The distribution and relative abundance of the invasive Northern blue mussel, *M. galloprovincialis,* in New Zealand: management implications

Ian Randall Blixt

A thesis submitted to Victoria University of Wellington in fulfillment of the requirements for the degree of Master of Science in Marine Biology

2020

ABSTRACT

Biological invasions are a growing problem on a global scale, are capable of causing moderate to severe damage, and are most often associated with human activity. This is particularly true in the marine environment, where there are many well documented cases of non-indigenous species (NIS) spreading via maritime traffic or undergoing a range expansion associated with ocean temperatures continuing to increase. The blue mussel Mytilus galloprovincialis, recognized a one of the most successful NIS globally, is one such species that is spreading in such ways. However, in the Southern hemisphere research over the past decade has shown that there are two lineages of *M. galloprovincialis* present, likely different species, one native to the Northern hemisphere and one native to the Southern hemisphere. In New Zealand, the Northern lineage of *M. galloprovincialis* (MgN) has successfully invaded and poses a unique threat to native biodiversity as there are higher rates of introgression with the native Southern lineage of *M. galloprovincialis* (MgS) than elsewhere in the Southern hemisphere. However, whether MgN is causing other ecological damage in New Zealand is still unknown. Therefore, the main objective of Chapter 2 is to provide a preliminary description of the distribution and relative abundance of MgN in New Zealand, and how abiotic factors may be influencing this invasion. Also, it was recently reported that *M. galloprovincialis* causes severe economic losses to the native greenshell mussel Perna canaliculus aquaculture industry. However, these reports did not differentiate between the native and invasive blue mussel lineages. The main objective of Chapter 3 then is to provide a description of the distribution and relative abundance of MgN in the Marlborough Sounds, the primary P. canaliculus aquaculture region in New Zealand. The results of Chapter 2 indicate that several abiotic factors significantly influence the relative abundance of MgN across New Zealand; and that intranational maritime traffic is a more important vector of spread than international maritime traffic. The results of Chapter 3 show that the relative abundance of MgN was approximately equal across aquaculture facility sites and adjacent shore sites, but that increased habitat space on the grow-lines of aquaculture facilities has resulted in significantly increased overall abundances of both MgN and MgS. This means that there is a much larger MgN population in this region than would be otherwise present due to the aquaculture facilities. Overall, these results indicate that human activity in New Zealand is significantly affecting the MgN invasion therein. These findings are important for both management purposes and for guiding future research.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my adviser, Dr. Jonathan Gardner, for the opportunity to pursue this qualification. To say that your guidance and patience over the past two years has been instrumental to my success would be a vast understatement.

Special thanks to the members of the Gardner lab, Lorenz, Sonali, Giulia, Calvin, and Jo. Very special thank you to Sonali for helping me so much in my first few months of study, and for your helpful discussion.

I would also like to thank Dr. Javier Atalah and the Cawthron Institute for their funding of this project. Dr. Atalah was a great help with statistics and coding and provided many fruitful conversations.

I am also extremely grateful to wife Morgan. I know that my career change and decision to go back to school has not been easy for either of us. Your support through these long years means a great deal to me.

Finally, without the support of my mother and late father I would not be here in so many ways. My father, Spencer, lost his battle with cancer last November during my studies here at VUW. My passion for marine biology and reason for pursing this degree are due to the years of my childhood spent fishing with him in the bays and rivers of the island on which we lived. Rest in peace dad, and thank you for everything.

TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF FIGURES	vi
LIST OF TABLES	vi
SUPPLEMENTARY FIGURES AND TABLES	vi
Chapter 1: Review of the Taxonomic Status of the Blue Mussel Mytilus galloprovincialis in New	Zealand 1
1.1 INTRODUCTION	1
1.2 PHENOTYPIC PLASTICITY, HYBRIDIZATION AND INTROGRESSION, INHERITANCE OF mtDN	JA4
1.2.1 PHENOTYPIC PLASTICITY	5
1.2.2 HYBRIDIZATION & INTROGRESSION	6
1.2.3 DOUBLY UNIPARENTAL INHERITANCE OF MTDNA	9
1.3 PHYLOGEOGRAPHY	
1.3.1 TRANS-ARCTIC MIGRATION	
1.3.2 TRANS-EQUITORIAL MIGRATIONS	
1.4 DISCUSSION	13
1.4.1 THESIS OUTLINE	
LITERATURE CITED	
Chapter 2: Distribution of and Invasion Vectors for Northern Mytilus galloprovincialis in New Z	ealand . 23
2.1 INTRODUCTION	23
2.1.1 MARINE BIOLOGICAL INVASIONS	
2.1.2 STUDY ORGANISM, M. GALLOPROVINCIALIS	26
2.1.3 NORTHERN <i>M. GALLOPROVINCIALIS</i> INVASION IN NEW ZEALAND	
2.2 MATERIALS AND METHODS	
2.2.1 ADDITION OF NEW SAMPLES	
2.2.1.1 DNA EXTRACTION AND PURIFICATION	

2.2.1.2 PCR AND RFLP ASSAY	31
2.2.2 ENVIRONMENTAL AND GEOSPATIAL DATA	32
2.2.2.1 ENVIRONMENTAL DATA	32
2.2.2 GEOSPATIAL DATA	34
2.2.3 MODELLING AND STATISTICAL ANALYSES	36
2.3 RESULTS	
2.3.1 PRESENCE – ABSENCE	
2.3.2 RELATIVE ABUNDANCE	40
2.3.3 SUMMARY OF RESULTS	44
2.4 DISCUSSION	44
2.4.1 PRESENCE - ABSENCE	44
2.4.2 ABUNDANCE	46
2.4.3 IMPLICATIONS	48
2.4.4 FUTURE RESEARCH	50
2.5 CONCLUSION	51
LITERATURE CITED	52
Chapter 3: Increased Habitat Availability on Mussel Aquaculture Farms Leads to Increased	Population
Sizes for both Native and Invasive Species	60
3.1 INTRODUCTION	61
3.2 METHODS	65
3.2.1 MgN RELATIVE ABUNDANCE	66
3.2.2 REGIONAL DISTRIBUTION OF <i>M. GALLOPROVINCIALIS</i> IN MARLBOROUGH SOUN	DS68
3.2.3 MODELLING AND STATISTICAL ANALYSES	69
3.3 RESULTS	71
3.3.1 MGN RELATIVE ABUNDANCE	71
3.3.2 DISTRIBUTION OF <i>M. GALLOPROVINCIALIS</i> IN NATURAL HABITATS	72
3.3.3 DISTRIBUTION OF <i>M. GALLOPROVINCIALIS</i> ACROSS NATURAL AND ARTIFICIAL H	ABITATS74

3.4 DISCUSSION
3.4.1 MGN RELATIVE ABUNDANCE77
3.4.2 REGIONAL DISTRIBUTION OF <i>M. GALLOPROVINCIALIS</i> IN THE MARLBOROUGH SOUNDS78
3.5 CONCLUSION
LITERATURE CITED
Chapter 4: Overview: The Invasive Northern Mytilus galloprovincialis in New Zealand
4.1 INTRODUCTION
4.1.1 PRIMARY RESEARCH FOUNDATION
4.2 CHAPTER 290
4.2.1 SUMMARY AND CONTEXT OF RESULTS90
4.2.2 IMPLICATIONS AND APPLICATIONS
4.3 CHAPTER 3
4.3.1 SUMMARY AND CONTEXT OF RESULTS96
4.4 SUMMARY
4.4.1 FUTURE RESEARCH
4.4.2 FUTURE METHODS
4.5 CONCLUSION
LITERATURE CITED
SUPPLEMENTARY MATERIAL

LIST OF FIGURES

- Figure 1.1 Phylogenetic tree for the *M. edulis* species complex
- Figure 1.2 Current global distribution of M. galloprovincialis
- Figure 2.1 Average proportion of Northern M. galloprovincialis by region
- Figure 2.2 PCA of sites with MgN present and absent
- Figure 2.3 Box and whisker plot of the significant variables identified by the PERMANOVA
- Figure 3.1 *M. galloprovincialis* coverage map for all sample sites, by site type
- Figure 3.2 Scatter plot of *M. galloprovincialis* coverage as a function of latitude
- Figure 3.3 *M. galloprovincialis* coverage as a function of the categorical variables

LIST OF TABLES

- Table 2.1 Existing data set from Westfall et al. (2010)
- Table 2.2 16s RFLP assay results
- Table 2.3 PERMANOVA results
- Table 2.4 Results of the Generalized Linear Model
- Table 3.1 16s RFLP assay results
- Table 3.2 PERMANOVA results
- Table 3.3 GLM numeric results

SUPPLEMENTARY FIGURES AND TABLES

Figure 1 Workflow diagram for the processing of satellite derived environmental data Figure 2 Volume of international shipping traffic for all primary and secondary ports Table 1 List of primary ports Table 2 List of secondary ports Table 3 List of places of first arrival Table 4 List of marinas

Chapter 1: Review of the Taxonomic Status of the Blue Mussel *Mytilus* galloprovincialis in New Zealand

ABSTRACT: The genus *Mytilus*, commonly referred to as blue mussels, is comprised of several species that are of ecological and economic importance. The presence of dense *Mytilus* mats are directly correlated with increased biodiversity and habitat complexity, and *Mytilus* aquaculture was a global \$2.5 billion USD industry in 2016. Surprisingly, given their importance, *Mytilus* spp. lack a clear and agreed upon phylogeny which is repeatedly revised, as recently as early 2018. Specifically, much of the current confusion is primarily around the *M. edulis* species complex, which consists of several closely related taxa. In the Southern hemisphere there is mounting evidence that one member of the *M. edulis* species complex, *M. galloprovincialis*, is in fact a monophyletic group consisting of two to three taxa. In New Zealand, two of these taxa have been confirmed to be present, the invasive Northern *M. galloprovincialis* lineage from the Northern hemisphere, and the native Southern lineage from the Southern hemisphere, which diverged from each other 0.84-1.2 mya. In the review presented here, current molecular and phylogeographic evidence is synthesized to address the taxonomic status of *M. galloprovincialis* in New Zealand. This review will then serve as a knowledge base for future research which will assess the ecology and invasion success of the Northern *M. galloprovincialis* in New Zealand.

1.1 INTRODUCTION

Blue mussels, members of the genus *Mytilus*, are important species both ecologically and economically. They are important members of intertidal and subtidal communities as habitat forming species, with the presence of mussel mats being directly correlated with increased biodiversity and habitat complexity (Lindahl et al. 2001; Norling & Kautsky, 2007; Gestoso et al. 2013). Moreover, they have several biological characteristics that make them useful for ecosystem monitoring of pollution and anthropogenic disturbance, or for bioremediation (Ostroumov, 1998; Peteiro et al. 2006; Beyer et al. 2017). Blue mussels are equally as important economically, with *Mytilus* spp. aquaculture being a global \$2.5 billion USD industry in 2016 (Vaccaro & Waite,

2001; FAO, 2017). However, the same traits that make these mussels ecologically and economically important have also enabled them to be some of the most invasive species in the world (Lowe et al. 2000).

Surprisingly, given their importance, there is still much that is unknown about blue mussels. Specifically, members of the *Mytilus edulis* species complex (Figure 1.1) lack a clear and agreed upon phylogeny and have thus undergone multiple reclassifications (McDonald & Koehn, 1988; McDonald et al; 1991; Gosling, 1992; Larraín et al. 2018). This is largely an artefact of the multitude of methodologies that researchers have used, whether it be morphology, allozyme data, nuclear and/or cytoplasmic sequence DNA data, or other comparable molecular techniques (McDonald & Koehn, 1988; Hilbish et al. 2000; Gardner et al. 2004; Gérard et al. 2008; Westfall & Gardner, 2010; Astorga et al. 2015).

The confusion regarding *Mytilus* phylogeny is due to many of these techniques leading to different phylogenetic topologies and time estimates on divergence and speciation (Hilbish et al. 2000; Gérard et al. 2008, Astorga et al. 2016; Larraín et al. 2018). A full review of the taxonomic changes (1758 to present) within the genus *Mytilus* is beyond the scope of this thesis. Therefore, in order to be in alignment with previous research on *M. galloprovincialis* in Australasia (Westfall et al. 2010; Westfall & Gardner, 2010; Gardner et al. 2016), two lineages are recognized and throughout this thesis, detailed below.

One member of the *M. edulis* species complex, *Mytilus galloprovincialis*, is of specific importance in the Southern hemisphere and warrants further investigation. *M. galloprovincialis* is a cosmopolitan species with a typical antitropical distribution (Figure 1.2) but its classification in the Southern hemisphere is complex as these mussels are considered both native and invasive. The

now native *M. galloprovincialis* (hereafter Southern *M. galloprovincialis*) populations performed a trans-equatorial migration roughly 0.84-1.2 mya, and spread across South America, Australasia, and several off-shore islands (Hilbish et al. 2000; Gardner, 2004; Gérard et al. 2008, Westfall et al. 2010; Westfall & Gardner, 2010, 2013; Astorga et al. 2015).



Figure 1.1 Phylogenetic tree for the *M. edulis* species complex, inferred from 16s rRNA RFLP and COI mtDNA (Hilbish et al. 2000; Gérard et al. 2008; Westfall & Gardner, 2010; Astorga et al. 2015). Note that the Southern *M. galloprovincialis* lineage is presented as a single taxon in this Figure, however there is mounting evidence that it is in fact a monophyletic group consisting of two sister taxa. This is discussed at length in the discussion section.

The invasive *M. galloprovincialis* populations (hereafter Northern *M. galloprovincialis*) are native to the Northern hemisphere and were introduced to the Southern hemisphere within the past several centuries, likely a human mediated process via biofouling of ships (Figure 1.2; Hilbish et al. 2000; Lee & Chown, 2007; Gérard et al. 2008; Murray et al. 2011). The amount of divergence that has occurred between these two lineages during their 0.84-1.2 million years of allopatric isolation is enough to be detected by most molecular techniques, and the two lineages are able to retain their genetic integrity in the presence of each other and other closely related taxa, likely making them identifiably different species (Hilbish et al. 2000; Gérard et al. 2008; Westfall & Gardner, 2010, 2013; Westfall et al. 2010; Larraín et al. 2018; Gardner et al. 2020). This is reported on by Gardner et al. (2020).

The first robust molecular technique for the differentiating between Northern and Southern *M. galloprovincialis* lineages was found by Westfall & Gardner (2010) using nuclear and mitochondrial markers comprised of blue mussel samples from Australia, Chile, and New Zealand. This study and subsequent studies have also confirmed that these two lineages are hybridizing and have relatively high levels of introgression compared to other locations where they occur sympatrically, meaning that the invasive Northern *M. galloprovincialis* is threatening the genetic diversity of the native Southern *M. galloprovincialis* in New Zealand more than elsewhere in the Southern hemisphere (Westfall et al. 2010; Westfall & Gardner, 2010, 2013; Gardner et al. 2016). Furthermore, Forrest & Atalah (2017) also found that *M. galloprovincialis* causes and estimated \$11 million USD of damage to the endemic greenshell mussel *Perna canaliculus* (hereafter *Perna*) aquaculture industry in New Zealand, however the authors did not differentiate between Northern and Southern *M. galloprovincialis*.

Therefore, the focus of the literature review presented here will be to form a knowledge base on the taxonomic status of *M. galloprovincialis* in New Zealand for this thesis. This thesis will then describe the relative abundance of MgN across New Zealand, assess how abiotic factors are affecting their invasion, and determine if there is a bias of Northern or Southern *M. galloprovincialis* causing economic damage to the *Perna* industry.

1.2 PHENOTYPIC PLASTICITY, HYBRIDIZATION AND INTROGRESSION, INHERITANCE OF mtDNA

Before addressing the taxonomic status of *M. galloprovincialis* in New Zealand, or more broadly in the Southern hemisphere, it must first be understood why there is confusion around it, how a nominal single species can be simultaneously native and invasive in an area, and why that warrants further investigation. The phylogeny of the *Mytilus edulis* species complex, which is comprised of the closely related species *M. edulis*, *M. galloprovincialis*, and *M. trossulus* (Figure 1.1), is confounded by certain biological characteristics. The primary characteristics in regard to this subject are phenotypic plasticity, hybridization, and a phenomenon referred to as doubly uniparental inheritance of mitochondrial DNA (DUI). The overall effect of these characteristics is to hinder the ability of researchers to accurately delimit species.

1.2.1 PHENOTYPIC PLASTICITY

Every member of the *M. edulis* species complex has been shown to display a high degree of phenotypic plasticity, often making separate species morphologically indistinguishable or members of the same species appear very different (Stirling & Okumus, 1993; Innes & Bates, 1999; Akester & Martel, 2000; Gardner, 2004; Katolikova et al. 2014; Illesca et al. 2018). Phenotypic plasticity in blue mussels can largely be viewed as a suite of physiological adaptations to biotic and abiotic factors, thus increasing the survivability of each mussel (Akester & Martel, 2000; Kovačić et al. 2017). These physiological adaptations are differentiated from evolutionary adaptations as they can be observed within a single generation (Stirling & Okumus, 1993; Kovačić et al. 2017).

Many factors such as sea surface temperature, salinity, tidal height, tidal velocity, and food availability have been shown to effect growth rate of blue mussels measured in shell length and weight, and ash free dry weight of meat (Stirling & Okumus, 1993; Akester & Martel, 2000; Kovačić et al. 2017; Illesca et al. 2018). One factor however, wave exposure, has been repeatedly shown to alter shell morphology. In sites that are highly exposed to wave action mussels develop shells that are more circular or tube-like in shape, which is also associated with increased shell thickness, hinge thickness, and changes to shell lustre and colour (Stirling & Okumus, 1993; Akester & Martel, 2000; Steffani & Branch, 2003; Kovačić et al. 2017). For example, Stirling & Okumus, 1993, demonstrated this well in their research by showing that *M. edulis* individuals transplanted as juveniles to a more exposed site displayed significantly different shell morphologies than members of the same cohort at the original less exposed site. Akester & Martel, 2000, also found this to be true in *M. trossulus* populations in British Columbia, Canada, and suggest that the adaptive alterations in shell morphology likely make individual mussels more resistant to hydrodynamic stress, thus increasing their survivability.

1.2.2 HYBRIDIZATION & INTROGRESSION

Morphological characteristics and subsequent phylogenetic analyses are further compounded by the ability of *Mytilus* spp. to hybridize with each other, which leads to morphologically and genetically intermediate individuals (McDonald et al. 1991; Daguin et al. 2001; Braby& Somero, 2006; Brannock et al. 2009; Brannock & Hilbish, 2010; Westfall et al. 2010; Katolikova et al. 2014; Saarman & Pogson, 2016; Mathiesen et al. 2017; Illesca et al. 2018). The degree to which they hybridize, along with hybrid fitness, is highly variable and appears to be depend on environmental factors and other selection pressures (Daguin et al. 2001; Braby & Somero, 2006; Springer & Crespi, 2007; Brannock et al. 2009; Brannock & Hilbish, 2010; Mathiesen et al. 2017). The level of hybridization and introgression that occurs is depends upon the species pair in question. For example, *M. edulis* and *M. galloprovincialis* have been shown to hybridize freely and have high relative rates of introgression in some regions, whereas *M. trossulus* and *M. galloprovincialis* hybrid zones can contain F1 hybrids in relatively large proportions, but

little to no introgression occurs due to partial sterility of F1 hybrids (Gardner and Skibinski, 1988; Brannock et al. 2009; Brannock & Hilbish, 2010, Saarman & Pogson, 2015; Oyarzún et al. 2016).

The degree to which hybridization and introgression occurs between Northern and Southern *M. galloprovincialis* is also highly variable, and the observed patterns appear to be unique to each hybrid zone location (Springer & Crespi, 2007; Westfall & Gardner, 2010, 2013; Gardner et al. 2016; Rahim et al. 2016; Larraín et al. 2018). In Chile, where Northern and Southern *M. galloprovincialis* occur sympatrically, barriers to gene flow appear to exist between the two lineages as seen in the occurrence of hybridization but little introgression (Borsa et al. 2012; Westfall & Gardner, 2013; Astorga et al. 2015; Oyarzún et al. 2016).

That being said, the taxonomic status of *Mytilus* in Chile has long been debated, but there is now a growing body of evidence to show that the native Southern *M. galloprovincialis* population in Chile and South America is divergent enough to be considered a separate species, *M. chilensis* (Borsa et al. 2012; Astorga et al, 2015; Oyarzún et al. 2016; Larraín et al. 2018; Zbawicka et al. 2018). There is also recent evidence to suggest that the Australasian populations of Southern *M. galloprovincialis* appear to be separate from *M. chilensis* within the Southern *M. galloprovincialis* lineage, which may help explain the variability in levels of hybridization and introgression (Gérard et al. 2008; Westfall & Gardner 2010, 2013; Astorga et al. 2015; Rahim et al. 2016).

In New Zealand, this is supported by evidence that the Northern and Southern *M.* galloprovincialis populations are also able to hybridize which is associated with introgression (Westfall & Gardner, 2010, 2013; Gardner et al. 2016). It is likely that this is a positive feedback



Figure 1.2 Current distribution of *M. galloprovincialis*. MgN Native is the Northern *M. galloprovincialis* lineage native range, although it should be noted that the arctic distribution could be from anthropogenic sources (Mathiesen et al. 2016). MgN Invasive is the area that the Northern *M. galloprovincialis* lineage invaded range. MgN & MgS is where the Northern and Southern *M. galloprovincialis* lineages occur sympatrically. Please note that in areas that lack adequate sampling density or have little available published research (i.e. China, the Korean Peninsula, and the arctic MgN range), entire geopolitical boundaries are highlighted (Territorial Sea boundaries), and that the actual distribution of *M. galloprovincialis* within those areas may be non-continuous.

loop, with more hybridization and introgression having a homogenizing effect, thus enabling to more hybridization and introgression to occur. This also implies that the Northern *M. galloprovincialis* poses a greater threat to the genetic diversity of the native populations than elsewhere in the Southern hemisphere (Westfall & Gardner, 2010, 2013; Gardner et al. 2016).

1.2.3 DOUBLY UNIPARENTAL INHERITANCE OF MTDNA

The molecular tests to that form the basis of most evidence for the above points typically use cytoplasmic DNA, specifically the 16s rRNA gene of mitochondria (Westfall et al. 2010; Westfall & Gardner, 2010, 2013; Astorga et al. 2015; Oyarzún et al. 2016; Larraín et al. 2018). However, it is important to note that all members of the *M. edulis* species complex have an unusual form of mitochondrial inheritance termed doubly uniparental inheritance of mitochondrial DNA, or DUI (Hoeh et al. 1991; Zouros et al. 1992, 1994; Rawson & Hilbish, 1995a, 1995b; Śmietanka, & Burzyński, 2017). DUI is an uncommon trait among some *Bivalvia* species, where there are distinct female (F) and male (M) mitotypes (Hoeh et al. 1991; Zouros et al. 1992; Rawson & Hilbish, 1995a, 1995b; Hilbish et al. 2000; Mizi et al. 2005; Doucet-Beaupré et al. 2010; Machordom et al. 2015; Gusman et al. 2016; Śmietanka, & Burzyński, 2017).

In taxa which have DUI, females inherit mitochondria from only their mother and are thus typically homoplasmic F type, and males inherit their mitochondria from both their mother and father and are thus typically heteroplasmic M and F types (Rawson & Hilbish, 1995; Brannock et al. 2013; Kyriakou et al. 2016). F and M mitotypes are also highly divergent, with up to 20% divergence in *Mytilus* spp. and up to 38% in other members of *Mytilidae*, and divergence of these mitotypes appears to predate the speciation event(s) that led to the *M. edulis* species complex (Rawson & Hilbish, 1995; Hilbish et al. 2000; Brannock et al. 2013; Kyriakou et al. 2017). This may have the effect of confounding phylogenetic

analyses and hindering species delimiting power, especially when considering the F and M mitotypes of hybrid individuals.

Consequently, caution must be taken when viewing results that use mtDNA, which is very common in taxonomic studies of *Mytilus* spp. In the context of the taxonomic status of *M. galloprovincialis* in New Zealand, the 16s rRNA RFLP assay that is most commonly used yields strong F mitotype bands and weak M mitotype bands, and only the F type bands are considered (Westfall et al. 2010; Westfall & Gardner, 2010). This technique then largely circumvents the complications posed by DUI, and also avoids gender biasing results.

1.3 PHYLOGEOGRAPHY

Even though the taxonomy of the *M. edulis* species complex is complicated, there is substantial evidence for the timing of arrivals in the Southern hemisphere (Hilbish et al. 2000; Gérard et al. 2008; Astorga et al. 2015). Furthermore, the historic and contemporary phylogeography of *M. galloprovincialis* present a clear picture on the divergence between the Northern and Southern lineages, and thus the taxonomic status of *M. galloprovincialis* in New Zealand.

1.3.1 TRANS-ARCTIC MIGRATION

The most recent common ancestor of the *Mytilus* genus originated from the northern Pacific during the middle Paleogene period (Vermiej, 1991, 1992; Hilbish et al. 2000; Liu et al. 2018). This *Mytilus* ancestor performed a trans-Arctic migration from the North Pacific to the North Atlantic after the opening of the Bering Strait in the early Pliocene, roughly 3.5 MYA, as did many other taxa (Vermeij, 1991; Hilbish et al. 2000; Wares & Cunningham, 2001). The timing of this migration is in conjunction with the evolutionary origin of *M. trossulus* and the most recent common ancestor of the *M. edulis* and *M. galloprovincialis* (Figure 1.1), an observation supported by molecular evidence and geographic distribution of each species (Hilbish et al. 2000; Gérard et al. 2008; Astorga et al. 2015). The later isolation of this *M. edulis* and *M. galloprovincialis* common ancestor in the Mediterranean Sea led to the divergence between the two, with *M. galloprovincialis* inhabiting the Mediterranean and *M. edulis* remaining in the North Atlantic (Hilbish et al. 2000; Gérard et al. 2008).

There is also strong evidence that during the last glacial maximum, roughly 18 kya, all Atlantic European *Mytilus* populations were forced into a single glacial refugium in the Bay of Biscay, while *M. galloprovincialis* survived in isolation in the Mediterranean Sea (Clark et al. 2009; Śmietanka et al. 2014). Both of these *M. galloprovincialis* populations, North Atlantic and Mediterranean, are both considered the invasive Northern hemisphere lineage currently present in the Southern hemisphere, including New Zealand.

1.3.2 TRANS-EQUITORIAL MIGRATIONS

After the trans-Arctic migration 3.5 mya, there is strong phylogenetic evidence that *Mytilus* performed two trans-equatorial migrations during the late Pleistocene, roughly 0.84 to 1.2 mya (Hilbish et al. 2000; Gérard et al. 2008; Astorga et al. 2015). However, there is no fossil evidence for how they managed to perform these two migrations. It was previously thought that some marine biota, possibly including *Mytilus* spp., were able to expand their range through the tropical zone of west Africa during the Pliocene, down the west African coast into the Southern hemisphere, then later died back in the tropic zone as it became inhabitable to them. This vicariance model has been largely refuted by Lindberg (1991), and furthermore there is no fossil evidence to suggest that this is true for *Mytilus* (Lindberg, 1991; Vermeij, 1992; Hilbish et al. 2000). What is more likely is that these migrations were rare, episodic dispersal events, as *Mytilus* spp. have been recorded to cross vast distances of ocean attached to flotsam (Miller et al. 2018).

The best current molecular evidence indicates that it was *M. galloprovincialis* from the Northern hemisphere, both Atlantic and Mediterranean forms, that performed these migrations (Hilbish et al. 2000; Gérard et al. 2008; Westfall & Gardner, 2010). However, it must be noted that there is some evidence that suggests that *M. edulis* could have performed one of these migrations as well, as Southern hemisphere mussels have been previously described as *M. edulis* like, subspecies of *M. edulis*, intermediate between *M. edulis* and *M. galloprovincialis*, etc. (Seed, 1990; McDonald et al. 1991; Gardner et al. 2004; Gérard et al. 2008; Rodriguez et al. 2008; Astorga et al. 2015; Gaitán-Espitia et al. 2016; Zbawicka et al. 2018).

After arrival in the Southern hemisphere it is likely that this first migration of blue mussels naturally expanded their range around South America and reached the Kerguelen Islands in the southern Indian Ocean. A second migration then managed to reach to Australasia (Hilbish et al. 2000; Gérard et al. 2008; Westfall & Gardner, 2010, 2013; Gardner et al. 2016), although there is no record of them having reached or at least established populations in southern Africa (Grant & Cherry 1985). Gérard et al. (2008) present strong evidence that these populations form two distinct clades within the Southern hemisphere lineage, i.e. South American / Kerguelen, and Australasian, however if there has been sufficient divergence between these taxa for the classification of separate species or subspecies is still debated, as previously discussed.

The Northern *M. galloprovincialis* lineage has likely performed regular human mediated trans-equatorial migrations, starting on early sailing vessels within the past several centuries (Carlton & Hodder, 1985; Murray et al. 2000; Gardner, 2004; Lee & Chown, 2007; Westfall & Gardner, 2010; Gardner et al. 2016). The regions that this lineage has been introduced to include the United States, Canada, New Zealand, Australia, Japan, China, the Korean Peninsula, Chile, Argentina, and South Africa (Figure 1.2; Lee & Morton, 1985;

McDonald, 1991; Rawson & Hilbish, 1995a; Daguin & Borsa, 2000; Hilbish et al. 2000; Bownes & McQuaid, 2006; Gérard et al. 2008; Seo & Lee, 2009; Colgan & Middlefart, 2011; Crego-Prieto et al. 2015; Gaitán-Espitia et al. 2016; Gardner et al. 2016; Rahim et al. 2016; Larraín et al. 2018). The propensity of *Mytilus* spp. to biofoul ships, both on hull exteriors and inside internal ship compartments and systems (Carlton & Hodder, 1985; Apte et al. 2000; Murray et al. 2000; Lee & Chown, 2007; Forrest & Atalah, 2017). Lourenço et al. (2015) has also reported a reciprocal invasion from the Southern hemisphere into the Northern hemisphere, where the Southern *M. galloprovincialis* may become invasive as well. This extensive invasion success of *M. galloprovincialis* has led to its status as one of the top 100 most invasive species in the world (Lowe, 2000).

1.4 DISCUSSION

Figure 1.1, while relatively limited in scope, neatly summarizes our current best understanding of the phylogeny of the *M. edulis* species complex. The simplicity and lack of resolution of this phylogeny is largely due to the challenges that *Mytilus* cellular and organismal biology present to delimiting closely related species. There are a number of possible approaches to enable researchers to delimit these closely related species, however if the taxonomic history *Mytilus* has taught us anything it is the importance of waiting for sufficient and robust scientific evidence before claiming or denying the existence of a species. This is the primary reason why in this review these mussels are simply referred to as Northern and Southern *M. galloprovincialis* lineages.

That being said, it is apparent that *M. galloprovincialis* in the Southern hemisphere is a monophyletic group that consists of several taxa; the invasive Northern lineage, Atlantic, Pacific, and Australasian (Hilbish et al. 2000; Gérard et al. 2008; Westfall & Gardner, 2010, 2013; Astorga et al. 2015; Gardner et al. 2016; Larraín et al. 2018). These separate taxa are

very likely separate species, a topic which is addressed at length in Gardner et al. (2020). As this Chapter was being written at the same time as Gardner et al. (2020), the terminology utilized throughout this thesis is still the Northern and Southern lineages, to be in alignment with Westfall et al. (2010), Westfall & Gardner (2010), Westfall & Gardner (2013), and Gardner et al. (2016).

With that point in mind, to summarize the taxonomic status of *M. galloprovincialis* in New Zealand, there are clearly two identifiably different lineages present, the native Southern lineage and the invasive Northern lineage. At this point in time there is sufficient evidence to show that these two taxa are distinct, however there is a lack of a sufficient body of evidence to support the claim that Southern *M. galloprovincialis* mussels, including the New Zealand populations, are a separate species (Gardner et al. 2020).

What has yet to be addressed specifically is the ecological niches that the Northern and Southern *M. galloprovincialis* lineages occupy in the Southern hemisphere, specifically in New Zealand. However, any future research that attempts to answer this question must be founded upon clear and robust scientific evidence that the Northern and Southern lineages of *M. galloprovincialis* are identifiably different taxa.

1.4.1 THESIS OUTLINE

Chapter 1, this chapter, is a literature review of the taxonomic status of *M*. *galloprovincialis* in the Southern hemisphere. The purpose of this chapter is to clearly state the taxonomic identities of the native and invasive blue mussels in New Zealand. This distinction, both evolutionary and taxonomic, underpins the need for new knowledge about invasion success, distribution and abundance, and also ecological and economic impact of the invasive MgN in New Zealand.

Chapter 2 is an assessment of the MgN invasion across New Zealand. This was done by assessing presence-absence and relative abundance of MgN as a function of environmental and geospatial data. In this chapter 500 samples were taken from the Marlborough Sounds to have a 16s mitochondrial rDNA RFLP assay performed, to determine the MgN or MgS identity of each sample. These data were then added to an existing data set (Westfall & Gardner, 2010) with sites across New Zealand to assess the MgN invasion on a national scale. These same 500 samples are also the focus of Chapter 3 but are assessed only on a regional scale to address the effect of aquaculture on MgN relative abundance. Therefore, there will necessarily be overlap between these two chapters, specifically in the methods and results sections.

Chapter 3 is an assessment of the MgN invasion in the Marlborough Sounds region. This chapter builds upon Forrest & Atalah (2017), and Atalah et al. (2017). Forrest & Atalah (2017) found that *M. galloprovincialis* causes extensive economic damage to the *Perna* aquaculture industry in this region, while Atalah et al. (2017) describe how environmental factors influence *M. galloprovincialis* abundance for the purposes of enabling aquaculture managers mitigate this economic damage. However, whilst both Forrest & Atalah (2017) and Atalah et al. (2017) acknowledge the existence of two lineages of *M. galloprovincialis* in New Zealand, they do not differentiate between them in their results. Therefore, the main object of this chapter is to describe MgN relative abundance in the Marlborough Sounds region, and in doing so also assess if increased niche space availability at aquaculture facilities is affecting MgN relative abundance. Dr. Atalah also had data from field surveys that needed to be processed that fit nicely with this chapter and is thus included.

Chapter 4 is a general discussion, integration and overview chapter. In this chapter the overall results of the thesis are interpreted in a broader context. To do this, the specific published research that forms the foundation of this thesis are discussed directly. With these

projects assessed, how the results of this thesis expand upon those papers is then discussed.

Finally, how these results can guide future research is discussed, with specific examples.

LITERATURE CITED

- Ab Rahim, E. S., Nguyen, T. T. T., Ingram, B., Riginos, C., Weston, K. J., & Sherman, C. D. H. (2016). Species composition and hybridisation of mussel species (Bivalvia: Mytilidae) in Australia. *Marine and Freshwater Research*, 67(12), 1955–1963. https://doi.org/10.1071/MF15307
- Akester, R. J., & Martel, A. L. (2000). Shell shape, dysodont tooth morphology, and hingeligament thickness in the bay mussel *Mytilus* trossulus correlate with wave exposure. *Canadian Journal of Zoology*, 78(2), 240–253. https://doi.org/10.1139/z99-215
- Apte, S., Holland, B. S., Godwin, L. S., & Gardner, J. P. A. (2000). Jumping ship: A stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. *Biological Invasions*, 2(1), 75–79. https://doi.org/10.1023/A:1010024818644
- Astorga, M. P., Cardenas, L., & Vargas, J. (2015). Phylogenetic Approaches to Delimit Genetic Lineages of the *Mytilus* Complex of South America: How Many Species are there? *Journal of Shellfish Research*, 34(3), 919–930. https://doi.org/10.2983/035.034.0322
- Atalah, J., Rabel, H., & Forrest, B. M. (2017). Modelling long-term recruitment patterns of blue mussels *Mytilus* galloprovincialis: A biofouling pest of green-lipped mussel aquaculture in New Zealand. *Aquaculture Environment Interactions*, 9(1), 103–114. https://doi.org/10.3354/aei00216
- Beyer, J., Green, N. W., Brooks, S., Allan, I. J., Ruus, A., Gomes, T., ... Schøyen, M. (2017). Blue mussels (*Mytilus* edulis spp.) as sentinel organisms in coastal pollution monitoring: A review. *Marine Environmental Research*, 130, 338–365. https://doi.org/10.1016/j.marenvres.2017.07.024
- Borsa, P., Rolland, V., & Daguin-Thiébaut, C. (2012). Genetics and taxonomy of Chilean smooth-shelled mussels, *Mytilus* spp. (Bivalvia: Mytilidae). *Comptes Rendus -Biologies*, 335(1), 51–61. https://doi.org/10.1016/j.crvi.2011.10.002
- Bownes, S. J., & McQuaid, C. D. (2006). Will the invasive mussel *Mytilus* galloprovincialis Lamarck replace the indigenous *Perna perna* L. on the south coast of South Africa? *Journal of Experimental Marine Biology and Ecology*, 338(1), 140–151. https://doi.org/10.1016/j.jembe.2006.07.006
- Braby, C. E., & Somero, G. N. (2006). Ecological gradients and relative abundance of native (*Mytilus* trossulus) and invasive (*Mytilus* galloprovincialis) blue mussels in the

California hybrid zone. *Marine Biology*, *148*(6), 1249–1262. https://doi.org/10.1007/s00227-005-0177-0

- Brannock, P. M., & Hilbish, T. J. (2010). Hybridization results in high levels of sterility and restricted introgression between invasive and endemic marine blue mussels. *Marine Ecology Progress Series*, 406, 161–171. https://doi.org/10.3354/meps08522
- Brannock, P. M., Roberts, M. A., & Hilbish, T. J. (2013). Ubiquitous heteroplasmy in *Mytilus* spp. resulting from disruption in doubly uniparental inheritance regulation. *Marine Ecology Progress Series*, 480, 131–143. https://doi.org/10.3354/meps10228
- Brannock, P. M., Wethey, D. S., & Hilbish, T. J. (2009). Extensive hybridization with minimal introgression in *Mytilus galloprovincialis* and m. trossulus in Hokkaido, Japan. *Marine Ecology Progress Series*, 383, 161–171. https://doi.org/10.3354/meps07995
- Carlton, J. T., & Hodder, J. (1995). Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th-century sailing vessel. *Marine Biology*, *121*(4), 721–730. https://doi.org/10.1007/BF00349308
- Clark, P. U., Dyke, A. S., Shakun, J. D., Carlson, A. E., Clark, J., Wohlfarth, B., ... McCabe, A. M. (2009). The Last Glacial Maximum. *Science*, 325(5941), 710–714. https://doi.org/10.1126/science.1172873
- Clarke Murray, C., Gartner, H., Gregr, E. J., Chan, K., Pakhomov, E., & Therriault, T. W. (2014). Spatial distribution of marine invasive species: Environmental, demographic and vector drivers. *Diversity and Distributions*, 20(7), 824–836. https://doi.org/10.1111/ddi.12215
- Clarke Murray, C., Pakhomov, E. A., & Therriault, T. W. (2011). Recreational boating: A large unregulated vector transporting marine invasive species. *Diversity and Distributions*, 17(6), 1161–1172. https://doi.org/10.1111/j.1472-4642.2011.00798.x
- Colgan, D., & Middelfart, P. (2011). *Mytilus* mitochondrial DNA haplotypes in southeastern Australia. *Aquatic Biology*, 12(1), 47–53. https://doi.org/10.3354/ab00323
- Crego-Prieto, V., Ardura, A., Juanes, F., Roca, A., Taylor, J. S., & Garcia-Vazquez, E. (2015). Aquaculture and the spread of introduced mussel genes in British Columbia. *Biological Invasions*, 17(7), 2011–2026. https://doi.org/10.1007/s10530-015-0853-z
- Daguin, C., Bonhomme, F., & Borsa, P. (2001). The zone of sympatry and hybridization of *Mytilus* edulis and *M. galloprovincialis*, as described by intron length polymorphism at locus mac-1. *Heredity*, 86(3), 342–354. https://doi.org/10.1046/j.1365-2540.2001.00832.x
- Daguin, C., & Borsa, P. (2000). Genetic relationships of *Mytilus* galloprovincialis Lamarck populations worldwide: Evidence from nuclear-DNA markers. *Geological Society Special Publication*, 177, 389–397. https://doi.org/10.1144/GSL.SP.2000.177.01.26
- Doucet-Beaupré, H., Breton, S., Chapman, E. G., Blier, P. U., Bogan, A. E., Stewart, D. T., & Hoeh, W. R. (2010). Mitochondrial phylogenomics of the Bivalvia (Mollusca):

Searching for the origin and mitogenomic correlates of doubly uniparental inheritance of mtDNA. *BMC Evolutionary Biology*, *10*(1), 1–19. https://doi.org/10.1186/1471-2148-10-50

- Forrest, B. M., & Atalah, J. (2017). Significant impact from blue mussel *Mytilus* galloprovincialis biofouling on aquaculture production of green-lipped mussels in New Zealand. *Aquaculture Environment Interactions*, 9(1), 115–126. https://doi.org/10.3354/aei00220
- Gaitán-Espitia, J. D., Quintero-Galvis, J. F., Mesas, A., & D'Elía, G. (2016). Mitogenomics of southern hemisphere blue mussels (Bivalvia: Pteriomorphia): Insights into the evolutionary characteristics of the *Mytilus* edulis complex. *Scientific Reports*, 6(January), 1–10. https://doi.org/10.1038/srep26853
- Gardner, J. P. A., Oyarzún, P., Toro, J., Wenne, R., & Zbawicka, M. (2020). Phylogeography of Southern hemisphere blue mussels of the genus *Mytilus*: evolution, biosecurity, aquaculture, and food labelling. *Oceanography and Marine Biology: Annual Reviews, in press.*
- Gardner, J. P. A., & Skibinski, D. O. F. (1988). Historical and size-dependent genetic variation in hybrid mussel populations. *Heredity*, 61(1), 93–105. https://doi.org/10.1038/hdy.1988.94
- Gardner, J. P. A. (2004). A historical perspective of the genus *Mytilus* (Bivalvia: Mollusca) in New Zealand: Multivariate morphometric analyses of fossil, midden and contemporary blue mussels. *Biological Journal of the Linnean Society*, *82*(3), 329–344. https://doi.org/10.1111/j.1095-8312.2004.00362.x
- Gardner, J. P. A., Zbawicka, M., Westfall, K. M., & Wenne, R. (2016). Invasive blue mussels threaten regional scale genetic diversity in mainland and remote offshore locations: the need for baseline data and enhanced protection in the Southern Ocean. *Global Change Biology*, 22(9), 3182–3195. https://doi.org/10.1111/gcb.13332
- Gérard, K., Bierne, N., Borsa, P., Chenuil, A., & Féral, J. P. (2008). Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from Northern and Southern hemispheres and strong genetic differentiation among southern populations. *Molecular Phylogenetics and Evolution*, 49(1), 84–91. https://doi.org/10.1016/j.ympev.2008.07.006
- Gestoso, I., Arenas, F., Rubal, M., Veiga, P., Peña, M., & Olabarria, C. (2013). Shifts from native to non-indigenous mussels: Enhanced habitat complexity and its effects on faunal assemblages. *Marine Environmental Research*, 90, 85–95. https://doi.org/10.1016/j.marenvres.2013.05.015
- Gosling, E. (1992). Systematics and geographic distribution of *Mytilus*. *Development in Aquaculture and Fisheries Science*, 25.
- Grant, S., & Cherry, M. (1985). MYTZLUS GALLOPROVZNCZALZS Lmk. IN SOUTHERN AFRICA. Journal of Experimental Marine Biology and Ecology, 90, 179– 191.

- Gusman, A., Lecomte, S., Stewart, D. T., Passamonti, M., & Breton, S. (2016). Pursuing the quest for better understanding the taxonomic distribution of the system of doubly uniparental inheritance of mtDNA. *PeerJ*, 2016(12), 1–27. https://doi.org/10.7717/peerj.2760
- Hilbish, T. J., Mullinax, A., Dolven, S. I., Meyer, A., Koehn, R. K., & Rawson, P. D. (2000). Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): Routes and timing of transequatorial migration. *Marine Biology*, 136(1), 69–77. https://doi.org/10.1007/s002270050010
- Hoeh, W. R., Blakley, K. H., & Brown, W. M. (1991). Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial DNA. *Science*, 251(5000), 1488–1490. https://doi.org/10.1126/science.1672472
- Illesca, A., Oyarzún, P. A., Toro, J. E., & Gardner, J. P. A. (2018). Morphometric variability of smooth-shelled blue mussels from the Pacific coast of South America. *Biological Journal of the Linnean Society*, 125(1), 194–209. https://doi.org/10.1093/BIOLINNEAN/BLY094
- Innes, D. J., & Bates, J. A. (1999). Morphological variation of *Mytilus* edulis and *Mytilus* trossulus in eastern Newfoundland. *Marine Biology*, 133(4), 691–699. https://doi.org/10.1007/s002270050510
- Kovačić, I., Pavičić-Hamer, D., Kanduč, T., & Hamer, B. (2018). Adaptation of cultured mussel *Mytilus* galloprovincialis Lamarck, 1819 from the northern Adriatic Sea to nearby aquaculture sites and translocationPrilagodba uzgojne dagnje *Mytilus* galloprovincialis Lamarck, 1819 u sjevernom Jadranu na uvjete u uzgajalištu. *Acta Adriatica*, 58(2), 285–296. https://doi.org/10.32582/aa.58.2.8
- Kyriakou, E., Kravariti, L., Zouros, E., & Rodakis, G. C. (2016). No sex-specific proteinbinding site in the VD1 of the F mitochondrial genome of the mussel *Mytilus* galloprovincialis. *Gene Reports*, 5, 148–150. https://doi.org/10.1016/j.genrep.2016.10.003
- Larraín, M. A., Zbawicka, M., Araneda, C., Gardner, J. P. A., & Wenne, R. (2018). Native and invasive taxa on the Pacific coast of South America: Impacts on aquaculture, traceability and biodiversity of blue mussels (*Mytilus* spp.). *Evolutionary Applications*, 11(3), 298–311. https://doi.org/10.1111/eva.12553
- Lee, J. E., & Chown, S. L. (2007). *Mytilus* on the move: Transport of an invasive bivalve to the Antarctic. *Marine Ecology Progress Series*, 339, 307–310. https://doi.org/10.3354/meps339307
- Lee, S., & Morton, Y. (1985). The introduction of the Mediterranean mussel *Mytilus* galloprovincialis into Hong Kong. *Malacological Review*, *18*(1–2), 107–109.
- Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L. O., Olrog, L., ... Syversen, U. (2005). Improving marine water quality by mussel farming: A profitable solution for Swedish society. *Ambio*, 34(2), 131–138. https://doi.org/10.1579/0044-7447-34.2.131

- Lindberg, D. R. (2010). Paleontological Society Marine Biotic Interchange Between the Northern and Southern hemispheres Marine biotic interchange between the northern and southern hemispheres. *Society*, *17*(3), 308–324.
- Liu, J., Liu, H., & Zhang, H. (2018). Phylogeny and evolutionary radiation of the marine mussels (Bivalvia: Mytilidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, 126(April), 233–240. https://doi.org/10.1016/j.ympev.2018.04.019
- Lourenço, C. R., Nicastro, K. R., Serrão, E. A., Castilho, R., & Zardi, G. I. (2014). Behind the mask: Cryptic genetic diversity of *Mytilus* galloprovincialis along southern European and northern African shores. *Journal of Molluscan Studies*, *81*(3), 380–387. https://doi.org/10.1093/mollus/eyv004
- Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). 100 of the World's Worst Invasive Species. *Aliens*, *12*, s1–s12.
- Machordom, A., Araujo, R., Toledo, C., Zouros, E., & Ladoukakis, E. D. (2015). Femaledependent transmission of paternal mtDNA is a shared feature of bivalve species with doubly uniparental inheritance (DUI) of mitochondrial DNA. *Journal of Zoological Systematics and Evolutionary Research*, 53(3), 200–204. https://doi.org/10.1111/jzs.12096
- Mathiesen, S. S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., ... Nielsen, E. E. (2017). Genetic diversity and connectivity within *Mytilus* spp. in the subarctic and Arctic. *Evolutionary Applications*, 10(1), 39–55. https://doi.org/10.1111/eva.12415
- McDonald, J. H., & Koehn, R. K. (1988). The mussels *Mytilus* galloprovincialis and M. trossulus on the Pacific coast of North America. *Marine Biology*, 99(1), 111–118. https://doi.org/10.1007/BF00644984
- McDonald, J. H., Seed, R., & Koehn, R. K. (1991). Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern hemispheres. *Marine Biology*, *111*(3), 323–333. https://doi.org/10.1007/BF01319403
- Mizi, A., Zouros, E., Moschonas, N., & Rodakis, G. C. (2005). The complete maternal and paternal mitochondrial genomes of the Mediterranean mussel *Mytilus* galloprovincialis: Implications for the doubly uniparental inheritance mode of mtDNA. *Molecular Biology and Evolution*, 22(4), 952–967. https://doi.org/10.1093/molbev/msi079
- Norling, P., & Kautsky, N. (2007). Structural and functional effects of *Mytilus* edulis on diversity of associated species and ecosystem functioning. *Marine Ecology Progress Series*, 351, 163–175. https://doi.org/10.3354/meps07033
- Palumbi, S. R. (1996). Nucleic acids II: the polymerase chain reaction. *Molecular Systematics*, 205–247.

- Peteiro, L. G., Babarro, J. M. F., Labarta, U., & Fernández-Reiriz, M. J. (2006). Growth of *Mytilus* galloprovincialis after the Prestige oil spill. *ICES Journal of Marine Science*, 63(6), 1005–1013. https://doi.org/10.1016/j.icesjms.2006.03.010
- Rawson, P. D., & Hilbish, T. J. (1995). Distribution of male and female mtDNA lineages in populations of blue mussels, *Mytilus* trossulus and *M. galloprovincialis*, along the Pacific coast of North America. *Marine Biology*, 124(2), 245–250. https://doi.org/10.1007/BF00347128
- Rawson, P. D., & Hilbish, T. J. (1995). Evolutionary relationships among the male and female mitochondrial DNA lineages in the *Mytilus* edulis species complex. *Molecular Biology and Evolution*, 893–901. https://doi.org/10.1093/oxfordjournals.molbev.a040266
- Robicheau, B. M., Powell, A. E., Del Bel, L., Breton, S., & Stewart, D. T. (2017). Evidence for extreme sequence divergence between the male- and female-transmitted mitochondrial genomes in the bivalve mollusc, Modiolus modiolus (Mytilidae). *Journal* of Zoological Systematics and Evolutionary Research, 55(2), 89–97. https://doi.org/10.1111/jzs.12160
- Rodríguez, F., Feist, S. W., Guillou, L., Harkestad, L. S., Bateman, K., Renault, T., & Mortensen, S. (2008). Phylogenetic and morphological characterisation of the green algae infesting blue mussel *Mytilus* edulis in the North and South Atlantic oceans. *Diseases of Aquatic Organisms*, 81(3), 231–240. https://doi.org/10.3354/dao01956
- Saarman, N. P., & Pogson, G. H. (2015). Introgression between invasive and native blue mussels (genus *Mytilus*) in the central California hybrid zone. *Molecular Ecology*, 24(18), 4723–4738. https://doi.org/10.1111/mec.13340
- Seed, R. (1990). Taxonomic and evolutionary relationships within the genus *Mytilus*. *Asian Marine Biology*, 7, 97.
- Seo, K. S., & Lee, Y. (2004). A first assessment of invasive marine species on Chinese and Korean coasts. In *Ecological Studies* (pp. 577–585).
- Śmietanka, B., Burzyński, A., Hummel, H., & Wenne, R. (2014). Glacial history of the European marine mussels *Mytilus*, inferred from distribution of mitochondrial DNA lineages. *Heredity*, 113(3), 250–258. https://doi.org/10.1038/hdy.2014.23
- Śmietanka, B., & Burzyński, A. (2017). Disruption of doubly uniparental inheritance of mitochondrial DNA associated with hybridization area of European *Mytilus* edulis and *Mytilus* trossulus in Norway. *Marine Biology*, 164(11), 1–11. https://doi.org/10.1007/s00227-017-3235-5
- Springer, S. A., & Crespi, B. J. (2007). Adaptive gamete-recognition divergence in a hybridizing *Mytilus* population. *Evolution*, 61(4), 772–783. https://doi.org/10.1111/j.1558-5646.2007.00073.x

- Steffani, C. N., & Branch, G. M. (2003). Effect of temperature on endogenous hormone levels and opposite phyllotaxy in maize leaf primordial. *Marine Ecology Progress Series*, 246(4), 197–209. https://doi.org/10.4238/2015.December.16.2
- Vaccaro, E., & Waite, J. H. (2001). Yield and post-yield behaviour of mussel byssal thread: A self-healing biomolecular material. *Biomacromolecules*, 2(3), 906–911. https://doi.org/10.1021/bm0100514
- Vermeij, G. J. (1991). Anatomy of an Invasion : The Trans-Arctic Interchange. *Paleobiology*, *17*(3), 281–307.
- Vermeij, G. J. (1992). Trans-equatorial connections between biotas in the temperate eastern Atlantic. *Marine Biology*, *112*(2), 343–348. https://doi.org/10.1007/BF00702481
- Wares, J. P., & Cunningham, C. W. (2001). Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution*, 55(12), 2455–2469. https://doi.org/10.1111/j.0014-3820.2001.tb00760.x
- Westfall, K. M. (2011). Molecular ecology and systematics of blue mussels (genus Mytilus) (Mytilidae; Bivalvia; Mollusca) in the Southern hemisphere. 285.
- Westfall, K. M., Wimberger, P. H., & Gardner, J. P. A. (2010). An RFLP assay to determine if *Mytilus* galloprovincialis Lmk. (Mytilidae; Bivalvia) is of Northern or Southern hemisphere origin. *Molecular Ecology Resources*, 10(3), 573–575. https://doi.org/10.1111/j.1755-0998.2009.02779.x
- Westfall, K. M., & Gardner, J. P. A. (2013). Interlineage *Mytilus* galloprovincialis Lmk. 1819 hybridization yields inconsistent genetic outcomes in the Southern hemisphere. *Biological Invasions*, 15(7), 1493–1506. https://doi.org/10.1007/s10530-012-0385-8
- Westfall, K. M., & Gardner, J. P. A. (2010). Genetic diversity of Southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. *Biological Journal of the Linnean Society*, 101(4), 898–909. https://doi.org/10.1111/j.1095-8312.2010.01549.x
- Zbawicka, M., Trucco, M. I., & Wenne, R. (2018). Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels:
 Hybridization with invasive European *Mytilus* galloprovincialis. *Genetics Selection Evolution*, 50(1), 1–14. https://doi.org/10.1186/s12711-018-0376-z
- Zouros, E. (1994). Mitochondrial DNA inheritance. Nature, 368(6474), 818.
- Zouros, E., Freeman, K. R., Ball, A. O., & Pogson, G. H. (1992). *mussel Mytilus*. 359(October), 412–414.

Chapter 2: Distribution of and Invasion Vectors for Northern *Mytilus galloprovincialis* in New Zealand

ABSTRACT: Introductions of non-indigenous species (NIS) are important environmental, economic and social issues on a global scale. One NIS, the blue mussel *M. galloprovincialis*, is of particular importance as it is recognized as one of most invasive species globally. In the Southern hemisphere, including New Zealand, they have a complicated taxonomic status as there are two lineages: one native to the Southern hemisphere and one invasive from the Northern hemisphere. Northern and Southern individuals are also morphologically indistinguishable, and thus molecular techniques are required for identification. A 16s mitochondrial rDNA RFLP assay was performed to identify 500 M. galloprovincialis individuals from New Zealand's main aquaculture region in the Marlborough Sounds. These data were then added to an existing dataset with 16s RFLP information from sample sites across the North and South Islands of New Zealand. The primary hypothesis, based on previous research, is that no variable except for distance to nearest primary port will correlate with MgN relative abundance, or describe MgN presence-absence. To test this, geospatial data and satellite based environmental data were collected at these sample sites to generate independent variables. Statistical analyses including a Principal Components Analysis, Permutational ANOVA, and Generalized Linear Model, were then performed on this data set. These tests revealed that Chlorophyll-A concentration (mg/m³), Sea Surface Temperature, and Distance to the nearest Marina (km) were significant in predicting the presence or absence of the invader, whilst and Turbidity (NTU), Distance to the nearest Primary Port (km), and Distance to the nearest Place of First Arrival (km) were significant in describing the relative abundance of the invader. These combined results suggest that anthropogenic disturbance is directly influencing the Northern M. galloprovincialis invasion in New Zealand.

2.1 INTRODUCTION

2.1.1 MARINE BIOLOGICAL INVASIONS

Many non-indigenous species (NIS) are capable of inflicting severe ecological and economic damage (Perrings et al. 2002; Didham et al. 2007; McGeoch et al. 2010, 2016;

Gardner et al. 2016; Forrest & Atalah, 2017; Bellard et al. 2017). This is true in both terrestrial and marine environments, with species that are highly capable of biofouling ships or that are associated with aquaculture being some of the most ecologically and economically damaging species currently recognized (Lowe et al. 2000; Bisson 2006; Chapman et al. 2013). While biofouling of ships' hulls and internal systems is increasingly recognized as the primary vector of NIS introduction, it is also essential to understand post initial invasion processes and mechanisms (Carlton & Hodder, 1995; Floerl & Coutts, 2009; Coutts et al. 2010; Carlton, 2011; Hopkins et al. 2011a). Unfortunately, it is often difficult if not impossible to eradicate a marine NIS after introduction, although a few notable exceptions do exist (Hopkins et al. 2011b). However, there are other viable biosecurity options available besides eradication that can slow the invasion if the invasion vectors are understood (Thresher & Kuris, 2004; Forrest et al. 2009).

When considering an NIS that is capable of biofouling ships, intranational boating traffic is an important vector for continued range expansion post initial invasion and early establishment (Forrest et al. 2009; Clarke Murray et al. 2011, 2012, 2014; Bracken et al. 2016). Broadly speaking, ships arriving from foreign ports are the most heavily regulated in regard to anti-fouling, but often recreational vessels or smaller commercial vessels are not (Hewitt et al. 2010). With this lack of regulation and/or enforcement, these recreational vessels and small commercial vessels can become heavily biofouled and greatly enable the spread of some NIS post initial invasion (Ardura et al. 2015, 2016). The Mediterranean blue mussel, *Mytilus galloprovincialis* (Lamarck 1819), is one such species that is known to spread in this manner (Apte et al. 2000; Bownes & McQuaid, 2006; Westfall & Gardner, 2010, 2013; Clarke Murray et al. 2012; Crego-Prieto et al. 2015).

M. galloprovincialis is currently regarded as one of the most invasive and damaging species globally (Lowe et al. 2000). The presence of this NIS has various negative effects depending upon the region being considered, however these effects can be severe (Bownes & McQuaid, 2006; Brannock et al. 2009; Brannock & Hilbish, 2010; Forrest & Atalah, 2017). The ecological damage that *M. galloprovincialis* has been reported to cause is to completely displace native species and cause localized extinction events of some taxa (Bownes & McQuaid, 2006), and they can threaten the genetic diversity of closely related taxa (Gardner et al. 2016; Oyarzún et al. 2016; Zbawicka et al. 2018). Furthermore, *M. galloprovincialis* can cause economic damage through biofouling of ships, requiring expensive delays for cleaning (Bracken et al. 2016); outcompete targeted aquaculture species causing reduction in crop yield (Forrest & Atalah, 2017); and even cause infrastructure damage through biofouling of seawater based cooling systems (Sakaguchi, 2003).

Through deliberate introduction via aquaculture, accidental introduction via vessel biofouling, and increasing niche space availability due to increasing ocean temperature, there are currently non-native populations of *M. galloprovincialis* in all continents except Antarctica (Gosling, 1992; Wonham, 2004; Brannock et al. 2009; Westfall & Gardner, 2010; Svane, 2011; Ab Rahim et al. 2016; Crego-Prieto et al. 2015; Gardner et al. 2016; Zardi et al. 2018). Furthermore, *M. galloprovincialis* is currently expanding into the Arctic Ocean, an expansion likely enabled by anthropogenic climate change, which suggests that the same may occur in the Antarctic in the near future (Oyarzún et al. 2016; Mathiesen et al. 2017; Thyrring et al. 2017; Leopold et al. 2019). While these points highlight the need to research and address *M. galloprovincialis* invasion, several unique biological characteristics of *Mytilus* spp. makes it challenging to delimit closely related taxa (Chapter 1). In some regions native and invasive taxa may be so similar that they are morphologically indistinguishable (Westfall et al. 2010) or at a minimum may require sophisticated analyses to tell them apart (Illesca et al. 2018).

2.1.2 STUDY ORGANISM, M. GALLOPROVINCIALIS

M. galloprovincialis and closely related species are members of the *M. edulis* species complex (Rawson & Hilbish, 1995; Westfall et al. 2010; Astorga et al. 2015), which consists of *M. galloprovincialis, M. edulis* (Linnaeus 1758), and *M. trossulus* (Gould 1850). Where any combination of these species occur sympatrically, hybridization and introgression of varying degrees is observed leading to morphologically and genetically intermediate individuals (Gardner et al. 2016, Brannock et al. 2009; Stuckas et al. 2009; Brannock & Hilbish, 2010). While degree of hybridization and associated introgression is variable between specific hybrid zones, these morphologically intermediate individuals have undoubtedly led to erroneous species classifications over time (Gardner et al. 2020). This means that where *M. galloprovincialis* is invasive it can hybridize with native *Mytilus sp.*, posing a unique form of ecological damage through loss of genetic diversity (Gardner et al. 2016).

The historic classifications of *Mytilus* spp., i.e. those taxonomic classifications based on morphology, are repeatedly revised due to the aforementioned hybridization and the high degree of phenotypic plasticity these mussels display with regard to environmental factors (Stirling & Okumus, 1994; García-Souto et al. 2017; Gardner et al. 2020). Even modern methods such as valve morphometric analysis (Akester & Martel, 2000; Gardner, 2004), and larval morphometry gamete (McDonald et al. 1991; Springer & Crespi, 2007), etc., have varying and often suboptimal degrees of effort and delimiting power. Given that these moderately effective methods are the foundation of our current understanding of *Mytilus* taxonomy, molecular techniques have become the most common tool for *Mytilus* research over the past two decades (Gérard et al. 2008; Westfall et al. 2010; Gardner et al. 2016; Astorga et al. 2018). Again, however, the apparently variability of hybridization and introgression, both contemporarily and historically, have led to patterns of genetic differentiation that are often difficult to interpret (Wilson et al. 2018; Zbawicka et al. 2019; Simon et al. 2019; Gardner et al. 2020).

These confounding biological characteristics have led to the blue mussels in the Southern hemisphere being interchangeably referred to as *M. edulis, M. galloprovincialis, M. chilensis* (Hupè 1854), *M. platensis* (d'Orbigny 1842), *M. aoteanus* (Powell 1958) and *M. planulatus* (Lamarck 1819); each with between 1 to 25 synonymous species or subspecies names (World Register of Marine Species). It should be noted that various authors use various combinations of one or more of these names or their synonyms, showing that there is not a clear consensus regarding Southern hemisphere *Mytilus* taxonomy. Importantly, the invasion ecology of *Mytilus* spp. in some regions of the Southern hemisphere is lacking due to this near continuous revision of *Mytilus* taxonomy. The most recent published research indicates that there are several distinct Southern hemisphere *Mytilus* taxa, and that Australasian mussels appear to be more related to *M. galloprovincialis* (Hilbish et al. 2000; Gérard et al. 2008; Gardner et al. 2020).

These Southern hemisphere *Mytilus* spp. are demonstrably different from each other and their northern siblings as they demonstrate the ability to maintain their genetic identity in the presence of other each other and other closely related taxa. However, as they lack a clear and agreed upon taxonomic identifier they will be referred to as the Southern hemisphere lineage of *M. galloprovincialis* (Southern *M. galloprovincialis*, or MgS) for the native blue mussel, and the Northern hemisphere lineage of *M. galloprovincialis* (Northern *M. galloprovincialis*, MgN, or the invader) for the invasive blue mussel. This is done to be in alignment with Westfall et al. (2010), Westfall & Gardner, (2010), and Gardner et al. (2016).

The Northern hemisphere mussels which gave rise to the native Southern *M*. *galloprovincialis* appear to have performed two trans-equatorial migration, arriving initially in

South America and then spreading to Australasia. These migrations were between 0.84 and 1.2 million years ago after the Northern *M. galloprovincialis* was isolated in the Mediterranean Sea during the last glacial maximum (Hilbish et al. 2000; Gérard et al. 2008). During this time in isolation, *M. galloprovincialis* became adapted to the warmer waters of the Mediterranean Sea, relative to the cooler North Atlantic and North Pacific waters that *M. edulis* and *M. trossulus* inhabit (Braby and Somero, 2006). They then underwent a rapid range expansion across the Southern hemisphere, likely facilitated by its life cycle, dispersal capacity and the Circum-Polar Current (Miller et al. 2018; Gardner et al. 2020). Currently the distribution of the Southern *M. galloprovincialis*, if considered a distinct taxon from other South American mussel(s), includes New Zealand, Australia including Tasmania, and associated offshore islands (Westfall & Gardner, 2013). The invasive Northern *M. galloprovincialis* appears to have arrived in this region more recently, again likely through anthropogenic means, e.g. biofouling of ships or for aquaculture.

2.1.3 NORTHERN M. GALLOPROVINCIALIS INVASION IN NEW ZEALAND

In New Zealand, where the Northern and Southern *M. galloprovincialis* lineages occur sympatrically as a result of the introduction of the Northern *M. galloprovincialis*, there is very little recourse in addressing the Northern *M. galloprovincialis* as an NIS both biologically and legislatively. MgN is both wide spread and morphologically indistinguishable from the native Southern *M. galloprovincialis*, making it difficult and expensive to detect (Westfall & Gardner, 2010, 2013; Ab Rahim 2016; Gardner et al. 2016); and the invasive Northern *M. galloprovincialis* likely arrived before 29 July 1998, meaning that it is not considered a new organism to New Zealand under the Hazardous Substances and New Organisms Act 1996 (HSNO Act, 1996). However, given that *M. galloprovincialis* is such a prolific and damaging NIS globally, it is insightful to study to contribute to the body of evidence regarding Northern
M. galloprovincialis invasions globally, and discover what damage it may be causing in New Zealand. Also, one common invasion vector for Northern *M. galloprovincialis* is to be introduced for aquaculture (Crego-Prieto et al. 2015). However, neither MgN nor MgS are purposefully grown in New Zealand for aquaculture, which presents a unique opportunity to study the effects of aquaculture on *M. galloprovincialis* invasion without the accompanying direct and deliberate introduction.

Hewitt et al. (2010), in an assessment of New Zealand's marine biosecurity policy, identified that knowledge of biological invasion mechanisms is one key area that is lacking. This shortcoming is important to address as it is important to understand factors that affect biological invasions, both biotic and abiotic. That is, in order to prevent introduction or slow spread after introduction requires effective policy, e.g. policy that effectively addresses those invasion mechanisms.

Therefore, the aim of this chapter is primarily to further describe the distribution and relative abundance of the MgN in New Zealand; begin to describe how abiotic variables affect the competition between the Northern and Southern *M. galloprovincialis;* and discover what is driving the invasion success of MgN in New Zealand, including invasion vectors. In doing so this research will contribute to the mounting body of evidence of *M. galloprovincialis* invasion ecology globally. To do this, a set of abiotic factors was tested, including geospatial factors, against both presence and abundance of Northern *M. galloprovincialis* across a range of sites in New Zealand and *Perna canaliculus* (hereafter *Perna*) aquaculture facilities sites therein. Based on previously published research, the primary hypothesis of this chapter is that distance to primary ports and no other environmental factors would correlate with Northern *M. galloprovincialis* presence / absence and abundance. The results of the sampling at aquaculture sites are considered in the chapter but are the primary subject of Chapter 3.

2.2 MATERIALS AND METHODS

This project uses a 16s mitochondrial rDNA RFLP assay developed by Westfall et al. (2010), to expand upon an existing data set from Westfall et al. (2010). This data set includes samples from across all of mainland New Zealand and several offshore islands (Table 2.1).

2.2.1 ADDITION OF NEW SAMPLES

This data set (Table 2.1) was expanded with n = 50 samples from 10 sites in the Marlborough Sounds, New Zealand, for a total of n = 500 new samples. The sites were *Perna* aquaculture sites (n = 5) and adjacent reef systems (n = 5). These samples were collected by Cawthron Institute staff, preserved in 90% ethanol, and sent to the lab at Victoria University of Wellington, New Zealand, for analysis.

Table 2.1 Existing data set utilized in this chapter, adapted from Westfall et al. (2010). Each of the 10 sites has n = 30 samples, meaning that there were 300 samples overall. For all samples at these sites, the 16s RFLP was utilized to determine the Northern or Southern lineage of each individual. MgN (%) was calculated as described in section 2.2.3. Offshore islands were removed from this data set, as were sites without 16s data.

Site Name	Latitude	Longitude	MgN (%)
Akaroa	43° 40.323′ S	172° 57.914′E	3.3%
Greymouth	42° 27.457′ S	171° 12.357′E	0.0%
Kaikoura	42° 24.260′ S	173° 41.103′ E	3.3%
Mussel Point	41° 43.989′ S	174° 16.052′ E	33.3%
Ringitingi Beach	46° 53.259′ S	167° 59.907' E	0.0%
TeTii Bay	35° 08.768′ S	174° 00.258' E	26.7%
Waiheke Island	36° 47.272′ S	175° 05.264′ E	57.1%
Waitangi	35° 16.718′ S	174° 05.383′ E	33.3%
Wellington Harbour	41° 17.000′ S	174° 50.000′ E	6.7%
Whakariki Beach	40° 30.223′ S	172° 39.592′ E	0.0%

2.2.1.1 DNA EXTRACTION AND PURIFICATION

DNA extraction and purification were performed with a Geneaid Genomic DNA Mini Kit (Tissue), following the manufacturer's instructions. 30 mg of mantle tissue was dissected from each sample and added to a microcentrifuge tube with 200 μ L of GT buffer. 20 μ L of proteinase K was added to each sample, then incubated at 60°C for a minimum of 30 minutes. 200 μ L of GT buffer was added, vortexed until homogenous, then incubated at 60°C for a minimum of 20 minutes. RNA degradation was not performed. 200 μ L of absolute ethanol was then added and vortexed until homogeneous. Each sample was then transferred to a GS column and centrifuged at 14,000 RPM for 2 minutes. The flow-through was then discarded, and 400 μ L of W1 buffer was added. Samples were then centrifuged again at 14,000 RPM for 2 minutes. Flow-through was again discarded. 600 μ L of Wash Buffer, with ethanol, was added and samples were then dried by centrifuging at 14,000 RPM for 3 minutes. GS columns were then transferred to a clean microcentrifuge tube. 100 μ L of Elution Buffer, heated to 60°C, was then added and left for a minimum of 5 minutes. Samples were then centrifuged at 14,000 RPM for 30 seconds, yielding the final DNA samples used.

DNA samples were then tested with a Nanodrop for DNA concentration and purity. A 260/280 wavelength ratio near 1.8 was considered to be clean. Samples with ratios outside of 1.7 to 1.9 had DNA extraction performed again. DNA concentrations were adjusted to near 50 ng/ μ L, then tested again to confirm correct concentrations for PCR.

2.2.1.2 PCR AND RFLP ASSAY

The 16s PCR and RFLP protocol described by Westfall et al. (2010) was followed. A 527 bp fragment of the mitochondrial 16s rDNA gene was amplified. A 25 µL PCR reaction was made using 12.5 µL of MyTaq Red Mix, 1 µL each of 16sAR and 16sBR primers (Palumbi,

1996), 8.5 μ L ddH₂O, and 2 μ L of DNA template of each sample. Samples were then amplified under the following PCR protocol. Initial denaturation for 3 minutes at 95°C; 30 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 52°C, and extension for 45 seconds at 72°C; and a final extension of 3 minutes at 72°C.

Following Westfall et al. (2010) the 16s RFLP assay was then performed. A 20 μ L double restriction digest reaction was made using 5 μ L ddH₂O, 2 μ L red buffer, 1 μ L loading buffer, 1 μ L each of the restriction enzymes Eco321 and Nhe1 (Westfall et al. 2010), and 10 μ L of PCR product for each sample. These samples were then incubated at 37°C for a minimum of 1 hour. Digestion products were scored on a 2% agarose gel using RedSafe nucleic acid staining solution. Southern hemisphere *M. galloprovincialis* individuals were determined by fragment lengths of 342, 167, and 28 bp; Northern hemisphere *M. galloprovincialis* were then used to generate the two response variables of presence-absence of Northern *M. galloprovincialis*, and relative abundance of Northern *M. galloprovincialis*. Relative abundance was calculated by dividing the number of MgN individuals by the total number of samples.

2.2.2 ENVIRONMENTAL AND GEOSPATIAL DATA

The independent variables tested were environmental data and geospatial data. It is also important to consider biological factors as well, such as predation pressure, biodiversity, biofilm composition, etc. Those data are unavailable for these sample sites as it was not collected during initial sampling and it is highly likely that these measures would not be accurate if collected now. This is addressed by assuming that biological factors are placing equal pressure on Northern and Southern *M. galloprovincialis* populations. This assumption should be explicitly tested in future research.

2.2.2.1 ENVIRONMENTAL DATA

Satellite data were collected by Dr. Matt Pinkerton at NIWA. This environmental data set includes monthly means from 2002 to 2018 for Chlorophyll-A concentration (Chl-A) in mg/m³, Turbidity (Turb) in Normalized Turbidity Units (NTU), and Sea Surface Temperature (SST) in °C. For each sample site an annual minimum, average, and maximum was calculated for each variable for each year (2002-2018). These annual data were then averaged across all years to yield the variables used for statistical testing; maximum, minimum, and average of Chl-A, Turb, and SST.

This simplification, i.e. going from a data set with monthly means to a data set with annual means, was performed because satellite data are not ideal for coastal sites (Zheng & DiGiacomo, 2017). This is because the satellite takes repeated images of a section of ocean to then be processed to yield the desired information about that section of ocean. If one of those images contains clouds, a piece of land, or if the sea floor is clearly visible, then that image is removed from the dataset (Martin, 2014). Therefore, a great deal of the images near coastlines were removed, resulting in a great deal of missing data in the environmental data set. To overcome this, the simplification from monthly means to annual means was performed. That said, these data are much more temporally consistent and robust than those that could have been collected manually.

To extract environmental data, each site was represented by a latitude-longitude coordinate. The desired environmental data were extracted from either the nearest 9 cells (images) or the nearest 25 cells in a square pattern around those coordinates. Extractions using the nearest 9 cells represent the site more accurately but having more missing data, while extractions using the nearest 25 cells represent the site less accurately but have fewer missing data. Four rounds of extractions were then performed sequentially for each site as described below. Initial data extraction started directly on the latitude/longitude of the sample site, with

subsequent rounds of extraction moving roughly 500 m seaward to avoid the aforementioned land clipping, cloud cover, or visible seafloor issues. This resulted in eight sets of extractions, i.e. extraction 1 - 9 cells, extraction 1 - 25 cells, extraction 2 - 9 cells ... extraction 4 - 25 cells.

To quantify the quality of data, an extraction rate was calculated for each site in each of the eight sets of extractions. The extraction rate was calculated by dividing the number of successful extractions divided by the total number of attempted extractions (Eqn. 1).

Extraction Rate = $\frac{\#$ Successful extractions # Attempted extractions

(Eqn. 1)

The final environmental data utilized was generated from the single best extraction for each site. The best extraction was determined by considering, in order of priority, extraction rate, round of extraction, and number of cells. A successful data extraction rate of \geq 70% was preferred, while rates of \geq 50% were used of necessary. Sites with extraction rates of \leq 50% were never utilized. Earlier extractions were preferred over later, i.e. extraction 1 was preferred over extraction 2, etc., as these data were taken from a closer location to the actual sample site. Finally, extractions using the nearest 9 cells was preferred over the nearest 25 cells. These steps were taken so that the environmental data utilized was as complete and accurate as possible. A workflow diagram for the above process is provided in Supplementary Figure 1.

2.2.2.2 GEOSPATIAL DATA

Geospatial data were collected from publicly available data sets from the New Zealand Ministry for the Environment, Ministry of Transport, or by measurement on Google Earth using shortest over-water distances. These data include distance to nearest port as a function of port type, distance to nearest source of freshwater, and wave data – refer to details below. Port types were primary ports, that receive more than 100 ships arriving from overseas ports per year; secondary ports, that receive less than 100 ships arriving from overseas ports per year but are still commercial in nature; places of first arrival, which are sites designated by Biosecurity NZ for recreational vessels to arrive whose voyage originated from overseas (Places of First Arrival, Biosecurity NZ); and marinas, which are ports or anchorages that are primarily recreational in nature. Shipping data were collected from the New Zealand Ministry of Transport (Supplementary Figure 2).

Distance from each mussel collection site to these ports was measured in Google Earth, using shortest overwater distance. Each site therefore had a value for distance to nearest primary port (DPP), distance to nearest secondary port (DSP), distance to nearest place of first arrival (DPFA), and distance to nearest marina (DM). If two or more ports were directly adjacent to each other, or simultaneously considered to be two or more port types, then only one port type was used for that group. For example, in Auckland there are multiple marinas and places of first arrival that are part of the same continuous waterfront as a primary port, and therefore the Port of Auckland is only considered to be a primary port. This step was taken to avoid biasing the data set by having the same value for multiple variables.

Distance to nearest source of freshwater input (DFW) was used as a proxy variable for salinity (Crego-Prieto et al. 2015), as salinity data were not available from the satellite data. This was calculated by measuring the shortest overwater distance to nearest coastal hydrosystem, which is typically where streams, rivers, or springs run into the marine coastal environment. Hydrosystems were located using a publicly available coastal hydrosystems data set from the New Zealand Ministry for the Environment (NZ Coastal Hydrosystems, Ministry for the Environment). This data set also included freshwater inflow data in cubic meters of freshwater inflow per tidal cycle, which was used to create an inflow index.

The inflow index was calculated by log transforming the inflow data, then dividing each individual value by the log of the maximum flow value. This effectively created a freshwater inflow index from 0 to 1, with smaller values representing lower inflow values, and vice versa. The DFW data were divided by this inflow index (DWF/II), which had a gradient effect of artificially increasing DFW values for sites near the mouths of small streams, while leaving DFW values for sites near the mouths of large rivers mostly unchanged. These steps were taken to make this index a more accurate proxy for salinity, although it should be noted that both DFW and DFW/II were ultimately dropped from the data set. This is explained in subsection 2.2.3.1.

Wave data were collected from a publicly available dataset, prepared by NIWA for the Ministry for the Environment (Extreme Wave Indices for New Zealand Coastal and Oceanic Waters, 2018). These data are average annual number of occurrences for which significant wave height exceeded 4 meters, 6 meters, and 8 meters, for 13 coastal regions representing all of mainland New Zealand. Sites were assigned to one of these coastal sections based on their latitude-longitude coordinates and had wave data from that section added to the data set. As such, each site now had the variables 4M, 6M, and 8M, as predictor variables of wave height frequency of occurrence events.

2.2.3 MODELLING AND STATISTICAL ANALYSES

2.2.3.1 DATA PROCESSING AND EXPLORATION

Data processing and exploration after this point was done in a manner similar to that described by Zuur et al. (2010). All data management and statistical analyses were performed in Microsoft Excel 2016, PRIMER v7, and R v3.6.3 (Clarke & Warwick, 1994; Clarke & Gorley, 2017; R Core Team, 2019).

After initial data exploration, a non-parametric autocorrelation analysis was performed. This revealed that all wave data variables were highly correlated with each other and with DM and DFW (R>0.8), and thus were eliminated from the data set. The autocorrelation analysis also revealed that the DFW and DFW/II data were highly correlated (R>0.99), and thus DFW/II was removed from the final data set.

Variance inflation factor (VIF) scores were then generated for each variable. This was done to further detect multicollinearity between the independent variables. The initial VIF scores were high, i.e. >10. The maximum and minimum variables were then eliminated from the data set with only annual averages being retained, yielding VIF scores of <10, which is not ideal but acceptable (Zuur et al. 2010, 2016; Montgomery et al. 2012). After further data exploration it was found that DFW was not an important variable to consider, i.e. it was not significant and/or retained in a final model. DFW was therefore dropped from the data set, which then yielded VIF scores of <5 for the remaining variables, which is a much more acceptable VIF score (Zuur et al. 2010, 2016; Montgomery et al. 2012).

The result of these steps was the removal of 12 of the independent variables. It is unfortunate that so many of these had to be removed, especially the maxima and minima of the environmental variables as it is important to consider variability and episodic extremes. However, these steps were critical as including those variables would have likely led to erroneous results.

2.2.3.2 FINAL DATA SET

This data set now contained the following variables for each site: dependent variables of MgN presence-absence and MgN relative abundance, ranging from 0.00 to 0.57, with 0.23 \pm 0.04 (mean \pm SE); and independent variables of Chl-A (mg/m³), ranging from 0.71 to 4.19, with 1.96 \pm 0.19 (mean \pm SE); SST (°C), ranging from 12.59 to 18.69, with 15.8 \pm 0.33 (mean

 \pm SE); TURB (NTU), ranging from 0.55 to 3.24, with 1.68 \pm 0.18 (mean \pm SE); DPP (km), ranging from 4.2 to 382, with 103.87 \pm 14.13 (mean \pm SE); DPFA (km), ranging from 29.7 to 880, with 162.44 \pm 35.48 (mean \pm SE); DSP (km), ranging from 0.17 to 435, with 121.73 \pm 18.96 (mean \pm SE); and DM (km), ranging from 4.52 to 587, with 97.63 \pm 28.33 (mean \pm SE). This reduced data set was then standardized (Z = (X- μ)/ σ), excluding the dependent variables. All further statistical testing and modelling was then performed on this independent and standardized data set.

After these initial data processing steps, statistical analyses were performed on two dependent variables, presence-absence of MgN and relative abundance of MgN. This was done to disentangle what factors affect MgN spread, i.e. invasion spread, and what factors affect MgN relative abundance, i.e. factors that affect MgN and MgS competition and/or MgN range expansion.

2.2.3.3 PRESENCE-ABSENCE

A Principle Components Analysis (PCA) was performed to visualize differences between sites with MgN present and absent (Figure 2.2). It should be noted that only 5 sites had no MgN present (Table 2.1; Table 2.2). Sites with MgN absent do not clearly cluster in the PCA plot (Figure 2). However, there is a distinct cluster in the PCA for sites with MgN present, suggesting that the independent variables are similar at these sites.

Presence-absence data were tested with a Permutational Multivariate Analysis of Variance (PERMANOVA), using the R package 'Vegan', with 999 permutations of the data (Oksanen et al. 2019). Homogeneity of dispersion, the statistical assumption of PERMANOVA, was tested using a permutation test for homogeneity of multivariate dispersions in the R package 'Vegan', again with 999 permutations (Oksanen et al. 2019). This

test indicated that the dispersions are homogeneous, e.g. that the statistical assumptions of PERMANOVA were met (Oksanen et al. 2019).

2.2.3.4 RELATIVE ABUNDANCE

Relative abundance of MgN data were modelled using a generalized linear model (GLM) in the R package 'Stats', using the binomial family and logistic regression (R Core Team, 2019; Crawley, 2012). MgN relative abundance data were modelled as a function of all predictor variables of the final data set. The final and most parsimonious model (Eqn. 2) was determined via stepwise reduction of AIC scores.

The overdispersion value of this model was 0.91, meaning the model was not overdispersed and the results and subsequent assumption tests were valid (Zuur et al. 2010, 2016). A Shapiro-Wilks test and Fligner-Killeen test were used to test for normality of residuals and homogeneity of variances, respectively, both in the R package 'Stats' (R Core Team, 2019). The P-values for these tests were 0.08 and 0.12 respectively, meaning that the statistical assumptions of the model were met.

2.3 RESULTS

2.3.1 PRESENCE – ABSENCE

The PERMANOVA test of Northern *M. galloprovincialis* presence-absence indicated that chlorophyll-a, sea surface temperature, and distance to the nearest marina were significant, with P-values of 0.010, 0.019, and 0.003, respectively (Table 2.3). Presence of the Northern *M. galloprovincialis* was associated with a lower and less variable average chlorophyll-a concentration (Figure 2.3). Sites with MgN present had an average annual chlorophyll- A density of 1.75 (mg/m³), while the sites with MgN absent had an annual average chlorophyll-

Table 2.2 16s RFLP assay results for the samples from sites within Marlborough Sounds, New Zealand. These results were added to the existing data set published in Westfall & Gardner, 2010. MgN (%) was calculated as described in section 2.2.3. These results are utilized in both Chapter 2 and Chapter 3, and as such the methods and results, including this table, appear in both chapters.

Site Name	Туре	Latitude	Longitude	MgN (%)
Keneperu	Farm	-41.2081	173.9213	14.0%
Keneperu	Shore	-41.2071	173.9353	25.0%
Crail Bay	Farm	-41.1259	173.9601	23.8%
Crail Bay	Shore	-41.1050	173.9819	28.9%
Tawhitinui Reach, Rams Head	Farm	-41.0551	173.8957	28.9%
Tawhitinui Reach, Maud Island	Shore	-41.0294	173.8693	34.1%
Port Ligar	Farm	-40.9361	173.9755	34.9%
Port Ligar	Shore	-40.9325	173.9727	26.1%
Catherine Cove	Farm	-40.8667	173.9005	34.8%
Catherine Cove	Shore	-40.8687	173.9033	18.8%

a density of 2.80 (mg/m³). Northern *M. galloprovincialis* presence was also associated with warmer sea surface temperature, with sites where they are present having an annual average of 0.96°C warmer than sites where they are absent (Figure 2.3). Distance to the nearest marina, the most significant variable identified by the PERMANOVA, indicated that sites near marinas were more likely to have the invader than those sites that were distant (Table 3). Sites with the invader present were on average 48.7 (km) from the nearest marina, while sites with the invader absent were 293.3 (km) from the nearest marina (Figure 2.3).

2.3.2 RELATIVE ABUNDANCE

$$MgN_i = -3.2697 + 0.2216*TURB_i + 0.8615*DPP_i - 6.2185*DPFA_i$$
(Eqn. 2).

 Table 2.3 Results of the PERMANOVA, testing differences in environmental and geospatial

 variables between sites with MgN present and absent.

Variable	Sum of Squares	\mathbb{R}^2	F-statistic	P-value
Chlorophyll-A	0.807	0.202	10.0	0.010
Sea Surface Temp.	0.508	0.127	6.3	0.019
Turbidity	0.046	0.012	0.6	0.460
DM	1.121	0.280	13.9	0.003
DPP	0.126	0.031	1.6	0.227
DPFA	0.015	0.004	0.2	0.666
DSP	0.008	0.002	0.1	0.731

Equation 2 shows the final and most parsimonious model, where MgN_i is the relative abundance of MgN at site *i*, TURB_i is the turbidity (NTU) at site *i*, DPP_i is the distance from site *i* to the nearest primary port (km), and DPFA_i is the distance from site *i* to the nearest place of first arrival (km). These results indicate that sites with MgN in higher abundance were generally more turbid, closer to a place of first arrival, and further from a primary port. It is interesting that DPP was negatively correlated with invader abundance, which is the opposite of what previous research indicated (Westfall & Gardner, 2010).

Table 2.4 Numeric results of the Generalized Linear Model after stepwise reduction of AICscores. The final model, i.e. the most parsimonious model, includes the variables Turbidity,DPP, and DPFA.

	Estimate	Standard Error	Z-value	P value
(Intercept)	-3.2697	0.352	-9.279	< 0.001
Turbidity	0.2216	0.103	2.149	0.032
DPP	0.8615	0.281	3.065	0.002
DPFA	-6.2185	0.938	-6.630	< 0.001



Figure 2.1 Relative proportion of Northern *M. galloprovincialis* by region. MgN relative abundance was calculated by dividing the number of MgN individuals by the total number of samples for each site, then averaging the MgN proportion values for all sites within each region. The region with the highest average MgN relative abundance was the Auckland region, with 57.1% MgN. The regions with the lowest MgN relative abundance were the Tasman, West Coast, and Southland regions, all with 0.0% MgN. The region on the South Island with the highest MgN relative abundance of MgN was the Marlborough region, with 33.3 % MgN. Regions not outlined in black do not contain any sample sites.



Figure 2.2 Principal Components Analysis of sites categorized by MgN present or absent. PC1 and PC2 together described 62.7% of the cumulative variation. PC3 explained and additional 13.5% of the variation. The largest eigenvectors for PC1 were DM, DPFA, and SST.Avg. The largest eigenvectors for PC2 were TURB.Avg, CHL.Avg, and DSP. These results are mostly in agreement with the results of the other statistical analyses performed.



Figure 2.3 Box and whisker plots of the significant variables identified in the PERMANOVA. These plots also show moderate kurtosis in the variable's SST and DM for sites with MgN absent, likely giving these data a non-normal distribution. This is addressed in the methods section.

2.3.3 SUMMARY OF RESULTS

The results of both the PERMANOVA and GLM indicate that there are separate variables that are driving presence-absence and relative abundance of the Northern *M. galloprovincialis* in New Zealand. Chlorophyll-A, sea surface temperature, and distance to nearest marina were significant for MgN presence-absence (Figure 2.3; Table 2.3); while turbidity, distance to nearest primary port and place of first arrival were significant for percent abundance (Table 2.4). It is interesting that there were no variables that were significant for both the PERMANOVA and retained in the final GLM, and that the only variable that was insignificant for both tests was distance to nearest secondary port.

2.4 DISCUSSION

Overall, the results indicate that environmental factors are more important for describing the invasion of Northern *M. galloprovincialis* in New Zealand than anticipated. It also appears that intranational vessel traffic, rather than international, is more important for the spread of Northern *M. galloprovincialis* in New Zealand. Therefore, the primary hypothesis that the MgN invasion would be best predicted by distance to the nearest primary port was not supported. It is also interesting to note that many of the factors that were found to be significant or included in the final model are associated with anthropogenic disturbance or climate change.

2.4.1 PRESENCE - ABSENCE

Distance to the nearest marina was the most significant factor in predicting the presence or absence of the invader, MgN. Given these findings and the biofouling ability of *M*. *galloprovincialis*, it is likely that MgN is spreading from marina to marina, effectively creating invasion hotspots across the North Island of New Zealand. These results thus indicate that recreational or intranational boating traffic is a more important vector of spread within New Zealand than international traffic. This finding is also reported by Hewitt et al. (2010) for other marine NIZ in New Zealand, as well as several other papers globally (Clarke Murray et al. 2011, 2012; Ardura et al. 2015). This is also supported by the finding that distance to nearest primary port was negatively correlated with Northern *M. galloprovincialis* relative abundance.

The results of the PERMANOVA indicate that Chlorophyll-A concentration and sea surface temperature are both environmental explanators of invasion success on a local scale. Sites where the invader was present had a lower, more stable annual chlorophyll-a concentration, and a higher annual average sea surface temperature. It is important to note that these variables do not appear to be important for describing the relative abundance of MgN. Therefore, Chl-A and SST appear to be stopping the spread of MgN in some way. It is likely that this pattern is from post-settlement processes, because for *Mytilus* communities post-settlement processes are typically more important to consider than rate of primary settlement when describing final adult distributions (Bayne, 1964; Seed, 1969; Bao et al. 2007; Azpeitia et al. 2019).

Chlorophyll-A concentration is an important factor contributing to the growth of *Mytilus* spp., post-settlement survival, and reproductive success (Gardner, 2000; Gardner & Thompson, 2001a, 2001b; Gardner, 2002; Helson & Gardner, 2007a, 2007b, Gardner, 2013). Therefore, these results indicate that either MgN is not able to survive at sites with a high and variable chlorophyll-a concentration; or that MgS is able to completely displace MgN during post-settlement competition. It is difficult to do more than speculate on these points given the results, however these finding do provide direction for future research.

Sea surface temperature is often a significant factor in describing *M. galloprovincialis* invasions globally (Leopold et al. 2019). Northern *M. galloprovincialis*, being adapted to warm Mediterranean Sea waters, tends to occupy relatively warmer waters compared to various

native species. The above results indicate that this is also true in New Zealand, where the native MgS has likely become more adapted to the relatively cooler waters therein. With higher rates of invader presence at warmer sites, it is likely that either the invader is better able to compete with the native MgS at warmer-water sites, or that the invader is occupying niche space previously unoccupied by the native. More research focused on the mechanisms behind these findings are needed to determine which of these scenarios is occurring.

Regardless, sea surface temperature is a significant factor in describing the Northern *M. galloprovincialis* invasion in New Zealand. This is important given increasing sea temperatures with respect to climate change (Hayward et al. 2012). The Intergovernmental Panel on Climate Change reported that ocean temperatures globally, including the Southern Ocean, are increasing and will continue to increase into the near future (IPCC, 2018). It is important to note that predicted sea surface temperature increase is less for the Southern Ocean than for the global mean (IPCC, 2018). However, episodic extremes, such as the marine heatwaves severely damaged the Great Barrier Reef are likely to increase (Hughes et al. 2018). This is important to consider when describing the MgN invasion, however this was not possible in this project as the sea surface temperature maximum values were autocorrelated with sea surface temperature mean, and thus were eliminated from the data set.

2.4.2 ABUNDANCE

The final MgN relative abundance model included turbidity, distance to nearest place of first arrival, and distance to nearest primary port. MgN relative abundance was positively correlated with turbidity and distance to nearest primary port, and negatively correlated with distance to nearest please of first arrival. This means that there are more MgN present in more turbid sites and near places of first arrival, and less MgN present near primary ports. This latter point is, perhaps, counter intuitive and may represent a bias in the data set. MgN relative abundance is positively correlated with turbidity. This may indicate that MgN is outcompeting MgS at relatively higher turbidity sites. Turbidity, as with chlorophyll-A concentration, is an important factor for growth rate of *Mytilus* spp. (Gardner, 2013). Turbidity is often also associated with anthropogenic inputs in both urban and rural areas (Gardner, 2013). In urban areas, turbidity is often representative of eutrophication and urban runoff (Nixon, 1995); while in rural areas, turbidity is associated with eutrophication from land erosion and modern agriculture practices (Cornelison et al. 2011). It is important to note that these results only show correlation, not causation. These results therefore should be taken as a guide for future research, and that anthropogenic disturbance is significantly affecting the MgN invasion in New Zealand.

MgN relative abundance was negatively correlated with distance to nearest place of first arrival, meaning that sites closer to places of first arrival have a higher MgN relative abundance than sites that are distance. That is, as DPFA increased MgN relative abundance decreased. Places of first arrival are sites that are designated by Biosecurity New Zealand for vessel to arrive whose voyages originated from an overseas port. These sites are effectively border nexuses where biosecurity and other rules and regulations can be applied, so it is logical that there is an increased level of marine NIS near these sites.

Distance to the nearest primary port was positively correlated with MgN relative abundance, indicating that sites near primary ports are likely to have less MgN than sites that are distant. That is, as DPP increased MgN relative abundance increased. These results are unexpected as they contradict of the previous research that the primary hypothesis was based upon (Westfall & Gardner, 2010). In interpreting these results it is important to again clarify the port classification scheme utilized. Ports were initially categorized based on the amount of international traffic they receive; place of first arrival are those ports or marinas with a PFA designation by Biosecurity New Zealand; and marinas are primarily recreational anchorages or mooring areas. Ports and marinas that are directly adjacent to each other, or those that have multiple categorizations, were only considered by a single category. After this process, primary ports are distributed roughly equally in a spatial context across the North and South Islands, while places of first arrival and marinas were largely on the North Island. This is largely because on the South Island of New Zealand, many of the marinas are directly adjacent to primary or secondary ports; and places of first arrival are often primary ports (Supplementary Figure 1).

Rather than the primary ports reducing the relative abundance of Northern *M. galloprovincialis* in some way, it is likely that the above results are an artefact of the data. Given that the North Island is more invaded than the South Island (Westfall & Gardner, 2010), primary ports are roughly equally distributed across both the North and South Islands, and marinas and places of first arrival are more concentrated on the North Island, these results are likely showing that the North Island has a larger MgN population than the South Island. This is in alignment with previous research (Westfall & Gardner, 2010; Gardner et al. 2016). These results also suggest that New Zealand's strict biosecurity policy in regard to international shipping traffic is largely effective (Hewitt et al. 2010).

2.4.3 IMPLICATIONS

The Northern *M. galloprovincialis* is recognized as one of the most invasive species globally (Lowe et al. 2000). In New Zealand, there is little direct recourse in addressing the Northern *M. galloprovincialis* invasion, as it is both widespread and morphologically indistinguishable from the native Southern *M. galloprovincialis* (Westfall et al. 2010; Westfall & Gardner, 2013). However, the above results generally suggest that anthropogenic disturbance is affecting the MgN invasion in New Zealand.

Various factors that represent anthropogenic disturbance are repeatedly found to be significant in describing biological invasions (Didham et al. 2007; McGeoch et al. 2010; Pyšek et al. 2010; Ardura et al. 2016). This is true for *M. galloprovincialis* invasions, in which human activity such as aquaculture and type and volume of vessel traffic act as primary invasion vectors (Lee & Chown, 2007; Crego-Prieto et al. 2015). Furthermore, rising ocean temperatures are also enabling *M. galloprovincialis* range expansion into areas previously uninhabitable to them (Mathiesen et al. 2017; Leopold et al. 2019). The results of this project are in alignment with these global trends and are important as they contribute to the body of evidence regarding *M. galloprovincialis* invasions globally and may reveal insights into the invasion ecology of other marine NIS in New Zealand.

The results of this project show that these factors representing anthropogenic disturbance are correlated with the MgN invasion in New Zealand. Given these results of MgN abundance throughout the North and South Islands, it is clear that limiting anthropogenic disturbance is an important post-border biosecurity measure for the MgN invasion (Forrest et al. 2009). Continuing to research these factors and discover the mechanisms that make them significant in describing the MgN invasion, will yield insights not only in the MgN invasion but likely other marine biological invasions as well. In doing so, policy makers will be enabled to design evidence based post-border biosecurity controls, thus preventing or controlling current or future biological invasions.

It is interesting that no variable was significant in the PERMANOVA results and included in the final relative abundance model (GLM). This indicates that some variables are more associated with the spread of MgN in New Zealand, while others are associated with the relative success of MgN versus the native MgS. In terms of applying these results, the PERMANOVA results and GLM results are both individually valid as all statistical assumptions have been met, however the results of the abundance model (GLM) are more likely to be robust. This is due to the extreme nature of the presence-absence transformation of the response variable.

Given New Zealand's reliance on international shipping and the socioeconomic importance of the marine environment, there are robust biosecurity policies regarding vessels arriving from overseas (Hewitt et al. 2010). That being said, control measures for recreational vessels already within New Zealand are less robust. Given that MgN presence is significantly predicted by distance to nearest marina, and that MgN abundance is correlated with distance to place of first arrival, these results suggest that implementing similar post-border biosecurity measures for recreational vessels would be effective at inhibiting further spread of MgN, and likely other marine NIS as well (Forrest et al. 2009; Ardura et al. 2015, 2016).

2.4.4 FUTURE RESEARCH

The scope of this project was to begin to identify invasion vectors and abiotic habitat selection of MgN in New Zealand. As such, the environmental and geospatial variables utilized are not an exhaustive list but are instead the factors that previous research indicate are important to consider, combined with the best methods available given time and budget restrictions. Three factors that are missing from this project and should be considered in future research are biological data, wave data, and salinity. Moreover, the interactive effects between these factors may prove to be insightful (Didham et al. 2007).

These factors were deliberately not considered in this project for several reasons. The pre-existing 16s RFLP dataset that was expanded upon was generated in 2010 (Westfall & Gardner, 2010) and did not include biological data. Given the temporal variability typically found in these biological indices (Murphy, 1970), it would be inappropriate to collect these data now and apply it to this data set. Wave data and salinity are both important factors when

Mytilus spp., as they are repeatedly significant in describing their ecology (Zardi et al. 2007; Crego-Prieto et al. 2015), and as such were both included in this project. Unfortunately, they were eliminated from the data set during data processing as the correlated with other independent variables. Including these wave and salinity data would have led to erroneous results then, and it would be inappropriate to re-run models which included them and the other variables that they correlated with. A future project that considers these variables again, perhaps in another form, would again likely be insightful.

The results also suggest that the Northern and Southern *M. galloprovincialis* may occupy different abiotic niche space. However, the results only show correlation, not causation. Therefore, organismal studies of the two lineages that test for the biological mechanisms behind these differences are necessary. Given that sea surface temperature was significant in predicting presence-absence of the invader, testing thermal tolerance or heat shock proteins of the two lineages will likely be a very useful study. Also, if these projects are able to resample at the same sites in the future, temporal data would then become available which will great enable researchers to describe MgN range expansion or contraction.

What is of primary important in this line of research though is a population genetics or seascape genetics project. The results that are presented in this project highlight the need to use SNPs (e.g., Gardner et al. 2016) or other tools to identify hybrid status of individuals, and also identify connectivity between populations. This will be particularly useful as it can identify source and sink populations for MgN, MgS, and their hybrids; and better assess the natural range expansion of the invader in New Zealand.

2.5 CONCLUSION

The Northern *M. galloprovincialis* invasion in New Zealand is more complex than previously published research indicates. The primary hypothesis was that only distance to the

nearest primary port would significantly predict presence and abundance of the invader MgN, which was not supported by the results. Sea surface temperature, chlorophyll-A concentration, turbidity, and distance to various port types were all found to be significant predictors of the invader presence or abundance. Broadly speaking, these results indicate that anthropogenic disturbance appears to be more important for the Northern *M. galloprovincialis* invasion in New Zealand than predicted; and that this invader is likely not solely passively spreading via natural processes.

LITERATURE CITED

- Ab Rahim, E. S., Nguyen, T. T. T., Ingram, B., Riginos, C., Weston, K. J., & Sherman, C. D. H. (2016). Species composition and hybridisation of mussel species (Bivalvia: Mytilidae) in Australia. *Marine and Freshwater Research*, 67(12), 1955–1963. https://doi.org/10.1071/MF15307
- Apte, S., Holland, B. S., Godwin, L. S., & Gardner, J. P. A. (2000). Jumping ship: A stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. *Biological Invasions*, 2(1), 75–79. https://doi.org/10.1023/A:1010024818644
- Ardura, A., Juanes, F., Planes, S., & Garcia-Vazquez, E. (2016). Rate of biological invasions is lower in coastal marine protected areas. *Scientific Reports*, 6(December 2015), 1–11. https://doi.org/10.1038/srep33013
- Ardura, A., Planes, S., & Garcia-Vazquez, E. (2015). Aliens in Paradise. Boat density and exotic coastal mollusks in Moorea Island (French Polynesia). *Marine Environmental Research*, 112, 56–63. https://doi.org/10.1016/j.marenvres.2015.08.007
- Astorga, M. P., Cardenas, L., & Vargas, J. (2015). Phylogenetic Approaches to Delimit Genetic Lineages of the *Mytilus* Complex of South America: How Many Species are there? *Journal of Shellfish Research*, 34(3), 919–930. https://doi.org/10.2983/035.034.0322
- Astorga, M. P., Vargas, J., Valenzuela, A., Molinet, C., & Marín, S. L. (2018). Population genetic structure and differential selection in mussel *Mytilus* chilensis. *Aquaculture Research*, 49(2), 919–927. https://doi.org/10.1111/are.13538
- Azpeitia, K., Rodríguez-Ezpeleta, N., & Mendiola, D. (2019). Settlement and recruitment pattern variability of the mussel *Mytilus* galloprovincialis Lmk. from SE Bay of Biscay

(Basque Country). *Regional Studies in Marine Science*, 27, 100523. https://doi.org/10.1016/j.rsma.2019.100523

- Bao, W. Y., Satuito, C. G., Yang, J. L., & Kitamura, H. (2007). Larval settlement and metamorphosis of the mussel *Mytilus* galloprovincialis in response to biofilms. *Marine Biology*, 150(4), 565–574. https://doi.org/10.1007/s00227-006-0383-4
- Bayne, B. L. (1964). Primary and Secondary Settlement in *Mytilus* edulis L. (Mollusca)
 Author (s): B. L. Bayne Source : Journal of Animal Ecology, Vol. 33, No. 3 (Oct., 1964), pp. 513-523 Published by : British Ecological Society Stable URL : https://www.jstor.o. *Journal of Animal Ecology*, 33(3), 513–523.
- Bellard, C., Rysman, J. F., Leroy, B., Claud, C., & Mace, G. M. (2017). A global picture of biological invasion threat on islands. *Nature Ecology and Evolution*, 1(12), 1862–1869. https://doi.org/10.1038/s41559-017-0365-6
- Biosecurity NZ. (n.d.). Places of first arrival. Retrieved February 5, 2020, from https://www.mpi.govt.nz/importing/border-clearance/places-of-first-arrival/
- Bisson, P. A. (2006). Assessment of the risk of invasion of national forest streams in the *Pacific Northwest by farmed Atlantic salmon.*
- Bownes, S. J., & McQuaid, C. D. (2006). Will the invasive mussel *Mytilus* galloprovincialis Lamarck replace the indigenous *Perna Perna* L. on the south coast of South Africa? *Journal of Experimental Marine Biology and Ecology*, 338(1), 140–151. https://doi.org/10.1016/j.jembe.2006.07.006
- Braby, C. E., & Somero, G. N. (2006). Ecological gradients and relative abundance of native (*Mytilus* trossulus) and invasive (*Mytilus* galloprovincialis) blue mussels in the California hybrid zone. *Marine Biology*, 148(6), 1249–1262. https://doi.org/10.1007/s00227-005-0177-0
- Bracken, J., Gust, N., Ross, J., & Coutts, A. (2016). An assessment of the efficacy of chemical descalers for managing non-indigenous marine species within vessel internal seawater systems and niche areas. *Management of Biological Invasions*, 7(3), 241–256. https://doi.org/10.3391/mbi.2016.7.3.04
- Brannock, P. M., & Hilbish, T. J. (2010). Hybridization results in high levels of sterility and restricted introgression between invasive and endemic marine blue mussels. *Marine Ecology Progress Series*, 406, 161–171. https://doi.org/10.3354/meps08522
- Brannock, P. M., Wethey, D. S., & Hilbish, T. J. (2009). Extensive hybridization with minimal introgression in *Mytilus* galloprovincialis and m. trossulus in Hokkaido, Japan. *Marine Ecology Progress Series*, 383, 161–171. https://doi.org/10.3354/meps07995
- Carlton, J. T. (2011). The Global Dispersal of Marine and Estuarine Crustaceans.
- Chapman, J. W., Breitenstein, R. A., & Carlton, J. T. (2013). Port-by-port accumulations and dispersal of hull fouling invertebrates between the Mediterranean Sea, the Atlantic

Ocean and the Pacific Ocean. *Aquatic Invasions*, 8(3), 249–260. https://doi.org/10.3391/ai.2013.8.3.01

- Clarke Murray, C., Pakhomov, E. A., & Therriault, T. W. (2011). Recreational boating: A large unregulated vector transporting marine invasive species. *Diversity and Distributions*, *17*(6), 1161–1172. https://doi.org/10.1111/j.1472-4642.2011.00798.x
- Clarke Murray, C., Therriault, T. W., & Martone, P. T. (2012). Adapted for invasion? Comparing attachment, drag and dislodgment of native and nonindigenous hull fouling species. *Biological Invasions*, 14(8), 1651–1663. https://doi.org/10.1007/s10530-012-0178-0
- Clarke, K. R., & Warwick, R. M. (1994). Change in marine communities. An Approach to Statistical Analysis and Interpretation. Marine Laboratory, Plymouth.
- Clarke, K. R., & Gorley, R. N. (2015). PRIMER v7. PRIMER-E: Plymouth, Plymouth Marine Laboratory.
- Cornelisen, C. D., Gillespie, P. A., Kirs, M., Young, R. G., Forrest, R. W., Barter, P. J., ... Harwood, V. J. (2011). Motueka River plume facilitates transport of ruminant faecal contaminants into shellfish growing waters, Tasman Bay, New Zealand. New Zealand Journal of Marine and Freshwater Research, 45(3), 477–495. https://doi.org/10.1080/00288330.2011.587822
- Coutts, A. D. M., Piola, R. F., Hewitt, C. L., Connell, S. D., & Gardner, J. P. A. (2010). Effect of vessel voyage speed on survival of biofouling organisms: Implications for translocation of non-indigenous marine species. *Biofouling*, 26(1), 1–13. https://doi.org/10.1080/08927010903174599
- Crawley, M. J. (2012). STATISTICS. In An introduction using R (Vol. 66).
- Crego-Prieto, V., Ardura, A., Juanes, F., Roca, A., Taylor, J. S., & Garcia-Vazquez, E. (2015). Aquaculture and the spread of introduced mussel genes in British Columbia. *Biological Invasions*, 17(7), 2011–2026. https://doi.org/10.1007/s10530-015-0853-z
- Didham, R. K., Tylianakis, J. M., Gemmell, N. J., Rand, T. A., & Ewers, R. M. (2007). Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology and Evolution*, 22(9), 489–496. https://doi.org/10.1016/j.tree.2007.07.001

Environment, N. Z. M. for the. Hazardous Substances and New Organisms act., (1996).

- Floerl, O., & Coutts, A. (2009). Potential ramifications of the global economic crisis on human-mediated dispersal of marine non-indigenous species. *Marine Pollution Bulletin*, 58(11), 1595–1598. https://doi.org/10.1016/j.marpolbul.2009.08.003
- Forrest, B. M., & Atalah, J. (2017). Significant impact from blue mussel *Mytilus* galloprovincialis biofouling on aquaculture production of green-lipped mussels in New Zealand. *Aquaculture Environment Interactions*, 9(1), 115–126. https://doi.org/10.3354/aei00220

- Forrest, B. M., Gardner, J. P. A., & Taylor, M. D. (2009). Internal borders for managing invasive marine species. *Journal of Applied Ecology*, 46(1), 46–54. https://doi.org/10.1111/j.1365-2664.2008.01544.x
- García-Souto, D., Sumner-Hempel, A., Fervenza, S., Pérez-García, C., Torreiro, A., González-Romero, R., ... Pasantes, J. J. (2017). Detection of invasive and cryptic species in marine mussels (Bivalvia, Mytilidae): A chromosomal perspective. *Journal* for Nature Conservation, 39, 58–67. https://doi.org/10.1016/j.jnc.2017.07.005
- Gardner, J. P. A., Oyarzún, P., Toro, J., Wenne, R., & Zbawicka, M. (2020). Phylogeography of Southern hemisphere blue mussels of the genus *Mytilus*: evolution, biosecurity, aquaculture, and food labelling. *Oceanography and Marine Biology: Annual Reviews, in press.*
- Gardner, J. P. A. (2004). A historical perspective of the genus *Mytilus* (Bivalvia: Mollusca) in New Zealand: Multivariate morphometric analyses of fossil, midden and contemporary blue mussels. *Biological Journal of the Linnean Society*, *82*(3), 329–344. https://doi.org/10.1111/j.1095-8312.2004.00362.x
- Gardner, J. P. A. (2013). Bottom-up control of temperate rocky intertidal community structure: Evidence from a transplant experiment. *Marine Ecology Progress Series*, 491, 137–151. https://doi.org/10.3354/meps10456
- Gardner, J. P. A. (2002). Effects of seston variability on the clearance rate and absorption efficiency of the mussels Aulacomya maoriana, Mytilus galloprovincialis and Perna canaliculus from New Zealand. 268, 83–101.
- Gardner, J. P. A. (2000). Where are the mussels on Cook Strait (New Zealand) shores? Low seston quality as a possible factor limiting multi-species distributions. *Marine Ecology Progress Series*, *194*, 123–132. https://doi.org/10.3354/meps194123
- Gardner, J. P. A., & Thompson, R. J. (2001). The effects of coastal and estuarine conditions on the physiology and survivorship of the mussels *Mytilus* edulis, M. trossulus and their hybrids. *Journal of Experimental Marine Biology and Ecology*, 265(2), 119–140. https://doi.org/10.1016/S0022-0981(01)00328-8
- Gardner, J. P. A., & Thompson, R. J. (2001). Naturally low seston concentration and the net energy balance of the greenshell mussel (*Perna* canaliculus) at Island Bay, Cook Strait, New Zealand. New Zealand Journal of Marine and Freshwater Research, 35(3), 457– 468. https://doi.org/10.1080/00288330.2001.9517015
- Gardner, J. P. A., Zbawicka, M., Westfall, K. M., & Wenne, R. (2016). Invasive blue mussels threaten regional scale genetic diversity in mainland and remote offshore locations: the need for baseline data and enhanced protection in the Southern Ocean. *Global Change Biology*, 22(9), 3182–3195. https://doi.org/10.1111/gcb.13332
- Gosling, E. (1992). Systematics and geographic distribution of *Mytilus*. *Development in Aquaculture and Fisheries Science*, 25.

- Hayward, B. W., Sabaa, A. T., Kolodziej, A., Crundwell, M. P., Steph, S., Scott, G. H., ... Grenfell, H. R. (2012). Planktic foraminifera-based sea-surface temperature record in the Tasman Sea and history of the Subtropical Front around New Zealand, over the last one million years. *Marine Micropaleontology*, 82–83, 13–27. https://doi.org/10.1016/j.marmicro.2011.10.003
- Helson, J. G., & Gardner, J. P. A. (2007). Variation in mussel scope for growth: a test of intertidal community structure. *Hydrobiologia*, 586, 373–392.
- Helson, J. G., Pledger, S., & Gardner, J. P. A. (2007). Does differential particulate food supply explain the presence of mussels in Wellington Harbour (New Zealand) and their absence on neighbouring Cook Strait shores? *Estuarine, Coastal and Shelf Science*, 72(1–2), 223–234. https://doi.org/10.1016/j.ecss.2006.10.015
- Hewitt, C. L., Willing, J., Bauckham, A., Cassidy, A. M., Cox, C. M. S., Jones, L., & Wotton, D. M. (2004). New Zealand marine biosecurity: Delivering outcomes in a fluid environment. *New Zealand Journal of Marine and Freshwater Research*, 38(3), 429– 438. https://doi.org/10.1080/00288330.2004.9517250
- Hilbish, T. J., Mullinax, A., Dolven, S. I., Meyer, A., Koehn, R. K., & Rawson, P. D. (2000). Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): Routes and timing of transequatorial migration. *Marine Biology*, 136(1), 69–77. https://doi.org/10.1007/s002270050010
- Hopkins, G. A., Forrest, B. M., Jiang, W., & Gardner, J. P. A. (2011). Successful eradication of a non-indigenous marine bivalve from a subtidal soft-sediment environment. *Journal* of Applied Ecology, 48(2), 424–431. https://doi.org/10.1111/j.1365-2664.2010.01941.x
- Hopkins, G. A., Forrest, B. M., Piola, R. F., & Gardner, J. P. A. (2011). Factors affecting survivorship of defouled communities and the effect of fragmentation on establishment success. *Journal of Experimental Marine Biology and Ecology*, 396(2), 233–243. https://doi.org/10.1016/j.jembe.2010.10.027
- Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., ... Torda, G. (2018). Global warming transforms coral reef assemblages. *Nature*, 556(7702), 492– 496. https://doi.org/10.1038/s41586-018-0041-2
- Lee, J. E., & Chown, S. L. (2007). *Mytilus* on the move: Transport of an invasive bivalve to the Antarctic. *Marine Ecology Progress Series*, 339, 307–310. https://doi.org/10.3354/meps339307
- Leopold, P., Renaud, P. E., Ambrose, W. G., & Berge, J. (2019). High Arctic *Mytilus* spp.: occurrence, distribution and history of dispersal. *Polar Biology*, *42*(1), 237–244. https://doi.org/10.1007/s00300-018-2415-1
- Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). 100 of the World'S Worst Invasive Species. *Aliens*, 12, s1–s12.

Martin, S. (2014). An Introduction to Ocean Remote Sensing.

- Mathiesen, S. S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., ... Nielsen, E. E. (2017). Genetic diversity and connectivity within *Mytilus* spp. in the subarctic and Arctic. *Evolutionary Applications*, 10(1), 39–55. https://doi.org/10.1111/eva.12415
- McDonald, J. H., Seed, R., & Koehn, R. K. (1991). Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern hemispheres. *Marine Biology*, *111*(3), 323–333. https://doi.org/10.1007/BF01319403
- McGeoch, M. A., Butchart, S. H. M., Spear, D., Marais, E., Kleynhans, E. J., Symes, A., ... Hoffmann, M. (2010). Global indicators of biological invasion: Species numbers, biodiversity impact and policy responses. *Diversity and Distributions*, 16(1), 95–108. https://doi.org/10.1111/j.1472-4642.2009.00633.x
- McGeoch, M. A., Genovesi, P., Bellingham, P. J., Costello, M. J., McGrannachan, C., & Sheppard, A. (2016). Prioritizing species, pathways, and sites to achieve conservation targets for biological invasion. *Biological Invasions*, 18(2), 299–314. https://doi.org/10.1007/s10530-015-1013-1
- Miller, J. A., Carlton, J. T., Chapman, J. W., Geller, J. B., & Ruiz, G. M. (2018). Transoceanic dispersal of the mussel *Mytilus* galloprovincialis on Japanese tsunami marine debris: An approach for evaluating rafting of a coastal species at sea. *Marine Pollution Bulletin*, 132(October 2017), 60–69. https://doi.org/10.1016/j.marpolbul.2017.10.040
- Ministry for the Environment. (n.d.). NZ Coastal Hydrosystems. Retrieved February 5, 2020, from https://data.mfe.govt.nz/layer/53565-nz-coastal-hydrosystems/
- Montgomery, D., Peck, E., & Geoffrey, G. (2012). *Introduction to Linear Regression Analysis* (4th ed.). John Wiley & Sons.
- Murphy, P. M. (1970). The temporal variability in biotic indices. *Environmental Pollution*, *17*(3), 227–236.
- NIWA. (2017). Extreme Wave Indices for New Zealand Coastal and Oceanic Waters. Retrieved from https://www.mfe.govt.nz/sites/default/files/media/Marine/extreme-waveindices-for-nz-coastal-and-oceanic-waters.pdf
- Nixon, S. (1995). Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia*, *41*(1), 199–219.
- Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... Wagner, H. (2019). *vegan: Community Ecology Package*. Retrieved from https://cran.r-project.org/package=vegan
- Oyarzún, P. A., Toro, J. E., Cañete, J. I., & Gardner, J. P. A. (2016). Bioinvasion threatens the genetic integrity of native diversity and a natural hybrid zone: Smooth-shelled blue mussels (*Mytilus* spp.) in the Strait of Magellan. *Biological Journal of the Linnean Society*, 117(3), 574–585. https://doi.org/10.1111/bij.12687

- Palumbi, S. R. (1996). Nucleic acids II: the polymerase chain reaction. *Molecular Systematics*, 205–247.
- Perrings, C., Williamson, M., Barbier, E. B., Delfino, D., Dalmazzone, S., Shogren, J., ... Watkinson, A. (2002). Biological invasion risks and the public good: An economic perspective. *Ecology and Society*, 6(1). https://doi.org/10.5751/es-00396-060101
- Pyšek, P., Jarošík, V., Hulme, P. E., Kühn, I., Wild, J., Arianoutsou, M., ... Winter, M. (2010). Disentangling the role of environmental and human pressures on biological invasions across Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 107(27), 12157–12162. https://doi.org/10.1073/pnas.1002314107
- R Core Team. (2019). *R: A language and environment for statistical computing*. Retrieved from https://www.r-project.org/
- Rawson, P. D., & Hilbish, T. J. (1995). Evolutionary relationships among the male and female mitochondrial DNA lineages in the *Mytilus* edulis species complex. *Molecular Biology and Evolution*, 893–901. https://doi.org/10.1093/oxfordjournals.molbev.a040266
- Sakaguchi, I. (2003). An Overview of the Antifouling Technologies in Power Plant Cooling Water Systems. *The Sessile Organisms Society of Japan*, 20(1), 15–19.
- Seed, R. (1969). The ecology of *Mytilus* edulis L. (Lamellibranchiata) on exposed rocky shores - I. Breeding and settlement. *Oecologia*, 3(3–4), 277–316. https://doi.org/10.1007/BF00390380
- Simon, A., Arbiol, C., Nielsen, E. E., Couteau, J., Sussarellu, R., Burgeot, T., ... Bierne, N. (2019). Replicated anthropogenic hybridisations reveal parallel patterns of admixture in marine mussels. In *bioRxiv*. https://doi.org/10.1101/590737
- Springer, S. A., & Crespi, B. J. (2007). Adaptive gamete-recognition divergence in a hybridizing *Mytilus* population. *Evolution*, 61(4), 772–783. https://doi.org/10.1111/j.1558-5646.2007.00073.x
- Stuckas, H., Stoof, K., Quesada, H., & Tiedemann, R. (2009). Evolutionary implications of discordant clines across the Baltic *Mytilus* hybrid zone (*Mytilus* edulis and *Mytilus* trossulus). *Heredity*, 103(2), 146–156. https://doi.org/10.1038/hdy.2009.37
- Svane, I. (2011). An overview of the blue mussel in Southern Australia A serial invader, a blind passenger, or just a welcome addition to the menu? *Transactions of the Royal Society of South Australia*, 135(2), 134–139. https://doi.org/10.1080/03721426.2011.10887153
- Thresher, R. E., & Kuris, A. M. (2004). Options for managing invasive marine species. *Biological Invasions*, 6(3), 295–300. https://doi.org/10.1023/B:BINV.0000034598.28718.2e
- Thyrring, J., Jensen, K. T., & Sejr, M. K. (2017). Gametogenesis of an intertidal population of *Mytilus* trossulus in NW Greenland: Not a limitation for potential Arctic range

expansion. *Marine Ecology Progress Series*, 574, 65–74. https://doi.org/10.3354/meps12179

- Westfall, K. M., & Gardner, J. P. A. (2013). Interlineage *Mytilus* galloprovincialis Lmk. 1819 hybridization yields inconsistent genetic outcomes in the Southern hemisphere. *Biological Invasions*, 15(7), 1493–1506. https://doi.org/10.1007/s10530-012-0385-8
- Westfall, K. M., & Gardner, J. P. A. (2010). Genetic diversity of Southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. *Biological Journal of the Linnean Society*, 101(4), 898–909. https://doi.org/10.1111/j.1095-8312.2010.01549.x
- Wilson, J., Matejusova, I., McIntosh, R. E., Carboni, S., & Bekaert, M. (2018). New diagnostic SNP molecular markers for the *Mytilus* species complex. *PLoS ONE*, 13(7), 1–13. https://doi.org/10.1371/journal.pone.0200654
- Wonham, M. J. (2004). Mini-review: Distribution of the Mediterranean mussel *Mytilus* galloprovincialis (Bivalvia: Mytilidae) and hybrids in the northeast Pacific. *Journal of Shellfish Research*, 23(2), 535–543.
- WoRMS Editorial Board. (2020). World Register of Marine Species. Retrieved February 4, 2020, from http://www.marinespecies.org
- Zardi, G. I., McQuaid, C. D., Jacinto, R., Lourenço, C. R., Serrão, E. A., & Nicastro, K. R. (2018). Re-assessing the origins of the invasive mussel *Mytilus* galloprovincialis in southern Africa. *Marine and Freshwater Research*, 69(4), 607–613. https://doi.org/10.1071/MF17132
- Zardi, G. I., McQuaid, C. D., & Nicastro, K. R. (2007). Balancing survival and reproduction: Seasonality of wave action, attachment strength and reproductive output in indigenous *Perna Perna* and invasive *Mytilus* galloprovincialis mussels. *Marine Ecology Progress Series*, 334, 155–163. https://doi.org/10.3354/meps334155
- Zbawicka, M., Gardner, J. P. A., & Wenne, R. (2019). Cryptic diversity in smooth-shelled mussels on Southern Ocean islands: connectivity, hybridisation and a marine invasion. *Frontiers in Zoology*, *16*(1), 1–12. https://doi.org/10.1186/s12983-019-0332-y
- Zbawicka, M., Trucco, M. I., & Wenne, R. (2018). Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels:
 Hybridization with invasive European *Mytilus* galloprovincialis. *Genetics Selection Evolution*, 50(1), 1–14. https://doi.org/10.1186/s12711-018-0376-z
- Zheng, G., & DiGiacomo, P. M. (2017). Uncertainties and applications of satellite-derived coastal water quality products. *Progress in Oceanography*, *159*(December 2016), 45–72. https://doi.org/10.1016/j.pocean.2017.08.007
- Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1(1), 3–14. <u>https://doi.org/10.1111/j.2041-210x.2009.00001.x</u>

Chapter 3: Increased Habitat Availability on Mussel Aquaculture Farms Leads to Increased Population Sizes for both Native and Invasive Species

ABSTRACT: Aquaculture is recognized as a primary mode of spread and secondary mode of introduction of marine non-indigenous species (NIS). The blue mussel Mytilus galloprovincialis, one of the most prolific invasive species globally, is one NIS that is highly likely to invade regions where it is grown for aquaculture. What is less understood is what effect aquaculture has on the spread and establishment of M. galloprovincialis distribution and abundance in regions where it is not farmed, such as the Marlborough Sounds, New Zealand, where *M. galloprovincialis* causes USD\$11.4 million per year to the native Perna canaliculus aquaculture industry. It has been speculated that an observed increase of M. galloprovincialis in this region is due to increasing provision of habitat by increased aquaculture activity. Another unknown is what amount of this economic damage is caused by the invasive Northern M. galloprovincialis versus the native Southern M. galloprovincialis. These unknowns are addressed in this chapter via two distinct lines of sampling and analysis: 1) M. galloprovincialis samples were collected from 5 aquaculture facilities and 5 adjacent rocky reef sites, with a total of 500 samples taken. A 16s mitochondrial rDNA RFLP assay was then performed to identify individuals were of Northern or Southern lineage. 2) Field surveys were conducted at 33 sites which were either aquaculture facilities, adjacent reef sites or distant to aquaculture facilities. These surveys were conducted during the austral summer and winter of 2018. They collected M. galloprovincialis coverage data as a proxy for population size, substratum type and slope for reef sites, and Secchi disk depth. The relative abundance data were tested with a Permutational ANOVA, while the field survey data were modelled with a generalized linear model. The results show that there was no significant difference for Northern or Southern lineage abundances between aquaculture facilities and adjacent reef sites; that there was significantly higher *M. galloprovincialis* coverage on aquaculture facilities than reef sites; and slope of the reef and percent cover of bedrock substratum were significantly associated to greater *M. galloprovincialis* coverage at reef sites. These results suggest that selection pressure is acting evenly on the two lineages of M. galloprovincialis, and also support the speculation that aquaculture farms provide an ideal habitat for the proliferation of blue mussels, which could promote the establishment and spread of *M. galloprovincialis* in the region.

3.1 INTRODUCTION

As aquaculture continues to grow on a global scale, it is increasingly important to assess its ecological impacts for both the protection of the marine environment and the continued financial stability of the aquaculture industry. With fisheries yield decreasing on a global scale (Zeller & Pauly, 2005; Worm et al. 2009; Pauly & Zeller, 2016, 2019; FAO, 2018), aquaculture has been expanding, having a positive effect by relieving pressure on wild fisheries (Naylor et al. 2002, FAO 2018). However, aquaculture is also associated with negative effects such as eutrophication and introduction of non-indigenous species (NIS) introduction (Naylor et al. 2001; Savini et al. 2010; Crego-Prieto et al. 2015). In some instances, these NIS also cause moderate to severe economic damage to the associated aquaculture crops (Forrest & Atalah, 2017). By implementing effective mitigation measures, aquaculture managers will both reduce negative ecological impacts and increase the profitability of their facilities. However, as these effects can be highly variable, the design and implementation of informed mitigation measures requires a detailed knowledge of the impacts of NIS on crops.

Aquaculture has been identified as a primary vector of biological invasion and postinvasion spread (Cook et al. 2008; Crego-Prieto et al. 2015). Often NIS are deliberately introduced for aquaculture production (Handley, 1995; Handley & Bergquist, 1997; Bersine et al. 2008; Kochmann et al. 2012). However, in most cases NIS are accidentally introduced by aquaculture associated vector of introduction, such as vessel movement, stock and gear transfer. In this case NIS are capable of inflicting considerable economic damage to the industry (Lee & Gordon, 2006; Forrest & Atalah, 2017; Soliman & Inglis, 2018) as in many cases aquaculture facilities create favourable habitat for proliferation of NIS (Banister et al. 2019). For example, in suspended shellfish culture systems this damage is typically through biofouling of an NIS pest, than can outcompete or directly displace the crop species (Fitridge et al. 2012; Soliman & Inglis, 2018; Banister et al. 2019). This is occurring in New Zealand, where the endemic greenshell mussel *Perna canaliculus* (hereafter *Perna*) aquaculture industry is suffering economic losses caused by the blue mussel *Mytilus galloprovincialis* (Forrest & Atalah, 2017).

The Marlborough Sounds is the primary region for *Perna* aquaculture in New Zealand, accounting for approximately 57% of national *Perna* production, with a value of NZD\$317 in 2018 (Forrest & Atalah, 2017; New Zealand Sustainable Aquaculture, 2018). These farms follow a typical shellfish culture design of suspended lines on which the crop is grown (Jeffs et al. 1999). The wild-caught *Perna* spat utilized by the industry comes from two main sources, either Ninety Mile Beach near Kaitaia, or the top of the South Island (TOS), which vary significantly in crop yield (Atalah et al. 2017; Forrest & Atalah, 2017).

M. galloprovincialis is a major biofouling pest for New Zealand's mussel industry, affecting all production stages. Forrest & Atalah (2017) reported that *M. galloprovincialis* causes roughly \$16 million USD in damage per year to the *Perna* aquaculture industry in the Marlborough Sounds region. Whilst there are multiple types of economic damage which *M. galloprovincialis* inflicts on the *Perna* industry in this region, USD\$11.4 million is from direct *Perna* crop loss (Forrest & Atalah, 2017). This is mainly from *M. galloprovincialis* displacing adult *Perna*, primarily in the top 6m of the crop line, but also negatively affects spat catch, harvest, and marketability. This is likely through secondary settlement competition between the two species, as secondary settlement is typically more important for final adult mussel distribution (Bayne, 1964; Seed, 1969; Bao et al. 2007; Azpeitia et al. 2019). Competitive outcome appears to be influenced by depth, likely in relation to the halocline (Forrest & Atalah, 2017).

Atalah et al. (2017) designed a web application for aquaculture managers to use to predict *M. galloprovincialis* settlement in the Marlborough Sounds, allowing them to optimize their plans to avoid over-settlement by blue mussels. They utilized a dataset generated by the New Zealand Marine Farming Association, with data dating back to when the *Perna* aquaculture began in the Marlborough Sounds in the 1970's. This data set includes *M. galloprovincialis* coverage as a proxy for population size at aquaculture facilities in the Marlborough Sounds. The authors showed that *M. galloprovincialis* spat abundance has been increasing since the 1970's but at a much more rapid rate since 1994 (Atalah et al. 2017).

While the model utilized by this web application and described by Atalah et al. (2017) is very good for describing short term trends in *M. galloprovincialis* settlement with respect to environmental variables, it was unable to account for the overall long-term increase of *M. galloprovincialis* coverage. The authors speculate that this is likely due to a dramatic increase in artificial habitat associated with the large number of suspended shellfish culture farms in the region, which have increased in area from roughly 100 hectares to 2500 hectares over the past 40 years (Atalah et al. 2017). That is, the aquaculture facilities create a favourable habitat not just for the *Perna* crop but also for biofouling pests such as *M. galloprovincialis*, promoting their establishment and spread into adjacent natural areas. Furthermore, this growing population supported by the farms constitute a reservoir of larvae, essentially creating a positive feedback loop that results in auto-recruitment (Warner & Cowen, 2002; Levin, 2006; Gilg et al. 2007).

Whilst *M. galloprovincialis* is well documented to cause losses at New Zealand aquaculture farms, and is also documented to be increasing in coverage at many locations, the situation is confused because *M. galloprovincialis* in New Zealand appears to be comprised of two morphologically indistinguishable lineages (Gardner et al. 2004; Westfall et al. 2010;

Westfall & Gardner, 2010, 2013; Gardner & Westfall, 2012; Gardner et al. 2016; Gardner et al. 2020). One lineage is native to the Southern hemisphere, including New Zealand (hereafter referred to as the Southern *M. galloprovincialis*, or MgS), and the other is native to the Northern hemisphere (hereafter referred to as the Northern *M. galloprovincialis*, or MgN). MgN is recognized as one of the most invasive species globally (Lowe et al. 2000). This is primarily through accidental introduction via the biofouling of international shipping traffic or intentional introduction for aquaculture (Apte et al. 2000; Bownes & McQuaid, 2006; Clarke Murray et al. 2012; Crego-Prieto et al. 2015). The taxonomy and systematics of these two lineages are presently unclear and receiving a great deal of research attention (Gardner et al. 2016, 2020).

Briefly, *M. galloprovincialis* is a member of the *M. edulis* species complex, which consists of several closely related smooth shelled blue mussel species *M. edulis* (Linnaeus 1758), and *M. trossulus* (Gould 1850) and *M. galloprovincialis* (Hilbish et al. 2000; Gérard et al. 2008; Westfall & Gardner, 2010; Astorga et al. 2015). Every member of this species complex is able to hybridize with every other member resulting in geospatially explicit regions referred to as hybrid zones or mosaic zone, but specific hybridization patterns and degree of introgression are unique to each hybrid zone (Shields et al. 2010; Oyarzún et al. 2016; Zbawicka et al. 2018). These species and their hybrids have been extensively studied in the Northern hemisphere, but much less so in the Southern hemisphere. This has led to an unclear understanding of *Mytilus* taxonomy in the Southern hemisphere, which is repeatedly revised, most recently by Gardner et al. (2020), and the reason for the taxonomic terminology utilized in this thesis. By referring to MgN and MgS in this chapter I follow the terminology employed by Westfall et al. (2010), Westfall & Gardner (2010), Westfall & Gardner (2013), and Gardner et al. (2016).
Westfall et al. (2010) developed a 16s mitochondrial rDNA RFLP assay that is diagnostic for delimiting MgN and MgS individuals. Westfall & Gardner (2010) then utilized this RFLP to describe MgN distribution across mainland New Zealand and its offshore islands and found that this invader is present in many areas but has a much larger population in the north of the North Island. Gardner et al. (2016) found that there is a higher degree of introgression between MgN and MgS in New Zealand than is reported elsewhere in the Southern hemisphere, and thus the invasive MgN is threatening the genetic diversity of the native MgS.

Both Atalah et al. (2017) and Forrest & Atalah (2017) acknowledge the existence two lineages of *M. galloprovincialis* in New Zealand but do not explicitly test them. Also, Northern *M. galloprovincialis* invasion is also often associated with aquaculture activity in regions where it is the targeted aquaculture species (Crego-Prieto et al. 2015). Given that MgN is invasive in New Zealand but is not grown for aquaculture directly, there is a unique opportunity to study the effects of aquaculture on MgN invasion in New Zealand. Therefore, the focus of this chapter is to: 1) explicitly test for differences in MgN and MgS relative abundance in the Marlborough Sounds, 2) Compare the prevalence and distribution of *M. galloprovincialis* between aquaculture facilities (farms) versus adjacent shallow rocky reef systems and 3) identify factors associated to the prevalence of *M. galloprovincialis* in natural habitats, irrespective of their lineage.

3.2 METHODS

The experimental design of this project consists of two distinct parts, the determination of MgN relative abundance, and the combined coverage of both MgN and MgS. For these two parts of the project, sample sites, methods, and statistical analyses are independent of each other.

3.2.1 MGN RELATIVE ABUNDANCE

A 16s rDNA RFLP assay was conducted according to Westfall et al. (2010), using the same samples and methods as described in Chapter 2. For Chapter 2, these samples were added to the existing data set generated by Westfall & Gardner (2010) and were utilized to assess the MgN invasion on a national scale. Here, these samples are considered on their own to assess the MgN invasion on a regional scale in the Marlborough Sounds, focusing on the effects of MgN and MgS on *Perna* aquaculture. Section 3.2.1 is the same as section 2.2.1 in Chapter 2 and is included here for completeness.

3.2.1.1 ADDITION OF NEW SAMPLES

M. galloprovincialis samples were collected from 10 sites, 5 *Perna* aquaculture facilities and 5 adjacent shallow rocky reef sites (Figure 3.1). 50 *M. galloprovincialis* were collected from each site, for a total of n = 500 samples. These samples were collected by Cawthron Institute staff, preserved in 90% ethanol, and sent to Victoria University of Wellington, New Zealand, for analysis.

3.2.1.2 DNA EXTRACTION AND PURIFICATION

DNA extraction and purification were performed with a Geneaid Genomic DNA Mini Kit (Tissue), following the manufacturer's instructions. 30 mg of mantle tissue was dissected from each sample and added to a microcentrifuge tube with 200 μ L of GT buffer. 20 μ L of proteinase K was added to each sample, then incubated at 60°C for a minimum of 30 minutes. 200 μ L of GT buffer was added, vortexed until homogenous, then incubated at 60°C for a minimum of 20 minutes. RNA degradation was not performed. 200 μ L of absolute ethanol was then added and vortexed until homogeneous. Each sample was then transferred to a GS column and centrifuged at 14,000 RPM for 2 minutes. The flow-through was then discarded, and 400

 μ L of W1 buffer was added. Samples were then centrifuged again at 14,000 RPM for 2 minutes. Flow-through was again discarded. 600 μ L of Wash Buffer, with ethanol, was added and samples were centrifuged at 14,000 RPM for 30 seconds. Flow-through was again discarded, and samples were then dried by centrifuging at 14,000 RPM for 3 minutes. GS columns were then transferred to a clean microcentrifuge tube. 100 μ L of Elution Buffer, heated to 60°C, was then added and left for a minimum of 5 minutes. Samples were then centrifuged at 14,000 RPM for 30 seconds, yielding the final DNA samples used.

DNA samples were then tested with a Nanodrop for DNA concentration and purity. A 260/280 wavelength ratio near 1.8 was considered to be clean. Samples with ratios outside of 1.7 to 1.9 had DNA extraction performed again. DNA concentrations were adjusted to near 50 ng/ μ L, then tested again to confirm correct concentrations for PCR.

3.2.1.3 PCR AND RFLP ASSAY

The 16s PCR and RFLP protocol described by Westfall et al. (2010) was followed. A 527 bp fragment of the mitochondrial 16s rDNA gene was amplified. A 25 μ L PCR reaction was made using 12.5 μ L of MyTaq Red Mix, 1 μ L each of 16sAR and 16sBR primers (Palumbi, 1996), 8.5 μ L ddH₂O, and 2 μ L of DNA template of each sample. Samples were then amplified under the following PCR protocol. Initial denaturation for 3 minutes at 95°C; 30 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 52°C, and extension for 45 seconds at 72°C; and a final extension of 3 minutes at 72°C.

Following Westfall et al. (2010) the 16s RFLP assay was then performed. A 20 μ L double restriction digest reaction was made using 5 μ L ddH₂O, 2 μ L red buffer, 1 μ L loading buffer, 1 μ L each of the restriction enzymes Eco321 and Nhe1 (Westfall et al. 2010), and 10 μ L of PCR product for each sample. These samples were then incubated at 37°C for a minimum

of 1 hour. Digestion products were scored on a 2% agarose gel using RedSafe nucleic acid staining solution. Southern hemisphere *M. galloprovincialis* individuals were determined by fragment lengths of 342, 167, and 28 bp; Northern hemisphere *M. galloprovincialis* were determined by fragments lengths of 342, 195, and 12 bp. These scores were then used to generate the two response variables of presence-absence of Northern *M. galloprovincialis*, and relative abundance of Northern *M. galloprovincialis*. Relative abundance was calculated by dividing the number of MgN individuals by the total number of samples.

3.2.2 REGIONAL DISTRIBUTION OF *M. GALLOPROVINCIALIS* IN MARLBOROUGH SOUNDS

Field surveys were conducted by Cawthron Institute staff to describe the cover of *M. galloprovincialis*, both MgN and MgS, across Marlborough Sounds. Coverage was described for both *Perna* aquaculture artificial structures and natural hard-bottom habitats, both adjacent and distant to farms (Figure 3.1). *M. galloprovincialis* data were recorded as percent cover as a proxy of population size, following the data set described in Atalah et al. (2017). Surveys were performed in 2018 during the Austral Summer (February 2018) and repeated in the Austral Winter (August 2018). Each survey collected Secchi Disk depth (m), which is the depth at which a Secchi disk was no longer visible, thus representing turbidity.

Within each of three regions within the Pelorus Sounds (inner, mid and outer) haphazardly selected bays with and without/few marine farms were surveyed between 0 - 5 m depth. Surveys on natural habitats were carried out on bedrock, boulder, or cobble habitats; surveys of blue mussels on *Perna* farms targeted crop lines and buoys. Sampling units consisted in 100 m² transects (2 x 50 m) sampled visually by snorkelers who observed 1 m on each side of the 50 m transect and estimated the percentage cover of blue mussels. Additionally, on natural habitats only, the reef slope and substratum type were recorded.

3.2.3 MODELLING AND STATISTICAL ANALYSES

Statistical testing was performed in three separate ways. This was because the MgN relative abundance and coverage data sets are separate from one another; and because for the coverage data set, substratum type and slope were only recorded for reef sites and thus could not be included in the model when farms were included. Therefore, the following statistical methods are broken into three sections; RFLP, which includes the data from the RFLP assays; COVERAGE – REEF, which does not include farm sites from the coverage surveys, and only tests the three substratum types and slope and *M. galloprovincialis* coverage; and COVERAGE – ALL, which includes all sites from the coverage surveys, farm and reef; and tests the variables of latitude, site type, and Secchi disk depth, against *M. galloprovincialis* coverage.

3.2.3.1 MGN RELATIVE ABUNDANCE

This data set consisted of 500 mussels from each of 10 sites, 5 farm and 5 reef (Table 3.1). The RFLP was able to identify 88.9% of the sampled mussels to Northern or Southern lineage type. The final data set consisted of 10 sites with MgN relative abundance (proportion) as a dependent variable, which was bounded between 0 and 1, and site type which is a categorical independent variable (farm or reef).

MgN relative abundance at farm and reef sites was tested with a one-way ANOVA. A Shapiro-Wilks test of normality was carried out and indicated that the data are not significantly different from a normal distribution.

3.2.3.2 DISTRIBUTION OF MYTILUS GALLOPROVINCIALIS IN NATURAL HABITATS

The data set for the *M. galloprovincialis* coverage, again both MgN and MgS, consisted of 33 sites with 1 to 4 transects each, and surveys in the summer and winter. This yielded a final data set with 68 samples. The dependent variable was *M. galloprovincialis* coverage,

which is a continuous variable and represented the proportion of substratum covered by M. galloprovincialis, ranging from 0.0 to 1.0. The independent variables were site type, season, latitude, longitude, Secchi disk depth, substratum type (3 categories - bedrock, boulder, cobble), and substratum slope (3 categories - gentle, moderate, steep). Site type is a categorical variable with three categories, farm, adjacent, and distant. Farm sites sampled the crop grow lines, adjacent sites were reef sites directly adjacent to a farm and sample natural substratum, and distant sites are reef sites that ranged from roughly 1 to 5 km away to the nearest farm site. At adjacent and distant sites, data for *M. galloprovincialis* coverage were tested as a function of substratum type and slope, and their interactive effect, at reef sites only as no such data exists for farm sites. Substratum type was represented by three continuous variables - boulder, bedrock, and cobble. Along each transect, the proportion of each of these substratum types was estimate by the snorkeler performing the transect survey, yielding 3 continuous variables. For example, if a 100 m long transect was half cobble and half boulder, then cobble would be 0.5, boulder would be 0.5, and bedrock would be 0.0 for that transect. The overall slope for each transect was also estimated, but due to a high degree of variable within each transect, slope was categorized as Gentle (0°- 30°), Moderate (30°- 60°), or Steep (60°- 90°), across the entire transect.

M. galloprovincialis coverage data were tested against these variables with a permutational ANOVA (PERMANOVA), using 999 permutations of the data. Each substratum type was tested with an interactive effect with slope. This was done in R v3.6.1, using the package Vegan v2.6 (Oksanen et al. 2019; R Core Team, 2019). Homogeneity of dispersion, the statistical assumption of PERMANOVA, was then tested using a permutation test for homogeneity of multivariate dispersions in the R package 'Vegan', again with 999 permutations (Oksanen et al. 2019). This test indicated that the dispersions are homogeneous, e.g. that the statistical assumptions of PERMANOVA have been met (Oksanen et al. 2019).

3.2.3.3 DISTRIBUTION OF *M. GALLOPROVINCIALIS* ACROSS ARTIFICAL AND NATURAL HABITATS

M. galloprovincialis coverage data were modelled as a function of latitude, season, site type, and Secchi disk depth. Initial data exploration indicated a geospatial pattern in *M. galloprovincialis* coverage with respect to latitude, which is why latitude was included in the model. Latitude, in decimal degrees, ranged from 40.85142°S to 41.20784°S. Season is a categorical variable, being either winter or summer. Site type is a categorical variable, being either farm or reef. Secchi disk depth is the depth at which a standard Secchi disk was no longer visible in the water column and is a continuous variable in meters.

To account for fixed and random effects, and as the dependent variable (proportion of *M. galloprovincialis* coverage) is bounded between 0 and 1, a generalized linear mixed model in the beta distribution family was utilized. First, a beta transformation was performed on the dependent variable. The initial model included all 4 independent variables, each with an interactive effect with site type, and site number as a random factor. A stepwise reduction of Akaike Information Criterion (AIC) was then performed to yield the final, most parsimonious model (Eqn. 1; Zuur et al. 2010, 2016).

$$C_i = -2.6565 + L_i: T_i + (1|site no.)$$
(Eqn. 1).

Where C_i is *M. galloprovincialis* coverage at site *i*, and L_i and T_i are latitude and site type at site *i*, with site number as a random factor. All other variables were not included in the final model. A histogram of the residuals was made to check for normality.

3.3 RESULTS

3.3.1 MGN RELATIVE ABUNDANCE

The 16s RFLP revealed that MgN relative abundances were roughly equal at farms (0.390) and at adjacent reef sites (0.368) (Table 1). ANOVA indicated that these values were not significantly different (P = 0.805). These results do not support the hypothesis that a difference exists in MgN relative abundance between farm and adjacent reef sites.

Table 3.1 Results of the 16s rDNA RFLP assay for sample sites within the Marlborough Sounds. Each site has n = 50 samples, for a total n = 500. As previously mentioned, this is the same Table as Table 2.2 and is included here for the readers convenience. Note that MgN:MgS composition is reported here as a percentage but was utilized as a proportion in statistical testing, i.e. bounded between 0 and 1.

Site Name	Туре	Latitude	Longitude	MgN (%)
Keneperu	Farm	-41.2081	173.9213	14.0%
Keneperu	Reef	-41.2071	173.9353	25.0%
Crail Bay	Farm	-41.1259	173.9601	23.8%
Crail Bay	Reef	-41.1050	173.9819	28.9%
Tawhitinui Reach, Rams Head	Farm	-41.0551	173.8957	28.9%
Tawhitinui Reach, Maud Island	Reef	-41.0294	173.8693	34.1%
Port Ligar	Farm	-40.9361	173.9755	34.9%
Port Ligar	Reef	-40.9325	173.9727	26.1%
Catherine Cove	Farm	-40.8667	173.9005	34.8%
Catherine Cove	Reef	-40.8687	173.9033	18.8%

3.3.2 DISTRIBUTION OF M. GALLOPROVINCIALIS IN NATURAL HABITATS

The PERMANOVA indicated that slope, bedrock, and the interactive effect between cobble and slope were significant in explaining *M. galloprovincialis* coverage at reef sites (Table 3.2). There was greater *M. galloprovincialis* coverage on steeper sloped substratum (mean coverage of 0.127), whereas moderate and gentle sloped sites had mean coverages of 0.048 and 0.037, respectively (Table 3.2). Proportion of bedrock substratum was also positively correlated with *M. galloprovincialis* coverage (Figure 3.4).



Figure 3.1 *M. galloprovincialis* coverage for sample sites of the field surveys, by site type. This Figure shows that 1) *M. galloprovincialis* coverage (%) is higher at farm sites than adjacent and distant reef sites, and 2) there is a geospatial pattern to *M. galloprovincialis* coverage at farm sites, with farms further south having higher coverage than those further north. Note that coverage is presented here as percentage, but which was transformed to proportions for statistical testing.

3.3.3 DISTRIBUTION OF *M. GALLOPROVINCIALIS* ACROSS NATURAL AND ARTIFICIAL HABITATS

The results of the coverage surveys showed that *Perna* farm sites had a much higher coverage of *M. galloprovincialis* (mean coverage of 0.543) than adjacent (0.042) and distant (0.067) sites, respectively (Figure 3.3). There was also an observed geospatial pattern amongst the farm sites, with a positive correlation between latitude (°S) and *M. galloprovincialis* coverage, resulting in increasing coverage at farms further south (Figures 3.1, 3.2). There was no observed pattern between *M. galloprovincialis* coverage and Secchi Disk depth: Secchi Disk depth was not included in the final model. Nor was there an observable difference in coverage between summer or winter, with mean *M. galloprovincialis* coverages of 0.232 and 0.192, respectively (Figure 3.3): season was not included in the final model, with mean *M. galloprovincialis* coverages of 0.232 and 0.192 respectively (Figure 3.3). Data exploration did not reveal any interactive effects between season and other variables tested, and interaction terms involving season were thus not considered further. The final model indicated that latitude and site type were the only variables which accurately predicted *M. galloprovincialis* coverage (Table 3.3).

Variable	Sum of Squares	R ²	F-statistic	P-value
Bedrock	756.59	0.398	53.5	0.001
Boulder	2.17	0.001	0.2	0.717
Cobble	8.72	0.005	0.6	0.416
Slope	170.57	0.090	6.0	0.007
Cobble:Slope	113.47	0.060	4.0	0.044

Table 3.2. Results of the PERMANOVA, testing the effect substratum type and slope, and their interactive effect, on *M. galloprovincialis* coverage.

	Estimate	Standard Error	Z-value	P-value
(Intercept)	-2.6565	0.131	-20.292	< 0.001
Latitude	0.2265	0.122	1.857	0.063
SiteTypeDistant	0.2447	0.146	1.679	0.093
SiteTypeFarm	2.8922	0.127	22.711	< 0.001
Latitude : SiteTypeDistant	-0.0400	0.140	-0.286	0.775
Latitude : SiteTypeFarm	-0.7826	0.114	-6.862	< 0.001

Table 3.3 Numeric results of the Generalized Linear Model after stepwise reduction of AIC scores. The final model is the interactive effect between latitude and site type, with site number as a random factor (Eqn. 1).



Figure 3.2 Scatter plot of *M. galloprovincialis* coverage at farm sites as a function of latitude. This same pattern was not observed at reef sites, which is why they are not included in this plot. The equation of the trendline is f(x) = 130.1x - 5286.2, with an $R^2 = 0.3917$. This trendline is not used for statistical analysis, but shows geospatial pattern observed in Figure 3.1, and provides a clearer understanding of Equation 1.





Figure 3.3 *M. galloprovincialis* coverage plotted as a function of the categorical variables collected during the field surveys. A) *M. galloprovincialis* coverage by site type, for all sites. B) *M. galloprovincialis* coverage by season, for all sites. C) *M. galloprovincialis* coverage by substrate slope, for reef sites only.



Figure 3.4 Scatter plot of *M. galloprovincialis* coverage at reef sites as a function of percent bedrock. The equation of the trendline is f(x) = 0.1449x + 1.421, with an $R^2 = 0.3981$. This trendline is not used for statistical analysis. Note that proportions were used in statistical analyses, which were transformed to percentages for this plot.

3.4 DISCUSSION

Aquaculture is an important source of accidental or intentional introductions of NIS (Naylor et al. 2001; Savini et al. 2010; Crego-Prieto et al. 2015). In areas where the NIS is introduced intentionally for aquaculture, there is a clear link between NIS presence or abundance and aquaculture activity (Crego-Prieto et al. 2015; Ju et al. 2020). However, in areas such as the Marlborough Sounds, New Zealand, where an invasive species is not cultivated, the way in which aquaculture affects the presence or abundance of that species is not clearly understood. Because *M. galloprovincialis* in this region causes substantial economic losses to the aquaculture industry (Forrest & Atalah, 2017), it is necessary to better understand this problem, and also to start to understand if and how the native (MgS) and the introduced (MgN) lineages of this mussel interact and affect the *Perna* aquaculture industry.

3.4.1 MGN RELATIVE ABUNDANCE

The results from the 16s RFLP show that MgN/MgS community composition is similar at farm and reef sites. This is likely due to the life cycle of *Mytilus* spp., specifically that they are broadcast spawning and have a high dispersal capacity (Miller et al. 2018). This likely has the effect of homogenizing sites with apparently equal rates of MgN and MgS settlement. That being said, there is an increased presence of MgN in the Marlborough Sounds in comparison to the rest of the South Island of New Zealand.

Given that there is no statistically significant difference in MgN abundance at farm versus reef sites, the findings cannot support the hypothesis that aquaculture is promoting MgN relative abundance. However, given that MgN relative abundance was similar at farm and reef sites, and *M. galloprovincialis* coverage is significantly higher at farm sites than reef sites, the findings show that aquaculture is promoting the overall abundance of MgN within the region. That is, the increased habitat availability on the suspended artificial habitat provided by the

of aquaculture facilities in the Marlborough Sounds is promoting an increase in the MgN population size. Given these findings and the findings of Chapter 2, it is likely that MgN has been introduced to the Marlborough Sounds through intranational vessel traffic, then settles alongside / competes with MgS and *Perna* on the grow-lines, and then MgN relative abundance between farm and reef sites is homogenizing through broadcast spawning and larval dispersal.

3.4.2 REGIONAL DISTRIBUTION OF *M. GALLOPROVINCIALIS* IN THE MARLBOROUGH SOUNDS

The results of the coverage surveys and coverage model indicate that site type (farm, adjacent, distant) and latitude are the most important factors to consider. The results indicate that there is much greater *M. galloprovincialis* coverage at farm sites than at reef sites, and that farms further south have a higher coverage than those further north. These findings support the speculation by Atalah et al. (2017), that aquaculture activity is associated with the *M. galloprovincialis* population increase over the past 20 years. This is also likely a positive feedback loop, as a larger adult *M. galloprovincialis* population will result in an increase in spawning activity and volume, thus increasing the population faster than it would have previously until carrying capacity is reached.

However, the geospatial pattern in coverage at farms sites, i.e. the positive correlation between latitude (°S) and coverage, cannot be explained by auto-recruitment. It is true that *M. galloprovincialis* larvae are not completely passive drifters, i.e. they do have some mobility (Levin, 2006), however it is very unlikely the observed pattern is from larval dispersal (Stuckas et al. 2017). The Marlborough Sounds is part of a fjord system, characterized by a large freshwater input from rainfall and the Pelorus River at the southern end of Pelorus Sound and with open sea (Cook Strait and the Tasman Sea) at the northern end (Gibbs et al. 1991; Proctor & Hadfield, 1998). As a result, the water column is characterized by a large degree of stratification relative to coastal sites (Proctor & Hadfield, 1998). As a consequence, Forrest & Atalah (2017) and Atalah et al. (2017) described that *M. galloprovincialis* is mostly present on the top few meters of the crop lines, where it is out-competing *Perna*. Atalah et al. (2017), also described that some aquaculture facilities avoid this problem by sinking their lines at specific times of the year to avoid *M. galloprovincialis* biofouling.

Given that *M. galloprovincialis* is more present on the top few meters of the grow-lines (Atalah et al. 2017), that the water column is characterized by thermal and saline stratification (Proctor & Hadfield, 1998), and that there is a much higher degree of *M. galloprovincialis* on farms than at adjacent reef sites, it is likely that the observed patterns of *M. galloprovincialis* distribution and relative abundance in the Marlborough Sounds is an interactive effect between habitat availability at aquaculture facilities, and temperate and/or salinity dependent competition with *Perna*.

3.5 CONCLUSION

Overall, the above results are in alignment with those of Chapter 2, e.g. that the ecology of *M. galloprovincialis* in New Zealand appears to be positively associated with anthropogenic disturbance. The above results show that *M. galloprovincialis* coverage is significantly greater at farm sites than at reef sites; and that the most important factor in describing *M. galloprovincialis* coverage is the interactive effect of site type and latitude. Given these results, it is likely that the *M. galloprovincialis* population increase observed in this region is associated with increasing aquaculture activity.

MgN relative abundance is nearly identical at farm and at reef sites, and there was significantly greater coverage at farm sites than at reef sites, meaning that aquaculture is promoting an increase in MgN overall abundance. That is, the invasive MgN and native MgS are both taking advantage of the available space on the grow-lines of the farms in the Marlborough Sounds. This then poses a biosecurity threat because more MgN individuals means more spawning, increasing the likelihood that they will spread to nearby areas. Furthermore, MgN relative abundance in the Marlborough Sounds is the highest in the South Island of New Zealand. These findings suggest that aquaculture is promoting MgN invasion in New Zealand.

For future research, our understanding of the above occurrences would greatly benefit from a population genetics, or seascape genetics project. Describing the genetic connectivity of the Northern and Southern *M. galloprovincialis* populations in the Marlborough Sounds, or New Zealand in general, would greatly increase our understanding of their spread.

LITERATURE CITED

- Apte, S., Holland, B. S., Godwin, L. S., & Gardner, J. P. A. (2000). Jumping ship: A stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. *Biological Invasions*, 2(1), 75–79. https://doi.org/10.1023/A:1010024818644
- Astorga, M. P., Vargas, J., Valenzuela, A., Molinet, C., & Marín, S. L. (2018). Population genetic structure and differential selection in mussel *Mytilus* chilensis. *Aquaculture Research*, 49(2), 919–927. https://doi.org/10.1111/are.13538
- Atalah, J., Rabel, H., & Forrest, B. M. (2017). Modelling long-term recruitment patterns of blue mussels *Mytilus* galloprovincialis: A biofouling pest of green-lipped mussel aquaculture in New Zealand. *Aquaculture Environment Interactions*, 9(1), 103–114. https://doi.org/10.3354/aei00216
- Azpeitia, K., Rodríguez-Ezpeleta, N., & Mendiola, D. (2019). Settlement and recruitment pattern variability of the mussel *Mytilus* galloprovincialis Lmk. from SE Bay of Biscay (Basque Country). *Regional Studies in Marine Science*, 27, 100523. https://doi.org/10.1016/j.rsma.2019.100523
- Bannister, J., Sievers, M., Bush, F., & Bloecher, N. (2019). Biofouling in marine aquaculture: a review of recent research and developments. *Biofouling*, 35(6), 631–648. https://doi.org/10.1080/08927014.2019.1640214

- Bao, W. Y., Satuito, C. G., Yang, J. L., & Kitamura, H. (2007). Larval settlement and metamorphosis of the mussel *Mytilus* galloprovincialis in response to biofilms. *Marine Biology*, 150(4), 565–574. https://doi.org/10.1007/s00227-006-0383-4
- Bayne, B. L. (1964). Primary and Secondary Settlement in *Mytilus* edulis L. (Mollusca)
 Author (s): B. L. Bayne Source : Journal of Animal Ecology, Vol. 33, No. 3 (Oct., 1964), pp. 513-523 Published by : British Ecological Society Stable URL : https://www.jstor.o. *Journal of Animal Ecology*, 33(3), 513–523.
- Bersine, K., Brenneis, V. E. F., Draheim, R. C., Rub, A. M. W., Zamon, J. E., Litton, R. K., ... Chapman, J. W. (2008). Distribution of the invasive New Zealand mudsnail (Potamopyrgus antipodarum) in the Columbia River Estuary and its first recorded occurrence in the diet of juvenile Chinook salmon (Oncorhynchus tshawytscha). *Biological Invasions*, *10*(8), 1381–1388. https://doi.org/10.1007/s10530-007-9213-y
- Bownes, S. J., & McQuaid, C. D. (2006). Will the invasive mussel *Mytilus* galloprovincialis Lamarck replace the indigenous *Perna Perna* L. on the south coast of South Africa? *Journal of Experimental Marine Biology and Ecology*, 338(1), 140–151. https://doi.org/10.1016/j.jembe.2006.07.006
- Clarke Murray, C., Therriault, T. W., & Martone, P. T. (2012). Adapted for invasion? Comparing attachment, drag and dislodgment of native and nonindigenous hull fouling species. *Biological Invasions*, 14(8), 1651–1663. https://doi.org/10.1007/s10530-012-0178-0
- Cook, E., Ashton, G., Campbell, M., Coutts, A., Gollash, S., Hewitt, C., ... Shucksmith, R. (2008). Non-native aquaculture species releases: implications for aquatic ecosystems. *Aquaculture in the Ecosystem*, 155–184.
- Cowen, R. K., & Warner, R. R. (2002). Local retention of production in marine populations: evidence, mechanisms, and consequences. *Bulletin of Marine Science*, 70(1), 245–249.
- Crego-Prieto, V., Ardura, A., Juanes, F., Roca, A., Taylor, J. S., & Garcia-Vazquez, E. (2015). Aquaculture and the spread of introduced mussel genes in British Columbia. *Biological Invasions*, 17(7), 2011–2026. https://doi.org/10.1007/s10530-015-0853-z
- Eads, A. R., Evans, J. P., & Kennington, W. J. (2016). Plasticity of fertilization rates under varying temperature in the broadcast spawning mussel, *Mytilus* galloprovincialis. *Ecology and Evolution*, 6(18), 6578–6585. https://doi.org/10.1002/ece3.2375
- Fitridge, I., Dempster, T., Guenther, J., & de Nys, R. (2012). The impact and control of biofouling in marine aquaculture: A review. *Biofouling*, 28(7), 649–669. https://doi.org/10.1080/08927014.2012.700478
- Forrest, B. M., & Atalah, J. (2017). Significant impact from blue mussel *Mytilus* galloprovincialis biofouling on aquaculture production of green-lipped mussels in New Zealand. *Aquaculture Environment Interactions*, 9(1), 115–126. https://doi.org/10.3354/aei00220

- Gardner, J. P. A., Oyarzún, P., Toro, J., Wenne, R., & Zbawicka, M. (2020). Phylogeography of Southern hemisphere blue mussels of the genus *Mytilus*: evolution, biosecurity, aquaculture, and food labelling. *Oceanography and Marine Biology: Annual Reviews, in press.*
- Gardner, J. P. A. (2004). A historical perspective of the genus *Mytilus* (Bivalvia: Mollusca) in New Zealand: Multivariate morphometric analyses of fossil, midden and contemporary blue mussels. *Biological Journal of the Linnean Society*, *82*(3), 329–344. https://doi.org/10.1111/j.1095-8312.2004.00362.x
- Gardner, J. P. A., & Westfall, K. M. (2012). Geographic distribution and molecular identification of a metapopulation of blue mussels (genus *Mytilus*) in northeastern New Zealand. *Journal of Molluscan Studies*, 78(1), 66–73. https://doi.org/10.1093/mollus/eyr037
- Gardner, J. P. A., Zbawicka, M., Westfall, K. M., & Wenne, R. (2016). Invasive blue mussels threaten regional scale genetic diversity in mainland and remote offshore locations: the need for baseline data and enhanced protection in the Southern Ocean. *Global Change Biology*, 22(9), 3182–3195. https://doi.org/10.1111/gcb.13332
- Gérard, K., Bierne, N., Borsa, P., Chenuil, A., & Féral, J. P. (2008). Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from Northern and Southern hemispheres and strong genetic differentiation among southern populations. *Molecular Phylogenetics and Evolution*, 49(1), 84–91. https://doi.org/10.1016/j.ympev.2008.07.006
- Gibbs, M. M., James, M. R., Pickmere, S. E., Woods, P. H., Shakespeare, B. S., Hickman, R. W., & Illingworth, J. (1991). Hydrodynamic and water column properties at six stations associated with mussel farming in Pelorus sound, 1984–85. New Zealand Journal of Marine and Freshwater Research, 25(3), 239–254. https://doi.org/10.1080/00288330.1991.9516476
- Gilg, M. R., Kirby, S. E., Sullivan, R., Knapp, L. W., & Hilbish, T. J. (2007). Dispersal vs. retention: Correspondence of species-specific reproductive cycles and settlement periods in a blue mussel hybrid zone. *Marine Ecology Progress Series*, 351, 151–161. https://doi.org/10.3354/meps07145
- Hilbish, T. J., Mullinax, A., Dolven, S. I., Meyer, A., Koehn, R. K., & Rawson, P. D. (2000). Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): Routes and timing of transequatorial migration. *Marine Biology*, 136(1), 69–77. https://doi.org/10.1007/s002270050010
- Ju, R. T., Li, X., Jiang, J. J., Wu, J., Liu, J., Strong, D. R., & Li, B. (2020). Emerging risks of non-native species escapes from aquaculture: Call for policy improvements in China and other developing countries. *Journal of Applied Ecology*, 57(1), 85–90. https://doi.org/10.1111/1365-2664.13521
- Kochmann, J., Carlsson, J., Crowe, T. P., & Mariani, S. (2012). Genetic evidence for the uncoupling of local aquaculture activities and a population of an invasive species-a case study of pacific oysters (Crassostrea gigas). *Journal of Heredity*, 103(5), 661–671. https://doi.org/10.1093/jhered/ess042

- Lee, D. J., & Gordon, R. M. (2006). Economics of aquaculture and invasive aquatic species -An overview. Aquaculture Economics and Management, 10(2), 83–96. https://doi.org/10.1080/13657300600694502
- Levin, L. A. (2006). Recent progress in understanding larval dispersal: New directions and digressions. *Integrative and Comparative Biology*, 46(3), 282–297. https://doi.org/10.1093/icb/icj024
- Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). 100 of the World'S Worst Invasive Species. *Aliens*, *12*, s1–s12.
- Miller, J. A., Carlton, J. T., Chapman, J. W., Geller, J. B., & Ruiz, G. M. (2018). Transoceanic dispersal of the mussel *Mytilus* galloprovincialis on Japanese tsunami marine debris: An approach for evaluating rafting of a coastal species at sea. *Marine Pollution Bulletin*, 132(October 2017), 60–69. https://doi.org/10.1016/j.marpolbul.2017.10.040
- Naylor, R. L., Williams, S. L., & Strong, D. R. (2001). Aquaculture A gateway for exotic species. Science, 294(5547), 1655–1656. https://doi.org/10.1126/science.1064875
- Naylor, R. L., Goldburg, R. J., Clay, J., Troell, M., Mooney, H., Primavera, J. H., ... Lubchenco, J. (2002). Effect of aquaculture on world fish supplies. *Nature*, 405(6790), 1017–1024.
- New Zealand Sustainable Aquaculture. (2018). *New Zealand Aquaculture: a sector overview with key facts and statistics*. Retrieved from https://www.aquaculture.org.nz/wp-content/uploads/2019/10/New-Zealand-Aquaculture-sector-overview-2019.pdf
- Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... Wagner, H. (2019). vegan: Community Ecology Package. Retrieved from https://cran.rproject.org/package=vegan
- Oyarzún, P. A., Toro, J. E., Cañete, J. I., & Gardner, J. P. A. (2016). Bioinvasion threatens the genetic integrity of native diversity and a natural hybrid zone: Smooth-shelled blue mussels (*Mytilus* spp.) in the Strait of Magellan. *Biological Journal of the Linnean Society*, 117(3), 574–585. https://doi.org/10.1111/bij.12687
- Palumbi, S. R. (1996). Nucleic acids II: the polymerase chain reaction. *Molecular Systematics*, 205–247.
- Pauly, D., & Zeller, D. (2017). Comments on FAOs State of World Fisheries and Aquaculture (SOFIA 2016). *Marine Policy*, 77(August 2016), 176–181. https://doi.org/10.1016/j.marpol.2017.01.006
- Pauly, D., & Zeller, D. (2019). Agreeing with FAO: Comments on SOFIA 2018. Marine Policy, 100(July 2018), 332–333. https://doi.org/10.1016/j.marpol.2018.12.009
- Proctor, R., & Hadfield, M. (1998). Numerical investigation into the effect of freshwater inputs on the circulation in Pelorus Sound, New Zealand. *New Zealand Journal of*

Marine and Freshwater Research, *32*(3), 467–482. https://doi.org/10.1080/00288330.1998.9516837

- R Core Team. (2019). *R: A language and environment for statistical computing*. Retrieved from https://www.r-project.org/
- Savini, D., Occhipinti-Ambrogi, A., Marchini, A., Tricarico, E., Gherardi, F., Olenin, S., & Gollasch, S. (2010). The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology*, 26(SUPPL. 2), 1–7. https://doi.org/10.1111/j.1439-0426.2010.01503.x
- Shields, J. L., Heath, J. W., & Heath, D. D. (2010). Marine landscape shapes hybrid zone in a broadcast spawning bivalve: Introgression and genetic structure in Canadian west coast *Mytilus. Marine Ecology Progress Series*, 399, 211–223. https://doi.org/10.3354/meps08338
- Soliman, T., & Inglis, G. J. (2018). Forecasting the economic impacts of two biofouling invaders on aquaculture production of greenlipped mussels *Perna* canaliculus in New Zealand. *Aquaculture Environment Interactions*, 10, 1–12. https://doi.org/10.3354/AEI00249
- Stuckas, H., Knöbel, L., Schade, H., Breusing, C., Hinrichsen, H. H., Bartel, M., ... Melzner, F. (2017). Combining hydrodynamic modelling with genetics: can passive larval drift shape the genetic structure of Baltic *Mytilus* populations? *Molecular Ecology*, 26(10), 2765–2782. https://doi.org/10.1111/mec.14075
- Thyrring, J., Jensen, K. T., & Sejr, M. K. (2017). Gametogenesis of an intertidal population of *Mytilus* trossulus in NW Greenland: Not a limitation for potential Arctic range expansion. *Marine Ecology Progress Series*, 574, 65–74. https://doi.org/10.3354/meps12179
- Westfall, K. M., Wimberger, P. H., & Gardner, J. P. A. (2010). An RFLP assay to determine if *Mytilus* galloprovincialis Lmk. (Mytilidae; Bivalvia) is of Northern or Southern hemisphere origin. *Molecular Ecology Resources*, 10(3), 573–575. https://doi.org/10.1111/j.1755-0998.2009.02779.x
- Westfall, K. M., & Gardner, J. P. A. (2013). Interlineage *Mytilus* galloprovincialis Lmk. 1819 hybridization yields inconsistent genetic outcomes in the Southern hemisphere. *Biological Invasions*, 15(7), 1493–1506. https://doi.org/10.1007/s10530-012-0385-8
- Westfall, K. M., & Gardner, J. P. A. (2010). Genetic diversity of Southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. *Biological Journal of the Linnean Society*, 101(4), 898–909. https://doi.org/10.1111/j.1095-8312.2010.01549.x
- Worm, B., Hilborn, R., Baum, J. K., Branch, T. A., Collie, J. S., Costello, C., ... Zeller, D. (2009). Rebuilding Global Fisheries. *Science*, 325(5940), 578–585. https://doi.org/10.1126/science.1173146

- Zbawicka, M., Trucco, M. I., & Wenne, R. (2018). Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels: Hybridization with invasive European *Mytilus* galloprovincialis. *Genetics Selection Evolution*, 50(1), 1–14. https://doi.org/10.1186/s12711-018-0376-z
- Zeller, D., & Pauly, D. (2005). Good news, bad news: Global fisheries discards are declining, but so are total catches. *Fish and Fisheries*, *6*(2), 156–159. https://doi.org/10.1111/j.1467-2979.2005.00177.x
- Zuur, A. F., & Ieno, E. N. (2016). A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, 7(6), 636–645. https://doi.org/10.1111/2041-210X.12577
- Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1(1), 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

Chapter 4: Overview: The Invasive Northern *Mytilus* galloprovincialis in New Zealand

ABSTRACT: M. galloprovincialis is recognized as one of the most prolific invasive species globally. One limitation in addressing M. galloprovincialis invasions is a lack of understanding of their taxonomy in the Southern hemisphere. A glaring example of this is in New Zealand, where there appears to be two closely related lineages, one invasive and one native. The purpose of this thesis was not to assess how much damage the invasive Northern *M. galloprovincialis* was causing in New Zealand, but instead to assess if it was likely enough that damage was occurring to warrant future research. In doing so, this thesis acts as a foundation for future research projects. The results of Chapters 2 and 3 broadly suggest that anthropogenic disturbance, both direct human activity and climate change, is influencing the Northern M. galloprovincialis invasion. These findings are interesting but unexpected, and the mechanisms behind the results are still unknown. In this Chapter I contextualize this thesis by describing the specific scientific papers which this thesis is based upon, place the results of Chapters 2 and 3 in a broader context, and discuss how this will impact future research. Also, there were several obstacles that were faced in this project which led to several shortcomings. I acknowledge these shortcomings and discuss how future research may address them.

4.1 INTRODUCTION

To ask the question "how much damage is the invasive Northern *M. galloprovincialis* causing in New Zealand?" is a very large, and very expensive question to answer. The purpose of the work presented here is not to answer this question, but to answer another; is there enough evidence to warrant asking that very large, and very expensive question? That is to say that, given the allotted time and funding for this work, it serves neither as a conclusive solution to the MgN problem, nor as an exhaustive list of the key abiotic factors affecting its invasion. This work is instead a preliminary investigation into the distribution and invasion mechanisms of the Northern *M. galloprovincialis* in New Zealand. This work may then serve as a foundation for future research and/or aid in marine biosecurity decisions.

Hewitt et al. (2010) described the progress of marine biosecurity policy in New Zealand since the Biosecurity Act of 1993. The authors acknowledge that while progress is being made there are several gaps in New Zealand's marine biosecurity policy, one of which is an absence knowledge of marine invasion mechanisms. This is especially true for the Northern *M. galloprovincialis* invasion in New Zealand, or more broadly, *Mytilus* spp. in the Southern hemisphere. While research regarding *Mytilus* in the Southern hemisphere is still lacking when compared to the Northern hemisphere (Gardner et al. 2020), our understanding is improving rapidly.

4.1.1 PRIMARY RESEARCH FOUNDATION

Mytilus taxonomy in the Southern hemisphere is repeatedly revised, which makes research regarding *Mytilus* spp. challenging (Gardner et al. 2020). Leaving those already discussed points aside, the line of research that forms the foundation of this thesis and the foundation of our current understanding of *Mytilus* taxonomy in the Southern hemisphere began in 2000. At this point, it was generally agreed that smooth shelled blue mussels in the Southern hemisphere were *Mytilus*, regardless of how many species names were used (Gardner et al. 2020).

Hilbish et al. (2000) followed by Gérard et al. (2008) began to clarify the situation by describing the two likely north to south trans-equatorial migrations events leading to the observed anti-tropical distribution of *Mytilus*. These authors also showed that the Southern and Northern hemisphere lineages of *Mytilus* spp. are related but appear to have existed in allopatric isolation from each other for 0.84-1.2 million years. The combined results of these papers are very influential as they provide a clear molecular evidence of the phylogeography of *Mytilus* in the Southern hemisphere. However, the taxonomic status and migration routes of these mussels is still not clear, constantly being reconsidered and is therefore undergoing revision

(Gardner et al. 2020). Regardless, the results described by Hilbish et al. (2000) and Gérard et al. (2008) are very important parts of the foundation of this thesis as they show that Northern and Southern hemisphere lineages of *Mytilus* spp. are distinct, and that Australasian mussels, i.e. the native blue mussel described throughout this thesis (MgS), share a more recent common ancestor with *M. galloprovincialis* rather than with other members of the *M. edulis* species complex (Gardner et al. 2020).

After these findings, a cheaper and more accurate method of identifying individuals from these lineages was needed. Westfall et al. (2010) achieved this by creating a 16s RFLP to identify Northern and Southern lineages of *M. galloprovincialis*, as well as *M. trossulus* and *M. edulis*. While this RFLP is effective and is within the research budget of a MSc project, it is limited in that it cannot determine hybridization status of individuals because it is a mitochondrial, not nuclear, DNA-based assay. Westfall & Gardner (2010) then utilized this new RFLP to describe the distribution of MgN across New Zealand and its offshore islands, as well as in Australia and Chile. The data set generated by these papers is the data described in Chapter 2 and was expanded on with the addition of new samples from the Marlborough Sounds. This thesis can largely be viewed as a direct continuation of the work of Westfall & Gardner (2010, 2013) and Gardner & Westfall (2012) because it expands on their data set with the addition of new mussel samples from the Marlborough Sounds as well as site specific environmental data, to begin to explore the distribution of MgN in NZ and how abiotic factors may be affecting its invasion.

At this point it was clear that the Northern and Southern lineages of *M. galloprovincialis* are distinct (Hilbish et al. 2000; Gérard et al. 2008), that the Northern *M. galloprovincialis* is invasive in New Zealand (Westfall et al. 2010;), and there had been a preliminary description of its distribution across New Zealand (Westfall & Gardner, 2010, 2013; Gardner & Westfall,

2012). What was lacking was an understanding of the impacts the MgN invasion was having. Gardner et al. (2016) addressed this to some extent in their findings that more introgression was occurring between MgN and MgS in New Zealand than elsewhere in the Southern hemisphere, e.g. MgN poses a unique threat to the genetic integrity and diversity of the native MgS. In New Zealand this has several important social, political, and economic implications.

Published in the following year and on the topic of MgN invasion impacts in New Zealand, Forrest & Atalah (2017) reported that M. galloprovincialis causes severe economic damage to the greenshell mussel Perna canaliculus (hereafter Perna) aquaculture industry in New Zealand. The authors reported that the majority of loss is from over-settlement, i.e. the displacement of *Perna* by *M. galloprovincialis*, resulting in reduced crop yield. Subsequently, Atalah et al. (2017) studied this over-settlement in the Marlborough Sounds region and described the environmental factors that affect M. galloprovincialis over-settlement in order to help aquaculture managers avoid the damaged caused by *M. galloprovincialis*. The authors also reported an increase in M. galloprovincialis coverage over the past 20 years, with coverage as a proxy for population size, however this increase could not be accounted for by the environmental factors considered. They speculated that this increase was due to the increased aquaculture activity in the region since the 1970's, and that the increased population size of M. galloprovincialis was from an increase in habitat availability at the aquaculture facilities. However, neither publications differentiated between MgN and MgS, meaning that the distinction between native and introduced blue mussels could not be considered. These two papers form the basis of the objectives of Chapter 3, which aimed to contribute evidence to test the speculation that the *M. galloprovincialis* population in the Marlborough Sounds has been increasing due to aquaculture activity; and to describe the MgN proportion of the M. galloprovincialis population at farm sites in comparison to neighboring reef sites.

On the Pacific coast of Canada, Crego-Prieto et al. (2015) studied the effects of aquaculture on *M. galloprovincialis* population size and relative abundance, although in a very different system than the one in the Marlborough Sounds. They described the distribution of introduced *M. galloprovincialis* near Vancouver, British Columbia, Canada. In this region the native blue mussels are *M. trossulus* and *M. californianus*, while *M. edulis* and Northern *M. galloprovincialis* are invasive species. Unlike in New Zealand, Northern *M. galloprovincialis* was introduced for aquaculture and is currently farmed. The authors tested population composition (i.e. degree of hybridization and introgression between members of the *M. edulis* species complex) against several anthropogenic disturbance and environmental factors and reported that NIS introgression primarily depended upon aquaculture facility density. Clearly these results are directly relevant to the research discussed in this thesis, but more generally Crego-Prieto et al. (2016) also provided an experimental design framework for Chapter 2. It highlighted the need to sample and study effects of aquaculture on MgN in New Zealand, and presented an interesting question; does aquaculture activity have an effect on a *M. galloprovincialis* population that is not the target aquaculture species?

This line of research forms the foundation of this thesis. These papers clearly show that MgN is distinct lineage of *M. galloprovincialis* in Australasia, that it is invasive in New Zealand, that it causes ecological and socioeconomic damage, and is associated with aquaculture. The next phase of research in this process is to continue to describe the distribution and relative abundance of MgN in New Zealand and begin to study what may be driving their invasion success.

4.2 CHAPTER 2

4.2.1 SUMMARY AND CONTEXT OF RESULTS

A preliminary description of the distribution and relative abundance of MgN in New Zealand, along with studying how some abiotic factors are affecting its invasion, is the primary objective of this thesis. Before this project, it was speculated that MgN and MgS would likely occupy the same abiotic niche due to their very similar evolutionary history, but repeated introduction from commercial vessels may create an MgN bias or hot spot near primary ports. Preliminary evidence from Westfall & Gardner (2010) suggest that this is the case. However, this is not what the results of Chapter 2 show.

Given the results of Westfall & Gardner (2010), the hypothesis tested in Chapter 2 was that only distance to primary ports and no other environmental factors would correlate with Northern *M. galloprovincialis* presence-absence and/or abundance. The results of Chapter 2 do not support this hypothesis and it is thus rejected. These results are interesting because they show that there is some degree of abiotic environmental influence in the interaction between MgN and MgS, further supporting their separate taxonomic status (Zbawicka et al. 2017). That is, some of the environmental and geospatial independent variables are significantly affecting the competition between the invasive and native lineages of *M*.*galloprovincialis* in New Zealand. Chlorophyll-A concentration (mg/m³), distance to the nearest marina (km), and Sea Surface Temperature (°C) were all significant in predicting the presence-absence of MgN (Table 2.3). These results indicate that MgN is more likely to be present in relatively warmer water with a low and stable Chlorophyll-A concentration, likely near a marina. What these results cannot show is whether MgN is in a previously unoccupied niche space, or if it is out competing and displacing the native MgS.

While the effect of these environmental factors on MgN occurrence are clear, the results regarding how anthropogenic factors affect MgN occurrence, specifically distances to various port types, are much less clear. Distance to the nearest marina was the only geospatial factor

that was significant in predicting the presence-absence of MgN, while distance to the nearest primary port and place of first arrival, i.e. those facilities which receive the vast majority of international maritime traffic in New Zealand, were significant in describing the relative abundance of MgN. These results are difficult to interpret because there is no overlap between the two statistical tests. What is likely occurring is that intranational vessel traffic, specifically smaller vessels that moor at and travel between marinas, is a more important vector of spread for MgN within New Zealand than international traffic. Another possible interpretation would be that the environment in marinas, i.e. protected areas with floating structure, is promoting MgN abundance just as aquaculture facilities are. Primary ports and places of first arrival, i.e. those facilities which receive the vast majority of international traffic in New Zealand (supplementary data), are likely the primary vectors of MgN introduction.

However, the results described in Chapter 2 - that distance to primary ports is positively correlated with MgN abundance - may reflect a geospatial bias in the data. It is very unlikely that primary ports are somehow repelling MgN or reducing its population size or distribution. It is important to note that MgN relative abundance is greater in the North Island than the South Island; that there are more marinas on the North Island than the South Island; and that primary ports and places of first arrival are roughly evenly spatially distributed across both islands. With this understanding of the data, it is possible that the results merely represent that the North Island is more invaded than the South Island. That said, these results provide a preliminary understanding of the distribution and relative abundance of MgN in New Zealand. Also, the issues of whether MgN is displacing MgS or if it is expanding into previously unoccupied niche space, as well as the geospatial bias, can be addressed with future research and is discussed in section 4.4.1. Overall, the major finding from Chapter 2 is the importance of various port types to the spread of MgN within New Zealand. These results highlight the importance of intranational vessel traffic to marine biological invasion, which can effectively break down internal barriers and borders to the invader thus accelerating the invasion (Forrest et al. 2009). These results are similar to those reported in several recent studies that together suggest that while international vessel traffic is important for the introduction of marine NIS, including *M. galloprovincialis*, intranational traffic is often more important for spread after initial NIS introduction (Hewitt et al. 2010; Clarke Murray et al. 2011, 2012, 2014; Zabin et al. 2014; Ardura et al. 2015; CSAS 2017). However, the results of Chapter 2 only show that distance to the nearest marina is significant, meaning that the mechanism as to why marinas are promoting MgN presence / absence in New Zealand is still unknown.

The taxonomic status of smooth shelled blue mussels in the Southern hemisphere has long been a contentious subject and any evidence that is contributed to this debate is important (Gardner et al 2020). While the above results do contribute to this body of evidence in the Southern hemisphere, given that this thesis focuses on New Zealand these results are better contributions toward the identity of the Australasian mussel(s). With this evidence that MgN and MgS competition is being significantly affected by abiotic factors, combined with Westfall & Gardner (2010) and Gardner et al. (2016), suggest that the Australasian mussel is distinct from its northern counterpart, and may also be distinct from other *Mytilus* in the Southern hemisphere. However, these results are far from conclusive on this, and there is a need for a broad taxonomic review to make these assertations, which is the purpose of Gardner et al. (2020).

4.2.2 IMPLICATIONS AND APPLICATIONS

On a global scale, the results of Chapter 2 show that SST, specifically relatively warmer SST, is significant in predicting MgN presence-absence in NZ. It is likely that this is a range expansion facilitated by warming ocean temperatures. This has implications for potential marine NIS invasions into the Antarctic region for both MgN and MgS. There is a similar phenomenon occurring in the North Atlantic, where the range expansion of *M. galloprovincialis* into the Arctic enabled by increasing ocean temperatures is well documented (Oyarzún et al. 2016; Mathiesen et al. 2017; Thyrring et al. 2017; Leopold et al. 2019). Several projects have modelled this with regard to increasing ocean temperature and anthropogenic climate change to predict future range expansions and contractions, with most predicting that *M. galloprovincialis* will continue to expand its range north. Equivalent research in the Southern hemisphere for *Mytilus* is lacking but is clearly needed.

Further future invasions are likely given that *M. galloprovincialis* has demonstrated the ability to cross vast oceanic distances episodically (Miller et al. 2018). This is often through water that should be uninhabitable for them, and therefore should act as an invasion barrier, which is an attribute that has enabled their capacity to be an invader on a global scale (Apte et al. 2000). For example, adult *M. galloprovincialis* were able to cross the entire northern Pacific Ocean on flotsam from the Fukushima tsunami in 2011 (Miller et a. 2018). With ocean temperatures increasing globally, including in the Southern Ocean, the Antarctic Circumpolar Current breaking down and an increasing volume of vessel traffic heading to Antarctica, it is becoming clear that Antarctica is not as protected from biological invasion as we once thought (Lee & Chown, 2007; McGeoch et al. 2015; Oyarzún et al. 2016; IPCC, 2018; Miller et al. 2018). There is no evidence to suggest that *M. galloprovincialis* would be able to make this jump south soon, however it is important to start research on this topic early so as to prevent invasion for as long as possible, and have a plan already in place for when it does happen.

In New Zealand, given the current widespread distribution and abundance of MgN and its nearly identical morphology to the native MgS, it is unlikely that there is an effective method to eradicate the invader or even greatly reduce its numbers. However, just because this invader cannot be eradicated does not mean that there are no available biosecurity options, or that there is nothing to be learned from researching the ongoing invasion. Hewitt et al. (2010), in a summary of the current status of New Zealand's marine biosecurity program since the Biosecurity Act of 1993, found that recreational boating traffic is a more important vector of spread of marine NIS within New Zealand than international traffic. Therefore, the results of Chapter 2 further support the findings of Hewitt et al. (2010), i.e. that intranational traffic a more important invasion vector for marine NIS in New Zealand, highlighting the need for recreational and intranational vessel biosecurity policy.

Relative to other countries, New Zealand has a robust biosecurity policy including regulations for international shipping traffic (Hewitt et al. 2010). Ships arriving from overseas ports are required to provide proof of having a clean hull and of exchange of their ballast water at sea rather than when they arrive in port (Hewitt et al. 2010). These preventative measures are extremely important as it is much more effective to prevent biological invasions than it is to try to eradicate after initial arrival and establishment (Lodge et al. 2006; Bailey et al. 2011). While the current biosecurity regulations for international vessels arriving in New Zealand are good, an equivalent policy for intranational vessels, both recreational and smaller commercial vessels, does not exist. While there are some regional scale regulations, such as the Marlborough District Council requiring recreational vessels to have proof of recent hull cleaning (Marlborough Sounds Marinas, 2019), having no national scale biosecurity policy for intranational vessel traffic will result in the continued spread of marine NIS in New Zealand.

However, it is also important to not overstate the impact MgN in New Zealand. Given that there are limited biosecurity options to address the MgN invasion, and that at this point most evidence suggests that the ecological damage MgN is causing in New Zealand is not as severe as other *M. galloprovincialis* invasions globally (Robinson et al. 2005; Elliot et al. 2008), policy and biosecurity efforts should quite reasonably be focused on other more damaging marine NIS. That said, the results reported in this thesis should be taken as a contribution to the body of evidence that shows more intranational vessel biosecurity policy is needed to prevent or slow marine NIS incursions in New Zealand. Furthermore, the preservation of native genetic diversity is one of New Zealand's governments biosecurity goals (Convention on Biological Diversity, 1992). Given that Gardner et al. (2016), reported that MgN is threating the genetic diversity of the native MgS, the New Zealand government should devote some resources to furthering our understanding of MgN in New Zealand.

4.3 CHAPTER 3

4.3.1 SUMMARY AND CONTEXT OF RESULTS

The purpose of Chapter 3 was to describe the relative abundance of MgN in the Marlborough Sounds, and discover if aquaculture is somehow affecting its invasion success in this region. The results of this chapter show that there is significantly greater *M. galloprovincialis* coverage at aquaculture facilities than at adjacent and distant shore sites; that *M. galloprovincialis* population size and latitude (°S) are positively correlated; and that MgN relative abundance is roughly equal across aquaculture and shore sites. The results of Chapter 2 also show that this region has the highest relative MgN abundance on the South Island.

Given that the relative abundance of MgN was equal at farm and adjacent shore sites, and that there was significantly greater *M. galloprovincialis* coverage at farm sites than at adjacent and distant shore sites, means that there is a much larger MgN population in this region than would exist without the aquaculture facilities. That is, both MgN and MgS are taking advantage of newly available habitat on the grow-lines of aquaculture facilities in this region and expanding their population sizes. This has important implications, as each facility is then acting as a hotspot for MgN invasion with an elevated population density. The results of Chapter 3 thus suggest that aquaculture in the Marlborough Sounds may be breaking down internal invasion barriers and borders within New Zealand.

4.3.2 IMPLICATIONS AND APPLICATIONS

The above results have implications on a global scale as they show that aquaculture is having a significant effect on *M. galloprovincialis* local abundances when it is not the target species. This is important because it has already been demonstrated that *M. galloprovincialis* is likely to become invasive in regions where it is farmed (Crego-Prieto et al. 2015), but the results of Chapter 3 show that it is also be the increased habitat availability at aquaculture that is promoting the spread of MgN, which Atalah et al. (2017) speculated is the case in the Marlborough Sounds. This is an important finding for aquaculture managers, as *M. galloprovincialis* and other NIS are also well documented to cause moderate to severe economic damage to the aquaculture industry (Forrest & Atalah, 2017; Bannister et al. 2019).

For aquaculture managers in New Zealand, the above results, specifically that MgN relative abundance is similar across farm and shore sites, means that there is no evidence to suggest that the invasive MgN is more important to focus on. That is, both the MgN and MgS are effectively a pest species on the grow-lines, and it would not be effective to focus on one rather than the other. However, whilst *Perna* is the target species of these aquaculture facilities, *M. galloprovincialis* is a valuable commodity as well (FAO, 2018). If the aquaculture industry in New Zealand were to sell these blue mussels as a secondary crop, as they have begun to do (Hutching, 2017; Tabuteau, 2018), then the taxonomic identification of their product will be

required to satisfy the FAO labeling and traceability regulations (Larraín et al. 2018). Therefore, it would be in the best interest of this industry to continue to monitor and fund research on the topic.

The results that show increased habitat availability on the grow-lines of aquaculture facilities is increasing MgN and MgS population sizes is also important to consider for biosecurity purposes. It is likely that other marine NIS are also taking advantage of the available habitat on these grow-lines as well, as is occurring in New Zealand and elsewhere (Bannister et al. 2019). If future research is able to confirm this, then this will confirm that aquaculture facilities are breaking down natural barriers to biological invasion by creating hotspots of invasion.

4.4 SUMMARY

In summary, the findings of this thesis have direct applications to management, as well as contributing to justification for future research. That is to say that the main objective of providing a preliminary description of the distribution and relative abundance of MgN in New Zealand, and how some abiotic factors are influencing that invasion, has been achieved. In doing so, a clear direction for future research has been provided as well.

The primary objective of this thesis was to provide a preliminary description of the distribution and relative abundance of MgN in New Zealand, and how some abiotic factors may be influencing their invasion. The above results accomplish this goal, and in doing so contribute to the body of scientific evidence of *M. galloprovincialis* invasion ecology globally. Overall, the above findings suggest that anthropogenic disturbance is affecting the distribution of MgN in New Zealand via aquaculture, intranational vessel traffic, and warming ocean temperatures, which has broad implications for *M. galloprovincialis* invasions globally.

4.4.1 FUTURE RESEARCH

The broad summary of the findings of this thesis is that anthropogenic disturbance is significantly promoting the MgN invasion in New Zealand, firstly via introductions, secondarily through direct effects, such as the effect of various types of ports and marinas, and thirdly through indirect effects, such as increasing ocean temperatures. As previously discussed, it is unlikely that there is an effective method to eradicate MgN from New Zealand now that it is so well established. However, it is likely that there are ways that the MgN invasion could be inhibited if it is further researched.

Specifically, understanding the mechanism(s) behind the importance of distance to marinas and ports is crucial for creating an effective biosecurity policy to inhibit the spread of MgN. There are several possible explanations of these findings; it could be boats and small commercial vessels could be acting as invasion vectors, spreading to marinas and ports and establishing populations there; or that modified habitats at marinas and ports such as floating structures, like the grow-lines of aquaculture facilities in the Marlborough Sounds, create favorable habitat for MgN thus making ports and marinas hot spots of invasion; In all likelihood both are occurring, however future research should explicitly test these possibilities.

The evidence suggests that the MgN invasion in New Zealand is not as severe as other *M. galloprovincialis* invasion elsewhere, likely due to the relatedness of MgN and MgS. Though the negative impact MgN is causing is likely moderate at most, it is important to continue research to find out if there are any impacts not considered or reported here. While this could be done in several ways, what would likely be the most efficient way is to expand upon the existing data set generated by Westfall et al. (2010), and this thesis. In doing so, additional sample locations could be added to get higher geospatial resolution, and re-sampling of previous locations would allow us to collect temporal data.

Broader temporal and geospatial sampling in future research will likely lead to valuable results. By increasing the number of samples on the north end of the South Island, the effect of aquaculture on MgN relative abundance could be better analyzed. Furthermore, temporal data would reveal if MgN is increasing, decreasing, or remaining stable. This change in MgN relative abundance over time would then better assess the competition or abiotic niche space differentiation between MgN and MgS. In additional sampling, biological data should also be collected, such as species diversity and/or species abundance of native species. This would greatly increase our understanding of the MgN invasion ecology in New Zealand and identify potential damage that they may be causing to native biodiversity.

The development of a panel of diagnostic SNPs for identifying MgN and MgS individuals, and the degree of hybridization and introgression, is going to be a key aspect of future research. With these SNPs, and additional sampling outlined above, a population genetics / seascape genetics project could be undertaken. This project would reveal a great deal of information, such as degree of hybridization, identify source and sink populations or population connectivity to describe any geospatial pattern of MgN invasion, etc. This will in turn greatly enable our ability to assess what damage MgN may be causing in New Zealand, as well as enable targeted management of source populations. These efforts would likely greatly slow the spread of MgN and potentially other marine NIS in New Zealand.

If this project outlined above were completed, it would greatly enable another future project modelling the range expansion of MgN in Australasia and address the possibility of MgN invading Antarctica. As stated above, the *M. galloprovincialis* range expansion into the arctic is well documented, but equivalent research in the Southern hemisphere is lacking. This is an important deficiency to acknowledge because it is likely that the primary factor inhibiting the spread of *M. galloprovincialis*, both MgN and MgS, is ocean temperature (Eads et al. 2016).
It is imperative that this research is started early, as increasing ocean temperatures will enable *M. galloprovincialis* range expansion further toward the poles (cote x100). With this in mind it is clear that a more robust biosecurity policy for the Antarctic is needed, which in turn needs more research (Lee & Chown, 2007; Hewitt et al. 2010)).

4.4.2 FUTURE METHODS

The above outline future research projects could also address several setbacks experienced in this thesis. I originally intended to use more robust statistical methods, specifically a Random Forest model in Chapter 2. Briefly, a Random Forest model is a machine learning technique where an algorithm attempts to make a decision, or decision tree, based on the data which it is given (Liaw & Wiener, 2003; Elith et al. 2008;). In the context of this project, the algorithm would have decided whether a site has MgN present or absent, or decide how abundant MgN would be, based on the independent variables provided. The algorithm does this many times, thus making a 'forest' of decision trees, and takes the average of the forest as the final result. This statistical technique is becoming much more popular in ecology , and other similar projects on *M. galloprovincialis* invasion have utilized it with good results (Bergström et al. 2015; Kijewski et al. 2019). (Not enough data points in this thesis, but future work with more samples and more environmental data would).

Another statistical set back that the methodology of future research could address would be to include some of the independent variables that were removed in Chapter 2. Distance to nearest source of freshwater input (DFW), wave data, and maxima and minima for Chl-A, SST, and TURB, were all removed for either covariance or multicollinearity, i.e. including them in the final data set would have led to erroneous results.

Salinity data were not included in the satellite data, as the satellite utilized is not equipped with the appropriate sensor. As a proxy variable, distance to the nearest source of freshwater (DFW) was calculated for each site. This was unfortunate as salinity was found to be an important variable in a similar project on *M. galloprovincialis* invasion (Crego-Prieto et al. 2015). Salinity also likely an important variable in describing the spatial pattern observed at aquaculture facilities in the Marlborough Sounds, where *M. galloprovincialis* outcompetes the native *Perna* on the top few meters of grow-line (Forrest & Atalah, 2017; Atalah et al. 2017). This could be addressed by pursuing other options to collect year-round salinity data other than satellite data, such as buoys fitted with sensors, or repeated manual measurements with a CTD. Another possibility would be to collect meta-data from other published research.

Wave data were also eliminated from the data set for covarying with other independent variables. Wave data were retrieved from a publicly available data set published by the Ministry for the Environment. These were count data in number of events were significant wave height exceed a specified height, across eight coastal regions. Before trimming this variable out of the data set, exploratory statistical analyses and found that these data were significant in predicting the presence-absence of MgN. There are several alternatives to these data, the most attractive of which would be a GIS based wave force model. There are several published papers based on projects like this (Burrows et al. 2008; Pepper & Puotinen, 2009; Wijkmark & Isæus, 2010). After reviewing these projects, it seemed that developing a wave model such as this would push this project beyond the scope of an MSc thesis. Therefore, a future project could attempt to make such a model or form a collaborative project with a person who could make such a model.

The dataset generated by Westfall & Gardner (2010) has samples from several of New Zealand's offshore islands, however they were not included in Chapter 2 as their locations were outside of the sampling area of the satellite utilized to retrieve environmental data. If these sites were included, they would have likely revealed interesting results as they reflect a different

marine environment than mainland New Zealand. As with salinity data, a possible method to overcome this obstacle would be to collect meta-data from other published research or place a buoy with the appropriate sensors at these locations.

4.5 CONCLUSION

In conclusion, the primary findings of this thesis are that abiotic variables are significantly affecting the relative abundance of MgN in New Zealand, thus affecting the invasion of MgN in NZ; Intranational maritime traffic is a more important invasion vector for MgN than international traffic, which emphasizes the need for a biosecurity policy solution; Aquaculture is promoting a large MgN population that would not exist otherwise, and if other marine NIS are doing the same, which is likely, then aquaculture is serving to breakdown borders; and the combined results indicate that human activity is likely driving the invasion success of MgN in New Zealand.

LITERATURE CITED

- Apte, S., Holland, B. S., Godwin, L. S., & Gardner, J. P. A. (2000). Jumping ship: A stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment. *Biological Invasions*, 2(1), 75–79. https://doi.org/10.1023/A:1010024818644
- Atalah, J., Rabel, H., & Forrest, B. M. (2017). Modelling long-term recruitment patterns of blue mussels *Mytilus* galloprovincialis: A biofouling pest of green-lipped mussel aquaculture in New Zealand. *Aquaculture Environment Interactions*, 9(1), 103–114. https://doi.org/10.3354/aei00216
- Bailey, S. A., Deneau, M. G., Jean, L., Wiley, C. J., Leung, B., & MacIsaac, H. J. (2011). Evaluating efficacy of an environmental policy to prevent biological invasions. *Environmental Science and Technology*, 45(7), 2554–2561. https://doi.org/10.1021/es102655j
- Bannister, J., Sievers, M., Bush, F., & Bloecher, N. (2019). Biofouling in marine aquaculture: a review of recent research and developments. *Biofouling*, 35(6), 631–648. https://doi.org/10.1080/08927014.2019.1640214

- Bergström, P., Lindegarth, S., & Lindegarth, M. (2015). Modelling and predicting the growth of the mussel, *Mytilus* edulis: Implications for planning of aquaculture and eutrophication mitigation. *Ecology and Evolution*, 5(24), 5920–5933. https://doi.org/10.1002/ece3.1823
- Burrows, M. T., Harvey, R., & Robb, L. (2008). Wave exposure indices from digital coastlines and the prediction of rocky shore community structure. *Marine Ecology Progress Series*, *353*(Thomas 1986), 1–12. https://doi.org/10.3354/meps07284
- Clarke Murray, C., Gartner, H., Gregr, E. J., Chan, K., Pakhomov, E., & Therriault, T. W. (2014). Spatial distribution of marine invasive species: Environmental, demographic and vector drivers. *Diversity and Distributions*, 20(7), 824–836. https://doi.org/10.1111/ddi.12215
- Clarke Murray, C., Therriault, T. W., & Martone, P. T. (2012). Adapted for invasion? Comparing attachment, drag and dislodgment of native and nonindigenous hull fouling species. *Biological Invasions*, 14(8), 1651–1663. https://doi.org/10.1007/s10530-012-0178-0
- Eads, A. R., Evans, J. P., & Kennington, W. J. (2016). Plasticity of fertilization rates under varying temperature in the broadcast spawning mussel, *Mytilus* galloprovincialis. *Ecology and Evolution*, 6(18), 6578–6585. https://doi.org/10.1002/ece3.2375
- Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, 77(4), 802–813. https://doi.org/10.1111/j.1365-2656.2008.01390.x
- Elliott, J., Holmes, K., Chambers, R., Leon, K., & Wimberger, P. (2008). Differences in morphology and habitat use among the native mussel *Mytilus* trossulus, the non-native *M. galloprovincialis*, and their hybrids in Puget Sound, Washington. *Marine Biology*, 156(1), 39–53. https://doi.org/10.1007/s00227-008-1063-3
- FAO. (2018). FAO Fisheries statistics 2016. Retrieved from www.fao.org/fishery/static/Yearbook/YB2016_USBcard/index.ht
- Fisheries and Oceans Canada. (2017). National risk assessment of recreational boating as a vector for aquatic invasive species. *Canadian Science Advisory Secretariat (CSAS) Science Advisory Report*, (June).
- Forrest, B. M., & Atalah, J. (2017). Significant impact from blue mussel *Mytilus* galloprovincialis biofouling on aquaculture production of green-lipped mussels in New Zealand. *Aquaculture Environment Interactions*, 9(1), 115–126. https://doi.org/10.3354/aei00220
- Forrest, B. M., Gardner, J. P. A., & Taylor, M. D. (2009). Internal borders for managing invasive marine species. *Journal of Applied Ecology*, 46(1), 46–54. https://doi.org/10.1111/j.1365-2664.2008.01544.x
- Gardner, J. P. A., Oyarzún, P., Toro, J., Wenne, R., & Zbawicka, M. (2020). Phylogeography of Southern hemisphere blue mussels of the genus *Mytilus*: evolution, biosecurity,

aquaculture, and food labelling. Oceanography and Marine Biology: Annual Reviews, in press.

- Gardner, J. P. A., & Westfall, K. M. (2012). Geographic distribution and molecular identification of a metapopulation of blue mussels (genus *Mytilus*) in northeastern New Zealand. *Journal of Molluscan Studies*, 78(1), 66–73. https://doi.org/10.1093/mollus/eyr037
- Gardner, J. P. A., Zbawicka, M., Westfall, K. M., & Wenne, R. (2016). Invasive blue mussels threaten regional scale genetic diversity in mainland and remote offshore locations: the need for baseline data and enhanced protection in the Southern Ocean. *Global Change Biology*, 22(9), 3182–3195. https://doi.org/10.1111/gcb.13332
- Gérard, K., Bierne, N., Borsa, P., Chenuil, A., & Féral, J. P. (2008). Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from Northern and Southern hemispheres and strong genetic differentiation among southern populations. *Molecular Phylogenetics and Evolution*, 49(1), 84–91. https://doi.org/10.1016/j.ympev.2008.07.006
- Hewitt, C. L., Willing, J., Bauckham, A., Cassidy, A. M., Cox, C. M. S., Jones, L., & Wotton, D. M. (2004). New Zealand marine biosecurity: Delivering outcomes in a fluid environment. *New Zealand Journal of Marine and Freshwater Research*, 38(3), 429– 438. https://doi.org/10.1080/00288330.2004.9517250
- Hilbish, T. J., Mullinax, A., Dolven, S. I., Meyer, A., Koehn, R. K., & Rawson, P. D. (2000). Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): Routes and timing of transequatorial migration. *Marine Biology*, 136(1), 69–77. https://doi.org/10.1007/s002270050010
- Hutching, C. (2017, April 23). Blue mussels breakthrough for industry. *Stuff*. Retrieved from https://www.stuff.co.nz/business/91663698/blue-mussels-breakthrough-for-industry
- Intergovernmental Panel on Climate Change. (2018). *IPCC report Global Warming of 1.5 C: Summary for Policymakers*.
- Kijewski, T., Zbawicka, M., Strand, J., Kautsky, H., Kotta, J., Rätsep, M., & Wenne, R. (2019). Random forest assessment of correlation between environmental factors and genetic differentiation of populations: Case of marine mussels *Mytilus. Oceanologia*, 61(1), 131–142. https://doi.org/10.1016/j.oceano.2018.08.002
- Larraín, M. A., Zbawicka, M., Araneda, C., Gardner, J. P. A., & Wenne, R. (2018). Native and invasive taxa on the Pacific coast of South America: Impacts on aquaculture, traceability and biodiversity of blue mussels (*Mytilus* spp.). *Evolutionary Applications*, 11(3), 298–311. https://doi.org/10.1111/eva.12553
- Lee, J. E., & Chown, S. L. (2007). *Mytilus* on the move: Transport of an invasive bivalve to the Antarctic. *Marine Ecology Progress Series*, *339*, 307–310. https://doi.org/10.3354/meps339307

- Leopold, P., Renaud, P. E., Ambrose, W. G., & Berge, J. (2019). High Arctic *Mytilus* spp.: occurrence, distribution and history of dispersal. *Polar Biology*, *42*(1), 237–244. https://doi.org/10.1007/s00300-018-2415-1
- Marlborough Sounds Marinas. (2019). Biosecurity Update. Retrieved February 8, 2020, from https://marlboroughmarinas.co.nz/environment/biosecurity-update/
- Mathiesen, S. S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., ... Nielsen, E. E. (2017). Genetic diversity and connectivity within *Mytilus* spp. in the subarctic and Arctic. *Evolutionary Applications*, 10(1), 39–55. https://doi.org/10.1111/eva.12415
- McGeoch, M. A., Shaw, J. D., Terauds, A., Lee, J. E., & Chown, S. L. (2015). Monitoring biological invasion across the broader Antarctic: A baseline and indicator framework. *Global Environmental Change*, 32, 108–125. https://doi.org/10.1016/j.gloenvcha.2014.12.012
- Of, A. C., & Knowledge, C. (2005). ESA Report. *America*, 75(July 2004), 3–35. https://doi.org/10.1890/04-0922
- Oyarzún, P. A., Toro, J. E., Cañete, J. I., & Gardner, J. P. A. (2016). Bioinvasion threatens the genetic integrity of native diversity and a natural hybrid zone: Smooth-shelled blue mussels (*Mytilus* spp.) in the Strait of Magellan. *Biological Journal of the Linnean Society*, 117(3), 574–585. https://doi.org/10.1111/bij.12687
- Pepper, A., & Puotinen, M. L. (2009). GREMO: A GIS-based generic model for estimating relative wave exposure. 18th World IMACS Congress and MODSIM09 International Congress on Modelling and Simulation: Interfacing Modelling and Simulation with Mathematical and Computational Sciences, Proceedings, 1964–1970.
- Robinson, T. B., Griffiths, C. L., McQuaid, C. D., & Rius, M. (2005). Marine alien species of South Africa - Status and impacts. *African Journal of Marine Science*, 27(1), 297–306. https://doi.org/10.2989/18142320509504088
- Sievers, M., Fitridge, I., Bui, S., & Dempster, T. (2017). To treat or not to treat: a quantitative review of the effect of biofouling and control methods in shellfish aquaculture to evaluate the necessity of removal. *Biofouling*, 33(9), 755–767. https://doi.org/10.1080/08927014.2017.1361937
- South, P. M., Floerl, O., & Jeffs, A. G. (2019). The role of biofouling development in the loss of seed mussels in aquaculture. *Biofouling*, 35(2), 259–272. https://doi.org/10.1080/08927014.2019.1596261
- Tabuteau, F. (2018). PFG invests in Blue Mussel processing plant in Marlborough. *Beehive.Govt.Nz*. Retrieved from https://www.beehive.govt.nz/release/pgf-invests-bluemussel-processing-plant-marlborough
- Thyrring, J., Jensen, K. T., & Sejr, M. K. (2017). Gametogenesis of an intertidal population of *Mytilus* trossulus in NW Greenland: Not a limitation for potential Arctic range

expansion. *Marine Ecology Progress Series*, 574, 65–74. https://doi.org/10.3354/meps12179

United Nations. (1992). Convention on biological diversity united nations 1992. UN.

- Westfall, K. M., Wimberger, P. H., & Gardner, J. P. A. (2010). An RFLP assay to determine if *Mytilus* galloprovincialis Lmk. (Mytilidae; Bivalvia) is of Northern or Southern hemisphere origin. *Molecular Ecology Resources*, 10(3), 573–575. https://doi.org/10.1111/j.1755-0998.2009.02779.x
- Westfall, K. M., & Gardner, J. P. A. (2010). Genetic diversity of Southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. *Biological Journal of the Linnean Society*, 101(4), 898–909. https://doi.org/10.1111/j.1095-8312.2010.01549.x
- Westfall, K. M., & Gardner, J. P. A. (2013). Interlineage *Mytilus* galloprovincialis Lmk. 1819 hybridization yields inconsistent genetic outcomes in the Southern hemisphere. *Biological Invasions*, 15(7), 1493–1506. https://doi.org/10.1007/s10530-012-0385-8
- Wijkmark, N., & Isæus, M. (2010). Wave exposure calculations for the Baltic Sea. Retrieved from http://www.aquabiota.se/wpcontent/uploads/ABWR_Report_2010_02_BaltExp1.pdf
- Zabin, C. J., Ashton, G. V., Brown, C. W., Davidson, I. C., Sytsma, M. D., & Ruiz, G. M. (2014). Small boats provide connectivity for nonindigenous marine species between a highly invaded international port and nearby coastal harbours. *Management of Biological Invasions*, 5(2), 97–112. https://doi.org/10.3391/mbi.2014.5.2.03
- Zbawicka, M., Trucco, M. I., & Wenne, R. (2018). Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels: Hybridization with invasive European *Mytilus* galloprovincialis. *Genetics Selection Evolution*, 50(1), 1–14. https://doi.org/10.1186/s12711-018-0376-z

SUPPLEMENTARY MATERIAL



Figure 1 Workflow for processing of satellite derived environmental data. This process is for one site and was performed for every site.



Figure 2 Volume of international shipping traffic, i.e. number of arrivals of ships whose voyages originated from a port outside of New Zealand, for every primary and secondary port. If an international ship visited multiple port on their voyage, then each port visit was counted.

Port Name	Latitude	Longitude
Auckland	-36.837247	174.775195
Bluff	-46.595648	168.334881
Dunedin	-45.812942	170.627286
Gisborne	-38.677107	178.021703
Lyttelton	-43.609905	172.726332
Napier	-39.472159	176.917048
Nelson	-41.260045	173.279463
Port Taranaki	-39.055566	174.04134
Port Chalmers	-45.812862	170.629868
Tauranga	-37.656293	176.177966
Timaru	-44.389656	171.258319
Wellington	-41.286807	174.783022
Whangarei	-35.757571	174.348665

 Table 1 List of primary ports.

Port Name	Latitude	Longitude
Akaroa	-43.809544	172.959953
Bay of Islands	-35.281848	174.094921
Fiordland	-44.677624	167.920565
Manukau	-36.932823	174.782143
Opua (Bay of Islands)	-35.315975	174.120245
Picton	-41.286555	174.007614
Stewart Island	-46.897174	168.132237
Taharoa	-38.066367	174.823336
Waitangi (Chathams)	-43.945709	-176.55949
Greymouth (NZGMN)	-42.448557	171.198743
Tarakohe Harbor (NZTKH)	-40.821879	172.896417
Wanganui (NZWAG)	-39.945387	174.992758
Westport (NZWSZ)	-41.745583	171.596357

 Table 2 List of secondary ports.

Port Name	Latitude	Longitude
Chelsea Port, Birkenhead	-36.823062	174.723858
Devonport (Royal New Zealand Naval Base)	-36.831432	174.785012
Kauri Point, Birkenhead, (Royal New Zealand Navy)	-36.823447	174.696408
Marsden Cove Marina	-35.836102	174.468025
Marsden Point Oil Refinery	-35.836333	174.499119
Napier Port	-39.472159	176.917048
Northport (Marsden Point Cargo Terminal)	-35.834338	174.490093
Picton Harbour	-41.286555	174.007614
Port Nelson	-41.260045	173.279463
South Port, Bluff	-46.595648	168.334881
Taharoa offshore buoy	-38.066367	174.823336
Timaru Port	-44.389656	171.258319
Vessel Works Marine Precinct	-37.673778	176.172515
Waikato (Taharoa)	-37.79427	174.880191

Table 3 List of places of first arrival

Port Name	Latitude	Longitude
Bayswater Marina	-36.822321	174.765549
Bucklands Beach Yacht Club Marina	-36.878659	174.899522
Chaffers Marina	-41.290303	174.78653
Clyde Quay Boat Harbour	-41.290303	174.78653
Eastland Port Marina	-38.670754	178.029174
Evans Bay Marina	-41.311976	174.799661
Gulf Harbour Marina	-36.624276	174.788362
Half Moon Bay Marina	-36.878659	174.899522
Havelock Marina	-41.274913	173.770776
Hobsonville Marina	-36.809417	174.649151
Kerikeri Marina	-35.194813	174.034271
Mana Marina	-41.101222	174.866124
Napier Marina	-39.483203	176.892646
Orakei Marina	-36.848584	174.80848
Outboard Boating Club	-36.854405	174.803061
Picton Marina	-41.287605	174.008386
Pine Harbour Marina	-36.887901	174.985654
Riverside Drive	-35.72468	174.327622
Sandspit	-36.390476	174.725657
Seaview Marina	-41.251494	174.901382
Tairua Marina	-37.003377	175.863071
Tauranga Bridge Marina	-37.659676	176.165009
Te Ana Lyttelton Marina	-43.605549	172.713403
Tutukaka Marina	-35.612088	174.528685
Waikawa Marina	-41.265013	174.039154
Whangamata Marina	-37.196595	175.866618
Whangaroa Marina	-35.049568	173.741471
Whitianga Marina	-36.836031	175.708037

Table 4 List of marinas