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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).