Population dynamics in hybridizing invasive blue mussels (Mytilus spp.) in British Columbia and the Atlantic provinces.

Jody Lorraine Shields
University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

Recommended Citation
https://scholar.uwindsor.ca/etd/6966

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.
POPULATION DYNAMICS IN HYBRIDIZING INVASIVE BLUE MUSSELS
(*MYTILUS* SPP.) IN BRITISH COLUMBIA AND THE ATLANTIC PROVINCES

by

Jody Lorraine Shields

A Dissertation
Submitted to the Faculty of Graduate Studies
Through Environmental Science
In partial fulfillment of the requirements for
the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada
2007

© Jody Lorraine Shields
NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.
This thesis includes materials reprinted from co-authored and submitted articles. In all cases the contribution of co-authors was primarily in an advisory capacity or through the provision of facilities and materials to complete the research. The primary contributions, experimental designs, data collection and interpretation as well as the preparation of all manuscripts were performed by the author except in the case of samples collected for Chapter 4, which was done by Barry MacDonald.

ABSTRACT

The Mytilus species complex provides a naturally occurring opportunity to investigate hybrid zone dynamics. The purpose of this thesis was to evaluate mechanisms contributing to hybrid zone stability, and factors responsible for the maintenance of genetic integrity among sympatric populations. First, variable hybrid fitness was found on Vancouver Island (VI), BC, which suggested that the hybrid distribution should expand north. However, hybrids have remained restricted to the south region of VI for over ten years, suggesting barriers to gene flow. A second study confirms the stable southern distribution but temporally variable abundance of non-native genotypes on VI, identifying oceanographic factors and marine landscape as contributing barriers to gene flow. A third study provides a contrasting view of hybrid zone dynamics. In the Atlantic Provinces low levels of hybridization demonstrate that barriers to gene flow are not related to dispersal limitation but rather, result from selection against hybridization.
For my sisters, whose inquisitive minds never cease and always keep me thinking, and Amber who never left my side.
I would like to thank all those who have helped along the way and made this research possible. I am grateful to my supervisor, Dr. Daniel Heath for accepting me into his lab before having ever met me, providing guidance and support, grooming me into a ‘hybrid person’, and reminding me it is okay to say no to side projects. I thank my co-supervisor Dr. Penny Barnes who introduced me to Daniel Heath and this project while I was diligently filtering sea-water in her lab as a Fisheries & Aquaculture student. I would like to thank my committee members: Dr. Hugh MacIsaac, Dr. Brian Fryer, and Dr. Trevor Pitcher for showing up to committee meetings and providing constructive criticism.

To several members of the Heath family, thank you for your assistance in field sampling. I am greatly appreciative to Drs. John and Ann Heath at Yellow Island Aquaculture Ltd. for allowing the use of their facilities and staff as well as their excitement and interest in this research. I am very grateful to Bill and Joka Wright for their assistance in field sampling, the use of their boat, and for providing some great photos of mussels collected. I also thank Dr. Ellen Kenchington and Barry MacDonald for their assistance with East coast sampling and providing much appreciated site descriptions.

I would also like to thank Jenn Bronnenhuber and Jimmy Hu for their hard work in the laboratory, measuring, dissecting, and extracting DNA from countless numbers of mussels; Bree Dixon and Aaron Burgoyne for enduring the endless west coast rain and helping to deploy mussel condos even over Christmas break; and Bill Callery for tending to the condos at YIAL all summer and promptly informing me of dropped cages. To the
friends I made at GLIER who stood next to me at conferences even though I sounded like I was going to cry during my talks – many thanks. I’d also like to thank JP Danko for his continued advice, support and encouragement throughout my academic career.

Finally I would like to thank those closest to me. My sisters, Angie and Melanie for being the hilarious girls that they are and making me laugh when the frustration built to extreme levels, for ‘helping’ count and measure mussels even if their skills were a bit lacking, for understanding my obsession with this project and not lecturing me about getting enough sleep, for the countless questions on long drives that kept me awake and thinking about biology, and most importantly for their support in everything I do. I’d like to thank Ryan Walter for reading and commenting on several ever-evolving versions of manuscripts and insisting that each one was far better than I gave myself credit for, leaving the door open so I could come home at 7am without fumbling with the keys in a haze, and for the necessary comedic relief and sound counsel required to make it through the writing process. Lastly, my Mom and Dad – the most reliable sources of funding and encouragement, for listening without necessarily understanding, and who constantly remind me of my early childhood dream to become a ‘marine biologist’.
TABLE OF CONTENTS

ABSTRACT .......................................................................................................................... IV
DEDICATION .......................................................................................................................... V
ACKNOWLEDGEMENTS ......................................................................................................... VI
TABLE OF CONTENTS .......................................................................................................... VIII
LIST OF TABLES ..................................................................................................................... X
LIST OF FIGURES ................................................................................................................ XII

1.0 GENERAL INTRODUCTION ......................................................................................... 1
  1.1 REPRODUCTIVE ISOLATION IN HYBRID ZONES ......................................................... 3
  1.2 GLOBAL MYTILUS HYBRIDIZATION ........................................................................... 6
  1.3 THESIS OBJECTIVE .................................................................................................... 8
  1.4 CHAPTER 2 OBJECTIVES ......................................................................................... 8
  1.5 CHAPTER 3 OBJECTIVES ........................................................................................ 9
  1.6 CHAPTER 4 OBJECTIVES ......................................................................................... 10
  1.7 REFERENCES ............................................................................................................ 11

2.0 RELATIVE FITNESS AMONG NATIVE, INTRODUCED AND HYBRID BLUE MUSSELS
  (MYTILUS spp.): GENOTYPE, ENVIRONMENT AND INTERACTION EFFECTS ............ 15
  2.1 INTRODUCTION ....................................................................................................... 15
  2.2 MATERIALS AND METHODS .................................................................................. 19
  2.3 RESULTS .................................................................................................................. 26
  2.4 DISCUSSION ............................................................................................................. 32
  2.5 REFERENCES ............................................................................................................ 37

3.0 GENE FLOW AND DISTRIBUTION PATTERNS IN A CANADIAN WESTCOAST MYTILUS
  HYBRID ZONE: MARINE LANDSCAPE SHAPES POPULATION STRUCTURE OF A
  BROADCAST SPAWNING BIVALVE ................................................................................. 42
  3.1 INTRODUCTION ....................................................................................................... 42
  3.2 MATERIALS AND METHODS .................................................................................. 45
  3.3 RESULTS .................................................................................................................. 56
  3.4 DISCUSSION ............................................................................................................. 66
  3.5 REFERENCES ............................................................................................................ 71

4.0 DISTRIBUTION AND GENETIC DIFFERENTIATION OF HYBRIDIZING BLUE MUSSELS
  (MYTILUS spp.) IN THE CANADIAN ATLANTIC PROVINCES ...................................... 75
  4.1 INTRODUCTION ....................................................................................................... 75
  4.2 MATERIALS AND METHODS .................................................................................. 78
  4.3 RESULTS .................................................................................................................. 86
  4.4 DISCUSSION ............................................................................................................. 91
  4.5 REFERENCES ............................................................................................................ 94

VIII
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 General Discussion</td>
<td>99</td>
</tr>
<tr>
<td>5.1 Hybrid Fitness</td>
<td>100</td>
</tr>
<tr>
<td>5.2 Dispersal</td>
<td>101</td>
</tr>
<tr>
<td>5.3 Management</td>
<td>103</td>
</tr>
<tr>
<td>5.4 Final Note</td>
<td>105</td>
</tr>
<tr>
<td>5.5 References</td>
<td>105</td>
</tr>
</tbody>
</table>

**Vita Auctoris**.............................. 108
Table 2.1 Survival of individually caged mussels at two sites (Ladysmith, BC; Quadra Island, BC) by genotype. At the Glu-5’ locus: MT = Mytilus trossulus, MG = Mytilus galloprovincialis, MT×MG = M. trossulus x M. galloprovincialis hybrid, MG×ME = M. galloprovincialis x M. edulis hybrid, MTb = M. trossulus backcross. At the ITS locus: N = native (M. trossulus), A = alien (either M. edulis or M. galloprovincialis), H = hybrid (either M. trossulus x M. galloprovincialis or M. trossulus x M. edulis).
Table 4.4 Genetic differentiation among *Mytilus edulis* populations in the Canadian Atlantic Provinces. Pairwise Pairwise Weir & Cockerham’s $F_{ST}$ below the diagonal. Pairwise Nei’s genetic distance ($D_s$) above the diagonal. Bold italics indicate significant Exact test P-values.

Table 4.5 Genetic differentiation among *Mytilus trossulus* populations in the Canadian Atlantic Provinces. Pairwise Weir & Cockerham’s $F_{ST}$ below the diagonal. Pairwise Nei’s genetic distance ($D_s$) above the diagonal. Bold italics indicate significant Exact test P-values.
LIST OF FIGURES

**Figure 1.1** Approximate distribution of *Mytilus* species and their hybrid zones in the Northern Hemisphere. Percent range refers to the abundance of hybrid genotypes within each hybrid zone (Koehn 1991; McDonald et al. 1991; Heath et al. 1995; Inoue et al. 1997; Suchanek et al. 1997; Wilhelm & Hilbish 1988; Comesana et al. 1999; Penney & Hart 1999; Rawson et al. 1999; Hilbish et al. 2000).........................................................................................7

**Figure 2.1** Map of Vancouver Island, British Columbia showing the deployment sites for the mussel cages. The source of the experimental mussels was the Ladysmith (local) site which is a commercial dock in the Ladysmith harbour, while the Quadra Island (transplanted) site is located at the Yellow Island Aquaculture Ltd fish farm site on the west central coast of Quadra Island.................................................................21

**Figure 2.2** Mean daily water temperature (°C) profile for Ladysmith and Quadra Island sites at 1m depth.........................................................................................................................26

**Figure 2.3** Percent survival of caged *Mytilus* mussels from May 27 – August 30 2006 calculated as the number of mussels of a genotype alive at the end divided by the number of mussels of that genotype to start. Lower case a and b designate significant differences. .................................................................................................................................28

**Figure 2.4** Volumetric growth and shell length. Panel A shows relative growth rate as measured by PCI from May – August 2006 at Ladysmith and Quadra Island among MT, MG, F₁, and BC genotypes. PCI combines length, width and depth into a single variable accounting for 95% of the variance in these metrics. Factor loadings are as follows: Length – 0.982031, Width – 0.984944, Depth – 0.968181. Overall, Quadra Island had significantly higher growth rate than Ladysmith (P < 0.05). Panel B shows final (August 2006) shell length (mm). Error bars represent standard error..................................................................30

**Figure 2.5** Fitness function of volume (cm³) and relative fitness of mussels reared at Ladysmith and Quadra Island. Panel A shows a linear relationship between survival and volume. Survival was arcsine square root transformed for percentage data. Panel B shows fitness calculated relative to Ladysmith MT. Error bars represent standard error. .................................................................................................................................31

**Figure 3.1** Sampling locations of Vancouver Island and area *Mytilus* populations. Names of sampling locations and year of collection are shown in Table 1. A – Vancouver Island, B – Discovery Islands, C – Gulf Islands......................................................................................... 46
Figure 3.2 Genotype frequency of *Mytilus* species within the VI hybrid zone based on two diagnostic species-specific nuclear genetic markers (ITS and GLU). All genotype frequencies were significantly different after Bonferroni correction. MT = *M. trossulus* pure genotype, MG = *M. galloprovincialis* pure genotype, ME = *M. edulis* pure genotype, F₁ = hybrid, BC = backcross. Bold italics indicate significant departure from HWE tested by Exact U tests of heterozygote deficiency in GENEPOP 3.4 (Raymond & Rousset 1995).

Figure 3.3 Temporal variation in frequency of non-native genotype mussel abundance at the Ladysmith site from May 2005 – February 2007. All comparisons were statistically significant after Bonferroni correction, as indicated by asterisks.

Figure 3.4 Barrier network identifying areas of limited gene flow as defined by AIS barrier analyses. Inset: AIS output - Solid circles represent relative genetic position of individual sampling sites. Black shading indicates presence of hybrids. Putative barriers are labeled from a to e. Solid thick lines (a, b) represent primary barriers and thin lines represent secondary barriers found among the Southern Gulf Islands (c - e). Barriers are overlayed on a map of Vancouver Island for identification of corresponding oceanographic features limiting gene flow.

Figure 3.5: Average summer (May – June) surface currents for southern Strait of Georgia. Adapted with permission from M. Foreman, Institute for Ocean Studies, Sydney, BC.

Figure 4.1 Sampling locations of *Mytilus* collected from the Canadian Atlantic Provinces. Names of sampling sites are given in Table 4.1.

Figure 4.2 Genotype frequencies of *Mytilus* spp. at ten sites in the Canadian Atlantic Provinces. Frequencies determined by a combination of two nuclear diagnostic species markers and four polymorphic microsatellite loci. ME = *Mytilus edulis*, MT = *Mytilus trossulus*, F₁ = hybrid, BC = backcross.
1.0 GENERAL INTRODUCTION

Generally, evolutionary change occurs slowly and as a consequence, direct observation of change over time often results in limited information about the process of evolution. While documentation of changes in the frequencies of phenotypes or genotypes within populations is relatively easy, direct observation of the process of lineage divergence and the origin of evolutionary novel lineages is rare. Therefore, evolutionary process is most often inferred through the characterization and interpretation of patterns of variation within and among populations, species and higher taxonomic groups. Hybrid zones are no exception, and provide a great arena to study patterns of variation.

Several definitions of 'hybrid', 'hybridization', and 'hybrid zone' exist. For the purpose of this thesis, I refer to each as resulting from successful mating (production of viable and at least partially fertile offspring) in nature between individuals from two spatially and temporally overlapping populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters (Arnold 1997). Thus, hybrid zones occur where genetically distinct groups meet, mate, and produce at least some offspring of mixed ancestry. The persistence of hybrid zones has generally been ascribed to either selection against hybrid individuals and dispersal of parental types into the contact zone (Barton & Hewitt 1985), or selection for certain hybrid genotypes across a heterogeneous habitat within the contact zone (Endler 1977; Moore 1977). In any case, hybridization can either hinder or promote evolutionary diversification through genome integration or the generation of novel genotypes (Bell & Travis 2005).
While natural hybridization is common among plants, much debate has traditionally surrounded the evolutionary significance of hybridization in animal taxa. Recognizing hybridization as a potentially important evolutionary process has led to investigations of the role of natural hybridization in generating novel genotypes that may lead to adaptive evolution and new evolutionary lineages (Anderson & Stebbins 1954; Grant & Grant 1992; Rieseberg & Wendel 1993; Arnold & Hodges 1995). Hybridization was viewed early on as an important mechanism for understanding the process of speciation in animal taxa, as hybrid zones provided a natural setting in which to test hypotheses concerning the evolution of reproductive barriers (Dobzhansky 1937; Mayr 1942). With zoologists' emphasis on hybridization being maladaptive, these early studies lead to the formulation of a speciation model in which mating between genetically distinct groups could result in the completion of reproductive barriers through a process termed 'reinforcement' (Dobzhansky 1940). Under this model, selection against the formation of hybrid individuals (due to their intermediacy rendering them less fit in certain ecological circumstances) results in the strengthening of pre-mating isolation between the two genetically distinct groups, thereby finalizing the process of speciation. Based on these early frameworks, natural hybridization among animal taxa is often thought to be of little long-term evolutionary importance. Despite the supposed rarity of successful hybridization in animals, much attention has been devoted to the study of discrete hybrid zones (Barton and Hewitt 1985; Harrison 1990; Arnold 1997; Seehausen 2004).

The development of molecular techniques has led to an increase in analyses of animal hybrid zones in recent years, and a shift away from the paradigm that hybridization in
animal taxa is an evolutionary dead end. It is becoming more widely accepted that hybridization between genetically distinct groups and closely related species is common and carries important evolutionary consequences (Seehausen 2004; Bell & Travis 2005). Hybridization events that result in individuals exhibiting novel or extreme phenotypes relative to parental types (transgressive segregation) generates diversity and may facilitate speciation (Seehausen 2004; Albertson & Kocher 2005) if the new phenotype confers an adaptive advantage (Lewontin & Birch 1996). Where transgressive hybrids are sufficiently divergent from their parental species, a new hybrid species may result which is able to coexist with both parental types (Bell & Travis 2005). The generation of such diversity via hybridization has been implicated as a contributing factor in the explosive adaptive radiation of African cichlids (Albertson & Kocker 2005) and Darwin’s finches (Grant & Grant 1992).

1.1 REPRODUCTIVE ISOLATION IN HYBRID ZONES

Generally, analyses of hybrid zones have a common goal – to understand the genetic make-up of taxa and how reproductive barriers originate (Arnold 1997). Whether hybridization events are common or rare does not necessarily predict the degree of evolutionary importance. Even in cases where hybridization is rare, positive selection may favour some of the hybrid genotypes in certain environments (Arnold 1997). The stability and maintenance of resulting hybrid zones is largely determined by various isolating mechanisms that determine the frequency and distribution of hybrid genotypes and regulate gene flow between them and parental forms (Jiggins & Mallet 2000).
Pre-mating isolating factors such as behaviour (e.g., mating calls and displays, mate choice), temporal and spatial separation of reproduction, gamete recognition and assortative fertilization all contribute significantly to reproductive barriers between genetically distinct groups. Such factors serve to reduce the likelihood of hybrid formation, thereby strengthening reproductive barriers and facilitating the cohesion of taxa. Where pre-mating reproductive isolation is sufficiently strong, sympatric populations of genetically distinct groups are able to maintain genetic integrity in the face of potential gene flow. The resulting ‘bimodal’ hybrid zone is characterized by populations consisting predominately of individuals genetically similar to either parental genotype, with relatively few intermediates (Harrison & Bogdanowicz 1997; Jiggins & Mallet 2000). There is considerable variation in the frequency of hybrid genotypes and the relative abundance of F₁ and backcross types in these types of hybrid zones. For example, in a hybrid zone between the butterflies *Heliconius himera* – *Heliconius erato*, approximately 10% of individuals are hybrids, half of which are F₁ (Jiggins et al. 1997). Alternatively, in the Louisiana *Iris* spp. hybrid zones, F₁ are absent in samples, although must occur as backcross genotypes are relatively common (Cruzan & Arnold 1993). Such bimodal hybrid zones are thought to occur where speciation of parental forms is nearly complete (Jiggins & Mallet 2000).

In some cases, pre-mating reproductive barriers are incomplete, resulting in the formation of at least some viable, fertile hybrid individuals after which post-mating reproductive barriers may act to determine the success and therefore abundance and distribution of hybrid progeny. Selection on hybrid genotypes results in varying outcomes of viability,
survivorship, and fertility. Selection can act in an environmentally dependent manner (exogenous selection) such that different genotypes are favoured in various environments (Endler 1977). Alternatively, selection can occur independently of the environment (endogenous selection) due to genomic interactions between distinct taxa, such that fitness is a result of an individual’s genotype regardless of habitat variation across the hybrid zone (Barton & Hewitt 1985). Hybrid zones characterized by predominately hybrid genotypes (‘unimodal’) often form via secondary contact of previously allopatric populations where assortative mating breaks down in sympatry (Jiggins & Mallet 2000). In these cases, post-mating factors including reduction in hybrid viability and fertility may act against the complete introgression of parental genomes, as seen in the hybrid zone between *Bombina variegata* – *Bombina bombina* (Szymura & Barton 1991), and *Chorthippus parallelus parallelus* – *Chorthippus parallelus erythropus* (Hewitt 1993); or by stabilizing selection on traits such as colour pattern as seen in *Heliconius erato* where strong assortative mating, no hybrid inviability, and a lack of mitochondrial DNA introgression is observed (Mallet et al. 1998).

Evidently, naturally occurring hybrid zones vary in the degree to which parental genomes maintain genetic integrity when in sympatry, creating a continuum from unimodal to bimodal hybrid zones. Both pre- and post-mating processes represent significant barriers that must be overcome for natural hybridization to occur. Once pre-mating barriers are overcome and hybrids are formed, endogenous and exogenous selection act either for or against successful reproduction of F₁ hybrids and the subsequent formation of backcross genotypes. Again, at this stage, selection may favour certain genotypes in various
environments leading to either the replacement of parental genotypes by fitter hybrids, or the occupation of novel habitats by hybrids, resulting in evolutionarily stable lineages. Furthermore, the genetic and spatial structure of hybrid zones is determined by a combination of environmentally dependent and independent selection.

1.2 GLOBAL MYTILUS HYBRIDIZATION

Natural hybridization among blue mussels (Mytilus) occurs globally wherever any two species come into contact (Gosling 1992). Despite extensive hybridization, each species has remained genetically distinct (Rawson et al. 2003). The Mytilus species complex consists of three morphologically similar species common in temperate intertidal marine communities: Mytilus trossulus, M. edulis, and M. galloprovincialis (Fig. 1.1). The taxonomic status of the Mytilus species complex has been debated for some time due to the natural production of viable and fertile hybrid offspring, although molecular evidence now supports the classification of three separate species. In the Northern hemisphere, M. trossulus is found in the Baltic Sea, northwestern Atlantic and northern Pacific Ocean; M. edulis occurs primarily in the eastern and western Atlantic; and M. galloprovincialis is the most widespread found in the Mediterranean Sea, the Atlantic coast of southern Europe, northern Africa and the Pacific coast of North America (Fig. 1.1). Where these species’ distributions overlap, extensive hybrid zones are formed with varying dynamics and characteristics (Fig. 1.1).
Figure 1.1 Approximate distribution of *Mytilus* species and their hybrid zones in the Northern Hemisphere. Percent range refers to the abundance of hybrid genotypes within each hybrid zone (Koehn 1991; McDonald et al. 1991; Heath et al. 1995; Inoue et al. 1997; Suchanek et al. 1997; Wilhelm & Hilbish 1988; Comesana et al. 1999; Penney & Hart 1999; Rawson et al. 1999; Hilbish et al. 2000).

The frequency of hybridization among these three species may be determined by their evolutionary relationships (Rawson et al. 2003). Phylogenetic evidence based on mitochondrial 16S gene sequences (Rawson and Hilbish 1995) and genomic DNA (Martinez-Lage et al. 2002) suggests that *M. edulis* and *M. galloprovincialis* are closely related, while *M. trossulus* is the most divergent of the three species. The frequency of hybridization between the closely related *M. edulis* and *M. galloprovincialis* in western Europe can reach 80% or more (Gardner 1994; Hilbish et al. 1994; Wilhelm and Hilbish 1998). In contrast, the combined frequency of F₁ and backcross genotypes between *M.*
edulis and M. trossulus in the northwest Atlantic ranges from 10% - 26% (Bates and Innes 1995; Comesana et al. 1999; Rawson et al. 2001), and a similar range has been observed in the northwest Pacific between M. trossulus (native) and M. galloprovincialis (Heath et al. 1995; Yanick 2002). The characteristics and dynamics of each hybrid zone vary geographically; however, their maintenance and stability – or lack thereof – is often attributed to environmental conditions such as water temperature, salinity, and wave exposure (Bates and Innes 1995; Mallet and Carver 1995; Comesana et al. 1999; Gardner and Thompson 2001), or physiological attributes such as asynchronous spawning and gamete incompatibility (Maloy et al. 2003). In any case, investigations into hybrid zone maintenance requires the evaluation of genotype-specific fitness in a variety of environments, the determination of barriers to gene flow, and an understanding of the balance between selection against specific genotypes and migration of alleles into and out of the hybrid zone.

1.3 Thesis Objective

The overall aim of this study was to evaluate the factors responsible for the dynamics of Mytilus hybrid zones, specifically assessing the environmentally-dependent, genotype-specific fitness of mussels and the level of connectivity among their populations on large and small geographic scales.

1.4 Chapter 2 Objective

In the mid 1990’s, non-native Mytilus genotypes were identified along the sheltered east coast of Vancouver Island, British Columbia (BC), Canada (Heath et al. 1995). Since
then, these genotypes have persisted with a stable distribution and varying abundance, creating a dynamic ‘hybrid zone’ on the southern coast of the island. This hybrid distribution does not conform to any existing hybrid zone model, and is not self-sustaining but, rather, is likely maintained by repeated introductions of non-native genotypes. Assessing the relative fitness of hybrid individuals is thus essential to understanding the maintenance of hybrid zones and the ability of the parental taxa to maintain genetic integrity in the face of hybridization.

A clearer understanding of the complex dynamics of the VI *Mytilus* hybrid zone will provide valuable insight into how reproductive barriers are eroded, as well as inform management efforts aimed at minimizing the potential impact of non-native species introduction. In Chapter 2 the relative fitness of introduced and hybrid genotypes on Vancouver Island were assessed to uncover the mechanisms contributing to the maintenance of this hybrid zone.

1.5 Chapter 3 Objective

On Vancouver Island, BC, Canada, small unstable localized populations of the introduced *M. galloprovincialis* and *M. edulis* and their hybrids can be found in varying relative abundances. This localized hybrid distribution has persisted for over ten years despite drastic seasonal population crashes, and has remained restricted to the southern portion of VI.
Mytilus are broadcast spawners with high fecundity (Thompson 1979), an extended pelagic larval phase lasting from several weeks to two months (Bayne 1965) and a dispersal capability ranging on average from 30 – 60km, in some cases greater than 100km (McQuaid & Phillips 2000; Gilg & Hilbish 2003). Such life history characteristics coupled with higher genotype-specific fitness in the north (Chapter 2) indicate that the distribution of hybrid and introgressed mussels should be expanding northward, yet to date there is no evidence to suggest that this has occurred.

The purpose of this study was to determine the factors maintaining the localized hybrid distribution within the south region of Vancouver Island, specifically identifying putative barriers to gene flow in relation to the marine landscape.

1.6 Chapter 4 Objective

On the East coast of Canada, there is little evidence of extensive hybridization and introgression between populations of M. edulis and M. trossulus, which is often attributed to environmental factors and incompatibility between their nuclear genomes (Comesana et al. 1999). Alternatively, a lack of gene flow among discrete basins may restrict contact, and thus hybridization, between the two species. The purpose of this study was to determine the level of gene flow among populations on a large (among discrete bays) and small (within a single bay) geographic scale to explain the distribution and maintenance of genetic integrity among M. edulis and M. trossulus in the Canadian Atlantic Provinces.
1.7 REFERENCES


Bayne BL. (1965) Growth and delay of metamorphosis of the larvae of Mytilus edulis (L.). Ophelia 2:1-47


Dobzhansky T. (1940) Speciation as a stage in evolutionary divergence. American Naturalist. 74:312-321


Harrison RG, Bogdanowicz SM. (1997) Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. Evolution 51:493-505


Hewitt GM. (1993) After the ice: *parallelus* meets *erythropus* in the Pyrenees. In Hybrid zones and the evolutionary process (Harrison RG, ed.) pp. 140-164, Oxford University Press


Holm ER, Bourget E. (1994) Selection and population genetic structure of the barnacle *Semibalanus balanoides* in the northwest Atlantic and Gulf of St. Lawrence. Marine Ecology Progress Series. 113:247-256


Rieseberg LH, Wendel JF. (1993) Introgression and its consequences in plants. In Hybrid zones and the evolutionary process (Harrison RG ed.) pp. 70-109, Oxford University Press, Oxford


Szymura JM, Barton NH. (1991) The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution. 45:237-261


Yanick JF (2002) Survival, growth and the possible environmental impacts of introduced blue mussels (*Mytilus* spp.) in Georgia Strait, British Columbia. Implications for mussel aquaculture. MSc Thesis. Department of Biology, University of Northern British Columbia, Prince George, BC.

14

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
2.0 RELATIVE FITNESS AMONG NATIVE, INTRODUCED AND HYBRID BLUE MUSSELS (Mytilus spp.): GENOTYPE, ENVIRONMENT AND INTERACTION EFFECTS*

2.1 INTRODUCTION

Historically, natural hybridization among species was thought to be an evolutionary dead end (Mayr 1942). However, hybridization is often observed across environmental gradients, creating steep transitions between genotypes, and these areas are known as hybrid zones (Barton & Hewitt 1985). Environment-genotype interactions may influence the structure and stability of hybrid populations, as environment-dependent selection can result in differential survival of genotypes (Slatkin 1973; Moore 1977; Springer & Heath 2007). Thus, spatial and temporal variability in the distribution and abundance of hybrid individuals will depend on the fitness of hybrid, relative to parental, genotypes (Albert et al. 2006). Characterization of such hybrid zone dynamics has primarily relied on three models which differ in their assumptions of the relative fitness of hybrid genotypes: the ecotonal (Endler 1977), tension (Barton & Hewitt 1985) and mosaic (Harrison & Rand 1989) models. The tension and mosaic models suggest that hybrids are uniformly less fit than parental genotypes due to their mixed ancestry and resulting genetic incompatibility, and that their distribution and abundance is maintained by a balance between dispersal and selection against hybrid individuals.

The ecotonal model, on the other hand, suggests that hybrid genotypes exhibit higher fitness relative to either parental genotype at some point along an environmental gradient (e.g., Rawson et al. 1999). Assessing the relative fitness of hybrid individuals is thus essential to understanding the maintenance of hybrid zones and the ability of the parental taxa to maintain genetic integrity in the face of hybridization (Day and Schluter 1995).

Marine blue mussels of the *Mytilus* species complex (*M. trossulus*, *M. edulis*, and *M. galloprovincialis*) occur globally in temperate waters and hybridize where they come into contact (Hilbish et al. 2000), making them an excellent system in which to investigate the interaction of genotype and environment on the breakdown of interspecific reproductive barriers. Several studies of *Mytilus* spp. have shown that environmental effects are large determinants of both growth and survival (Dickie et al. 1984; Mallet & Carver 1989; Johannesson et al. 1990; Kautsky et al. 1990; Stirling & Okumus 1994). Environment-dependent effects on indirect measures of fitness such as physiological performance, reproductive investment, fecundity, strength of attachment to the substrate and susceptibility to parasitic infections have been shown in mussels (e.g., Rawson et al. 1999; Ringos & Cunningham 2005; Gardner 1994). Water temperature, salinity and wave exposure appear to be the most important environmental factors driving fitness differences in *Mytilus* hybrid zones (Riginos & Cunningham 2005). Such variance in fitness measures among genotypes may reflect underlying genetic incompatibilities, which may be conditionally expressed in an environment-dependent manner (Rundle & Whitlock 2001; Springer & Heath 2007).
Since divergent taxa tend to become fixed for different alleles at multiple genes, their hybrid offspring are expected to be highly heterozygous, which may result in the masking of hybrid breakdown in early generations due to heterosis (Rhode & Cruzan 2005). This effect would play a critical role in the establishment of hybrid zones, and perhaps in the subsequent introgression between the two parental taxa (Arnold 1997; Burke & Arnold 2001), since it will serve to create novel genotypes that are more fit than their parents in certain environments. However, local adaptation may contribute to fundamental genetic incompatibilities, perhaps masked in F1 hybrids, which may result in variation in the fitness of backcross individuals that will depend unpredictably on the environment (Whitlock et al. 2000; Burke & Arnold 2001; Rundle & Whitlock 2001).

If increased heterozygosity in early generation hybrids serves to mask deleterious alleles due to heterosis, a measurable performance advantage relative to parental individuals is expected. Hybrids have been found to be equally or more fit than their parents in both laboratory and field studies among several taxa (reviewed in Arnold & Hodges 1995; Bombina: Nurnberger et al. 1995; Geospiza: Grant & Grant 1992). However, attempts to relate growth (a fitness-related trait) and heterozygosity (a metric of hybridization), independent of environmental factors, have produced inconsistent results. Several studies of hybrid populations of bivalves failed to find a positive correlation between heterozygosity and growth due to problems with replication (Skibinksi & Roderick 1989) and with sampling from a limited number of parental populations (Beaumont et al. 1983; Gaffney & Scott 1984; Adamkewicz et al. 1984). Studies showing a positive growth-heterozygosity correlation in several natural populations of mussels (Koehn & Gaffney 1984; Diehl & Koehn 1985; Hawkins et al. 1986) have
focused on the well established hybrid zones of northwestern Europe. To date, little is known of the structure and dynamics of the relatively young hybrid zones on the Pacific coast of North America (Braby & Somero 2006).

All three sibling *Mytilus* species are found on Vancouver Island (VI), British Columbia (BC), Canada where *M. trossulus* is the native species. The three species form hybrids, with an unstable abundance that does not appear to fit any one particular model (Yanick 2002; JL Shields, unpublished data). This lack of stability makes it difficult to define the VI introgression areas as a true “hybrid zone”, as the abundance of hybrid mussels on VI does not appear to be self-sustaining but rather, is likely maintained by repeated introductions (Yanick 2002). The hybrid zone found on VI has a unique localized structure where parental individuals are found intermixed with hybrid, backcrossed, and higher order introgressed individuals, whose relative fitness is unknown but is likely highly variable. The VI hybrid zone differs from those in California where environmentally dependent selection influences the hybrid zone structure, as *Mytilus* spp. genotypes correlate with both temperature and salinity along an estuarine-oceanic gradient (Rawson et al. 1999, Braby & Somero 2006). Although, in a transplant study, Yanick et al. (2003) demonstrated local adaptation effects in the native *M. trossulus* on VI, there is no known environmental gradient that can explain the dynamics of the VI hybridization and introgression. Thus, the *Mytilus* hybrid zone off the east coast of VI (Heath et al. 1995) provides a unique opportunity to examine the contribution of genetics, environment and genotype-environment interactions to the distribution and abundance of hybrid and backcross offspring. A first step is to estimate the relative fitness of parental
taxa and their hybrids in various environments since such data are key to uncovering the mechanisms of hybrid zone stability, or lack thereof (Rolan-Alvarez et al 1997). In this study, I used growth and survival measures to estimate relative fitness of *Mytilus* genotypes in the VI hybrid zone. I predicted environmentally-dependent fitness differences between parental and hybrid groups, and that the pattern of those differences would explain the distribution and dynamics of the *Mytilus* hybrid zone on Vancouver Island. A clearer understanding of the complex dynamics of the VI *Mytilus* hybrid zone will provide valuable insight into how reproductive barriers are eroded, as well as inform management efforts aimed at minimizing the potential impact of non-native species introduction.

### 2.2 Materials and Methods

**Site Selection and Sample Collection**

In May of 2005 and 2006, a genetic survey of *Mytilus* spp. distribution on the east coast of Vancouver Island indicated that Ladysmith, BC had an abundance of introduced and hybrid mussels. Ladysmith was chosen as the collection site for this study to increase the likelihood of sampling native, introduced and hybrid mussels (Fig. 2.1). A total of 864 small (average shell length (± 1 SEM) = 18.6 ± 1.0 mm) mussels were collected from the underside of the Ladysmith Harbour Maritime Society dock. Half of the collected mussels (n = 432) were reared locally at Ladysmith, while the other half were transported to a site on Quadra Island, BC (Fig. 2.1). All mussels were placed in a container of seawater and shell measurements (length, width, and depth) were taken at the largest part of the shell on each mussel. Length was measured from the tip of the beak to the base of
the posterior edge; width was measured across the widest portion of the mussel from the umbo to the byssal opening; and depth was measured at the centre of the valve at the peak curvature when held dorso-ventrally. In addition, a hemolymph sample was taken from each mussel following the protocol of Yanick and Heath (2000). The mussels were then placed in individual compartments in the cages (see below). To standardize for the travel time to Quadra Island (approximately 3 hours), the mussels deployed at Ladysmith were held in the cages (out of direct sunlight) for 3 hours prior to final submergence.

**Rearing**

Growth and survival of mussels at the local (Ladysmith, BC) and remote (Quadra Island, BC) sites were evaluated in a common environment cage experiment carried out from May 26 – August 27 2006. The cages used were as described in Yanick et al (2003), and consisted of a 60 cm x 60 cm x 5.0 cm polyethylene sheet, 0.5 mm mesh screen on either side of the center sheet and 1.0 cm polyethylene sheets on both outer sides. One hundred and forty-four 5.0 cm holes (in a 12 x 12 grid) held individual mussels. Each cage thus housed 144 mussels in individual cells designed to keep mussels separate for identification purposes while allowing sufficient water flow for obtaining food and flushing wastes. Fouling organisms were removed from the cages and screens regularly throughout the experiment. Three cages (housing a total of 432 mussels) were deployed at each site, suspended at approximately 1.0 m below the surface. Temperature loggers (StowAway Tidbit; TBI32-05+37; Onset Computer Corporation) were attached to the rope of one cage at each site. Temperature was recorded at 15-minute intervals from May to August 2006 and was compared between sites using a t-test.
Figure 2.1 Map of Vancouver Island, British Columbia showing the deployment sites for the mussel cages. The source of the experimental mussels was the Ladysmith (local) site which is a commercial dock in the Ladysmith harbour, while the Quadra Island (transplanted) site is located at the Yellow Island Aquaculture Ltd fish farm site on the west central coast of Quadra Island.
Survival and growth of the mussels were monitored monthly for the experimental period, May 27, 2006 to August 30, 2006. Survival was assessed by retrieving the cages and identifying all mussels as either alive or dead. The shells from the dead mussels were removed from the cages and preserved in 50 mL tubes containing 95% EtOH. To determine growth, digital calipers (+/- 0.1 mm) were used to take shell measurements (length, width, depth) at the start and end of the experiment. Mussels that died during the experiment were not used in growth comparisons, since I could not identify the date on which mussels died.

Species identification

DNA was extracted from the hemolymph samples (see Yanick & Heath 2000) to determine the proportion of hybrid and non-native mussels in the cages. When the experiment was terminated in August 2006, mantle tissue was taken from all surviving mussels, stored in 95% EtOH, and transported to the laboratory for DNA extraction (following Elphinstone et al. 2003). DNA fragments were amplified via PCR at two species-specific co-dominant marker loci: the internal transcribed spacer region of ribosomal DNA (ITS - diagnostic between native and alien mussels and their hybrids) following the protocol described in Heath et al. (1995), and the adhesive byssal thread protein (Glu-5’ - diagnostic among all three mussel species and their hybrids) following a modified protocol of that described in Rawson et al. (1996). Individual mussels were genotyped at each locus via diagnostic restriction fragment length polymorphisms.
(RFLPs) for ITS (Heath et al. 1995) and using an automated DNA analyzer (LiCor 4300) to determine PCR fragment length polymorphisms for Glu-5' (Rawson et al. 1996). Mussels were genetically identified as homozygote (pure native *Mytilus trossulus* (MT), introduced *M. galloprovincialis* (MG), or introduced *M. edulis* (ME)) or heterozygote (MTxMG, MTxME or MGxME) at the Glu-5' marker locus. The ITS marker locus identified mussels as homozygous (native MT or pure introduced ME or MG), or as heterozygote (MTxME or MTxMG). Hybrid mussels were classified as F1 hybrids when heterozygous at both ITS and GLU. Backcross (BC) and higher-order hybrid crosses were identified whenever the markers disagreed. With only two diagnostic markers, error in hybrid classification by genotype is unavoidable, hence only the backcross (BC) classification is certain.

**Analyses**

Fitness was estimated using survival and growth as indicators. Survival was calculated as counts (dead or alive) for each genotype, where dead mussels were identified to species using DNA from the hemolymph sample taken before deployment. For survival comparisons among genotypes, percent survival was calculated as the total number of mussels of a particular genotype alive at the end of the study divided by the total number of mussels of that genotype at the start of the study. To determine if survival was independent of site and genotype, survival was calculated for the duration of the experiment (May – August 2006) for each site and genotype and a 2-way contingency table ($\chi^2$) was used to determine if there was a significant difference in survival between the local and transplanted mussels. Contingency tables were used to determine
differences in survival among genotypes within and between sites. Log-likelihood ratio
tests (G test for independence) were used when small sample sizes among mussel
genotypes precluded contingency table analyses (Sokal & Rohlf, 1995).

Relative growth rate (RGR) was calculated for each mussel at each site and compared
between locally reared (Ladysmith) and transplanted (Quadra Island) mussels, as well as
among genotypes (Native (MT), Introduced (MG), Hybrid (F1), and Introgressed (BC)) to
determine if growth was independent of site and genotype. RGR was calculated as:

\[
RGR = \frac{(SL_2 - SL_1)}{(SL_1 \times t)}
\]

where \(SL_1\) = shell length at time 1, \(SL_2\) = shell length at time 2, and \(t\) = number of days
between the two dates. This equation was applied to each shell measure (length, width
and depth), resulting in three RGR values for each mussel. Although shell length is
generally the standard measure used in growth studies of bivalves, here I measured
length, width and depth of each mussel, thereby allowing the estimation of volumetric
growth. Principal components analysis (PCA factor analysis, Statistica 6.0) reduces
variables that may be autocorrelated into a series of orthogonal variables; the first few
principal components usually account for the majority of the observed variation in the
original variables. RGR of the three shell measurements were combined into a single
‘volumetric growth’ variable using PCA, as length, width and depth are correlated due to
isometric growth in mussels. This reduced the data set from three variables to a single
variable for each mussel, which was used in comparisons of growth among individuals.
A 2-Factor ANOVA (Statistica 6.0) was used to determine if there was a significant difference (P < 0.05) in growth (PC1) between sites and among genotypes, as well as genotype-site interaction effects. Post-hoc Tukey tests with subsequent Bonferroni corrections were used to discern significant differences among genotypes. To determine if the much larger sample size of native (MT) mussels was affecting the results, I adjusted the MT sample size by randomly selecting 10 individuals, from those surviving at each site, using bootstrap re-sampling techniques with 100 iterations.

I estimated total mussel volume (cm$^3$) as the volume of an ellipsoid ($\frac{3}{4}\pi r^2 LWD$) and, because fecundity is related to volume in marine bivalves (David 1998), total mussel volume provides an important fitness variable. Volume and survival were used to calculate a fitness metric (average volume (cm$^3$) of a genotype x average survival of a genotype) from which relative fitness was estimated among genotypes. Native MT mussels reared at Ladysmith were set as the reference to which all other genotype fitnesses were compared, such that genotype specific fitness was relative to native mussels in their home environment. Relative fitness was calculated as the fitness metric of one genotype divided by the fitness metric of the native MT genotype reared at Ladysmith ($FM_d/FM_{MT}$). An ANOVA was used to partition the variance in genotype specific fitness metrics between sites and among genotypes within sites.
2.3 RESULTS

Of the 432 mussels reared at Ladysmith (local) and 432 mussels transplanted to Quadra Island, 307 (71.1%) and 347 (80.3%) mussels, respectively, survived until the end of the experiment. Of the original 864 mussels used in this experiment, 705 (alive and dead) were successfully identified to species, with the majority being pure native (Table 2.1). The proportions of genotypes sampled deviate significantly from Hardy-Weinberg Equilibrium (HWE) expectations ($\chi^2 = 325.07, P << 0.001$). No pure *M. edulis* were identified, perhaps not surprisingly as according to HWE, only two of 100,000 are expected. Genotype proportions were similar among sites (Table 2.1). Average water temperature (at 1 metre depth) at Quadra Island (11.5°C +/- 0.0165) was significantly cooler than Ladysmith (19.7°C +/- 0.0178) for the duration of the experiment (Fig. 2.2; $t = 1.66, P < 0.001$).

**Figure 2.2** Mean daily water temperature (°C) profile for Ladysmith and Quadra Island sites at 1m depth.
Table 2.1. Survival of individually caged mussels at two sites (LS = Ladysmith, BC; QI = Quadra Island, BC) by genotype. At the Glu-5' locus: MT = *Mytilus trossulus*, MG = *Mytilus galloprovincialis*, MT×MG = *M. trossulus* x *M. galloprovincialis* hybrid, MG×ME = *M. galloprovincialis* x *M. edulis* hybrid, MTb = *M. trossulus* backcross. At the ITS locus: N = native (*M. trossulus*), A = alien (either *M. edulis* or *M. galloprovincialis*), H = hybrid (either *M. trossulus* x *M. galloprovincialis* or *M. trossulus* x *M. edulis*), BC = backcross (an F1 hybrid x parental *M. trossulus*, or *M. edulis*, or *M. galloprovincialis*).

<table>
<thead>
<tr>
<th>Site</th>
<th>Genotype</th>
<th>GLU</th>
<th>Classification</th>
<th>Alive</th>
<th>Dead</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>MT</td>
<td>N</td>
<td>MT</td>
<td>282</td>
<td>35</td>
<td>317</td>
</tr>
<tr>
<td></td>
<td>MG</td>
<td>A</td>
<td>MG</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MT×MG</td>
<td>H</td>
<td>FI</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>MG×ME</td>
<td>H</td>
<td>FI</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MG×ME</td>
<td>A</td>
<td>BC</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>A</td>
<td>BC</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Site</td>
<td>Total</td>
<td></td>
<td></td>
<td>307</td>
<td>41</td>
<td>348</td>
</tr>
<tr>
<td>QI</td>
<td>MT</td>
<td>N</td>
<td>MT</td>
<td>329</td>
<td>10</td>
<td>339</td>
</tr>
<tr>
<td></td>
<td>MG</td>
<td>A</td>
<td>MG</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>MT×MG</td>
<td>H</td>
<td>FI</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>MG×ME</td>
<td>H</td>
<td>FI</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>MG×ME</td>
<td>A</td>
<td>BC</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>A</td>
<td>BC</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Site</td>
<td>Total</td>
<td></td>
<td></td>
<td>347</td>
<td>10</td>
<td>357</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>654</td>
<td>51</td>
<td>705</td>
</tr>
</tbody>
</table>

**Survival**

At Ladysmith, survival varied with genotype as follows: 88.9% (282/317) for the native, 75.0% (6/8) for the introduced, 88.9% (8/9) for the F1 hybrid, and 78.6% (11/14) for the backcrossed mussels. Genotype-specific survival was less variable at Quadra Island:
97.1% (329/339) for the native, 100% (6/6) for the introduced, 100% (10/10) for the F₁ hybrid, and 100% (2/2) for the backcrossed mussels. Overall survival (all mussels regardless of genotype) was significantly higher at Quadra Island ($\chi^2_{(1)} = 9.58$, $P < 0.05$) than at Ladysmith. Survival among mussel genotypes (both sites combined) did not differ significantly, ($G_{(2)} = 3.21$, $P > 0.05$). There were also no significant differences among genotype-specific survival within sites; Ladysmith $G_{(3)} = 2.19$, $P > 0.05$, and Quadra Island $G_{(3)} = 1.42$, $P > 0.05$ (Fig. 2.3). However, the lack of statistical significance within sites may be due to small sample sizes in some genotype classes.

**Figure 2.3.** Percent survival of caged *Mytilus* mussels from May 27 – August 30 2006 calculated as the number of mussels of a genotype alive at the end divided by the number of mussels of that genotype to start. Lower case a and b designate significant differences.
Growth

Principal Component 1 (PC1) accounted for 95% of the variance in shell measures. Using PC1, volumetric growth was compared between sites and among genotypes. Overall, mussels reared at Quadra Island exhibited faster growth than those reared at Ladysmith (Fig. 4a $F_{(1,57)} = 12.59$, $P = 0.00078$). These results held true regardless of adjustment for unequal sample size. Significant site-genotype interactions were observed ($F_{(3,55)} = 3.95$, $P = 0.013$), indicating that growth patterns among genotypes differed between sites. At Ladysmith, where the water temperature was warmer and more variable (Fig. 2.2), post-hoc Tukey tests revealed that growth among genotypes was not significantly different after Bonferroni correction (Fig. 2.4a). At Quadra Island, where water temperature was cooler and more stable (Fig. 2.2), Tukey tests revealed growth among genotypes was not significantly different after Bonferroni correction (Fig. 2.4a). There were no significant differences in shell length among genotypes at Ladysmith or Quadra Island (Fig. 2.4b).

The relationship between mean final shell volume (cm$^3$) and survival (arcsine square root transformed for percentage data) was found to be linear (Fig. 2.5a)). At both Ladysmith and Quadra Island, the F$_1$ genotype had the highest fitness relative to the native MT in its home environment (Fig. 2.5b). The fitness metric (volume x survival) revealed significant differences between sites ($F_{(7,1)} = 26.27$, $P < 0.05$), but not among genotypes within a site ($F_{(7,3)}= 1.28$, $P = 0.42$) confirming growth patterns as indicated by PC1 and survivorship.
Figure 2.4. Volumetric growth and shell length. Panel a shows relative growth rate as measured by PC1 from May – August 2006 at Ladysmith and Quadra Island among MT, MG, F1, and BC genotypes. PC1 combines length, width and depth into a single variable accounting for 95% of the variance in these metrics. Factor loadings are as follows: Length – 0.982031, Width – 0.984944, Depth – 0.968181. Overall, Quadra Island had significantly higher growth rate than Ladysmith (P < 0.05). Panel b shows final (August 2006) shell length (mm). Error bars represent standard error.
Figure 2.5. Fitness function of volume (cm$^3$) and relative fitness of mussels reared at Ladysmith and Quadra Island. Panel a shows a linear relationship between survival and volume. Survival was arcsine square root transformed for percentage data. Panel b shows fitness calculated relative to Ladysmith MT. Error bars represent standard error.
2.4 DISCUSSION

My results indicate that relative fitness varies among genotypes and between environments. Relative fitness is a critical hybrid zone parameter since the structure and maintenance of hybrid zones is defined by the relative fitness of hybrid individuals. Where hybrids exhibit a fitness advantage, introgression is likely and may lead to the breakdown of reproductive barriers between closely related species, resulting in extensive hybrid zones and potentially the extinction of parental taxa (Burke & Arnold 2001). In contrast, where hybrid fitness is reduced relative to parental types, the cohesion of taxa is reinforced via ecological selection or strict genetic incompatibilities (Barton & Hewitt 1985). Alternatively, hybrid fitness may be expressed in an environment dependent manner (Rundle & Whitlock 2001), resulting in transient and unstable hybrid zones. My data demonstrate varying degrees of fitness differentially expressed in an environment-dependent manner, and indicate that F₁ mussels are not universally less fit than the pure parental types due to inherent genetic incompatibilities (Burke & Arnold 2001; Springer & Heath 2007).

Native, introduced, hybrid and introgressed mussels differ in growth and survival patterns over the summer months May – August 2006, a time in which environmental conditions, including water temperature, vary considerably. The high survival and greater relative growth of F₁ hybrids reared at Quadra Island suggests a considerable fitness advantage over the summer months. Elevated fitness of non-native geonotypes is not consistent with the observed maintenance of the pure native species over much of VI; however, spatial and temporal variation in fitness among genotypes may address this apparent anomaly.
F₁ genotypes exhibit greater relative fitness than all other genotypes at both Ladysmith and Quadra Island; however, genotype specific fitness patterns were not consistent between sites, suggesting a genotype-environment interaction (growth showed a significant genotype-environmental interaction effect in the ANOVA). Environment-dependent selection has been shown for Pacific Mytilus zones in California (Rawson et al. 1999) and recently in Chemainus, BC (Springer & Heath 2007). Thus, environment-dependent variation in mussel fitness likely contributes to the incomplete reproductive isolation between Mytilus species and the spatial limitation of the VI hybrid zone.

Based on my results, selection is predicted to favour F₁ genotypes at Quadra Island; however, there is no incidence of hybridization at this site, or in the general area (Heath et al. 1995; Yanick 2002). I transplanted mussels from an area of high temperature to an area of lower temperature at the beginning of summer when water temperature may approach the upper thermal tolerance of Mytilus off southern VI. Thus, the transfer in this study may have served to alleviate thermal stress, allowing the transplanted mussels to invest more energy into growth. This would explain the reduced growth and survival of M. galloprovincialis at Ladysmith during the summer: perhaps M. galloprovincialis are released from the upper thermal limit when transplanted to the cooler water temperatures of Quadra Island, resulting in 100% survival and intermediate growth. However, my study estimated fitness during the summer months only and the fitness advantage may change through the winter months. Indeed, previous studies have documented dramatic seasonal declines in the abundance of introduced and introgressed genotypes from summer to winter months in the VI hybrid zone (Yanick 2002, JL Shields Chapter 3).
Such a seasonal decline suggests a seasonally variable selection against introduced and introgressed individuals which may be related to temperature as thermal stress has been implicated as a cause of mortality in mussels (Tremblay et al. 1998). Replication of my experiment through the winter months may provide valuable insight into the seasonal variation of mussel fitness at these sites, as MG and introgressed individuals would be expected to experience considerable mortality and reduced growth at both Ladysmith and Quadra Island, thereby limiting northern expansion.

Local adaptation is expected for many marine species, including broadcast spawners with high dispersal capacity, due to the extreme nature of intertidal environments, which drives strong selection pressures that may counteract gene flow (Hilbish 1985; Schmidt & Rand 1999). Among closely related species, heterosis of early generation hybrids is greatest in those resulting from the crossing of populations that exhibit local adaptation, as the heterozygote offspring resulting from parental fixed alleles may mask genetic incompatibilities (Whitlock et al. 2000). Therefore, the observed differences in performance among populations of the native *M. trossulus* upon transplantation experiments on VI (i.e., evidence for local adaptation; Yanick et al. 2003) may contribute to the observed pattern of hybrid fitness. Although heterosis resulting from hybridization is expected to facilitate the establishment and expansion of hybrid zones (Whitlock et al. 2000; Burke & Arnold 2001), heterosis should only be expressed in the $F_1$ hybrids. Thus, the $F_1$ heterosis should be transient, as the level of multilocus heterozygosity will decline in subsequent hybrid and backcross generations (Burke & Arnold 2001). I observed
growth and fitness advantages in all introgressed genotypes, however, indicating that heterosis alone cannot explain my results.

Although the apparent fitness advantage exhibited by introgressed mussels helps explain the presence and sometimes high abundance of hybrid mussels on VI, it does not explain the lack of hybrid zone expansion beyond southern VI (Yanick 2002). A number of possible factors may contribute to the limited spatial distribution of the VI hybrid zone. Perhaps the southern VI hybrid zone is driven by specific introduction vectors that do not exist farther north, and dispersal is limited by unknown physical barriers. Alternatively, the localized distribution of hybrids on southern VI may be due to hybrids favouring marginal habitats (Choler et al. 2004); Ladysmith is highly impacted by industry and urbanization and, hence, hybrid mussels may experience early-life habitat advantages. Furthermore, the hybrid population at Ladysmith suffers considerable seasonal declines in abundance. Variation in selection pressures, therefore, may be critical in the establishment and maintenance of introduced and introgressed genotypes.

Introduced genotypes have been identified on VI for over 10 years, despite there being no record of an established natural population of MG north of California (Heath et al. 1995). Intermittent seeding of M. galloprovincialis from aquaculture tenures in Puget Sound and the Strait of Georgia, as well as shipping, likely play a critical role in maintaining hybrid and introgressed genotypes on southern VI (Heath et al. 1995). The lack of an established, stable introgressed population suggests some inherent genetic incompatibilities that may be conditionally expressed in various environments (Rundle &
While the persistence of introduced and introgressed genotypes on VI is likely to continue (Heath et al. 1995), the abundance of hybrids is highly unstable and more information is required on environmental gradients that may exist along the east coast of VI and that may explain the lack of an established self-sustaining hybrid zone. Seasonal fluctuations in hybrid and introgressed proportions at Ladysmith may be related to temperature: the winter months may be too cold to allow the establishment of a self-sustaining population.

I found that the sampled mussels did not conform to Hardy-Weinberg equilibrium. This is not surprising given that it has been reported previously (Springer & Heath 2007) and that departures from random mating among species are expected. Introduced and hybrid genotypes are outnumbered by native genotypes. The low frequency of MG relative to MT should result in relatively high proportions of MTxMG F$_1$ individuals because the relatively rare MG gametes will come into contact with MT gametes more frequently than with MG gametes. This should also be true for MTxME F$_1$ individuals but this genotype was not observed. Furthermore, the low frequency of pure MG genotypes and the lack of pure ME genotypes suggests that MGxME individuals should be very rare but, surprisingly; a number of MGxME individuals were sampled. The observed frequency of MGxME individuals, and the lack of MTxME individuals, may be a result of slightly asynchronous spawning (Secor et al. 2001; Gilg & Hilbish 2003). Higher gametic compatibility may also play a role, as MG and ME are more closely related to each other than to MT (Martinez-Lage et al. 2002). Alternatively, MGxME individuals may be the result of artificial production at nearby aquaculture tenures. Despite gene flow and
elevated hybrid fitness, *Mytilus* species have retained genetic isolation, suggesting there may be sufficiently strong temporal or ecological reproductive isolating barriers, such as asynchronous spawning and gamete recognition, to overcome the homogenizing effects of gene flow (Albert et al. 2006).

This study adds to the growing body of literature documenting examples of elevated hybrid fitness, suggesting hybridization may play an important role in the evolution of locally-adapted populations or species (Seehausen 2004, Whitlock et al. 2000). Hybrid fitness is habitat specific and assessment of hybrid fitness is essential in understanding the mechanisms of hybrid zone maintenance. My data suggest that introduced and introgressed genotypes should expand their range to northern VI and yet, this has not occurred. While the temporal instability in abundance of introduced and introgressed genotypes may be related to temperature variation, the mechanism maintaining the constant localized distribution of this hybrid zone is not fully understood. As a result, further investigation into potential dispersal barriers away from the hybrid zone are warranted. Understanding the habitat-mediated variation of genotype-specific fitness may help uncover the dynamics required to create and maintain hybrid zones.

2.5 REFERENCES


Slatkin M (1973) Gene flow and selection in a cline. Genetics, 75:733-756


Yanick JF (2002) Survival, growth and the possible environmental impacts of introduced blue mussels (*Mytilus* spp.) in Georgia Strait, British Columbia. Implications for mussel aquaculture. MSc Thesis. Department of Biology, University of Northern British Columbia, Prince George, BC.

3.0 Gene flow and distribution patterns in a Canadian westcoast Mytilus hybrid zone: Marine landscape shapes population structure of a broadcast spawning bivalve

3.1 Introduction

Hybrid zones occur where genetically distinct groups of individuals come into contact and interbreed, producing at least some offspring of mixed ancestry (Barton & Hewitt 1989; Harrison 1990). Stable hybrid zones exist among several taxa (reviewed by: Barton & Hewitt 1985; Harrison 1990; Arnold 1997) and are thought to reflect the balance between selection and dispersal (Barton & Hewitt 1985; Endler 1977; Moore & Price 1993), where selection may be exogenous (due to ecological circumstance) or endogenous (due to inherent genetic incompatibilities). Alternatively, genetic incompatibilities may be conditionally expressed in an environmentally dependent manner (Harrison & Rand 1989; Rundle & Whitlock 2001). Both selection against hybrids and dispersal limitation represent barriers to gene flow (exchange of genetic material), and the relative strength of each component defines the dynamics and determines the stability of a hybrid zone.

Gene flow and dispersal capacity are correlated in many organisms (Burton 1983; Bohonak 1999) including marine invertebrates (Shaklee & Bentzen 1998). Most marine invertebrates share high fecundity and a pelagic larval stage with high dispersive capacities, and consequently high levels of gene flow are expected to result in little genetic population structure (Palumbi 1994). However, selective pressures may reduce the success of long distance dispersers, resulting in a much lower realized level of gene
flow (e.g. *Crassostrea virginica*: Reeb & Avise 1990; *Sebastes auriculatus* – Buonaccorsi et al. 2005). Additionally, pelagic larval dispersal may be constrained and directed by oceanic circulation patterns, shoreline topology and local hydrodynamic patterns (Archambault & Bourget 1999). Such features may act to limit gene flow by driving local larval retention facilitating genetic differentiation even among species with high dispersal potential (e.g., Kenchington et al. 2006). Thus, genetic population structure of broadcast spawning marine invertebrates likely results from a combination of selection and barriers to dispersal resulting in reduced gene flow.

Despite the expectation for panmixia among coastal habitats resulting from extensive larval dispersal, the highly intricate benthic topology and hydrology of the Canadian west coast suggests complex pelagic larval dispersal potentially resulting in localized population subdivision of marine invertebrates. In British Columbia (BC), Canada, the Strait of Georgia is a long (222 km), narrow (28 km), deep (50 – 420 m) fjord estuary separating Vancouver Island from mainland BC. The Strait is characterized by numerous peninsulas, narrow passes, islands, and shallow sill areas which restrict surface water flow among basins (Herlinveaux & Tully 1961; Emmet et al. 2000, Rabinovich et al. 2003; Masson & Cummins 2004) thereby potentially limiting pelagic larval dispersal and resulting in genetic differentiation on small spatial scales via local retention of pelagic larvae. Therefore, in this area genetic population structure of broadcast spawning marine invertebrates may well be defined by the marine landscape.
The most common sedentary marine species with pelagic larvae in the Strait of Georgia is the blue mussel, consisting of three species of the genus *Mytilus*, occurring sympatrically on the east coast of Vancouver Island (VI). The native mussel, *Mytilus trossulus*, is the most abundant and widely distributed, and found intermixed with unstable localized populations of the introduced mussels *Mytilus galloprovincialis* and *Mytilus edulis*. Introduced and hybrid genotypes were first identified along the southeastern coast of VI the mid-1990’s (Heath et al. 1995). Since then, these genotypes have persisted with a stable southern distribution and temporally varying abundance, creating a dynamic ‘hybrid zone’ restricted to the south (Heath et al. 1995; Yanick 2002; Chapter 2). This hybrid population does not appear to be self-sustaining since it experiences dramatic declines in abundance over time (Yanick 2002), but rather is likely maintained by repeated introductions and fluctuating relative fitness of the non-native genotypes (Chapter 2). *Mytilus* are broadcast spawners with high fecundity (Thomson 1976), an extended pelagic larval phase lasting from several weeks to two months (Bayne 1965) and a dispersal capability estimated to range on average from 30 – 60km, in some cases greater than 100km (McQuaid & Phillips 2000; Gilg & Hilbish 2003). Such dispersal potential coupled with high non-native genotype survival and growth in the north (Chapter 2) indicates that the distribution of introduced and hybrid mussels should be expanding northward on VI, yet to date there is no evidence to suggest this has occurred.

With the high fecundity, long pelagic larval stage and extensive dispersal potential in *Mytilus*, genetic homogeneity and large-scale panmixia is the logical prediction; however the observed spatially restricted hybrid zone on VI is not consistent with that expectation.
Here, I estimate the extent of the *Mytilus* hybrid zone on VI using two nuclear species identification markers, and measure population differentiation among those sites using four polymorphic microsatellite loci, and replicate the analysis over two years (2005 and 2006). The analysis of the VI mussel hybrid zone provides a remarkable example of unexpected, but severe barriers to gene flow in a species with very high dispersal capabilities. I use a genetic landscape analytical approach to identify barriers to gene flow in relation to the marine landscape that helps explain the maintenance of the southern Vancouver Island hybrid zone.

### 3.2 MATERIALS AND METHODS

*Field sampling*

A total of 1433 mussels were collected from the underside of docks and from subtidal pilings at 11 sites on the east coast of VI and 2 sites in Vancouver, BC, Canada in May and June of 2005 (n = 809) and 2006 (n = 624) (Fig. 3.1; Table 3.1). In the summer of 2005, an additional 3 sites (96 mussels) were sampled west of Campbell River, BC, and in 2006 an additional 4 sites (192 mussels) were sampled among the Southern Gulf Islands, BC for a total collection of 1721 mussels over the two years (Fig. 3.1; Table 3.1). At each site mussels were haphazardly selected from samples of mussel clusters. A small piece of mantle tissue was taken from each mussel and stored in 95% EtOH and stored at -20°C. I performed additional sampling at Ladysmith (December 2005 (N = 64), December 2006 (N = 64), February 2007 (N = 32)) to test for a seasonal-associated drop in non-native genotype abundance (Yanick 2002).
Figure 3.1 Sampling locations of Vancouver Island and area *Mytilus* populations. Names of sampling locations and year of collection are shown in Table 1. A – Vancouver Island, B – Discovery Islands, C – Gulf Islands
Table 3.1 Summary of microsatellite marker statistics for Mytilus trossulus populations by locus for 2005 and 2006, latitude and longitude is given for each sample site. Bold italics of $F_{IS}$ indicate significant departure from Hardy-Weinberg equilibrium based on Fisher’s Exact tests. N = sample size, $N_A$ = number of alleles, $H_O/H_E$ = observed and expected heterozygosities, $F_{IS}$ = inbreeding coefficient.

<table>
<thead>
<tr>
<th>Population</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mgu3</td>
<td>Mgu5</td>
</tr>
<tr>
<td><strong>North Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Port Hardy (PH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50°42'44&quot;N, 127°29'20&quot;W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>48</td>
</tr>
<tr>
<td>$N_A$</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.182</td>
<td>0.288</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.651</td>
<td>0.613</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.721</td>
<td>0.530</td>
</tr>
<tr>
<td>Sayward (SA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50°23'31&quot;N, 125°57'27&quot;W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>$N_A$</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.373</td>
<td>0.412</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.666</td>
<td>0.458</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.441</td>
<td>0.102</td>
</tr>
<tr>
<td><strong>Central Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadra Island (QI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50°09'33&quot;N, 125°20'10&quot;W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>86</td>
<td>48</td>
</tr>
<tr>
<td>$N_A$</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.621</td>
<td>0.242</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.751</td>
<td>0.595</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.173</td>
<td>0.593</td>
</tr>
<tr>
<td>Union Bay (UB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49°35'03&quot;N, 124°59'08&quot;W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>48</td>
</tr>
<tr>
<td>$N_A$</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.281</td>
<td>0.203</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.738</td>
<td>0.387</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.619</td>
<td>0.475</td>
</tr>
<tr>
<td>Gorge Harbour 1 (GH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50°03'04&quot;N, 124°59'43&quot;W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.063</td>
<td>0.219</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.374</td>
<td>0.371</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.833</td>
<td>0.410</td>
</tr>
<tr>
<td>Gorge Harbour 2 (GG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50°04'26&quot;N, 124°58'54&quot;W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.156</td>
<td>0.219</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.577</td>
<td>0.371</td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.729</td>
<td>0.410</td>
</tr>
<tr>
<td>Location</td>
<td>Coordinates</td>
<td>N</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------</td>
<td>----</td>
</tr>
<tr>
<td>Cascade Creek (CC)</td>
<td>49°59'45&quot;N, 125°13'42&quot;W</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>South Vancouver (SV)</td>
<td>49°16'18&quot;N, 123°10'11&quot;W</td>
<td>80</td>
</tr>
<tr>
<td>West Vancouver (WV)</td>
<td>49°22'09&quot;N, 123°17'02&quot;W</td>
<td>64</td>
</tr>
<tr>
<td>French Creek (FC)</td>
<td>49°40'28&quot;N, 124°58'42&quot;W</td>
<td>60</td>
</tr>
<tr>
<td>Nanaimo (NA)</td>
<td>49°11'52&quot;N, 123°58'56&quot;W</td>
<td>95</td>
</tr>
<tr>
<td>Ladysmith (LS)</td>
<td>48°59'39&quot;N, 123°48'42&quot;W</td>
<td>69</td>
</tr>
<tr>
<td>Chemainus (CH)</td>
<td>48°55'35&quot;N, 123°42'52&quot;W</td>
<td>60</td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
<table>
<thead>
<tr>
<th>Location</th>
<th>Coordinates</th>
<th>N</th>
<th>N&lt;sub&gt;A&lt;/sub&gt;</th>
<th>H&lt;sub&gt;0&lt;/sub&gt;</th>
<th>H&lt;sub&gt;E&lt;/sub&gt;</th>
<th>F&lt;sub&gt;IS&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crofton (CR)</td>
<td>48°51'40&quot;N, 123°38'37&quot;W</td>
<td>87</td>
<td>9  4  16  5  6  5  20  4</td>
<td>0.838  0.203  0.419  0.149  0.188  0.188  0.354  0.146</td>
<td>0.825  0.550  0.841  0.559  0.769  0.651  0.908  0.138</td>
<td>-0.016  0.632  0.502  0.734  0.756  0.712  0.610  -0.055</td>
</tr>
<tr>
<td>Maple Bay (MB)</td>
<td>48°48'33&quot;N, 123°36'40&quot;W</td>
<td>88</td>
<td>5  5  17  6  6  5  22  8</td>
<td>0.031  0.094  0.563  0.266  0.292  0.271  0.792  0.250</td>
<td>0.766  0.241  0.875  0.607  0.742  0.614  0.900  0.408</td>
<td>0.959  0.611  0.357  0.562  0.607  0.559  0.121  0.387</td>
</tr>
<tr>
<td>Moses Point (MP)</td>
<td>48°41'23&quot;N, 123°29'00&quot;W</td>
<td>69</td>
<td>7  5  15  4  5  5  10  4</td>
<td>0.484  0.281  0.672  0.141  0.063  0.292  0.333  0.167</td>
<td>0.801  0.566  0.856  0.458  0.698  0.592  0.784  0.639</td>
<td>0.395  0.503  0.215  0.693  0.911  0.507  0.575  0.739</td>
</tr>
<tr>
<td>Princess Cove (PC)</td>
<td>48°56'55&quot;N, 123°33'26&quot;W</td>
<td>48</td>
<td>-  -  -  -  5  6  13  4</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
</tr>
<tr>
<td>Georgeson Passage (GP)</td>
<td>48°49'71&quot;N, 123°13'76&quot;W</td>
<td>48</td>
<td>-  -  -  -  4  5  9  5</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
</tr>
<tr>
<td>Browning Canal (BC)</td>
<td>48°46'55&quot;N, 123°15'43&quot;W</td>
<td>48</td>
<td>-  -  -  -  5  5  10  4</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
</tr>
<tr>
<td>Cabbage Island (CI)</td>
<td>48°47'68&quot;N, 123°05'26&quot;W</td>
<td>48</td>
<td>-  -  -  -  3  3  2  4</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
<td>-        -        -        -        -        -        -        -</td>
</tr>
</tbody>
</table>
**DNA Extraction**

Approximately 50mg of mantle edge tissue were removed from the 95% EtOH, blotted dry, and coarsely chopped. The tissue was digested in 500µL of lysis buffer (50mM Tris-HCl pH 8.0; 1.0% SDS; 25mM EDTA) and 200µL (20µg/mL) proteinase K, shaking at room temperature overnight. The solution was then extracted using the Promega WIZARD® DNA extraction kit (Promega, Madison, WI, USA) following the standard ‘isolation from animal tissue’ protocol on 2005 samples. DNA was resuspended in 100µL of Tris-EDTA buffer (10mM Tris, 1.0mM EDTA, pH 8.0). Mussels collected in 2006 were extracted using the plate-based DNA extraction protocol for animal tissue developed by Elphinstone et al. (2003) and DNA was resuspended in 50µL of Tris-EDTA buffer (10mM Tris, 1.0mM EDTA, pH 8.0).

**Species Identification**

Each mussel was identified to species using two co-dominant nuclear loci, the polyphenolic adhesive protein GLU developed by Rawson et al. (1996) and the internally transcribed spacer (ITS) developed by Heath et al. (1995). These two markers identify members of the *Mytilus* species complex with different levels of resolution. The ITS marker is a co-dominant diagnostic species marker from the internal transcribed spacer regions between the 18S and 28S nuclear rDNA coding regions (Heath et al. 1995). The ITS PCR amplifies a single band of 1250bp and produces diagnostic banding patterns upon digestion with restriction endonuclease *HhaI* (Heath et al. 1995). These diagnostic RFLP banding patterns distinguish *M. trossulus* from other members of the *Mytilus*
species complex as well as hybrids, but are unable to distinguish between *M. edulis* and *M. galloprovincialis*. Mussels were thus classified as native (*M. trossulus*), introduced (*M. edulis* or *M. galloprovincialis*) and hybrid (*M. trossulus × M. edulis* or *M. trossulus × M. galloprovincialis*). All PCR amplifications and subsequent RFLPs were performed using the ITS protocol as described in Heath et al. (1995). RFLP products were visualized on 2.0% agarose gels stained with ethidium bromide and individual mussels were scored for genotype on the basis of diagnostic RFLP banding patterns.

GLU is a co-dominant diagnostic species marker from the polyphenolic adhesive protein used in the attachment of byssal threads to the substrate (Rawson et al. 1996). PCR amplification produces species-specific banding patterns identifying *M. trossulus*, *M. edulis*, *M. galloprovincialis* and hybrids between any of the three species (Rawson et al. 1996). All PCR amplifications were performed following the GLU protocol described in Rawson et al. (1996) using one dye-labeled primer, and the PCR fragments were visualized using an automated DNA analyzer (LiCOR 4300; Lincoln Nebraska, USA).

Individuals were assigned a genotype based on the two-locus genotype. Pure types (MT, MG, ME) were identified as consensus homozygous parental at both loci. First generation hybrid individuals (MT×MG, MT×ME, MG×ME) were identified as heterozygous at both loci. Backcross and introgressed individuals were identified where the two markers disagreed. With only two diagnostic markers, error in identifying hybridization and introgression by genotype is unavoidable. Additional sampling at Ladysmith (May 2005, December 2005, May 2006, December 2006, February 2007)
allowed quantification of the large variation in the abundance of non-native genotypes over the winter months. Differences in abundance between years at each site were tested by Chi-square in STATISTICA 6.0. Single locus Exact-U tests of Hardy-Weinberg equilibrium and $F_{IS}$ were tested in GENEPOP 3.4 (Raymond & Rousset 1995).

**Microsatellite Analyses**

All microsatellite analyses included only the native *M. trossulus* genotype as the inclusion of genetically distinct groups invalidates several assumptions of population genetic analysis assumptions and the incidence of non-native genotypes was not high (39 in 2005 and 41 in 2006). Four polymorphic *Mytilus* microsatellite loci ($Mgu3$; $Mgu5$; $Mgu6$; $Mgu7$; Presa et al. 2002) were amplified by polymerase chain reaction (PCR) using an Eppendorf ep gradient S Mastercycler (Brinkmann Instruments, Inc., Westbury USA) following conditions specified by Presa et al. (2002) to characterize *Mytilus* populations on VI. Reactions were carried out in 25µL volumes containing approximately 50ng DNA template, 32µM dye-labeled forward primer (IR-700, IR-800; NWG Biotech, High Point NC), 0.5µM reverse primer, 200µM each dNTP, 2.5mM MgCl$_2$, and 0.5U Taq DNA polymerase (Applied Biosystems) in a 1X PCR buffer supplied by the manufacturer. The number of individuals genotyped from each site ranged from 32 to 74 (Table 1). The PCR protocol consisted of one cycle at 95°C for 2 minutes followed by 35 cycles at 95°C for 15s, 55°C (annealing temperature for all four loci) for 15s, 72°C for 30s followed by a final extension at 72°C for 2 minutes and a 4°C hold. Following PCR, microsatellite alleles were visualized on a LiCOR 4300 DNA analyzer and scored using GENE IMGR 4.05 (Scanalytics Inc., Rockville, MD, USA)
imaging software. Alleles were binned to the nearest whole repeat. To reduce scoring error, each gel was read twice with at least a two-week interval between readings, and three control samples of known species were run on each gel as reference points.

Statistical Analyses

I used **micro-checker** 2.2.3 (Shipley 2003) software to test for null alleles, private alleles, and error due to scoring, stuttering, and large allele drop out. Linkage disequilibria between pairs of loci were tested overall and within populations using **GENEPOP** 3.4 (Raymond & Rousset 1995) software. I tested for departure from Hardy-Weinberg equilibrium (HWE) within each population for each locus using **GENEPOP** (Raymond & Rousset 1995). Hybrid populations violate assumptions of HWE due to varying degrees of assortative mating, interspecific gene flow and selective effects on introgressed genotypes, thus all of my calculations and analyses that assume HWE must be interpreted with caution. Violations of HWE make many statistical methods using genotype frequencies invalid, and in such cases methods using allele frequencies, such as Weir & Cockerham’s $F$ statistics (1984) are more appropriate (Kenchington et al. 2006), although still sensitive to such deviations. Furthermore, non-equilibrium parameters, such as Nei’s standard genetic distance ($D_s$), are preferred when HWE is violated. Pairwise Fisher’s Exact tests were also used to test for significant population differentiation in **TFPGA** 1.3 (Miller 1997).
**Temporal variation**

To determine the temporal stability of the VI hybrid zone and whether genetic population patterns are maintained over time, Fisher’s Exact tests were used to test variation in allele frequency data for both microsatellite loci and diagnostic species markers at the 13 VI sites sampled in both 2005 and 2006 using TFPGA 1.3 (Miller 1997).

**Geographic differentiation**

Spatial patterns of genetic divergence were determined using landscape genetics analyses, specifically, isolation by distance models and genetic discontinuity (boundary) analyses. As an alternative hypothesis to panmixia, isolation by distance (IBD) was tested among all populations using geographic distance (km) estimated by: 1) shoreline distance, and 2) oceanographic distance (closest ocean current pathway connecting the sampling locations) versus Nei’s standard genetic distance ($D_s$). The significance of the correlation was tested using a Mantel test with 1000 iterations in GENEPOP 3.4 (Raymond & Rousset 1995). Finally, I tested for gene flow barriers to identify geographic boundaries to dispersal using Monmonier’s algorithm (Monmonier 1973) to visualize genetic distances between samples in relation to their geographical position without defining populations in advance. First, latitude and longitude coordinates (Table 3.1) were converted to UTM coordinates and used in combination with genetic data to generate a connectivity network of genetic distances based on Delaunay triangulation (Brassel & Reif 1979). Nei’s standard genetic distance was applied to all pairs of individuals to generate a genetic matrix and genetic distances between samples were then associated to each edge of the network using AIS 1.0 (Miller 2005) software.
Monmonier’s maximum difference algorithm (1973) identified putative genetic barriers. The locations of putative barriers to gene flow are determined by iteratively identifying sets of contiguous, large genetic distances along connectivity networks (Manel et al. 2003). The maximum number of barriers was identified as five, above which the stability of the program declined. Monmonier’s algorithm was then applied to this geometric network to identify ‘barriers’ — areas where genetic differences between pairs of populations were greatest. This approach identifies significant discontinuities in the genetic distance matrix, and allows straightforward identification of corresponding geographic and oceanographic features that may drive restrictions of gene flow among populations.

To test the statistical significance of putative barriers identified by AIS 1.0 (Miller 2005) software, an hierarchical multilocus analysis of molecular variance (AMOVA) was used to partition total variance among large-scale regions defined by the barrier analysis, among populations within regions and within populations using ARLEQUIN 3.11 (Schneider et al. 2000) software. To determine if population diversity varied among regions identified by the large-scale barriers, average values of $H_0$, $H_E$ and $F_{IS}$ were calculated within each region. Genetic differentiation among regions ($F_{ST}$ and $D_s$) was estimated using a permutation test in GENEPOP 3.4 (Raymond & Rousset 1995) and the significance was tested by Fisher’s Exact tests in TFPGA 1.3 (Miller 1997).
3.3 RESULTS

Species distribution

The pure native *M. trossulus* genotype was most abundant in my combined samples in both years, making up 95.8% in 2005 and 93.4% in 2006 of the total sample. Individual sampling sites were dominated by the pure *M. trossulus* genotype as well, with the lowest frequency at LS (65.2% in 2005, and 72.9% in 2006; Fig. 3.2). The frequency of introduced, F₁ and backcross individuals in the hybrid sites varied significantly between years site-by-site (Fig. 3.2), although overall the proportion of non-native mussels did not statistically differ from 2005 to 2006 ($\chi^2 = 7.37, P = < 0.05$). Dramatic variation in abundance of non-native genotypes was observed across seasons at Ladysmith (all comparisons significant at the $P = 0.001$ level except Dec 2005 – Feb 2007, which contained only a single non-native mussel; Fig. 3.3) supporting previous data suggesting that the localized Vancouver Island hybrid zone suffers – and recovers – from severe declines in abundance of non-native genotypes (Yanick 2002). Both ITS and Glu-5’ deviated from HWE in several populations (Fig. 3.2), resulting from heterozygote deficiency and a paucity of certain expected genotypes (e.g. MT × ME: Chapter 2).

Microsatellite analyses

All microsatellite loci were out of HWE in at least one population (Table 3.1) after Bonferroni correction in both 2005 and 2006. Weir & Cockerham’s (1984) within-population $F_{IS}$ across all loci was significant in at least one population within the 16 and 17 populations in 2005 and 2006 respectively (Table 3.1). Values of $F_{IS}$ across populations ranged from -0.01 in *Mgu3* to 0.95 in *Mgu3* for 2005 and from -0.05 in *Mgu7* to 1.00 in *Mgu5* for 2006 (Table 3.1). MICROCHECKER 2.2.3 software (Shipley 2003) did
not identify stuttering, null alleles, or large allele dropout as contributors to the departure from HWE for any population in either 2005 or 2006. Significant linkage disequilibrium was found between \textit{Mgu5 - Mgu6} across all populations in both 2005 and 2006 (P = 0.03436 in 2005, P = 0.0327 in 2006), as is expected of physically linked loci (Presa et al. 2002). Despite linkage disequilibrium, these loci provide additional information as each contains different repeat motifs (tetranucleotide repeat vs. trinucleotide repeat) and thus mutate at different rates. No other loci were found to show significant linkage disequilibrium. All four loci were polymorphic, with the number of alleles ranging from 12 to 49 in 2005 and 9 to 63 in 2006. Allelic richness across populations ranged between 2 and 29 in 2006, with no population consistently allele-rich, or poor, across loci (Table 3.1). Of the 107 and 104 alleles detected with the four loci in 2005 and 2006, 34 and 33, respectively, were private, and the frequency of private alleles never exceeded 0.05. Nine populations (CR, MP, QI, GH, GG, CCC, PC, BC, and GP) had no private alleles.
Figure 3.2: Genotype frequency of *Mytilus* species within the VI hybrid zone based on two diagnostic species-specific nuclear genetic markers (ITS and GLU). All genotype frequencies were significantly different after Bonferroni correction. MT = *M. trossulus* pure genotype, MG = *M. galloprovincialis* pure genotype, ME = *M. edulis* pure genotype, F1 = hybrid, BC = backcross. Bold italics indicate significant departure from HWE tested by Exact U tests of heterozygote deficiency in GENEPOP 3.4 (Raymond & Rousset 1995).
Figure 3.3: Temporal variation in frequency of non-native genotype mussel abundance at the Ladysmith site from May 2005 – February 2007. All comparisons were statistically significant after Bonferroni correction, as indicated by asterisks.

Temporal variation

Pairwise Fisher’s Exact tests revealed significant temporal variation in microsatellite allele frequency distributions for all comparisons of the 13 VI sites sampled in 2005 and 2006. Because temporal replicates were significantly different from each other, sites sampled in both years could not be pooled in further analyses. Consequently, 2005 and 2006 data were analyzed separately.

Geographic differentiation

No significant isolation by distance pattern was detected overall, nor within regions in 2005 or 2006 using either geographic (overall: 2005 – r = 0.03, P = 0.99; 2006 – r = 0.13,
P = 1.00; within regions: 2005 – North $r = 0.02$, P = 0.89, Central $r = 0.02$, P = 0.92, South $r = 0.001$, P = 0.99; 2006 – North $r = 0.09$, P = 0.94, Central $r = 0.05$, P = 0.96, South $r = 0.01$, P = 0.99) or oceanographic distances (overall: 2005 – $r = 0.06$, P = 0.98; 2006 – $r = 0.13$, P = 0.99; within regions: 2005 – North $r = 0.07$, P = 0.96, Central $r = 0.09$, P = 0.989, South $r = 0.004$, P = 0.99; 2006 – North $r = 0.13$, P = 0.97, Central $r = 0.15$, P = 0.94, South $r = 0.03$, P = 0.92). Two genetic discontinuities were identified by AIs 1.0 (Miller 2005) software in both 2005 and 2006, based on those results, barriers to gene flow were inferred, grouping my sample sites into three broad scale geographic regions – North, Central and South VI (Fig. 3.4). Region 1 was comprised of two sites on the North coast of VI – Port Hardy (PH) and Sayward (SA). Region 2 was comprised of sites along the Central coast of VI and two sites in Vancouver on the mainland of BC – Quadra Island (QI), Gorge Harbour (GH and GG), Cascade Creek (CCC), Union Bay (UB), South Vancouver (SV), and West Vancouver (WV). Region 3 was comprised of sites along the South coast of VI as well as sites among the Southern Gulf Islands – French Creek (FC), Nanaimo (NA), Ladysmith (LS), Chemainus (CH), Crofton (CR), Maple Bay (MB), Moses Point (MP), Princess Cove (PC), Browning Canal (BC), Georgeson Passage (GP), and Cabbage Island (CI). Small-scale boundaries were also identified that correspond to secondary barriers detected among sites in narrow channels among the Southern Gulf Islands (Fig. 3.4). Fisher’s Exact tests revealed significant genetic differentiation among the three large scale regions defined by the barrier analyses (North – Central P < 0.001, Central – South P < 0.001, North – South P < 0.001). A hierarchical AMOVA revealed a small but significant among-region component, explaining 2.77% (P = 0.007) of the total variance. Most of the variance was among individuals.
within populations (88.44%, P < 0.001) and some variance was distributed among populations within regions (8.79%, P < 0.001). Among-regions, pairwise Nei’s genetic distance $D_s$ and Weir & Cockerham (1984) $F_{ST}$ reflect the relatively large differentiation identified by the barrier analysis, with the greatest differentiation between the North and Central regions ($D = 0.267$, $P < 0.001$ in 2005 and $D = 0.301$, $P < 0.001$ in 2006; $F_{ST} = 0.052$, $P < 0.001$ in 2005 and $F_{ST} = 0.096$, $P < 0.001$ in 2006). Differentiation between the Central and South region was less pronounced yet still significant ($D = 0.048$, $P < 0.001$ in 2005 and $D = 0.051$, $P < 0.001$ in 2006; $F_{ST} = 0.01$, $P < 0.001$ in 2005 and $F_{ST} = 0.015$, $P < 0.001$ in 2006). Within regions, genetic differentiation was high (Table 3.2) and secondary barriers to gene flow were identified in the south region using AIS 1.0 (Miller 2005), which correspond to geographic and oceanographic features of the area such as mean surface currents and narrow channels separating the Gulf Islands (Fig. 3.5). Two sites (LS, GP) within the southern region were isolated by the barrier analysis into discrete populations. Pairwise $F_{ST}$ and $D$ among sites within the southern region were compared and found to be greatest between Cabbage Island and all other sites ($F_{ST}$ ranging from 0.19 to 0.34 and $D$ ranging from 0.29 to 0.66). Of all 2006 pairwise comparisons in the south, differentiation was greatest between Ladysmith (LS) – a known hybrid population – and Cabbage Island (CI) – an isolated pure-type $M. trossulus$ population ($F_{ST} = 0.27$, $P < 0.001$ and $D = 0.66$, $P < 0.001$). Small-scale population connectivity (i.e., non-significant differentiation) among CH, CR, and MB was identified (Table 3.3).
Figure 3.4: Barrier network identifying areas of limited gene flow as defined by AIS barrier analyses. Inset: AIS output - Solid circles represent relative genetic position of individual sampling sites. Black shading indicates presence of hybrids. Putative barriers are labeled from a to e. Solid thick lines (a, b) represent primary barriers and thin lines represent secondary barriers found among the Southern Gulf Islands (c - e). Barriers are overlayed on a map of Vancouver Island for identification of corresponding oceanographic features limiting gene flow.
Figure 3.5: Average summer (May – June) surface currents for southern Strait of Georgia. Adapted with permission from M. Foreman, Institute for Ocean Studies, Sydney, BC.
Table 3.2. Pairwise microsatellite genetic distance and $F_{ST}$ values for all mussel sample sites off VI and area for 2005. Pairwise Weir & Cockerham's $F_{ST}$ above the diagonal, pairwise Nei’s genetic distance $D_s$ below the diagonal. Bold italics indicate significant Fisher’s Exact P-values (above diagonal), or significant $F_{ST}$ value after table-wide Bonferroni correction.

<table>
<thead>
<tr>
<th></th>
<th>PH</th>
<th>SA</th>
<th>SV</th>
<th>WV</th>
<th>UB</th>
<th>QI</th>
<th>NA</th>
<th>FC</th>
<th>MB</th>
<th>CR</th>
<th>CH</th>
<th>MP</th>
<th>LS</th>
<th>GH</th>
<th>GG</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>0.189</td>
<td>0.046</td>
<td>0.056</td>
<td>0.172</td>
<td>0.188</td>
<td>0.017</td>
<td>0.156</td>
<td>0.063</td>
<td>0.080</td>
<td>0.097</td>
<td>0.155</td>
<td>0.051</td>
<td>0.105</td>
<td>0.089</td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>0.373</td>
<td></td>
<td>0.255</td>
<td>0.349</td>
<td>0.325</td>
<td>0.191</td>
<td>0.270</td>
<td>0.263</td>
<td>0.241</td>
<td>0.086</td>
<td>0.300</td>
<td>0.237</td>
<td>0.337</td>
<td>0.310</td>
<td>0.253</td>
<td></td>
</tr>
<tr>
<td>SV</td>
<td>0.097</td>
<td>0.595</td>
<td>0.031</td>
<td>0.104</td>
<td>0.113</td>
<td>0.045</td>
<td>0.132</td>
<td>0.045</td>
<td>0.056</td>
<td>0.104</td>
<td>0.097</td>
<td>0.036</td>
<td>0.081</td>
<td>0.067</td>
<td>0.080</td>
<td></td>
</tr>
<tr>
<td>WV</td>
<td>0.113</td>
<td>0.704</td>
<td>0.081</td>
<td>0.064</td>
<td>0.089</td>
<td>0.053</td>
<td>0.130</td>
<td>0.030</td>
<td>0.053</td>
<td>0.150</td>
<td>0.071</td>
<td>0.018</td>
<td>0.039</td>
<td>0.020</td>
<td>0.071</td>
<td></td>
</tr>
<tr>
<td>UB</td>
<td>0.387</td>
<td>1.232</td>
<td>0.247</td>
<td>0.142</td>
<td>0.028</td>
<td>0.185</td>
<td>0.167</td>
<td>0.086</td>
<td>0.107</td>
<td>0.267</td>
<td>0.017</td>
<td>0.087</td>
<td>0.058</td>
<td>0.034</td>
<td>0.089</td>
<td></td>
</tr>
<tr>
<td>QI</td>
<td>0.501</td>
<td>1.235</td>
<td>0.315</td>
<td>0.224</td>
<td>0.062</td>
<td>0.198</td>
<td>0.148</td>
<td>0.113</td>
<td>0.100</td>
<td>0.251</td>
<td>0.009</td>
<td>0.101</td>
<td>0.116</td>
<td>0.081</td>
<td>0.098</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>0.039</td>
<td>0.406</td>
<td>0.106</td>
<td>0.115</td>
<td>0.460</td>
<td>0.588</td>
<td>0.154</td>
<td>0.064</td>
<td>0.092</td>
<td>0.079</td>
<td>0.164</td>
<td>0.056</td>
<td>0.138</td>
<td>0.120</td>
<td>0.089</td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td>0.419</td>
<td>0.893</td>
<td>0.445</td>
<td>0.389</td>
<td>0.470</td>
<td>0.460</td>
<td>0.451</td>
<td>0.146</td>
<td>0.147</td>
<td>0.203</td>
<td>0.133</td>
<td>0.079</td>
<td>0.192</td>
<td>0.169</td>
<td>0.130</td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>0.124</td>
<td>0.692</td>
<td>0.106</td>
<td>0.070</td>
<td>0.175</td>
<td>0.268</td>
<td>0.136</td>
<td>0.419</td>
<td>0.046</td>
<td>0.154</td>
<td>0.088</td>
<td>0.035</td>
<td>0.076</td>
<td>0.047</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0.182</td>
<td>0.737</td>
<td>0.165</td>
<td>0.142</td>
<td>0.262</td>
<td>0.278</td>
<td>0.234</td>
<td>0.536</td>
<td>0.110</td>
<td>0.144</td>
<td>0.084</td>
<td>0.045</td>
<td>0.092</td>
<td>0.067</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>0.213</td>
<td>0.152</td>
<td>0.299</td>
<td>0.430</td>
<td>1.000</td>
<td>1.049</td>
<td>0.185</td>
<td>0.808</td>
<td>0.419</td>
<td>0.473</td>
<td>0.224</td>
<td>0.144</td>
<td>0.244</td>
<td>0.218</td>
<td>0.146</td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>0.397</td>
<td>1.072</td>
<td>0.282</td>
<td>0.186</td>
<td>0.041</td>
<td>0.034</td>
<td>0.465</td>
<td>0.425</td>
<td>0.212</td>
<td>0.244</td>
<td>0.896</td>
<td>0.079</td>
<td>0.084</td>
<td>0.055</td>
<td>0.073</td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>0.116</td>
<td>0.710</td>
<td>0.121</td>
<td>0.062</td>
<td>0.221</td>
<td>0.307</td>
<td>0.141</td>
<td>0.258</td>
<td>0.086</td>
<td>0.144</td>
<td>0.471</td>
<td>0.253</td>
<td>0.067</td>
<td>0.047</td>
<td>0.071</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>0.184</td>
<td>0.414</td>
<td>0.990</td>
<td>1.002</td>
<td>0.664</td>
<td>0.466</td>
<td>0.382</td>
<td>0.291</td>
<td>0.154</td>
<td>0.179</td>
<td>0.802</td>
<td>0.430</td>
<td>0.705</td>
<td>-0.012</td>
<td>0.111</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>0.402</td>
<td>0.193</td>
<td>0.034</td>
<td>0.032</td>
<td>0.128</td>
<td>0.227</td>
<td>0.120</td>
<td>0.269</td>
<td>1.061</td>
<td>0.445</td>
<td>0.416</td>
<td>0.166</td>
<td>0.167</td>
<td>0.086</td>
<td>0.155</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.195</td>
<td>0.067</td>
<td>0.203</td>
<td>0.297</td>
<td>0.114</td>
<td>0.120</td>
<td>0.206</td>
<td>0.132</td>
<td>0.743</td>
<td>0.140</td>
<td>0.292</td>
<td>0.069</td>
<td>0.135</td>
<td>0.221</td>
<td>0.468</td>
<td></td>
</tr>
</tbody>
</table>

64
Table 3.3. Pairwise microsatellite genetic distance and $F_{ST}$ values for all mussel sample sites off VI and area for 2006. Pairwise Weir & Cockerham's $F_{ST}$ above the diagonal, pairwise Nei's genetic distance $D_s$ below the diagonal. Bold italics indicate significant Fisher's Exact P-values (above diagonal), or significant $F_{ST}$ value after table-wide Bonferroni correction.

<table>
<thead>
<tr>
<th></th>
<th>PH</th>
<th>SA</th>
<th>SV</th>
<th>WV</th>
<th>UB</th>
<th>QI</th>
<th>NA</th>
<th>FC</th>
<th>MB</th>
<th>CR</th>
<th>CH</th>
<th>MP</th>
<th>LS</th>
<th>PC</th>
<th>BC</th>
<th>GP</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>0.062</td>
<td>0.161</td>
<td>0.124</td>
<td>0.115</td>
<td>0.188</td>
<td>0.165</td>
<td>0.143</td>
<td>0.140</td>
<td>0.166</td>
<td>0.146</td>
<td>0.131</td>
<td>0.088</td>
<td>0.168</td>
<td>0.200</td>
<td>0.141</td>
<td>0.340</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>0.185</td>
<td>0.126</td>
<td>0.101</td>
<td>0.126</td>
<td>0.124</td>
<td>0.145</td>
<td>0.115</td>
<td>0.097</td>
<td>0.150</td>
<td>0.083</td>
<td>0.074</td>
<td>0.082</td>
<td>0.202</td>
<td>0.122</td>
<td>0.085</td>
<td>0.313</td>
<td></td>
</tr>
<tr>
<td>SV</td>
<td>0.406</td>
<td>0.273</td>
<td>0.012</td>
<td>0.102</td>
<td>0.018</td>
<td>0.055</td>
<td>0.032</td>
<td>0.010</td>
<td>0.033</td>
<td>0.030</td>
<td>0.081</td>
<td>0.060</td>
<td>0.051</td>
<td>0.036</td>
<td>0.076</td>
<td>0.194</td>
<td></td>
</tr>
<tr>
<td>WV</td>
<td>0.564</td>
<td>0.362</td>
<td>0.052</td>
<td>0.083</td>
<td>0.024</td>
<td>0.037</td>
<td>0.013</td>
<td>0.006</td>
<td>0.013</td>
<td>0.036</td>
<td>0.074</td>
<td>0.025</td>
<td>0.067</td>
<td>0.064</td>
<td>0.087</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td>UB</td>
<td>0.452</td>
<td>0.445</td>
<td>0.274</td>
<td>0.339</td>
<td>0.134</td>
<td>0.139</td>
<td>0.098</td>
<td>0.100</td>
<td>0.110</td>
<td>0.123</td>
<td>0.049</td>
<td>0.048</td>
<td>0.168</td>
<td>0.179</td>
<td>0.151</td>
<td>0.294</td>
<td></td>
</tr>
<tr>
<td>QI</td>
<td>0.618</td>
<td>0.309</td>
<td>0.061</td>
<td>0.050</td>
<td>0.419</td>
<td>0.041</td>
<td>0.032</td>
<td>0.005</td>
<td>0.044</td>
<td>0.020</td>
<td>0.089</td>
<td>0.075</td>
<td>0.112</td>
<td>0.037</td>
<td>0.079</td>
<td>0.208</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>0.522</td>
<td>0.308</td>
<td>0.094</td>
<td>0.152</td>
<td>0.443</td>
<td>0.108</td>
<td>0.029</td>
<td>0.021</td>
<td>0.045</td>
<td>0.024</td>
<td>0.101</td>
<td>0.064</td>
<td>0.078</td>
<td>0.086</td>
<td>0.133</td>
<td>0.271</td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td>0.455</td>
<td>0.305</td>
<td>0.042</td>
<td>0.084</td>
<td>0.303</td>
<td>0.070</td>
<td>0.077</td>
<td>0.011</td>
<td>0.012</td>
<td>0.047</td>
<td>0.090</td>
<td>0.039</td>
<td>0.069</td>
<td>0.086</td>
<td>0.116</td>
<td>0.251</td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>0.465</td>
<td>0.268</td>
<td>0.032</td>
<td>0.048</td>
<td>0.322</td>
<td>0.031</td>
<td>0.067</td>
<td>0.036</td>
<td>0.018</td>
<td>0.012</td>
<td>0.074</td>
<td>0.040</td>
<td>0.044</td>
<td>0.053</td>
<td>0.071</td>
<td>0.222</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0.511</td>
<td>0.390</td>
<td>0.045</td>
<td>0.094</td>
<td>0.322</td>
<td>0.104</td>
<td>0.100</td>
<td>0.042</td>
<td>0.050</td>
<td>0.060</td>
<td>0.116</td>
<td>0.043</td>
<td>0.080</td>
<td>0.094</td>
<td>0.123</td>
<td>0.251</td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>0.578</td>
<td>0.238</td>
<td>0.120</td>
<td>0.101</td>
<td>0.498</td>
<td>0.060</td>
<td>0.081</td>
<td>0.134</td>
<td>0.069</td>
<td>0.168</td>
<td>0.075</td>
<td>0.061</td>
<td>0.020</td>
<td>0.020</td>
<td>0.061</td>
<td>0.204</td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>0.481</td>
<td>0.210</td>
<td>0.208</td>
<td>0.231</td>
<td>0.163</td>
<td>0.228</td>
<td>0.275</td>
<td>0.242</td>
<td>0.218</td>
<td>0.304</td>
<td>0.242</td>
<td>0.067</td>
<td>0.099</td>
<td>0.120</td>
<td>0.105</td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>0.332</td>
<td>0.267</td>
<td>0.085</td>
<td>0.191</td>
<td>0.213</td>
<td>0.193</td>
<td>0.175</td>
<td>0.110</td>
<td>0.121</td>
<td>0.105</td>
<td>0.236</td>
<td>0.255</td>
<td>0.104</td>
<td>0.110</td>
<td>0.100</td>
<td>0.266</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>0.705</td>
<td>0.269</td>
<td>0.119</td>
<td>0.160</td>
<td>0.353</td>
<td>0.063</td>
<td>0.167</td>
<td>0.156</td>
<td>0.107</td>
<td>0.177</td>
<td>0.059</td>
<td>0.249</td>
<td>0.290</td>
<td>0.206</td>
<td>0.067</td>
<td>0.206</td>
<td></td>
</tr>
<tr>
<td>BC</td>
<td>0.473</td>
<td>0.226</td>
<td>0.198</td>
<td>0.240</td>
<td>0.539</td>
<td>0.183</td>
<td>0.340</td>
<td>0.305</td>
<td>0.194</td>
<td>0.316</td>
<td>0.175</td>
<td>0.305</td>
<td>0.327</td>
<td>0.156</td>
<td>0.067</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>0.656</td>
<td>0.283</td>
<td>0.083</td>
<td>0.145</td>
<td>0.560</td>
<td>0.081</td>
<td>0.178</td>
<td>0.184</td>
<td>0.119</td>
<td>0.199</td>
<td>0.053</td>
<td>0.296</td>
<td>0.293</td>
<td>0.062</td>
<td>0.144</td>
<td>0.212</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>1.118</td>
<td>0.760</td>
<td>0.315</td>
<td>0.436</td>
<td>0.790</td>
<td>0.324</td>
<td>0.514</td>
<td>0.470</td>
<td>0.403</td>
<td>0.457</td>
<td>0.364</td>
<td>0.574</td>
<td>0.666</td>
<td>0.318</td>
<td>0.294</td>
<td>0.361</td>
<td></td>
</tr>
</tbody>
</table>
3.4 DISCUSSION

Despite having an extended pelagic larval stage with high dispersive capacity, *Mytilus* populations in BC are highly localized, likely a consequence of oceanographic features resulting in local larval retention coupled with settlement habitat preference and the potential for strong selection at early life stages. Genotype-specific fitness patterns in the VI *Mytilus* hybrid zone suggest that selection against hybrids is limited, at least during the summer months, and indicates that non-native genotypes (including pure introduced, F₁ and backcross) have the potential to thrive in northern habitats (Chapter 2). However, the VI hybrid zone has not expanded its range, persisting with essentially the same geographic range for over ten years (Heath et al. 1995), despite my and previous data indicating severe non-native genotype abundance declines over short time periods (Yanick 2002). Since hybrid zone stability is dependent upon a balance between selection and dispersal; environmental gradients and spatial heterogeneity that result in varying relative fitness coupled with temporal variation in dispersal across hybrid zone boundaries should lead to changes in genotype frequencies over time (Arnold 1997; Endler 1998). Thus the spatial stability of the VI hybrid zone indicates this hybrid zone is not self-sufficient, nor at equilibrium, but rather is maintained by repeated introductions of non-native genotypes coupled with complex barriers to dispersal or gene flow. Contrary to expectations for many marine species, oceanographic barriers to dispersal may restrict high gene flow.

In marine taxa, life history traits and large-scale oceanographic circulation patterns facilitate the potential for long-distance dispersal (Kinlan et al. 2005). Long-distance
dispersal is often associated with genetic homogeneity of interconnected local populations across vast geographic scales (Ayre et al. 1997), however, increasingly, strong genetic population structure is being documented in taxa expected to fit the panmixia paradigm (e.g. urchins – Palumbi 1994; corals – Ayre & Hughes 2000; herring – Jorgensen et al. 2005; scallops – Kenchington et al. 2006) as physical oceanographic features restrict dispersal promoting local larval retention, resulting in reduced gene flow among populations (Hedgecock 1994; Palumbi 1994; Kinlan et al 2005). Consequently, local hydrodynamics may accurately predict the scale and direction of larval dispersal (McQuaid & Phillips 2000; Gilg & Hilbish 2003; Cowen et al. 2006).

In BC, the Strait of Georgia is characterized by warm, low salinity water with estuarine stratification and circulation (LeBlond 1983; Levings & Thom 1994), and is heavily influenced by outflow from the Fraser River creating a low salinity plume during its maximum outflow in May and June (Thomson 1981; Beamish et al. 1999). As a result of this estuarine circulation, the long-term average surface flow is seaward with an estimated velocity of 6 km/day (Thompson 1981). However, this circulation can be reversed in the winter if strong westerly winds increase sea level at the western extreme of the Juan de Fuca Strait (Beamish et al. 1999), which may be important for local retention of pelagic larvae. In addition to the large-scale circulation reversal, retention of local larvae may also be promoted by tidal currents and eddies, shallow sills, and narrow constrictions that can entrain and redistribute buoyant particles, including larvae present in high densities throughout the spring and summer (LeBlond 1983). Therefore, the
complex hydrodynamic features of the Strait of Georgia may limit the northward dispersal of mussels.

Population genetic patterns reflect spatial variation in allelic frequencies and hence gene flow, and can be used to infer barriers to dispersal. Genetic structure has been shown to be an accurate reflection of persistent marine landscape features, which effectively limit the level of dispersal and thus gene flow among populations (Manel et al. 2003; Jorgensen et al. 2005; Kenchington et al. 2006). Three large-scale geographic regions were identified in BC: the North, Central, and South coasts. The North coast from Sayward to Port Hardy is effectively isolated from the Central and South coast of VI by a series of narrow shallow channels through the Discovery Islands between the Strait of Georgia and Johnstone Strait. The Central coast from Campbell River to French Creek is less isolated from the South coast; however, a pair of gyres at depths to 290m and 15km wide within the central Strait of Georgia (cyclonic in the south and anticyclonic in the north) persists throughout the year with increased strength during the summer (Masson & Cummins 2004), which may restrict gene flow between the central and south coast populations. Such a pattern of genetic structure is consistent with my identified barriers (in 2005 and 2006), and explains the southern spatial limitation of the hybrid zone. Although the abundance of non-native genotypes on southern VI varied over time, the large-scale barrier position did not, thereby suggesting that biologically significant barriers to dispersal persist that are independent of non-native genotype abundance.
Several scenarios may explain the lack of non-native genotypes in the central and north regions of VI. First, physical oceanographic features may limit dispersal along the coast from the south to north, and hence larvae of non-native genotypes may be rare in the water column outside of the south region. Although oceanographic features tend to persist, and have accurately predicted population structure in many marine taxa (e.g., herring – Jorgensen et al. 2005; scallops – Kenchington et al. 2006; reef fish – Cowen et al. 2006), the likelihood that such processes alone have entirely blocked the spread of non-native mussel genotypes to the north seems unlikely. Over the 10+ years of the hybrid zone’s existence, some non-native larvae would be expected to eventually pass through the dispersive barrier, and since relatively few migrants are required to modify genetic realtionships among populations (Slatkin 1985), non-native mussels genotypes should be found in the central and north regions of VI. There is evidence to suggest this has occurred to a limited degree (Heath et al. 1995; Yanick 2002), indicating while some larvae may pass through the dispersal barriers, either settlement success is limited or post-settlement survival is low. Indications of elevated hybrid fitness in the north (Chapter 2), suggest that selection against dispersers is likely to be limited, at least in the summer months. Consequently, marine circulation patterns may have a greater influence on the spatial limitation of the VI hybrid zone than selection, although its maintenance is likely due to a balance between dispersal and selection.

Within the south region, the distribution of non-native genotypes varies remarkably among sites. Such a patchy distribution of the introduced and hybrid genotypes on small spatial scales may be a result of chance settlement of related cohorts (“sweepstakes-
chance matching" hypothesis Hedgecock 1994) as the scale of mating in marine broadcast spawners is small (metres: Yund 1990; Littikhuizen et al 2003). Such a scenario would also explain the small-scale patterns of population differentiation among sites within the south region that were detected by microsatellite marker analyses. Dispersal among sites within the south region may be limited by seaward flow from the Fraser River, strong tidal currents and vertical mixing throughout the constricted narrow and shallow passages (Foreman, personal communication). Similarly, genetic differentiation among sites within all regions was significant, suggesting some degree of local larval retention. However, surface currents are greatly influenced by tidal and wind forces, creating daily variation in mean surface flow, consequently, the genetic structure of *Mytilus*, and thus the VI hybrid zone, may vary considerably over time. Such variation is evidenced by the temporal changes in hybrid abundance and the degree of gene flow among sites. Hybrid genotypes should occur at all south sites if gene flow was high. However, low gene flow was found among most south sites, and the distribution of hybrid mussels was restricted to only a few areas. Consequently, sites with non-significant differentiation (CH – CR – MB) all contain varying proportions of hybrid individuals. Furthermore, interspecific gene flow resulting in varying abundance of hybrid individuals at only a few sites within the south region confirms the low gene flow among sites, further exemplifying the independence of site connectivity and variation in hybrid abundance.

For marine organisms with sessile adult forms, the role of larval dispersal in population genetic structure is critical, and hydrographic processes driving larval dispersal can be
inferred through landscape genetics. Geographic patterns of genetic differentiation in blue mussels on the east coast of Vancouver Island are predominately structured by oceanographic features, including currents facilitating local larval retention via restricted surface flow among the southern Gulf Islands. Such barriers to gene flow provide a plausible explanation for the maintenance of the localized hybrid zone along the southern portion of VI; however, other factors such as variation in larval settlement success and survival must also be acting. Furthermore, the potential for temporal variation in oceanographic features on a small geographic scale suggests that the genetic structure of the VI Mytilus, and thus the hybrid zone, may vary considerably over time. The combination of population genetics and hybrid zone analyses in relation to the marine landscape provides a novel approach to uncovering the mechanisms driving hybrid zone dynamics.

3.5 References


Bayne BL. (1965) Growth and delay of metamorphosis of the larvae of Mytilus edulis (L.). Ophelia 2:1-47

Beamish RJ, McFarlane GA, Thomson RE. (1999) Recent declines in the recreational catch of coho salmon (Oncorhynchus kisutch) in the Strait of Georgia are related to climate. Canadian Journal of Fisheries and Aquatic Sciences, 56:506-515


Foreman M. (2007) Institute of Ocean Sciences, Fisheries and Oceans Canada, Sydney, BC – personal communication


72


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Schneider S, Roessli D, Excoffier L. (2000) Arlequin ver 2.000: A software for population genetics data analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva


74

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
4.0 DISTRIBUTION AND GENETIC DIFFERENTIATION OF HYBRIDIZING BLUE MUSSELS (\textit{Mytilus} spp.) IN THE CANADIAN ATLANTIC PROVINCES

4.1 INTRODUCTION

Natural hybridization involves incomplete reproductive isolation and hybrid zones therefore afford opportunities for studying the mechanisms that maintain species boundaries (Arnold 1997; Riginos & Cunningham 2005). Among closely related taxa, reproductive isolation may be eroded as a result of interspecific gene flow and high levels of hybridization (Arnold & Hodges 1995). Alternatively, selection against hybridization may reinforce reproductive barriers leading to the maintenance of genetic integrity in the face of gene flow (Dobzhanski 1937). In the latter case, low levels of hybridization may result in a sympatric distribution of nearly equally abundant parental genotypes.

Speciation among the three closely related \textit{Mytilus} sister species \textit{M. edulis}, \textit{M. trossulus}, and \textit{M. galloprovincialis} was most likely allopatric, and subsequent hybridization is due to secondary contact (Mallet 1995; Riginos & Cunningham 2005). Hybridization among species of the \textit{Mytilus} complex is common wherever they come into contact, creating hybrid zones characterized by varying degrees of gene introgression and patterns of ecological assortment of parental genotypes (Riginos & Cunningham 2005). The degree of hybridization varies geographically, as indicated by differences in the characteristics of two \textit{M. edulis} – \textit{M. trossulus} hybrid zones on opposite sides of the Atlantic Ocean. In the Baltic Sea, \textit{M. edulis} and \textit{M. trossulus} hybridize to such a great extent that they are considered ‘semi-species’ with a frequency of hybridization near 80% (Vainola and Hvilsom 1991). In the Northwest Atlantic, the frequency of \textit{M. edulis} – \textit{M. trossulus}...
hybrid genotypes is much lower, ranging from 12% to 26% (Koehn et al. 1984; Varvio et al. 1988; Bates and Innes 1995; Mallet and Carver 1995; Saavedra et al. 1996; Comesana et al. 1999; Rawson et al. 2001). While variation in species identification technique (morphological analyses, allozyme electrophoresis, mitochondrial DNA, or nuclear DNA) may account for some of the reported variation in the frequency of hybridization, these studies suggest that hybridization is less prevalent among the blue mussels on the Atlantic coast of North America than in the Baltic (Innes and Bates 1999; Malloy et al. 2003).

In the Canadian Atlantic Provinces, Mytilus species have maintained genetic integrity despite sympatry, hybridization and considerable larval dispersal potential. The zone of sympatry is large, extending from Newfoundland, Canada (Bates & Innes 1995) to Maine, USA (Rawson et al. 2001), and is characterized by a bimodal genotype frequency distribution, where parental genotypes vastly outnumber the introgressed genotypes, in Newfoundland (Bates & Innes 1995; Penney et al. 2002; Toro et al. 2004 Nova Scotia (Saavedra et al. 1996, Comesana et al. 1999) and Maine (Rawson et al. 2001). Levels of hybridization are low, consisting mostly of backcross genotypes and F1 genotypes are quite rare (0% - 2.5%: Saavedra et al. 1996; Rawson et al. 2001). While factors responsible for maintaining this bimodal distribution are currently unknown, many studies suggest some assortment of parental genotypes by habitat (reviewed in Riginos & Cunningham 2005).
The abundance and distribution of blue mussels may be largely a reflection of environmental conditions coupled with biotic interactions, as salinity, temperature, and wave exposure appear to be the primary drivers of mussel population dynamics in general (Westerbom et al. 2002). Furthermore, habitat-specific selection creating patchy species distributions can limit the degree of hybridization, as fertilization among close neighbours is more favoured in *Mytilus* (Maloy et al. 2003). While environmental factors have been correlated with distribution patterns of blue mussels in the *M. edulis – M. galloprovincialis* hybrid zone of Western Europe (Hilbish et al. 1994; Gardner 1996; Gilg and Hilbish 2000; Hilbish et al. 2002) the *M. edulis – M. trossulus* hybrid zone of the Baltic Sea (Johannesson et al. 1990) and the *M. trossulus – M. galloprovincialis* hybrid zone of Pacific North America (Sarver and Foltz 1993), there is little evidence to suggest a direct link between salinity and wave exposure with either the distribution or relatively low frequency of hybrids between *M. edulis – M. trossulus* in the northwest Atlantic zone (Maloy et al. 2003). Moreover, throughout the northwest Atlantic mussel zone, synchronous spawning of *M. edulis* and *M. trossulus* has been observed (Freeman et al. 1992, Mallet and Carver 1995; Toro et al. 2002; Maloy et al. 2003), suggesting that the low frequency of hybridization may be a consequence of endogenous hybrid incompatibility between the distantly related *M. trossulus* and *M. edulis* (Bierne et al. 2006).

Past studies have correlated genetic population structure among marine taxa in the northwest Atlantic with physical features of the marine landscape. Larval retention along the Scotian Shelf has resulted in significant genetic population differentiation in several
species, including those with high dispersal potential and adult mobility (Reiss et al 2000; Pogson et al 2001; Kenchington et al 2006). The complex oceanography of the Atlantic Provinces coastline, characterized by deep channels and basins, post-glaciation features such as moraines and ice scour, coupled with very strong tidal forces and bathymetric features, is conducive to persistent retention systems (Townsend et al 2006). These retention systems have contributed to microgeographic structuring for many species (barnacles - Holm and Bourget 1994; larval fish - Reiss et al 2000; Atlantic cod - Pogson et al 2001; scallops - Kenchington et al 2006).

The unusual structure of the Mytilus hybrid zone in the Canadian Atlantic provinces’ likely reflects a balance between dispersal and selection across environmental gradients. Here, I use microsatellite and species identification genetic markers to analyze gene flow and the abundance and distribution of parental and hybrid genotypes of Mytilus in the Canadian Atlantic Provinces. My analyses show restricted gene flow on large and small geographic scales, patterns that are consistent with limited dispersal, and assortative mating between the two species in this hybrid zone.

4.2 MATERIALS AND METHODS

Study sites and sampling

Mussels were collected May – August 2006 from 10 sites in the Atlantic Provinces (Fig. 4.1). Forty-eight mussels from eight sites in Nova Scotia, one site on Prince Edward Island, and one site in New Brunswick were collected from the underside of docks and
pilings for a total of 480 mussels. A small piece of mantle tissue was taken from each mussel and stored in 95% EtOH.

Figure 4.1 Sampling locations of *Mytilus* collected from the Canadian Atlantic Provinces. Names of sampling sites are given in Table 4.1.

**DNA extraction**

Approximately 50mg of mantle edge tissue was removed from the 95% EtOH, blotted dry, and coarsely chopped. DNA was extracted following the plate based DNA extraction protocol for animal tissue developed by Elphinstone et al. (2003) and DNA was resuspended in 50μL of Tris-EDTA buffer (10mM Tris, 1.0mM EDTA, pH 8.0).
Diagnostic species markers

Each mussel was identified to species with two nuclear loci, the polyphenolic adhesive protein Glu-5' developed by Rawson et al. (1996) and the internally transcribed spacer (ITS) developed by Heath et al. (1995). All ITS – PCR amplifications and subsequent RFLP were performed as in Heath et al. (1995). RFLP products were visualized on 2.0% agarose gels stained with ethidium bromide and individual mussels were scored for genotype on the basis of diagnostic RFLP banding patterns. All Glu-5' – PCR amplifications were performed as in Rawson et al. (1996) and PCR fragment length polymorphisms were visualized using an automated DNA analyzer (LiCOR 4300, Lincoln Nebraska, USA).

Microsatellite analyses

Four polymorphic microsatellite loci (Mgu3; Mgu5; Mgu6; Mgu7; Presa et al. 2002) were amplified by polymerase chain reaction (PCR) using a Eppendorf ep gradient S Mastercycler (Brinkmann Instruments, Inc.) to characterize Mytilus populations in the Canadian Atlantic Provinces (see Chapter 2 for detailed microsatellite amplification methods). Forty-eight individuals were scored from each site (Table 4.1).
Table 4.1 Microsatellite summary statistics and sampling locations for the 10 sampled *Mytilus* populations in the Canadian Atlantic Provinces. Sample size (N), number of alleles ($N_A$), observed/expected heterozygosity ($H_O/H_E$) and inbreeding coefficient ($F_{IS}$) are given. Bold italic font for $F_{IS}$ indicate significant departure from Hardy-Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample Size</th>
<th>$N_A$</th>
<th>$N$</th>
<th>$M_{gul}3$</th>
<th>$M_{gul}5$</th>
<th>$M_{gul}6$</th>
<th>$M_{gul}7$</th>
<th>$M_{trol}3$</th>
<th>$M_{trol}5$</th>
<th>$M_{trol}6$</th>
<th>$M_{trol}7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cove Head, PEI</td>
<td>N</td>
<td>6</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46°24'42&quot;N, 63°02'32&quot;W</td>
<td>N</td>
<td>6</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N_A$</td>
<td>6</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$H_O$</td>
<td>0.35</td>
<td>0.60</td>
<td>0.68</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$H_E$</td>
<td>0.57</td>
<td>0.62</td>
<td>0.86</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_{IS}$</td>
<td>0.39</td>
<td>0.04</td>
<td>0.21</td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malagash, NS</td>
<td>N</td>
<td>3</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45°46'15&quot;N, 63°23'17&quot;W</td>
<td>N</td>
<td>6</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N_A$</td>
<td>3</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$H_O$</td>
<td>0.04</td>
<td>0.33</td>
<td>0.62</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$H_E$</td>
<td>0.041</td>
<td>0.59</td>
<td>0.90</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_{IS}$</td>
<td>-0.005</td>
<td>0.47</td>
<td>0.32</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Country Harbour, NS</td>
<td>N</td>
<td>5</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>45°16'25&quot;N, 61°52'59&quot;W</td>
<td>N</td>
<td>8</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$N_A$</td>
<td>5</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$H_O$</td>
<td>0.09</td>
<td>0.15</td>
<td>0.52</td>
<td>0.63</td>
<td>0.00</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>$H_E$</td>
<td>0.45</td>
<td>0.62</td>
<td>0.92</td>
<td>0.89</td>
<td>0.50</td>
<td>0.62</td>
<td>0.62</td>
<td>0.62</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>$F_{IS}$</td>
<td>0.80</td>
<td>0.75</td>
<td>0.44</td>
<td>0.29</td>
<td>1.00</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ship Harbour, NS</td>
<td>N</td>
<td>6</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44°47'01&quot;N, 62°50'00&quot;W</td>
<td>N</td>
<td>6</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N_A$</td>
<td>6</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$H_O$</td>
<td>0.27</td>
<td>0.27</td>
<td>0.79</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$H_E$</td>
<td>0.67</td>
<td>0.59</td>
<td>0.91</td>
<td>0.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_{IS}$</td>
<td>0.60</td>
<td>0.54</td>
<td>0.14</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location, NS</td>
<td>N</td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bedford Basin, NS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44°42'05&quot;N, 63°39'40&quot;W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>3</td>
<td>5</td>
<td>20</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>13</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.09</td>
<td>0.32</td>
<td>0.54</td>
<td>0.32</td>
<td>0.20</td>
<td>0.26</td>
<td>0.53</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.39</td>
<td>0.64</td>
<td>0.92</td>
<td>0.79</td>
<td>0.73</td>
<td>0.65</td>
<td>0.88</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.76</td>
<td>0.51</td>
<td>0.41</td>
<td>0.60</td>
<td>0.74</td>
<td>0.61</td>
<td>0.42</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shad Bay, NS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44°31'08&quot;N, 63°46'43&quot;W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>3</td>
<td>5</td>
<td>21</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.04</td>
<td>0.26</td>
<td>0.77</td>
<td>0.60</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.46</td>
<td>0.57</td>
<td>0.91</td>
<td>0.82</td>
<td>0.00</td>
<td>0.00</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.90</td>
<td>0.54</td>
<td>0.16</td>
<td>0.28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blind Bay, NS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44°29'41&quot;N, 63°49'04&quot;W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>5</td>
<td>5</td>
<td>18</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.30</td>
<td>0.62</td>
<td>0.58</td>
<td>0.72</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.56</td>
<td>0.62</td>
<td>0.89</td>
<td>0.80</td>
<td>0.37</td>
<td>0.62</td>
<td>0.37</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.47</td>
<td>0.01</td>
<td>0.35</td>
<td>0.11</td>
<td>-0.33</td>
<td>0.50</td>
<td>-0.33</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Corkum's Island, NS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45°46'15&quot;N, 63°23'17&quot;W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.45</td>
<td>0.20</td>
<td>0.66</td>
<td>0.50</td>
<td>0.50</td>
<td>0.25</td>
<td>0.75</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.65</td>
<td>0.53</td>
<td>0.81</td>
<td>0.86</td>
<td>0.78</td>
<td>0.71</td>
<td>0.78</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.31</td>
<td>0.62</td>
<td>0.20</td>
<td>0.44</td>
<td>0.47</td>
<td>0.72</td>
<td>0.18</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Digby, NS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44°37'19&quot;N, 63°23'17&quot;W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_A$</td>
<td>5</td>
<td>6</td>
<td>14</td>
<td>16</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_O$</td>
<td>0.41</td>
<td>0.36</td>
<td>0.47</td>
<td>0.50</td>
<td>0.71</td>
<td>0.14</td>
<td>0.28</td>
<td>0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.65</td>
<td>0.61</td>
<td>0.84</td>
<td>0.89</td>
<td>0.62</td>
<td>0.52</td>
<td>0.84</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{IS}$</td>
<td>0.37</td>
<td>0.42</td>
<td>0.45</td>
<td>0.45</td>
<td>-0.07</td>
<td>0.76</td>
<td>0.70</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. Andrew's, NB</td>
<td>( N )</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45°04'45&quot;N, 67°03'30&quot;W</td>
<td>( N_A )</td>
<td>5</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>16</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( H_O )</td>
<td>0.28</td>
<td>0.14</td>
<td>0.85</td>
<td>0.28</td>
<td>0.25</td>
<td>0.22</td>
<td>0.71</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( H_E )</td>
<td>0.73</td>
<td>0.35</td>
<td>0.85</td>
<td>0.68</td>
<td>0.72</td>
<td>0.58</td>
<td>0.88</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( F_{IS} )</td>
<td>0.65</td>
<td>0.64</td>
<td>0.07</td>
<td>0.63</td>
<td>0.65</td>
<td>0.61</td>
<td>0.21</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Statistical Analyses**

*Species identification markers*

Tests for departure from Hardy Weinberg equilibrium at each species identification locus was performed by Exact-U tests in *GENEPOP 3.4* (Raymond & Rousset 1995).

*Species classification*

*NEWHYBRIDS 1.1* (Anderson and Thompson 2002) was used to determine the posterior probability that individuals in a sample fall into different hybrid categories (pure type 1, pure type 2, F₁, F₂, BC₁, BC₂) using Markov chain Monte Carlo (MCMC) simulation with a combination of six loci (two diagnostic species markers: ITS, Glu-5'; four polymorphic microsatellite loci: *Mgu3, Mgu5, Mgu6, Mgu7*). The likelihood of misclassifying backcross and higher order hybrid genotypes with less than 10 loci is high (Vaha & Primmer 2006). Consequently, F₂ and BC individuals were combined into a single ‘introgressed’ group for further analyses.

To determine the level of reproductive isolation between parental genotypes, genetic differentiation between parental, hybrid and backcross genotypes was evaluated by pairwise comparisons of Nei’s genetic distance (*Dₛ*) and Weir & Cockerham’s *Fₛₜ* with *GENEPOP 3.4* and tested by Fisher’s Exact tests in *ARLEQUIN 3.11*.

*Microsatellite analyses*

Departure from Hardy-Weinberg equilibrium (HWE) within each population for each locus and overall as well as evidence for genotypic linkage disequilibrium between pairs
of loci were tested overall and within populations in GENEPOP 3.1 (Raymond & Rousset 1995). Evidence of null alleles, private alleles, and error due to scoring, stuttering, and large allele drop out were examined using MICRO-CHECKER 2.2.3 (Shipley 2003) software.

Non-equilibrium parameters, such as Nei’s standard genetic distance ($D_s$) were used to quantify differentiation among hybrid populations, since such values are insensitive to departure from HWE due to varying degrees of assortative mating, interspecific gene flow and differential selection in hybrid populations. Differentiation among species, among regions and among populations was also tested using Weir & Cockerham’s (1984) $F_{ST}$ with GENEPOP 3.1 (Raymond & Rousset 1995) software. Pairwise Fisher’s Exact tests were also used to test for significant population differentiation in TFPGA 1.3 (Miller 1997). Spatial patterns of genetic divergence were determined using isolation by distance. Isolation by distance (IBD) was tested among all populations using geographic distance (km) estimated by shoreline distance and Nei’s standard genetic distance ($D_s$). The significance of the correlation was tested using a Mantel test with 1000 iterations in GENEPOP 3.4 (Raymond & Rousset 1995).
4.3 Results

Species classification

While most locations were primarily comprised of *M. edulis*, genotype frequencies varied among sites ranging from pure *M. edulis* populations in the north (Cove Head, PEI, and Malagash, NS) to high frequency *M. trossulus* populations in the south (Digby NS, and St. Andrew’s, NB) and mixed populations in between (Shad Bay, Blind Bay, Corkum’s Island NS). Despite the coexistence of both parental genotypes at several sites, the degree of hybridization was low with relatively few $F_1$ individuals identified (Fig. 4.2, Table 4.2). Furthermore, most mixed genotypes were classified as backcrosses. Significant genetic differentiation was found between parental genotypes *M. edulis* – *M. trossulus*, and between backcross and both parental genotypes (Table 4.3). However, differentiation between $F_1$ and both parental genotypes and between $F_1$ – backcross genotypes was not significant (Table 4.3).
Figure 4.2 Genotype frequencies of *Mytilus* spp. at ten sites in the Canadian Atlantic Provinces. Frequencies determined by a combination of two nuclear diagnostic species markers and four polymorphic microsatellite loci. ME = *Mytilus edulis*, MT = *Mytilus trossulus*, F₁ = hybrid, BC = backcross.
Table 4.2 Single locus Exact-U tests of heterozygote deficiency/excess and Weir & Cockerham's $F_{IS}$ estimates in *GENEPOP* 3.4 (Raymond & Rousset 1995). Bold italics indicate statistically significant departures from Hardy-Weinberg equilibrium. E/E = *M. edulis* at both loci, T/T = *M. trossulus* at both loci, E/T = *M. edulis* at one loci and *M. trossulus* at the other (hybrid).

<table>
<thead>
<tr>
<th>Site</th>
<th>Locus</th>
<th>N</th>
<th>E/E</th>
<th>E/T</th>
<th>T/T</th>
<th>$F_{IS}$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>GLU</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>MA</td>
<td>GLU</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>CH</td>
<td>GLU</td>
<td>48</td>
<td>45</td>
<td>2</td>
<td>1</td>
<td>0.38</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>45</td>
<td>2</td>
<td>1</td>
<td>0.38</td>
<td>0.10</td>
</tr>
<tr>
<td>SH</td>
<td>GLU</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>BB</td>
<td>GLU</td>
<td>48</td>
<td>31</td>
<td>2</td>
<td>15</td>
<td>0.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>31</td>
<td>2</td>
<td>15</td>
<td>0.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SB</td>
<td>GLU</td>
<td>48</td>
<td>46</td>
<td>1</td>
<td>2</td>
<td>0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>45</td>
<td>1</td>
<td>1</td>
<td>0.62</td>
<td>0.03</td>
</tr>
<tr>
<td>BL</td>
<td>GLU</td>
<td>48</td>
<td>45</td>
<td>3</td>
<td>2</td>
<td>0.55</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>44</td>
<td>2</td>
<td>2</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CI</td>
<td>GLU</td>
<td>48</td>
<td>31</td>
<td>1</td>
<td>16</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>32</td>
<td>1</td>
<td>15</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DI</td>
<td>GLU</td>
<td>48</td>
<td>37</td>
<td>4</td>
<td>7</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>37</td>
<td>2</td>
<td>9</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SA</td>
<td>GLU</td>
<td>48</td>
<td>7</td>
<td>4</td>
<td>37</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ITS</td>
<td>48</td>
<td>7</td>
<td>6</td>
<td>35</td>
<td>0.63</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Table 4.3 Genetic differentiation among *Mytilus* genotypes based on variation at four microsatellite loci. Pairwise Weir & Cockerham’s $F_{ST}$ below the diagonal, Nei’s genetic distance $D$ above the diagonal. Bold italics indicate significant Exact test P-values. ME = *Mytilus edulis*, MT = *Mytilus trossulus*, F1 = hybrid, BC = backcross.

<table>
<thead>
<tr>
<th></th>
<th>ME</th>
<th>MT</th>
<th>F1</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>0.142</td>
<td>0.108</td>
<td>0.154</td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td>0.032</td>
<td>0.081</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>0.003</td>
<td>-0.010</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>BC</td>
<td>0.027</td>
<td>0.008</td>
<td>-0.02</td>
<td></td>
</tr>
</tbody>
</table>

Microsatellite analyses

All microsatellite loci deviated significantly from Hardy-Weinberg equilibrium (HWE) in at least one population after Bonferroni correction for multiple simultaneous tests (Table 4.1). Linkage disequilibrium was found between $Mgu5 - Mgu6$ in all populations, as is expected of physically linked loci (Presa et al. 2002). No other loci showed significant linkage disequilibrium. Most pairwise Fisher’s Exact tests were significant among pure *M. edulis* individuals (Table 4.4). However, among pure *M. trossulus*, most pairwise Fisher’s Exact tests were generally low and not significant, likely due to small sample sizes (Table 4.4). Pairwise Nei’s genetic distance among pure *M. edulis* ranged from $D_s = 0.027$ between CO – BL to $D_s = 0.170$ between SA – MA; and from $D_s = 0.348$ between SA – CI to $D_s = 1.579$ between BL – CH among pure *M. trossulus* (Table 4.5).
Table 4.4 Genetic differentiation among *Mytilus edulis* populations in the Canadian Atlantic Provinces. Pairwise Weir & Cockerham’s $F_{ST}$ below the diagonal. Pairwise Nei’s genetic distance ($D_S$) above the diagonal. Bold italics indicate significant Exact test P-values.

<table>
<thead>
<tr>
<th></th>
<th>CO</th>
<th>MA</th>
<th>CH</th>
<th>SH</th>
<th>BB</th>
<th>SB</th>
<th>BL</th>
<th>CI</th>
<th>DI</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>0.141</td>
<td>0.128</td>
<td>0.130</td>
<td>0.116</td>
<td>0.029</td>
<td>0.027</td>
<td>0.057</td>
<td>0.064</td>
<td>0.127</td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>0.141</td>
<td>0.051</td>
<td>0.102</td>
<td>0.037</td>
<td>0.164</td>
<td>0.124</td>
<td>0.084</td>
<td>0.143</td>
<td>0.170</td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>0.104</td>
<td>0.055</td>
<td>0.067</td>
<td>0.052</td>
<td>0.148</td>
<td>0.127</td>
<td>0.093</td>
<td>0.113</td>
<td>0.115</td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td>0.106</td>
<td>0.106</td>
<td>0.053</td>
<td>0.079</td>
<td>0.149</td>
<td>0.112</td>
<td>0.083</td>
<td>0.111</td>
<td>0.037</td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>0.097</td>
<td>0.038</td>
<td>0.040</td>
<td>0.065</td>
<td>0.127</td>
<td>0.095</td>
<td>0.068</td>
<td>0.124</td>
<td>0.127</td>
<td></td>
</tr>
<tr>
<td>SB</td>
<td>0.020</td>
<td>0.165</td>
<td>0.123</td>
<td>0.122</td>
<td>0.110</td>
<td>0.017</td>
<td>0.063</td>
<td>0.067</td>
<td>0.113</td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>0.017</td>
<td>0.127</td>
<td>0.103</td>
<td>0.090</td>
<td>0.080</td>
<td>0.007</td>
<td>0.052</td>
<td>0.066</td>
<td>0.110</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>0.041</td>
<td>0.089</td>
<td>0.073</td>
<td>0.063</td>
<td>0.052</td>
<td>0.050</td>
<td>0.037</td>
<td>0.056</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>0.049</td>
<td>0.141</td>
<td>0.088</td>
<td>0.086</td>
<td>0.099</td>
<td>0.054</td>
<td>0.051</td>
<td>0.037</td>
<td>0.116</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>0.084</td>
<td>0.161</td>
<td>0.074</td>
<td>0.000</td>
<td>0.088</td>
<td>0.077</td>
<td>0.070</td>
<td>0.046</td>
<td>0.070</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.5 Genetic differentiation among *Mytilus trossulus* populations in the Canadian Atlantic Provinces. Pairwise Weir & Cockerham’s $F_{ST}$ below the diagonal. Pairwise Nei’s genetic distance ($D_S$) above the diagonal. Bold italics indicate significant Exact test P-values.

<table>
<thead>
<tr>
<th></th>
<th>CO</th>
<th>MA</th>
<th>CH</th>
<th>SH</th>
<th>BB</th>
<th>SB</th>
<th>BL</th>
<th>CI</th>
<th>DI</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.868</td>
<td>1.088</td>
<td>1.578</td>
<td>0.942</td>
<td>0.794</td>
<td>0.879</td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.155</td>
<td>0.629</td>
<td>0.836</td>
<td>0.645</td>
<td>0.589</td>
<td>0.217</td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>-</td>
<td>0.287</td>
<td>-</td>
<td>0.076</td>
<td>0.067</td>
<td>0.091</td>
<td>0.059</td>
<td>0.630</td>
<td>0.347</td>
<td></td>
</tr>
<tr>
<td>SB</td>
<td>-</td>
<td>0.298</td>
<td>-</td>
<td>0.127</td>
<td>0.245</td>
<td>0.692</td>
<td>1.105</td>
<td>0.685</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>-</td>
<td>0.132</td>
<td>-</td>
<td>0.067</td>
<td>0.091</td>
<td>0.059</td>
<td>0.630</td>
<td>0.347</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>-</td>
<td>0.155</td>
<td>-</td>
<td>0.098</td>
<td>-0.015</td>
<td>0.175</td>
<td>0.066</td>
<td>0.389</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>-</td>
<td>0.185</td>
<td>-</td>
<td>0.045</td>
<td>0.082</td>
<td>0.130</td>
<td>0.038</td>
<td>0.081</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.082</td>
<td>0.130</td>
<td>0.038</td>
<td>0.081</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
No significant isolation by distance pattern was detected along shoreline distances for either pure *M. trossulus* populations ($r = 0.09; P = 1.00$), or pure *M. edulis* populations ($r = 0.11, P = 1.00$). Among pure *M. edulis*, pairwise comparisons revealed significant genetic differentiation between most distinct bays, with the exception of SA – SH and CI – SA, likely due to small sample sizes. Small-scale population connectivity was found within a single semi-enclosed coastal area (SB – BL). Among pure *M. trossulus*, pairwise comparisons revealed significant genetic differentiation between SA and most other bays (CH, BB, BL, DI) as well as between SA – DI (Table 4.4). Overall, genetic differentiation among *M. edulis* individuals appears to be greater than among *M. trossulus* within the same populations.

4.4 DISCUSSION

Despite considerable range overlap of *M. edulis* and *M. trossulus*, these species maintain genetic integrity in the Canadian Atlantic Provinces, with little evidence of extensive hybridization and introgression, which is often attributed to environmental factors and incompatibility between their nuclear genomes (Comesana et al. 1999). Consequently, levels of hybridization in Atlantic Canada are much lower than observed in European and Pacific North American *Mytilus* hybrid zones (Comesana et al. 1999). My use of two species-specific marker loci combined with four microsatellite marker loci that all show high levels of differentiation between species, ensured that my assignment of hybrid status was robust, relative to most previous studies. However, I found low levels of hybridization (<10% at most sites), with an often higher frequency of backcross
genotypes than F\textsubscript{1} genotypes, in accordance with previous results reported for Atlantic Canada (Toro et al. 2004).

Many sites were characterized by a bimodal species distribution, indicative of significant reproductive isolation (Jiggins & Mallet 2000). Although spawning periods of \textit{M. edulis} and \textit{M. trossulus} overlap (Toro et al. 2002; Maloy et al. 2003), interspecific fertilization between sympatric groups is highly inefficient, as evidenced by the rarity of F\textsubscript{1} hybrids, implicating effective reproductive isolating barriers (Rawson et al. 2003) acting to maintain cohesive parental genomes. However, the presence, albeit low, of backcross hybrids suggests interspecific matings must be successful at least some of the time. Slight spatial or temporal isolation of spawning may contribute to such a genotype frequency distribution, as the subsequent spawning of a few F\textsubscript{1} hybrids will result in relatively high frequencies of backcross genotypes as the likelihood of gamete encounter with one or the other parental species is high.

\textit{Mytilus} populations are significantly differentiated along the tidally energetic Scotian Shelf, where glacial scouring has created a complex incised coastline and rocky headlands separate frequent small bays (Roman et al. 2000; Townsend et al. 2006).

Within these bays, local estuarine circulation patterns effectively entrain the majority of suspended particles (Townsend et al. 2006), which can potentially result in local retention of pelagic larvae, while those larvae that do escape from coastal areas are likely transported away from the shoreline along the continental shelf in the slow moving Nova Scotia current (Pogson et al 2001), resulting in each bay constituting genetically distinct
populations. While dispersal among discrete bays may be limited, there is significant gene flow within bays (e.g. BL – SB). Interestingly, this gene flow between sites within a bay does not appear to facilitate elevated levels of hybridization, suggesting that gene flow among populations is assortative between species. Additionally, characteristically warmer northern sites were comprised primarily of pure *M. edulis* populations, while the conspicuously cooler south (Townsend et al. 2006) contained high frequencies of pure *M. trossulus*. High frequencies of *M. trossulus* have been associated with the cooler waters in studies of mussel distributions (Moreau 2005), however the distribution of hybrid genotypes is less predictable. The observed genotype distribution bimodality was largely confined to sites south of Bedford Basin, suggesting that the environmental preferences of the parental species contribute to the genotype frequency distribution.

Geographic patterns of genetic differentiation in blue mussels on the East coast of Canada indicate restricted gene flow among the many discrete bays of this highly convoluted coastline. Such genetic structuring has important implications for population studies of many broadcast spawners, as each bay consists largely of genetically isolated self-sustaining populations, which may exhibit a high degree of local adaptation. Without obvious physical barriers to dispersal within bays, reproductive isolation between sympatric populations of *M. edulis* and *M. trossulus* has resulted in a bimodal distribution of genetically distinct parental groups and low levels of hybridization throughout the species range overlap in the Canadian Atlantic Provinces. Habitat preferences combined with inherent genetic incompatibilities and dispersal limitations likely contribute to the observed distribution and abundance of *Mytilus* genotypes in the
northwest Atlantic hybrid zone. This hybrid zone provides a marked contrast to the VI hybrid zone, and thus provides an important opportunity to explore the relative roles of physical, behavioural, and genetic reproductive barriers in the closely related *Mytilus* sibling species.

### 4.6 REFERENCES


94

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Holm ER, Bourget E. (1994) Selection and population genetic structure of the barnacle Semibalanus balanoides in the northwest Atlantic and Gulf of St. Lawrence. Marine Ecology Progress Series, 113:247-256


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


97


5.0 General Discussion

The data presented in this thesis characterizes the dynamics of *Mytilus* hybrid zones in Canada. Genetic and ecological data were used to demonstrate the factors contributing to the various characteristics of these two different hybrid zones. Reproductive isolation among *Mytilus* species is only partially complete, as interspecific gene flow regularly produces viable, fertile hybrid offspring, which often leads to the formation of hybrid zones (Rawson et al. 2003). Several *Mytilus* hybrid zones occur in the temperate northern hemisphere, each of which is characterized by various degrees of stability. Relative fitness of hybrids among *Mytilus* species varies not only across environments, but also differs depending which two parental species are involved, as hybridization between *M. edulis* – *M. galloprovincialis* is much more common and successful than hybridization with the more distantly related *M. trossulus*, as a result of their evolutionary histories (Rawson and Hilbish 1995; Bierne et al. 2006).

On the west coast of Canada, hybrid genotypes have been identified for over ten years (Heath et al. 1995; Yanick 2002) in varying abundances. The factors driving the variability in hybrid abundance are not fully understood, nor are the mechanisms responsible for the spatial limitation of this hybrid zone. Chapters 2 and 3 of this thesis use a suite of ecological and genetic approaches to directly address those questions. Additionally, in Chapter 4 the characterization of a limited hybrid zone in the Canadian Atlantic Provinces lends support to the findings of Chapters 2 and 3, and further explains the mechanisms that drive hybrid zone dynamics, specifically highlighting the role of reproductive isolation in maintaining species boundaries.
Generally, the results of this thesis indicated that hybrid zone maintenance relies on a balance between selection and dispersal, with that balance differing among the various *Mytilus* hybrid zones. This chapter briefly summarizes the major findings of this thesis and discusses general implications for the Canadian hybrid zones. Finally, the results of this thesis are used to identify future research avenues and suggestions for management.

### 5.1 Hybrid Fitness

Although the *Mytilus* hybrid zone off southern Vancouver Island had been previously identified, patterns of gene flow throughout had not been characterized. To uncover the mechanisms that create and maintain hybrid zones, genotype-specific fitness was assessed in two different environments in the Strait of Georgia. The results of Chapter 2 indicate that hybrid fitness varies in an environmentally-dependent fashion, where at some times (summer months) in some surprising places (North Vancouver Island), hybrid and introduced genotypes exhibit elevated relative fitness (measured as an increase in volumetric growth and survival). Elevated fitness may have contributed to the establishment of the hybrid and introduced mussel populations on Vancouver Island; however, the variability of genotype-specific fitness has resulted in an unstable hybrid population that experiences drastic declines in abundance over the winter months. Such dynamics suggest some degree of selection against non-native genotypes. Despite this, the hybrid population continues to recover, with a stable southern distribution, implying that this hybrid zone is maintained by repeated introductions of non-native genotypes.
Genotype-specific fitness was estimated only on the west coast of Canada; however, reduced hybrid fitness can be inferred from spatial distribution and abundance data on the east coast of Canada, where the occurrence of hybrid genotypes is rare relative to expected equilibrium levels. The rarity of F₁ hybrids indicates that interspecific fertilization between the more distantly related *M. edulis* and *M. trossulus* sister species is highly inefficient, implicating strong reproductive isolating barriers (Rawson et al. 2003), despite considerable overlap in the spawning periods of sympatric *M. edulis* and *M. trossulus* populations (Toro et al. 2002; Maloy et al. 2003). Consequently, many sites were characterized by bimodal genotype frequency distributions, indicative of nearly complete reproductive isolation (Jiggins & Mallet 2000).

This contrasting view of two Canadian *Mytilus* hybrid zones further exemplifies the environmentally dependent context of relative fitness among these species (Springer & Heath 2007), and highlights differences in the relative importance of dispersal and selection in maintaining each hybrid zone. Further investigation of environmental factors as well as the stage and degree of reproductive isolation is required to reveal the relative contribution of selection against hybridization to the persistence yet dynamic character of the Canadian *Mytilus* hybrid zones.

### 5.2 Dispersal

Although the results of Chapter 2 indicated that introduced and hybrid genotypes should thrive in the northern section of the Strait of Georgia, there is no evidence to suggest such a range expansion has occurred. Based on that apparent anomaly, Chapter 3 examines
the mechanisms maintaining the stable southern distribution of hybrid and introduced genotypes on Vancouver Island.

While the varying abundances of introduced and hybrid mussels in the Strait of Georgia may well be explained by genotype-specific environmentally-dependent fitness, the spatial limitation of the hybrid zone cannot. If restricted dispersal is a factor contributing to the spatially limited distribution of hybrid genotypes on Vancouver Island, major regions of reduced gene flow should be observed among the native *M. trossulus* populations, which have similar dispersal characteristics to those of non-native species but are less likely to encounter selection against dispersing within their native environment. Interestingly, those sites experiencing significant gene flow from nearby locations also regularly contained relatively high frequencies of hybrid genotypes. Evidently, barriers were identified among the native species, suggesting dispersal limitation does in fact contribute to the spatial confinement of the Vancouver Island hybrid zone.

Although dispersal was also limited among populations on the east coast of Canada, the patterns of gene flow were fundamentally different. Among populations of pure *M. edulis*, nearly all bays were genetically distinct, while within pure *M. trossulus* populations, a greater degree of gene flow was observed among each bay. Additionally, two sites within a single bay were genetically similar, indicating that dispersal was not limited on small spatial scales. Without obvious barriers to dispersal within bays
containing high proportions of both parental genotypes, reproductive barriers must be a major factor in maintaining the genetic integrity of each parental species.

Such marked differences in the Canadian hybrid zones among *Mytilus* species may be a result of the relative age of each zone. The Vancouver Island hybrid zone is relatively young, having been first identified in the mid-1990's (Heath et al. 1995), and thus may still be in a dynamic state where reproductive barriers and habitat specialization have not had sufficient time to develop.

5.3 MANAGEMENT

Results from this thesis have direct implications for management of the economically important *Mytilus* on both the west and east coast of Canada. As the world-wide demand for shellfish increases, so does the expansion of the aquaculture industry. On the West coast of Canada, mussel aquaculture relies on hatchery production of non-native *M. galloprovincialis* and *M. edulis*. Within the Strait of Georgia, introduced *Mytilus* (*M. edulis* and *M. galloprovincialis*) species are being hatchery-reared for local grow-out operations, to circumvent the undesirable characteristics of the native *M. trossulus*. As such, whether intentional or not, non-native and hybrid mussels are likely being introduced to the southern region of Vancouver Island, potentially contributing to the maintenance of this localized hybrid zone. It has been shown that under some environmental conditions, the introduced and hybrid genotypes perform better than the native mussels, indicating potential for a successful invasion, although non-native genotypes appear to be susceptible to high seasonal mortality, which hinders the
establishment of a self-sustaining population. It is unlikely that the distribution of non-native and hybrid mussels will expand its range in the absence of human-mediated dispersal. Therefore, further development of mussel aquaculture in the Strait of Georgia should proceed with caution. Specifically, expansion of non-native mussel aquaculture should not proceed north of Nanaimo, as data suggest that non-native genotypes should do well in the natural environment.

Alternatively, on the east coast of Canada, mussel aquaculture is a lucrative business, which relies on natural seeding from wild *Mytilus* populations. In contrast to the west coast of Canada, the threat of introgressive hybridization is low, as a high degree of reproductive isolation has allowed these parental species to maintain genetic integrity even in sympatry, with no obvious barriers to gene flow. Because many areas have a high frequency of mixed *M. edulis* and *M. trossulus*, identifying areas of pure *M. edulis* populations has attracted increased attention for the purpose of collecting pure stocks for grow-out. However, low gene flow among all sites suggests each bay may constitute an isolated self-sustaining population, with potentially high degrees of local adaptation. Therefore, the translocation of mussels for grow-out in other bays may have unforeseen negative implications on growth and survival of cultured *Mytilus*. Under such circumstances, a shift toward hatchery-reared pure *M. edulis* spat may prove beneficial to the east coast aquaculture industry.
5.4 Final Note

Previous research has done little to characterize the extent of gene flow among *Mytilus* species, and thus not much was known in terms of their population genetic structure and the factors contributing to the spatial distribution of their hybrid zones. The abundance and distribution of *Mytilus* species is primarily a reflection of environmental conditions and biotic interactions (Westerbom et al. 2002). Genotype-specific fitness is evidently conditionally expressed in an environmentally dependent manner (Springer & Heath 2007; Chapter 2), resulting in variation in abundances of *Mytilus* species and genotypes (Chapter 2). Traditionally, it has often been assumed that recruits are in plentiful supply and that population size and distribution is determined largely by post-settlement interactions (Underwood & Keough 2001). However, this thesis provides evidence to suggest that pre-settlement factors such as barriers to dispersal and strong reproductive isolation are also significant contributors to the abundance and distribution of *Mytilus* species on the west and east coasts of Canada.

5.5 References


Holm ER, Bourget E. (1994) Selection and population genetic structure of the barnacle Semibalanus balanoides in the northwest Atlantic and Gulf of St. Lawrence. Marine Ecology Progress Series, 113:247-256


Pogson GH, Taggart CT, Mesa KA, Boutilier RG. (2001) Isolation by distance in the Atlantic Cod, Gadus morhua, at large and small geographic scales. Evolution, 55:131-146


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Yanick JF (2002) Survival, growth and the possible environmental impacts of introduced blue mussels (*Mytilus* spp.) in Georgia Strait, British Columbia. Implications for mussel aquaculture. MSc Thesis. Department of Biology, University of Northern British Columbia, Prince George, BC.
Vita Auctoris

NAME: Jody Lorraine Shields

PLACE OF BIRTH: Kamloops, British Columbia, Canada

DATE OF BIRTH: October 2, 1982

EDUCATION:
- Sardis Senior Secondary School, Chilliwack, BC, 2000 – Highschool
- University of Victoria, Victoria, BC, 2000 – 2004, BSc. Biology and Environmental Studies
- Malaspina University – College, Nanaimo, BC, Fisheries & Aquaculture post-degree Diploma
- Great Lakes Institute for Environmental Research: University of Windsor, Windsor, Ontario, 2005 – 2007, MSc. Environmental Science