Population Dispersal of Native B.C. Blue Mussel Species *M. trossulus* and non-native B.C. Blue Mussel Species *M.edulis* and *M. galloprovincialis* along B.C Coastlines

Alexandra Dimtriou, Adan Moallemi, Yasmin Mohammadian Bozorgi, Toktam Movassagh, Alexandra Schmidt

Abstract

Blue mussel species are abundant across the coast of British Columbia, but the distribution and proportion of native versus non-native populations is yet to be explored in depth. The bay mussel, *M. trossulus*, is native to British Columbia but the population has recently become threatened by the presence of the non-native common blue mussels, *M.edulis*, and Mediterranean blue mussels, *M. galloprovincialis*. Non-native species are able to withstand more harsh conditions and outcompete vital native species for resources, which ultimately causes a shift in delicate marine ecosystem dynamics. In order to better understand the distribution of these three key species in British Columbia, we collected mussels at random, and identified samples from Ambleside, Harbourside place, English Bay, and Jericho Beach. We also collected and identified mussels from The Lobster Man Seafood Market on Granville Island, which were marketed as *M. galloprovincialis* to act as a control. We then isolated the DNA of five mussels from each location, performed Polymerase Chain Reaction (PCR) on the 25 samples and identified the mussels through gel electrophoresis. Our findings indicate the presence of non-native species *M.galloprovincialis* and native species *M.trossulus* at English Bay, Jericho Beach and Harbourside place. Samples from Ambleside indicate only the presence of *M.trossulus* and, as expected, the samples from The Lobster Man in Granville Island were *M.galloprovincialis*. Our results did not indicate the presence of hybrid species or the common blue mussel, *M.edulis*. This study collected and analysed observational data from the blue mussel species in the Greater Vancouver area to show the distribution and abundance of the native and non-native *Mytilus spp.* blue mussel species across the B.C. coast.

Introduction

British Columbia's coastal waters are home to an array of unique and complex marine ecosystems. One ecologically important family are mussels, a group of marine bivalves that are often found residing on the rocky substrate of the intertidal zone. *Mytilus edulis* complex are a group of mussels that are phenotypically indistinguishable, requiring DNA analysis to differentiate between them, and comprise of *Mytilus trossulus*, *Mytilus edulis*, *Mytilus galloprovincialis*, and their hybrids. Blue mussels provide habitat and food for a multitude of

marine life. Additionally, as filter-feeders, they act as vehicles for the transfer of anthropogenic pollutants to higher level consumers (Beyer et al., 2017). This group of invertebrates are not only ecologically valuable but have economic value to B.C. residents as a food source (Zippay et al., 2012). *M. trossulus* (bay mussel) is native to British Columbia but now shares its habitat with non-native *M.edulis* (common blue mussel) and *M. galloprovincialis* (mediterranean blue mussel).

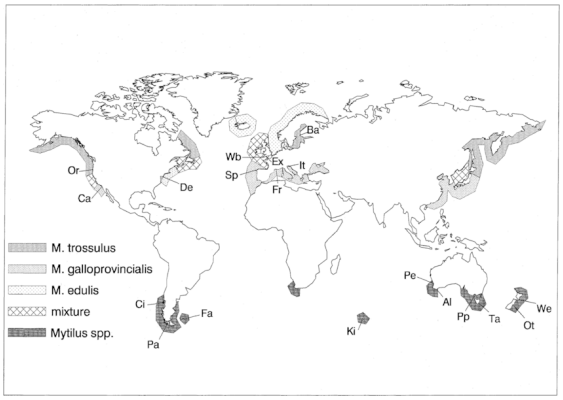

Figure 1. Global Distribution of *Mytilus* (Hilbish et al., 2000)

This study aims to investigate the distribution and abundance of blue mussel species across the B.C. coast. Little is known about the current abundance and impact of invasive mussels in British Columbia, however a prior foundation of research by Del Rio Wheatley and colleagues (2021) has paved the way for our analysis in invasive mussel colonisation. We are looking to fill the current knowledge gap by re-examining this research question at a greater scale. Compared to previous foundational work, this study has a greater sample size and broader spatial scale (expanding from Kitsilano to the North Shore).

Invasive mussels were introduced to the British Columbia coastline by shipping vessels and were later bred and used for aquaculture (Pickett & David, 2018). According to Zippay and colleagues (2012), *M. galloprovincialis* is a “dominant invader”, as it can withstand high salinity, increased water temperatures, and intense wave activity (Skibinski et

al., 1983), allowing it to out-compete native *M. trossulus*. Significantly, invasive species and climate change are the two main drivers of biodiversity loss globally (Mainka & Howard, 2010). As the ocean continues to warm, invasive mussel species are better equipped to survive the changing conditions than native species, and so the delicate balance of marine ecosystems becomes under greater threat. Monitoring and evaluating the threat of bioinvasions such as in the case of *M. galloprovincialis* are essential to the protection of B.C.'s coastline ecosystems.

We strive to accurately assess the current distribution of species within the *Mytilus edulis* complex across the lower Mainland of British Columbia, to determine the relative abundance of invasive and native species. Genetic analyses were used to identify and compare specimens collected from the collection sites. This observational study is predicted to identify the presence of all three mussel species at each collection site. Due to the labelling at the Granville Island site, The Lobster Man, we predict that all specimens at this site will be *M. galloprovincialis*.

Methods

A. Collection

Mussels were collected from five different locations; English Bay, Jericho Beach, Harbourside Place, Ambleside and a fish market on Granville Island, The Lobster Man. A total of five mussels from each location were selected at random with no phenotypic bias given based on colour or shape. Some mussels were selected above the surface of the water and others from beneath the water, but all were selected with at least a 1 metre distance from one another. The water temperature of the intertidal zone at each location was recorded, the mussels were placed into a plastic bag, labelled, and then stored in the freezer after collection.

Figure 2. Mussels at Ambleside Beach (photograph by Toktam Movassagh)

Table 1: Mussel Collection Data

Number of Specimen	**Date & Time**	**Location**	**Water Temperature (°C)**	**Collector Name**
5	March 6 @ 10:47 AM	English Bay	6.7	Adan, Ali, Alex, Yasmin
5	March 6 @ 12:02 PM	Jericho Beach	7.2	Adan, Ali, Alex, Yasmin
5	March 6 @11:22 AM	The Lobster Man	4.0	Adan, Ali, Alex, Yasmin
5	March 7 @ 3:15 PM	Ambleside	7.5	Toktam
5	March 7 @ 3:45 PM	Harbourside	8.4	Toktam

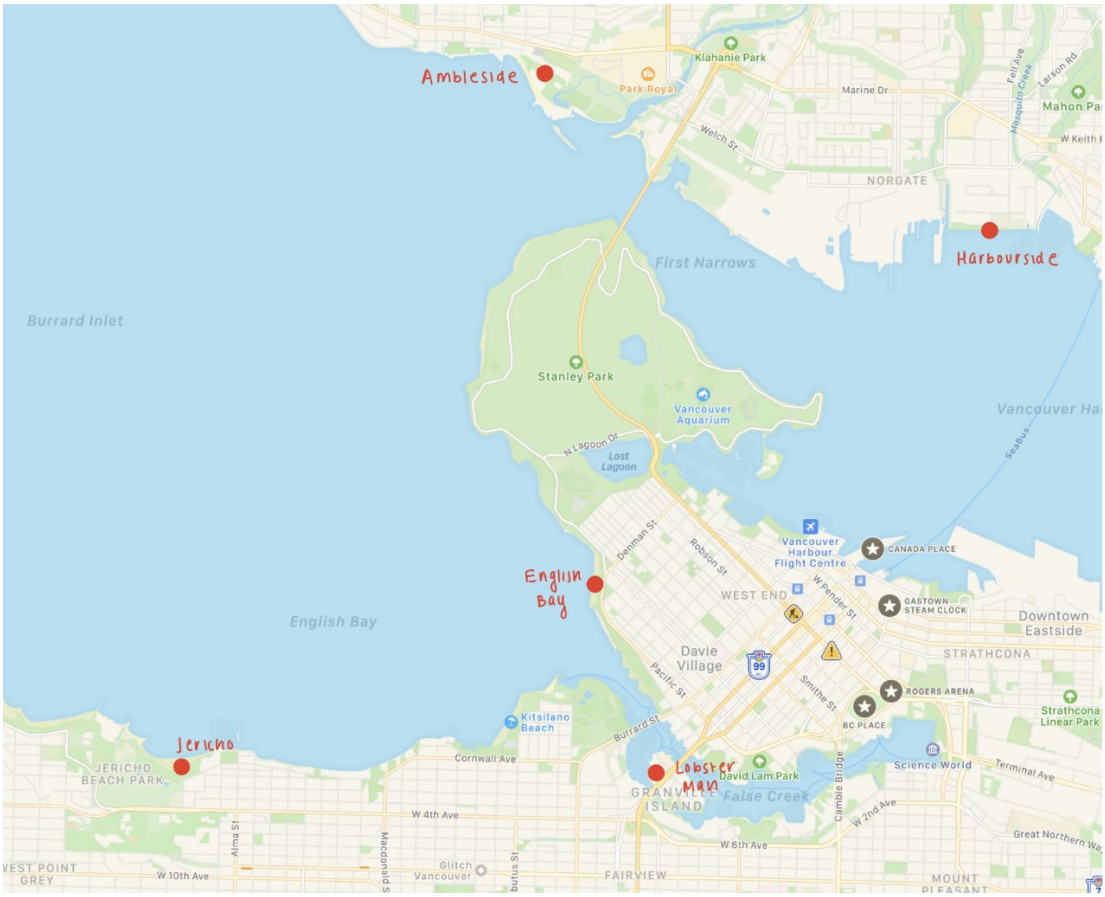

Figure 3. Partial map of Greater Vancouver and North Shore indicating all five collection sites; Jericho, English Bay, Harbourside, Ambleside and The Lobster Man

B. DNA Isolation

Twenty-six sterile 1.5 mL MCF tubes were labelled following Table 2. The mussels were pried open with gloves and a pinky nail size of mussel tissue was cut out with scissors and transferred to an appropriately labelled tube. Only some mussel pieces were mashed with a toothpick as the mussels were initially mushy in consistency. To each tube, 300 µl of "Cell Lysis Solution with Proteinase K " was added followed by a 15 minute incubatation period at 65^{o}C in 5 minute intervals and vortexed between each interval to yield a cloudy solution with some mussel tissue. The samples were then placed on ice for 5 minutes and 150 µl of "Protein Precipitate Reagent" was added to each tube. Samples were then vortexed again for 10 seconds followed by centrifugation at maximum speed for 10 minutes. The supernatant

from each sample was then transferred to a new, accordingly labelled 1.5 mL MCF tube and 500 µl of ice cold isopropanol was added. The tubes were then inverted 30-40 times. After inversion, white strings were observed in some tubes signifying the presence of DNA. The tubes were centrifuged for a second time for 10 minutes. The isopropanol was carefully poured off and 500 µl of ethanol was added to the pellet remaining at the bottom of the tube. The ethanol was poured off and this process was repeated twice in order to rinse away any remaining salts. Finally, the tubes were left to dry on their side overnight with the caps open to evaporate any remaining ethanol.

Table 2: Labelling Procedure

Location	**Sample #1**	**Sample #2**	**Sample #3**	**Sample #4**	**Sample #5**
Ambleside	AB1	AB2	AB3	AB4	AB5
English Bay	E1	E2	E3	E4	E5
Granville Island (The Lobster Man)	G1	G2	G3	G4	G5
Harbourside	HB1	HB2	HB3	HB4	HB5
Jericho Beach	J1	J2	J3	J4	J5
Control	dH_20				

C. PCR

To resuspend the dry pellet afforded from DNA isolation, 30 µl of TE Buffer was added to each pellet. New PCR tubes were then labelled accordingly for all 26 samples. The master mix was then prepared in an eppendorf tube following the recipe in table 3 and all reagents were kept on ice during the preparation and were mixed before adding to the eppendorf. Although there were only 26 samples, enough master mix was made for 28 samples to avoid making another master mix if there was not enough for all of the samples.

The dH_20 was added first as it had the largest volume followed by the remaining agents. With the help of our teaching assistant, the Taq polymerase was added. Finally 23 µl of mastermix was pipetted into all 26 labelled tubes, however our control tube, containing distilled water, only had 15 µl of master mix as there was not enough even after attempting to spin it down to recover more. The DNA was resuspended and added to the corresponding PCR tube containing the mastermix. dH_20 was added to the control tube in place of DNA. The PCR tubes were kept on ice until they were added to the PCR machine. The PCR tubes were placed in the PCR machine and run at the settings listed in Table 3. After PCR was complete, the tubes were stored in the freezer overnight.

Table 3: Master Mix Recipe for 28 Samples

Component	**Amount per tube**	**MM for group samples**
10X PCR Buffer	2.5 µl	70 µl
10 mM dNTPs	0.5 µl	14 µl
25 mM $MgCl_2$	1 µl	28 µl
5’ Primer 10uM (Me15)	1 µl	28 µl
3’ Primer 10uM (Me16)	1 µl	28 µl
Taq Polymerase	0.5 µl	14 µl
50% Glycerol	5.0 µl	140 µl
dH_2O (added first)	11.5 µl	322 µl
Total	**23 µl**	644 µl
DNA (added last) or sterile dH2O	2 µl	Not part of MM; added last

Table 4: PCR Cycle

Temperature	**Time**
95^{o}C	2 min.
95^{o}C	30 sec. x35

54°C	40 sec. x35
72°C	90 sec. x35
72°C	5 min.
4°C	Overnight

D. Electrophoresis

3% agarose gels were prepared and 2.7 µl of 10X loading dye were added to each PCR tube. Immediately before loading the samples into the gel, the solution was mixed by pipetting up and down. 20 µl of the sample and 20 µl of the DNA ladder were added onto the green coloured wells corresponding to the chart. Once all of the samples were loaded, the gels were run at 50V for 5 minutes and 150V for 75 minutes.

Results

Using the results from the gel electrophoresis, we analyzed the DNA banding patterns to extrapolate information regarding the population distribution of blue mussels (Figure 5). We distinguished blue mussel species based on the number of DNA base pairs. The classification was as follows: *M. edulis* (180 base pairs), *M. trossulus* (168 base pairs), *M. galloprovincialis* (126 base pairs) (Inoue et al., 1995).

.

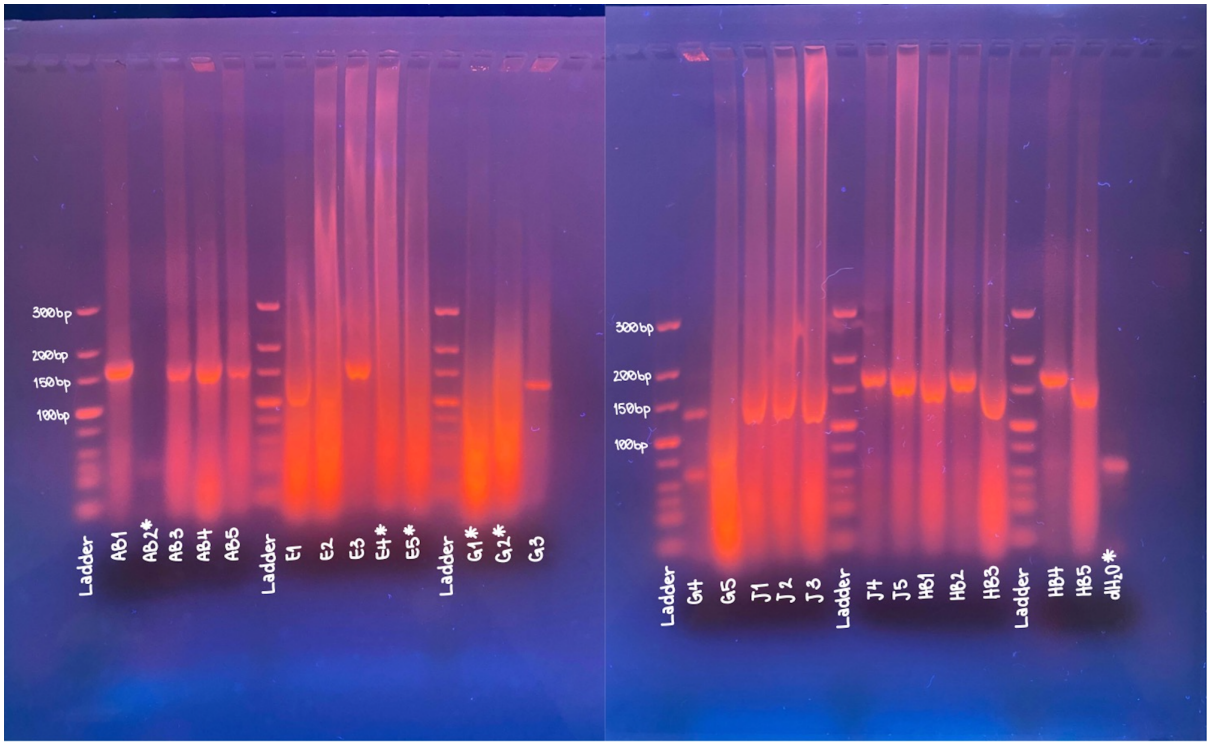

Figure 4. Gel electrophoresis depicting *Mytilus* species classification. AB1-5 = Ambleside; E1-5 = English Bay; G1-5 = Granville Island (The Lobster Man); J1-5 = Jericho Beach; HB1-5 = Harbourside; dH_2O = distilled water (control). Asterisk (*) depict samples showing contaminated results. Ladders serve as comparisons for banding patterns and are labelled based on relative number of base pairs, respectively.

At the Ambleside location, we observed banding patterns at 168 base pairs in four out of five of our samples. These patterns are consistent with the classification *M. trossulus*. One of the samples (AB2) lacks a clear banding pattern, which is indicative of contamination. Therefore, a conclusion regarding its species classification could not be made. At English Bay, E1 and E2 show banding patterns at 126 base pairs, consistent with what we would expect of *M. galloprovincialis*. E3 shows banding patterns at 168 base pairs, consistent with *M. trossulus*. E4 and E5 also show contamination, and therefore, were not classified. At the Granville Island location, we expected all of our classifications to be *M. galloprovincialis*, as they were advertised in this manner. Indeed, our results show banding patterns at 126 base pairs for G3 and G4, suggesting *M. galloprovincialis* classification. G1, G2, and G5 showed contaminated results, and were therefore not identified. At Jericho Beach, J1, J2, and J3 had a

clear banding pattern at 126 base pairs, and were classified as *M. galloprovincialis*. J4 and J5 had a band of 168 base pairs, indicative of *M.* trossulus. At Harbourside, HB1, HB2, HB4, and HB5 showed banding patterns at 168 base pairs. Thus, they were classified as *M. trossulus*. However, HB3 had a band of 126 base pairs, and was classified as *M. galloprovincialis*. Our control sample (dH_2O) also showed contaminated results.

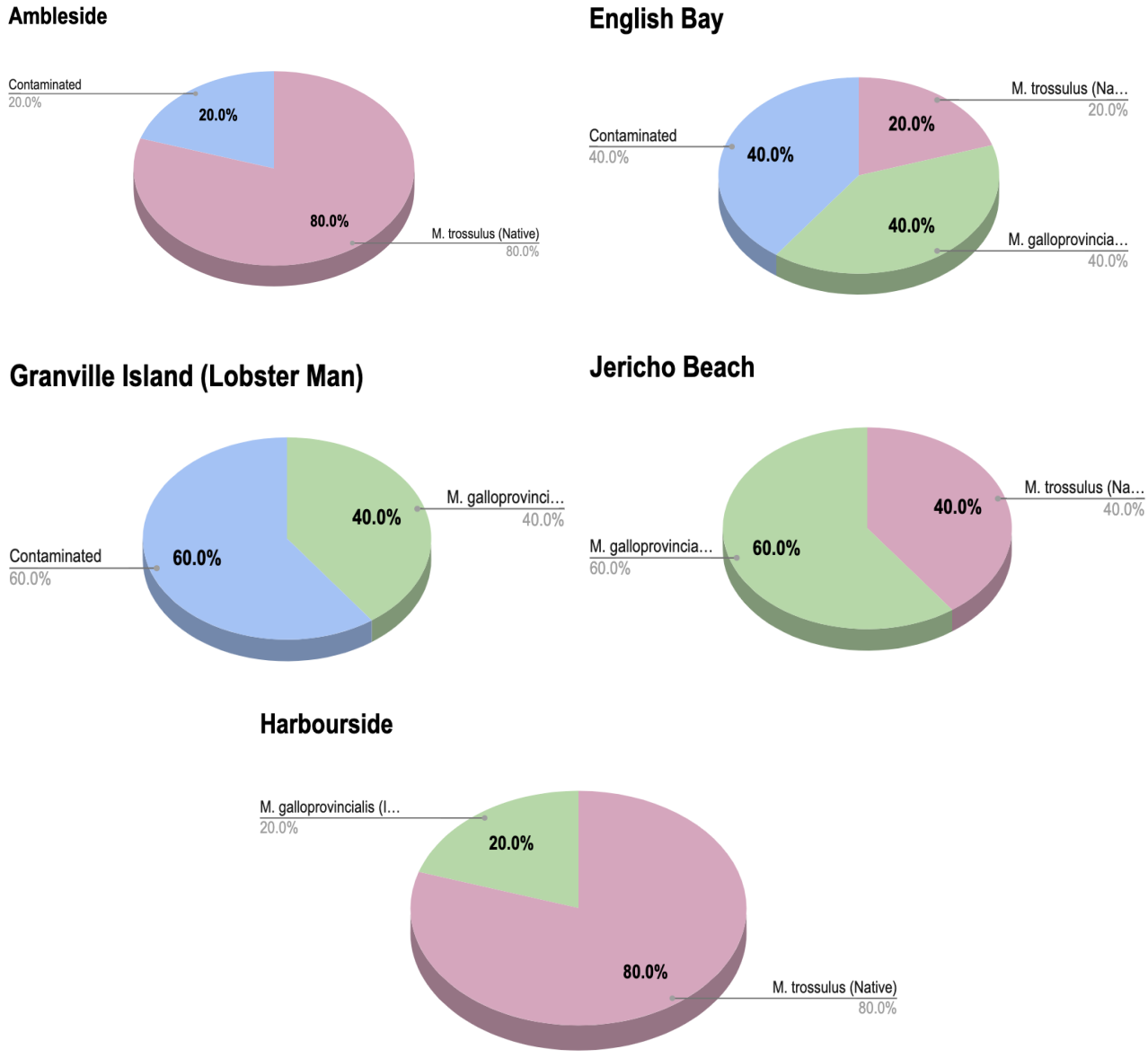

Figure 5. Graphs depicting species distribution of *Mytilus* based on gel electrophoresis analysis at each location. Pink = *M. trossulus*; green = *M. galloprovincialis*; blue = contamination.

Discussion

We predicted that we would find all blue mussel species at each collection site. Our findings suggest great variation in blue mussel species distribution amongst our collection sites, with the two most populous species being *M. galloprovincialis* and *M. trossulus*. Additionally, we failed to identify any *M. edulis* at our collection sites. Our findings contradict our earlier prediction, as we only identified *M. galloprovincialis* and *M. trossulus* with no *M. edulis* in our samples.

The samples collected from The Lobster Man were labelled as "Salt Spring Island" mussels, therefore we expected them to all be identified as *M. galloprovincialis* as most mussels harvested from Salt Spring Island belong to the *M. galloprovincialis* species (Saltspring Island Mussels, n.d.). Thus, identifying the non-contaminated samples from The Lobster Man as *M. gallopronvicialis* was expected and served as an effective control to compare with gel electrophoresis data from mussels collected at our sampling sites. It is well accepted amongst marine biologists studying the *M. edulis* complex that the invasive *M. galloprovincialis* often outcompetes *M. edulis* (Crego-Prieto et al., 2015). Based on the lack of *M. edulis* and hybrid mussel species identification, our data appears to be consistent with the literature. Surprisingly, Del Rio-Wheatley and colleagues (2021) reported markedly different findings. Their study only identified *M. edulis* invasive mussels and no *M. galloprovincialis* at their sampling locations. This observational difference could be attributed to a number of factors. First, in the aforementioned paper, only three mussels were used and analyzed from each collection site. Additionally, the collection sites were within a narrower geographic range; they were all in the local Vancouver area - with no mussels collected from the North Shore (Del Rio-Wheatley et al., 2021). Further, it is unclear why there was no trace

of *M. galloprovincialis* amongst their samples; this certainly warrants further investigation by expanding sample sizes beyond five and ensuring an even broader geographic range. Since mussel species are often found in a 'patchy' distribution (Koivisto & Westerbom, 2012), it is important to ensure adequate distance between each sample collected.

The importance of sampling from a wide geographic range was apparent in our results since both sampling sites from North Shore Vancouver, Ambleside beach and Harbourside place, had four out of five mussels collected identified as the native *M. trossulus* species. Such a high proportion of the native *M. trossulus* was not observed with mussels collected from Jericho Beach or English Bay since samples from these areas had more of a widespread distribution, which certainly favoured the invasive *M. galloprovincialis*. Past research has shown that *M.galloprovincialis* is more common in areas with high wave exposure (Suchanek et al., 1997). While we did not measure wave exposure at the collection sites, it is possible that Jericho and English Bay regions have a higher wave exposure, which could serve as a potential mechanism for the increased abundance of *M.galloprovincialis* in these regions compared to our North Shore collection sites. Studying the relative wave exposure of blue mussels in North Shore beaches compared to other tidal zones in Greater Vancouver would be an interesting avenue for further investigation. Moreover, studies have demonstrated that invasive species are selected for by exposure to open habitats, salinity, and temperature (Crego-Prieto et al., 2015). While this may serve to explain population distributions and variation in some regions, both salinity and water temperatures are consistent amongst all of the sampling regions in this study (Vancouver Coastal Health, 2021a & 2021b). Thus, our results may be representative of our sampling procedures or other mechanistics.

Gene amplification from six out of our twenty-five samples was unsuccessful, likely due to contamination as observed by the lack of discrete bands appearing in the gels. Despite

using sterile practice, contamination may have occurred following DNA isolation and upon exposure to the environment during PCR mix preparation. Additionally, contamination may have been introduced into the sample prior to sample collection through the mussel's natural environment. Future studies should first seek to reduce any error due to contamination by performing trial runs to identify the source of contamination and create an optimized protocol. Furthermore, a standardised method for mussel tissue isolation technique should be created and mussel tissue should be rinsed with sterile saline solution prior to DNA isolation to reduce contamination from the mussel environment.

Conclusion

Based on our experimental findings, we gained greater knowledge on the distribution and abundance of *Mytilus spp.* species across the B.C. coast. Our results indicate that there was presence of invasive species *M. galloprovincialis* and native species *M. trossulus* at English Bay, Jericho Beach and Harbourside locations. At the Ambleside location, samples only indicated the presence of only *M.trossulus.* As expected, the collection from The Lobster Man in Granville Island only indicated the presence of *M. galloprovincialis*. The collected samples showed no presence of *M. edulis* or hybrid species. Our results were consistent with the notion that *M. galloprovincialis* is a dominant non-native species that is present in the same niches as the native *M. trossulus* species. We suggest further analysis on shorelines along the B.C. coast with large sample sizes and diverse locations to gain further knowledge about the distributions of native and non-native blue mussel species. Ultimately, we hope for greater knowledge about the distribution of *Mytilus spp.* species will yield intervention against invasive mussels in order to prevent competitive exclusion of the native *M. trossulus*.

Acknowledgments

The authors express their sincere thanks to Dr. Celeste Leander for support and valuable advice in this study, to lab technician Mindy Chow for experimental setup and guidance, and to our teaching assistant Tessa Blanchard for assistance in planning the project. This work was performed as a part of the Biology 342 Integrated Laboratory Course supported by the University of British Columbia. We extend our acknowledgements to the University of British Columbia for the project resources. We acknowledge that we collected our samples on the traditional, ancestral and unceded territory of the Squamish Nation, Tsleil-Waututh Nation and Musqueam Nation, and conducted our experiment on the traditional, ancestral, and unceded territory of the Musqueam people. Finally, we give our gratitude to The Lobster Man seafood market located on Granville Island for providing the Granville Island set of mussels for the experiment.

References

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.

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

Del Rio-Wheatley, A., Gill, A. S., Le, C., & Wong, C. (2021). Determining the population distribution of invasive species Mytilus galloprovincialis and Mytilus edulis in comparison to the native species Mytilus trossulus in the Greater Vancouver area. The Expedition, 12.

Hilbish, Thomas & Mullinax, A. & Dolven, Sarah & Meyer, Axel & Koehn, Richard & Rawson, Paul. (2000). Origin of the antitropical distribution pattern in marine mussels (Mytilus spp.): routes and timing of transequatorial migration. First publ. in: Marine Biology 136 (2000), 1, pp. 69-77. 136. 10.1007/s002270050010.

Inoue, K., Waite, J.H., Matsuoka, M., Odo, S., Harayama, S. (1995). Interspecific variations in adhesive protein sequences of *Mytilus edulis, M. galloprovincialis, and M. trossulus*. *The Biological bulletin, 189*(3), 370-375. doi: 10.2307/1542155

Koivisto, M., & Westerbom, M. (2012). Invertebrate communities associated with blue mussel beds in a patchy environment: a landscape ecology approach. Marine Ecology Progress Series, 471, 101–110. https://doi.org/10.3354/meps10010

Mainka, S. A., & Howard, G. W. (2010). Climate change and invasive species: double jeopardy. *Integrative Zoology*, *5*(2), 102-111.

Pickett, T., & David, A. A. (2018). Global connectivity patterns of the notoriously invasive mussel, Mytilus galloprovincialis Lmk using archived CO1 sequence data. BMC Research Notes, 11(1), 1-7.

Saltspring Island Mussels. (n.d.). Saltspringislandmussels.Com. Retrieved March 27, 2022, from https://saltspringislandmussels.com/our_mussels.html

Skibinski DOF, Beardmore JA, Cross TF (1983). As-pects of the population-genetics of Mytilus (Mytilidae, Mollusca) in the British-Isles. Biological Journal of the Linnean Society 19, 137–83.

Suchanek, T. H., Geller, J. B., Kreiser, B. R., & Mitton, J. B. (1997). Zoogeographic distributions of the sibling species Mytilus galloprovincialis and M. trossulus (Bivalvia: Mytilidae) and their hybrids in the north Pacific. The Biological Bulletin, 193(2), 187–194. https://doi.org/10.2307/1542764

Vancouver Coastal Health. (2021a). Beach water quality Routine water test results 10/1/2021 10:52 AM. Vch.Ca. http://www.vch.ca/Documents/VCH-beach-route3.pdf

Vancouver Coastal Health. (2021b). Beach water quality Routine water test results 10/1/2021 10:53 AM. Vch.Ca. http://www.vch.ca/Documents/VCH-beach-route1.pdf

Zippay, Mackenzie & Helmuth, Brian. (2012). Effects of temperature change on mussel, Mytilus. *Integrative zoology. 7*(3). 312-27. doi: 10.1111/j.1749-4877.2012.00310.x