include differential adaptation to hatchery conditions (FAO, 2008), varying survival of genotypes influenced by genotype-environment interactions (Slatkin, 1973; Springer and Heath, 2007) or the relatively high contribution to progeny of some contributors (Hedgecock, 1994; Lallias et al., 2010). Such effects would produce skewed broodstock species compositions and subsequent offspring, and be compounded over time with successive generations. That ISF produces a hybrid *M. edulis*: *M. galloprovincialis* line may have contributed to that farm having the highest level of cross-species hybridization in this study. The proportions of *M. edulis*, *M. galloprovincialis* and *M. edulis* × *M. galloprovincialis* hybrids in the ISF population conform to random mating expectations (χ^2 goodness of fit $p = 0.96$), rather than an excess of hybrids. This is consistent with the tested lines not being purpose-generated hybrids as it suggests that hybridization occurred randomly due to lines of both species being co-cultured. The prevention of hybrid introgression into pure species parental stocks can be a major challenge for aquaculture operations (FAO, 2008). A further study examining the potential excess of hybrids in hybrid production lines would provide useful indicators of the efficiency in hybrid generation, employing mitochondrial markers to determine reciprocity and potential disruption in mitotype regulation in persistently crossed populations.

Species in the *Mytilus edulis* complex are known to readily hybridize, and indeed non-native hybrid genotypes have been observed on the east coast of Vancouver Island (Heath et al., 1995; Shields et al., 2010). In the 1980s there were reports of *M. edulis* growing alongside *M. trossulus* aquaculture lines, indicating that non-native species may have been present in BC waters for an extended period (N. Bourne, Fisheries and Oceans Canada, *pers. comm.*), but these observations were not genetically confirmed. While environmental selection against hybrids is believed to contribute to reproductive isolation in *Mytilus* species, the selection for hybrid performance through the broodstock program may have increased the hybrid stability (Springer and Heath, 2007), perhaps also explaining the high numbers of hybrids in the ISF population. However, the TSC population displayed the highest level of hybridization between *M. edulis* and *M. galloprovincialis* (53%), and does not employ a hybrid breeding program, although both species are grown at operation sites in BC so cross-contamination of lines may have occurred. Blue Frontier Adventures Inc. are selecting for a 'golden' colour morph of *M. edulis*, but to date have not reached farm-scale production, with golden colour mussels being maintained on a smaller scale. Therefore subsequent broodstock generations may have had contributions of wild *M. trossulus* individuals (which also naturally display 'blond' colour morphs) through larvae settling upon broodstock rope lines, which are then inadvertently used as broodstock. This may account for the small degree of *M. edulis* × *M. trossulus* hybrids (2%) observed at that farm in this study, and it would be interesting to determine if these proportions alter with full-scale production. Overall, the very low levels of *M. trossulus* detected in all studied populations highlights the successful preferential selection of the other species by aquaculture operators. Another factor in the low levels of observed *M. trossulus* may be a function of differential, and even asymmetric, gamete compatibility within the *M. edulis* complex. Laboratory studies have shown a strong maternal effect between *M. edulis and M. trossulus* fertilization success (Rawson et al., 2003), and pure species of *M. edulis* and *M. galloprovincialis* larvae were observed to grow faster than hybrid larvae (Beaumont et al., 2004), which in combination with the routine size-culling employed in shellfish hatcheries to select faster-growing individuals (Taris et al., 2006) may then have assisted in maintaining the level of pure species observed.

Although not the focus of the current study, there have been a number of studies examining the occurrence of different species within the *M. edulis* complex in British Columbia over the past two decades, with likely introductions through aquaculture and ship traffic (Crego-Prieto et al., 2015; Heath et al., 1995; Heath et al., 1996; Shields et al., 2008; Shields et al., 2010; Springer and Heath, 2007; Yanick et al., 2003). With the routine exclusion of the native *M. trossulus* from aquaculture operations in BC, shellfish culture can be a source of repeated introductions of non-natives to wild populations (Yanick, 2002). Numbers of non-indigenous species have increased from 6.2% (Heath et al., 1995) to 6.6% in 2005 in 2006 (Shields et al., 2010), to 8.7% in 2012 according to the most recent study (Crego-Prieto et al., 2015). This creates hybrid zones, where the different pure mussel species are found in close proximity and can produce hybrid offspring. Genotype-environment interactions can affect hybrid performance so if these hybrids were to exhibit a significant fitness advantage then excessive hybrid zones would have developed. However, the southern hybrid zone on Vancouver Island (VI) has not extended to the north, likely due to physical dispersal barriers such as oceanographic and hydrographic features in the Strait of Georgia (Shields et al., 2010). This implies that the hybrid zone within southern VI is relatively stable (due to biological barriers such as gamete incompatibility or spawning synchrony), and that in open areas *M. galloprovincialis* has a higher probability of hybridization than *M. edulis* (Crego-Prieto et al., 2015).

An excess of homozygotes was seen in over half of the locus / population comparisons, and null alleles were often the suspected cause of observed deviations from the Hardy–Weinberg model. Homozygote excess and null alleles is a common observation in bivalves in both allozyme (Gaffney, 1994; Zouros and Foltz, 1984) and microsatellite markers (Launey et al., 2002; McGoldrick et al., 2000; Reece et al., 2004). No corresponding heterozygote excess was observed, suggesting no distortion of the microsatellite loci used (Reece et al., 2004). Significant differences in heterozygosity between the wild and two of the farmed populations were observed, as has been found in other shellfish microsatellite (Li et al., 2004; Xiao et al., 2011) and allozyme (Smith and Conroy, 1992) studies. Both the ISF and TSC populations visually select broodstock for progeny generation based upon large size, not using genetic trait selection or accounting for environmental interactions in animals that have relatively short generational times, like mussels. Overtime siblings may have inadvertently been used to produce progeny. The initial founding effect of the mussel introduction from which all these aquaculture populations originated followed by genetic drift during hatchery propagation, may be causes for the differential decreased heterozygosity of these two 'quantitative' populations.

All three aquaculture populations were significantly genetically differentiated from the wild population they are thought to have arisen from through a single introduction event in 1992 (Kenn Renaud, Blue

Frontier Adventures Inc., pers. comm.). The reduction in the number of alleles and rare alleles in the aquaculture populations is consistent with a founder effect (Allendorf, 1986; Frost et al., 2006; Yu and Li, 2007) and genetic drift in hatchery populations (Hedgecock and Sly, 1990). Indeed, estimates of N_e based on linkage disequilibrium gave strong evidence for a very small number of breeders in farmed populations (average 43). This method is thought to have similar power to the BOTTLENECK test for heterozygosity excess for detecting population decline (Luikart et al., 2010) the latter of which did not generate a signal in this data set presumably due to homozygote deficiency potentially caused by null alleles. Despite that the LD- N_e estimator is susceptible to potential bias caused by other population-level processes (e.g. substructure, admixture; Luikart et al., 2010), it has been shown to be a precise way of obtaining N_e for populations with <200 breeders (Waples and Do, 2010).

Without multigenerational analysis and details of broodstock spawning, the role of selection or inbreeding can only be speculated at this time. The BFA population selecting for the colour morph was not found to have significantly different levels of heterozygosity compared to the wild population. Allelic diversity is more sensitive to bottleneck events than heterozygosity and therefore there is often delay until measureable reductions in heterozygosity are observed (Xiao et al., 2011). Routine size-culling operations in the hatchery may preserve heterozygotic fitness through eliminating smaller inbred individuals (Taris et al., 2007; Taris et al., 2006) and hatchery propagated marine bivalves have been observed to lose only allelic diversity (Carlsson et al., 2006; Hedgecock and Sly, 1990; Yu and Guo, 2004). In addition, selection processes operating prior to their sampling as adult individuals may preserve levels of heterozygotes (Bierne et al., 1998), with the selection of animals that perform best in hatchery conditions (FAO, 2008).

In contrast to the ISF and TSC populations, where larger broodstock individuals were visually 'selected' for progeny generation, the BSF operation was employing qualitative selection criteria (mussel shell colour morph) and had the highest mean allele number, heterozygosity levels comparable to the wild population and lowest F_{IS} of all the farmed populations. The initial *M. edulis* broodstock BSF population had 5% "blond" individuals, which had increased to 99% by the F_2 generation (Kenn Renaud, Blue Frontier Adventures Inc., *pers. comm.*) indicating that the colour morph was highly hereditary. Some report that discrete traits such as colour polymorphism are related to few genes (Beaumont and Hoare, 2003), such as the Manila clam *Ruditapes philippinarum* where two major genes control the symmetry and ornamentation of observed shell patterns, and in golden colour variation of the carp *Cyprinus carpio* (Beaumont et al., 2010). The selection of rare blond individuals in this case may have imposed an especially severe bottleneck: this population had the lowest effective number of breeders for the generation studied. The number of initial broodstock that effectively contributed to the first progeny is unknown.

We can only speculate about whether differences in qualitative versus quantitative hatchery propagation has led to differential domestication impacts on genetic structure. Broodstock selection for polygenic production traits are controlled by numerous genes, and aquaculture product desirable traits commonly include growth rate (Ward et al., 2000), age at maturation, disease resistance and marketability (Beaumont et al., 2010). As the ISF and TSC operations employ visual indicators of production success, such as yield, growth survival and bysuss thread strength, rather than comprehensive genetically-based pedigree assessment programs, they therefore do not take into account annual environmental variations to performance results and subsequent breeding cohorts. Based on results generated here of low N_e, it is highly plausible that genetic drift over multiple generations has reduced the population genetic variation, which is commonly observed in hatchery stocks (Allendorf and Phelps, 1980; Hedgecock and Sly, 1990; Manzi et al., 1991; Smith and Conroy, 1992; Xiao et al., 2011). This study used only one sampling time point, and temporal sampling would be needed to understand whether specific artificial selection practices contributed to the observed patterns of low genetic diversity in hatchery populations.

This study highlights the need for the effective management of shellfish hatchery operations, propagation protocols and broodstock populations, including the monitoring and maintaining of species purity and genetic diversity, especially in non-native species where limited access to wild broodstock may create genetic bottlenecks. The difficulty identifying mussels by morphology was reinforced here by the degree of non-target species and hybrids seen in the three studied aquaculture populations and highlights the need for rigorous site inventory control. This would include employing molecular methods of identification and the maintenance of pure species stocks, to prevent either the desired or undesired presence of hybrid individuals. In addition routine temporal sampling at locations around aquaculture sites would be useful in determining the levels of non-native introgression and the stability of any hybrid zones. The adaptive capacity of the aquaculture industry is in part a function of scientific knowledge and support. The use of new genomic, genetic, husbandry, gene banking and health methods in the future will help optimize practices for improved long-term production and mitigation to a changing culture environment.

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References

Aho, T., Johanna, R., Piironen, J., Bjorklund, M., 2006. Impacts of effective population size on genetic diversity in hatchery reared brown trout (*Salmo trutta* L.) populations. Aquaculture 253, 244–248.
Allendorf, F.W., 1986. Genetic drift and loss of alleles versus heterozygosity. Zoo Biol. 5, 181–190.
Allendorf, F.W., Phelps, S.R., 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. Trans. Am. Fish. Soc. 109, 537–543.
Beaumont, A.R., Hoare, K., 2003. Artifical Selection in the Hatchery, Biotechnology and Genetics in Fisheries and Aquaculture. Blackwell Publishing, Oxford, U.K., pp. 91–113.
Beaumont, A.R., Turner, G., Wood, A.R., Skibinski, D.O.F., 2004. Hybridisations between *Mytilus edulis* and *Mytilus galloprovincialis* and performance of pure species and hybrid veliger larvae at different temperatures. J. Exp. Mar. Biol. Ecol. 302, 177–188.
Beaumont, A.R., Boudry, P., Hoare, K., 2010. Genetic Variation of Traits, Biotechnology and Genetics in Fisheries and Aquaculture. Wiley-Blackwell, United Kingdom, pp. 99–128.
Bierne, N., Launey, S., Naciri-Graven, Y., Bonhomme, F., 1998. Early effect of inbreeding as revealed by microsatellite analysis on *Ostrea edulis* larvae. Genetics 148, 1893–1906.
Boudry, P., Collet, B., Cornette, F., Hervouet, V., Bonhomme, F., 2002. High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. Aquaculture 204, 283–296.
Bower, S.M., 1989. The summer mortality syndrome and haemocytic neoplasia in blue mussels (*Mytilus edulis*) from British Columbia. Canadian Technical Reports of Fisheries and Aquatic Sciences 1703 pp. 1–65.
Brownstein, M.J., Carpten, J.D., Smith, J.R., 1996. Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. BioTechniques 20, 1004–1010.
Carlsson, J., Morrison, C.L., Reece, K.S., 2006. Wild and aquaculture populations of the eastern oyster compared using microsatellites. J. Hered. 97, 595–598.
Chakraborty, R., Nei, M., 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. Evolution 31, 347–356.
Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.
Crego-Prieto, V., Ardura, A., Juanes, F., Roca, A., Taylor, J.S., Garcia-Vazquez, E., 2015. Aquacutlure and the spread of introduced mussel genes in British Columbia. Biol. Invasions 17, 2011–2026.

Do, C., Waples, R.S., Peel, D., Macbeth, G., Tillett, B.J., Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Mol. Ecol. Resour. 14, 209–214.
Emmett, B., Thompson, K., Popham, J.D., 1987. The reproductive and energy storage cycles of two populations of *Mytilus edulis* (Linne) from British Columbia. J. Shellfish Res. 6, 29–36.
Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.
FAO, 2008. Aquaculture development. 5 Genetic resource management. FAO Technical Guidelines for Responsible Fisheries. FAO, Rome, p. 125.
Fisher, R.A., 1922. On the interpretation of χ^2 from contingency tables, and the calculation of P. J. R. Stat. Soc. 85, 87–94.
Frost, L.A., Evans, B.S., Jerry, D.R., 2006. Loss of genetic diversity due to hatchery culture practices in barramundi (*Lates calcarifer*). Aquaculture 261, 1056–1064.
Gaffney, P.M., 1994. Heterosis and heterozygote deficiencies in marine bivalves: more light? In: Beaumont, A.R. (Ed.), Genetics and Evolution of Aquatic Organisms. Chapman and Hall, London, pp. 146–153
Gaffney, P.M., Bernat, C.M., Allen, S.K., 1993. Gametic incompatibility in wild and cultured populations of the eastern oyster, *Crassostrea virginica* (Gmelin). Aquaculture 115, 273–284.
Hadley, N.H., Dillon Jr., R.T., Manzi, J.J., 1991. Realized heritability of growth rate in the hard clam *Mercenaria mercenaria*. Aquaculture 93, 109–119.
Heath, D.D., Rawson, P.D., Hilbish, T.J., 1995. PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp) genotypes on the west coast of Canada. Can. J. Fish. Aquat. Sci. 52, 2621–2627.
Heath, D.D., Hatcher, D.R., Hilbish, T.J., 1996. Ecological interaction between sympatric *Mytilus* species on the west coast of Canada investigated using PCR markers. Mol. Ecol. 5, 443–447.
Hedgecock, D., 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont, A.R. (Ed.), Genetics and Evolution of Aquatic Organisms. Chapman & Hall, London pp, pp. 122–134
Hedgecock, D., Sly, F., 1990. Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster, *Crassostrea gigas*. Aquaculture 88, 21–38.
Hedgecock, D., Launey, S., Pudovkin, A.I., Naciri, Y., Lapegue, S., Bonhomme, F., 2007. Small effective number of parents (N_b) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. Mar. Biol. 150, 1173–1182.
Ibarra, A.M., Cruz, P., Romero, B.A., 1995. Effects of inbreeding on growth and survival of self-fertilized catarina scallop larvae, *Argopecten circularis*. Aquaculture 134, 37–47.
Ibarra, A.M., Ramirez, J.L., Ruiz, C.A., Cruz, P., Avila, S., 1999. Realized heritabilities and genetic correlation after dual selection for total weight and shell width in catarina scallop (*Argopecten ventricosus*). Aquaculture 175, 227–241.
Lallias, D., Stockdale, R., Boudry, P., Lapegue, S., Beaumont, A.R., 2009. Characterization of ten microsatellite loci in the blue mussel *Mytilus edulis*. J. Shellfish Res. 28, 547–551.
Lallias, D., Taris, N., Boudry, P., Bonhomme, F., Lapegue, S., 2010. Variance in the reproductive success of flat oyster *Ostrea edulis* L. assessed by parentage analyses in natural and experimental conditions. Genet. Res. 92, 175–187.
Langdon, C., Evans, F., Jacobson, D., Blouin, M., 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. Aquaculture 220, 227–244.
Launey, S., Ledu, C., Boudry, P., Bonhomme, F., Naciri-Graven, Y., 2002. Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. J. Hered. 93, 331–351.
Li, Q., Park, C., Endo, T., Kijima, A., 2004. Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). Aquaculture 235, 207–222.
Luikart, G., Ryman, N., Tallmon, D.A., Schwartz, M.K., Allendorf, F.W., 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conserv. Genet. 11, 355–373.
Manzi, J.J., Hadley, N.H., Dillon, R.T., 1991. Hard clam, *Mercenaria mercenaria*, broodstocks: growth of selected hatchery stocks and their reciprocal crosses. Aquaculture 94, 17–26.
McGoldrick, D.J., Hedgecock, D., English, L.J., Baoprasertkul, P., Ward, R.D., 2000. The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. J. Shellfish Res. 19, 779–788.
Nei, M., Maruyama, T., Chakraborty, R., 1975. The bottleneck effect and genetic variability in populations. Evolution 29, 1–10.
Peakall, R.P., Smouse, E., 2006. GENELEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295.
Piry, S., Luikart, G., Cornuet, J.M., 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J. Hered. 90, 502–503.
Rawson, P.D., Joyner, K.L., Meetze, K., Hilbish, T.J., 1996. Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. Heredity 77, 599–607.
Rawson, P.D., Slaughter, C., Yund, P.O., 2003. Patterns of gamete incompatability between the blue mussels *Mytilus edulis* and *M. trossulus*. Mar. Biol. 143, 317–325.
Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.
Reece, K.S., Ribiero, W.L., Gaffney, P.M., Carnegie, R.B., Allen, S.K., 2004. Microsatellite marker development and analysis in the eastern oyster (*Crassostrea virginica*):confirmation of null alleles and non-Mendelian segregation ratios. J. Hered. 95, 346–352.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.
Schneider, S., Kueffer, J.M., Roessli, D., Excoffier, L., 2000. ARLEQUIN, version 2.000. A Software Program for Population Genetic Data Analysis. University of Geneva, Geneva, Genetics and Biometry Laboratory.
Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18, 233–234.
Shields, J.L., Barnes, P., Heath, D.D., 2008. Growth and survival differences among native, introduced and hybrid blue mussels (*Mytilus* spp.): genotype, environment and interaction effects. Mar. Biol. 154, 919–928.
Shields, J.L., Heath, J.W., Heath, D.D., 2010. Marine landscape shapes hybrid zone in a broadcast spawning bivalve: introgression and genetic structure in Canadian west coast *Mytilus*. Mar. Ecol. Prog. Ser. 399, 211–223.
Slatkin, M., 1973. Gene flow and selection in a cline. Genetics 75.
Smith, P.J., Conroy, A.M., 1992. Loss of genetic variation in hatchery-produced abalone, *Haliotis iris*. N. Z. J. Mar. Freshw. Res. 26, 81–85.
Springer, S.A., Heath, D.D., 2007. Environment-specific heterozygote deficiency and developmental instabilty in hybrid *Mytilus*. Mar. Biol. Res. 3, 182–187.
Taris, N., Ernande, B., McCombie, H., Boudry, 2006. Phenotypic and genetic consequences of size selection at the larval stage in the Pacific oyster (*Crassostrea gigas*). J. Exp. Mar. Biol. Ecol. 333, 147–158.
Taris, N., Batista, F.M., Boudry, 2007. Evidence of response to unintentional selection for faster development and inbreeding depression in *Crassostrea gigas* larvae. Aquaculture 272S1, S69–S79.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Waples, R.S., Do, C., 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol. Ecol. Resour. 8, 753–756.
Waples, R.S., Do, C., 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol. Appl. 3, 244–262.
Ward, R.D., English, L.J., McGoldrick, D.J., Maguire, G.B., Nell, J.A., Thompson, P.A., 2000. Genetic improvement of the Pacific oyster *Crassostrea gigas* (Thunberg) in Australia. Aquac. Res. 31, 35–44.
Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Xiao, J., Cordes, J.F., Moss, J.A., Reece, K.S., 2011. Genetic diversity in U.S. hatchery stocks of *Crassostrea ariakensis* (Fujita, 1913) and comparison with natural populations in Asia. J. Shellfish Res. 30, 751–760.
Yanick, J.F., 2002. Survival, growth and the possible environmental impacts of introduced (*Mytilus* spp.) in Georgia Strait, British Columbia. Implications for Mussel Aquaculture. Department of Biology. University of Northern British Columbia, Prince George, BC, p. 105.
Yanick, J.F., Heath, J.W., Heath, D.D., 2003. Survival and growth of local and transplanted blue mussels (*Mytilus trossulus*, Lamark). Aquac. Res. 34, 869–875.
Yu, Z., Guo, X., 2004. Genetic analysis of selected strains of eastern oyster (*Crassostrea virginica* Gmelin) using AFLP and microsatellite markers. Mar. Biotechnol. (N.Y.) 6, 575–586.
Yu, H., Li, Q., 2007. Genetic variation of wild and hatchery populations of the Pacific oyster *Crassostrea gigas* assessed by microsatellite markers. J. Genet. Genomics 34, 1114–1122.
Zheng, H., Zhang, G., Liu, X., Zhang, F., Guo, X., 2004. Different responses to selection in two stocks of the bay scallop, *Argopecten irradians irradians* Lamarck (1819). J. Exp. Mar. Biol. Ecol. 313, 213–223.
Zheng, H., Zhang, G., Liu, X., Guo, X., 2006. Sustained response to selection in an introduced population of the hermaphroditic bay scallop *Argopecten irradians irradians* Lamarck (1819). Aquaculture 255, 579–585.
Zheng, H., Zhang, G., Guo, X., Liu, X., 2008. Inbreeding depression for various traits in two cultured populations of the American bay scallop, *Argopecten irradians irradians* Lamarck (1819) introduced into China. J. Exp. Mar. Biol. Ecol. 364, 42–47.
Zheng, H., Li, L., Zhang, G., 2012. Inbreeding depression for fitness-related traits and purging the genetic load in the hermaphroditic bay scallop *Argopecten irradians irradians* (Mollusca: Bivalvia). Aquaculture 366–367, 27–33.
Zouros, E., Foltz, D.W., 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25, 583–591.