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

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Abstract

The common blue mussel, *Mytilus edulis*, and the Mediterranean mussel, *Mytilus galloprovincialis*, are both invasive species that are thinning out the population of our native species the Pacific Blue mussel, *Mytilus trossulus*, due to their ability to survive in harsh environments. They compete with our native mussels for resources which causes a shift in nutrient dynamics and marine ecosystems. We collected mussels from English Bay, False Creek, Jericho Pier, Kitsilano Beach, and the Lobster Man seafood market on Granville Island to determine the population distribution throughout the Greater Vancouver region. We performed DNA isolation and polymerase chain reaction (PCR) on the mussel samples, then analyzed the samples using gel electrophoresis. Our results indicated that we were only able to observe the presence of *M. edulis* and *M. trossulus* at English Bay, Jericho Pier, False Creek, and Kitsilano Beach. At the Lobster Man, the mussels were *M. galloprovincialis* as they advertised. No hybrids were present in our samples. Our results show a potential population distribution of the mussel population along the coast of British Columbia.

Introduction

British Columbia's marine ecosystem contains a combination of mussel species. These species of mussels include *Mytilus trossulus, Mytilus galloprovincialis,* and *Mytilus edulis,* more commonly known as the Pacific Blue mussel, Mediterranean mussel, and Common Blue mussel respectively. *M.galloprovincialis* outcompetes the other mussels due to its invasiveness, potentially impacting British Columbia's marine ecosystems. They are frequently found in the intertidal zone, where their byssal threads bind them to the rocky substrate (White *et al*., 2014).

In comparison to the other species listed above, *M.galloprovincialis* is more adaptable towards warmer temperatures, growing faster with a reproductive output of 20-200% (Zippay *et al*., 2012). There is limited information on how the invasive mussels affect B.C.'s coastal waters.

According to Branch *et al*. (2004), invasive species displace benthic creatures and inhabit their habitat, which tends to alter the makeup of native benthic ecosystems.

This experiment aims to identify the proportion of the three species from five different locations around Vancouver. The three species are morphologically identical, and thus, *M. trossulus, M.galloprovincialis,* and *M. edulis* can only be distinguished through genetic testing. This study's objective is to use DNA isolation, PCR, and gel electrophoresis to determine the population distribution of the three mussel species in Vancouver's waters and markets. Being an observational study, our experiment doesn't have a hypothesis but we predict the presence of *M. trossulus, M. edulis,* and *M. galloprovincialis* at all our collection sites, although *M. galloprovincialis* should be the most prevalent. When primers Me15 and Me16 are used, bands for *M. trossulus* are seen around 168 bp, bands for *M. edulis* at 180 bp, and bands for *M. galloprovincialis* around 126 bp (Inoue *et al*., 1995). The hybrids show numerous bands based on their genetic makeup.

The immediate impact of the *M.galloprovincialis* invasion on British Columbia's salmon is unknown. Intertidal benthic ecosystems are being altered, native biodiversity is being lost, and food webs are being modified as a result of their expansion. Mussels are considered "ecosystem engineers" because they transform their habitat by removing food from the water they dwell in, making it healthier for themselves and other creatures (Importance of mussels, 2021). As a result, they are ideal for monitoring water quality in the environment and tracking bioaccumulation in aquatic food webs. However, invasive species may cause extinctions of native species thus reducing biodiversity. They compete with native organisms for resources, which causes nutrient dynamics and physical habitats to change. Therefore, it is essential to monitor the effects of

invasive species that put native species at risk.

Methods

Given the fact that mussels were found to be sparse at the sample sites, convenience sampling was used for the collection of mussels. Between three to a handful of mussels were collected depending on the collection site. The five collection sites were the buoys from two separate boat parking spots within the Heather Civic Marina in False Creek, rocks in English Bay near the Inukshuk, rocks in Kitsilano near the Maritime Museum, rocks next to the pier as well as the pier itself near the Jericho Sailing Club, and finally three mussels were purchased from the Lobster Man seafood market on Granville Island. The temperature was measured at each collection site using a thermometer. Once in the lab, three mussels from each of the collection sites were randomly selected, for a total of 15 mussels. A caliper was used to measure the length of each mussel.

A. DNA Isolation

16 sterile 1.5 mL eppendorf tubes were labeled with numbers 1 through 16, with tubes 1 to 3 representing samples of mussels from Kitsilano, 4 to 6 representing samples of mussels from Jericho, 7 to 9 representing samples of mussels from False Creek, 10 to 12 representing samples of mussels from English Bay, 13 to 15 representing samples of mussels from Lobster Man, and sample 16 representing a negative control of water.

A small piece of mussel tissue, about one quarter of a pinky nail, was placed in each corresponding tube using sterile toothpicks along with 300 µL of "Cell Lysis Solution with Proteinase K." The samples were incubated at 65ºC for 15 minutes and taken out every 5 minutes

to vortex until the solution was cloudy. Afterwards, the samples were placed on ice for 5 minutes. 150 µL of "Protein Precipitate Reagent" was added to each sample tube, and each sample was then vortexed for 10 seconds. The centrifuge machine was loaded and balanced with the samples, which then underwent centrifusion at maximum speed for 10 minutes.

16 new sterile 1.5 mL eppendorf tubes were labeled the same way as previously while the samples were undergoing centrifusion. Once 10 minutes of centrifusion was complete, a pipette was set to 335 µL and the supernatant of each sample was transferred into new corresponding 1.5 mL eppendorf tubes. 500 µL of ice cold ethanol (see discussion) was added to the supernatant and the tubes were inverted 30-40 times. The tubes were then centrifuged at maximum speed for 10 minutes. The ethanol was carefully poured off without disrupting the pellet and 50 µL of ethanol was used to rinse leftover salts from each sample. The liquid was poured off, and this was repeated once more for a total of two rinses.

The tubes were left on their sides with their caps open at room temperature for 24 hours to evaporate any ethanol that was left. Finally, 30 µL of TE Buffer was added to each dry DNA pellet, and the samples were pipetted up and down to resuspend the DNA.

B. PCR

COMPONENT	AMOUNT	AMOUNT x 20
1. Sterile distilled water	11.5 µL	230 µL
2. 50% glycerol	5.0 µL	100 µL
3. 10 µM forward primer (Me15)	1.0 µL	20 µL
4. 10 µM reverse primer (Me16)	1.0 µL	20 µL

7. 10X PCR buffer	2.5 µL	50 µL
8. 10 mM dNTP	0.5 µL	10 µL
9. 25 mM $MgCl_2$	1.0 µL	20 µL
10. Taq polymerase	0.5 µL	10 µL
Final volume	23 µL	460 µL
Sample DNA or sterile dH_2O	2µL	---

Figure 1. Master Mix Recipe for one sample and for 20 samples.

The next day, PCR was performed. To begin, 16 new and sterile eppendorf tubes were labelled as before. To account for pipetting errors, 20 samples worth of the Master Mix recipe shown in Figure 1 was prepared with the largest volumes being added first. After Taq polymerase was added by an instructor, the final volume was mixed using a pipette. Once ready, 23 µL of master mix was added to each of our new eppendorf tubes. Then, 2 µL of DNA from the corresponding tubes were added to the new tubes. The 16th sample was filled with 2 µL of distilled water in place of DNA to serve as a negative control. The tubes were then placed in the PCR machine overnight following the thermal cycler procedure seen below in Figure 2.

TEMPERATURE	TIME
95°C	2 min
95°C for 30 sec 54°C for 40 sec 72ºC for 90	x 35
72°C for 5 min	x1
4°C	overnight
Store in freezer	

Figure 2. Thermal cycler procedure.

C. Electrophoresis

On the last day, gel electrophoresis was performed. To begin, 2.7 µL of "10x loading buffer" (an orange dye), was loaded on parafilm 16 times, leaving us with 16 dots of the buffer on the parafilm. The samples were thawed using heat emanating from gloved hands. The pipette was set to 15 µL and the 2.7 µL buffer dot was added to a sample tube. The sample was then mixed using the pipette and this was repeated 16 times for each sample. 6 µL of green DNA loading dye was added twice in the gel to act as ladders. Our samples were then loaded into the gel and run at 50 Volts for 5 minutes, 150 Volts for 75 minutes, and then 50 Volts for 5 minutes.

Results

We first looked at the sizes of the mussels collected. We noticed that the English Bay mussels were particularly small and mussels from the Lobster Man Seafood Market were large.

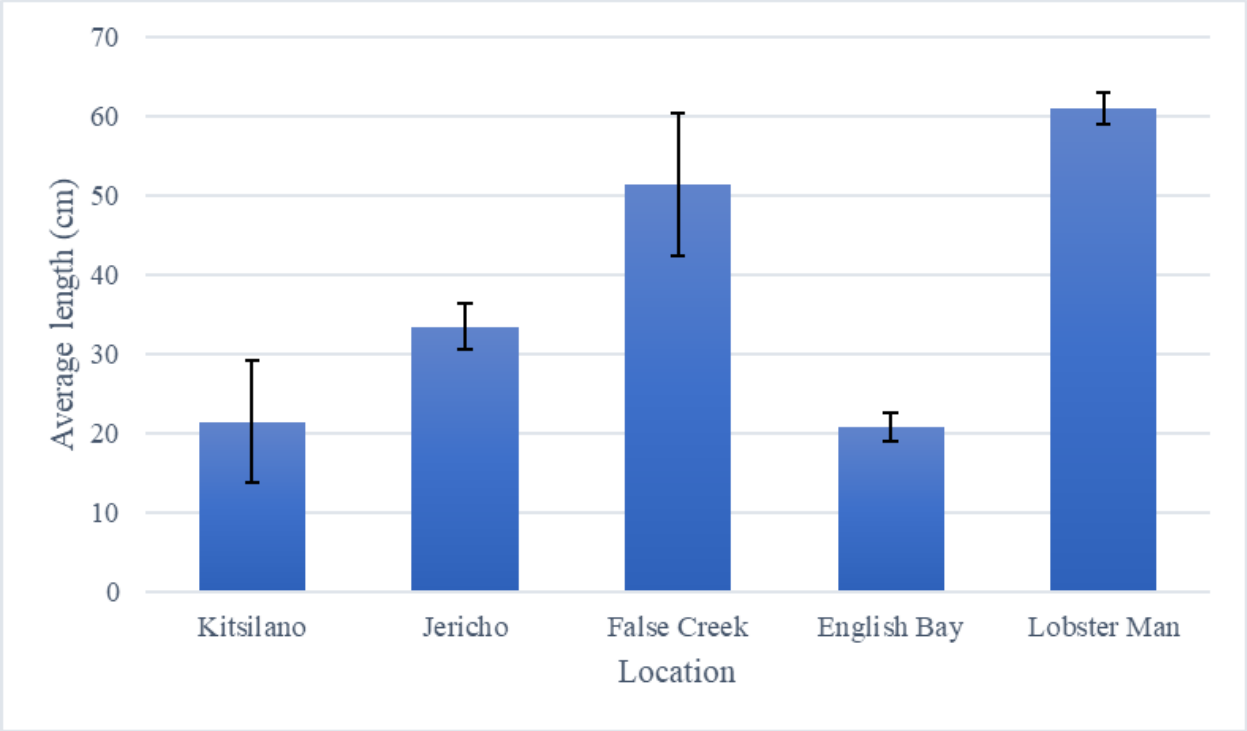

Figure 3. Average length in centimetres of all mussel samples by location. Error bars represent 95% confidence intervals. Three replicates were gathered at each site for a total of 15 replicates. Mussels were collected on October 31, 2021.

The population distribution of the mussels collected were analyzed using gel electrophoresis and their DNA band patterns. Figure 4 below shows the results of our gel electrophoresis run. From this, we can determine which species were present at each location by comparing band patterns to previous studies. At 126 bp, the species is *M. galloprovincialis;* 168 bp represents *M. trossulus,* and 180 bp indicates *M. edulis* (Inoue *et al*., 1995).

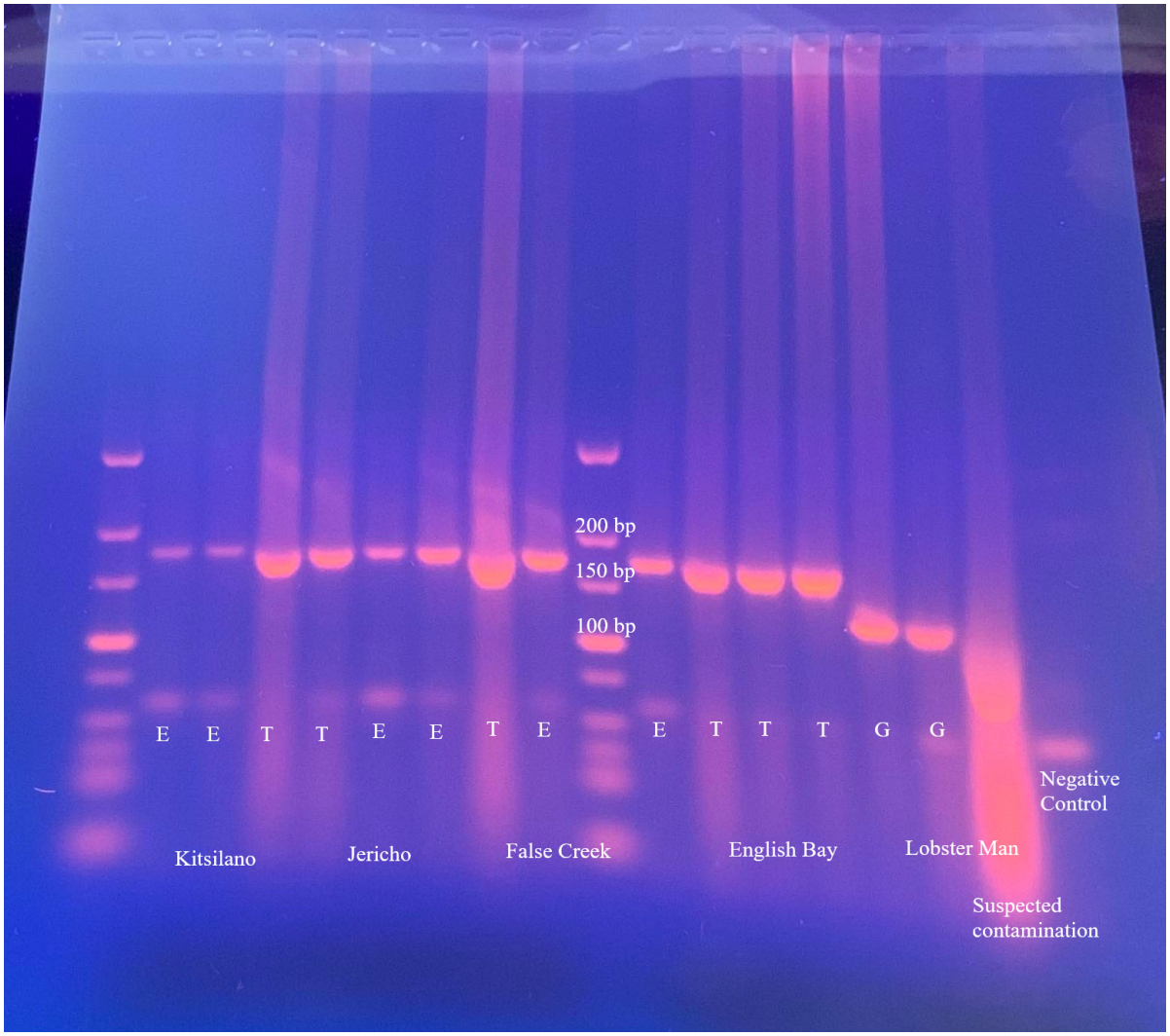

Figure 4. Gel electrophoresis on 16 mussel samples and a water control. E represents *M. edulis*, T represents *M. trossulus*, and G represents *M. galloprovincialis*. Each of the mussel samples are shown as single bands and the DNA ladder is marked in the middle. Bands around 126 bp suggest *M. galloprovincialis*, 168 bp suggests *M. trossulus*, and 180 bp suggests *M. edulis*. Mussels were collected on October 31, 2021 and electrophoresis was run on November 9, 2021.

From Figure 4 we can see that in Kitsilano, there were two of the invasive species *M. edulis* and one of the native species *M. trossulus* present. At Jericho and False Creek, there were two individuals of *M. edulis* and one of *M. trossulus*. At English Bay, all three were identified as *M.*

trossulus. Finally, at Lobster Man Seafood Market, there were only two successful funs in the gel electrophoresis and they were *M. galloprovincialis* as advertised.

Discussion

After analyzing the gel electrophoresis samples, we were able to draw some conclusions on the population distribution of the invasive species *M. galloprovincialis* and *M. edulis* in comparison to the native species *M. trossulus.* While there is no hypothesis to support or fail, the two successful runs on the mussels from Granville Island did meet our expectations of being *M. galloprovincialis* since they were labelled as so at Lobster Man. However, our prediction of seeing both invasive species with a majority of *M. galloprovincialis* at our collection sites was incorrect since we only saw the presence of *M.edulis*. Ultimately, extracting results from all samples allows us to determine that there is the presence of both the invasive species *M. edulis* and native species *M. trossulus* as well as no hybrids in the Greater Vancouver region.

Unexpectedly, we found that there was an absence of *M. galloprovincialis* from all four of our collection sites. While we cannot state that there is absolutely no presence of this invasive species in the Greater Vancouver region, this is unusual due to the fact that *M. galloprovincialis* is more likely to outcompete *M. edulis* (Crego-Prieto *et al*., 2015).

Although we can make some conclusions from our gel electrophoresis, there are potential errors that could have been rooted in our sampling method and our procedures. First, our decision of using only three mussels from each location did not allow us to have a good representation of all of the Greater Vancouver area or even the specific collection sites in general. Furthermore, our selection of mussels were limited at each location due to the fact that we were

unable to sample from an entire area. Therefore, our method of sampling was convenience sampling which is ultimately limited in its degree of randomization and representation.

Our sample method can explain the absence of *M. galloprovincialis* in our results but it also limits our ability to extrapolate our results over the whole Greater Vancouver area. A previous study found that *M. trossulus* and *M. galloprovincialis* also thrive in different environments, where the latter has grown in regions that have higher salinity and warmer temperatures with less variation in seasons (Braby, 2005). The significant difference in the environment here in comparison to the Mediterranean where *M. galloprovincialis* originates from is likely a key factor that influenced the distribution of this species in our samples.

Another factor that limited our ability to reflect the population distribution of the Greater Vancouver area is the fact that many mussels collected were small (see results) and presumably juvenile in age. This is likely due to the heatwave that befell onto our shores this summer, killing a concerning amount of marine life (Migdal, 2021). This could influence the results given that only mussels with higher thermal tolerances might have been able to survive and furthermore, indicating mussels sold at Lobster Man were significantly greater in size and were more likely to be fully developed.

While our experiment was able to extract some information about population distribution, we did yield one unsuccessful run which likely occurred due to human error. Smudging underneath the band indicates protein contamination, possibly occurring during DNA isolation or PCR. Although it was ensured that the environment was sterile at all times, contamination could have occurred during tube transfer when samples were vulnerable to open environments. In addition to this, inconsistencies in pipetting and mussel tissue extraction could have both

contributed to these failed results. In terms of potential errors in our methods, ethanol was accidentally added to the supernatant instead of isopropanol during DNA isolation. In future studies, it would be ideal to have more replicates and ensure greater consistency across samples in terms of mussel size and environment. Moreover, experiments would yield more valuable results if practices were more consistent in DNA isolation and PCR methods.

Conclusion

From our experiment, we were able to gain some insight on the population distribution of *Mytilus* spp. in the Greater Vancouver region. Results show that there is the presence of the invasive species *M. edulis* and native species *M. trossulus* at Kitsilano Beach, Jericho Beach, and False Creek. Samples from English Bay only showed the presence of *M. trossulus*. Our findings also determined that there was an absence of *M. galloprovincialis* and hybrids at all collection sites. As predicted, samples at the Lobster Man sold as "Gallo Mussels" from Saltspring Island were all confirmed as *M. galloprovincialis*.

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