Determining the species distribution of *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus* across different vendors in the Greater Vancouver area in British Columbia

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Abstract

This study aims to determine the species distribution of mussels across different vendors in the Greater Vancouver area in British Columbia. To evaluate such distribution, a cohort of 15 individual mussels were gathered from 5 vendors and screened for a genetic barcode that corresponds to different variants in one of their adhesive protein genes. The test consisted of the isolation of mussel DNA and subsequent polymerase chain reaction (PCR) amplification to obtain amplicons of different sizes that can be attributed to a given species. Our results indicate that 11 of 15 samples could be successfully identified as either *M. trosullus*, *M. galloprovincialis* or *M. edulis* and that the invasive species *M. galloprovincialis* was the most abundant with 47% of confirmed individuals. *M. trossulus* is the only endemic species to the coast of BC with 20% in abundance.

Introduction

The Pacific Coast along British Columbia, Canada is home to three Mytilus species, more commonly known as the mussel. *M. trossulus* (bay mussel) is native to this region, while *M. galloprovincialis* (mediterranean mussel) and *M. edulis* (blue mussel) are both invasive species originating from Europe (Crego-Prieto *et al.*, 2015). Mussels are morphologically very similar, which can make it very difficult to differentiate the mussel species by their morphology alone. The most variable features of these species are only accessible after dissection and optical magnification (Paulus *et al.*, 2018). The objective of our study aims to determine the species distribution of *Mytilus* across different vendors in the Greater Vancouver area in British Columbia. We predict to find all three species of mussels within our samples and hypothesize that our mussel population will not be homogeneous in species distribution. The mussels were collected from five different vendors across Greater Vancouver, BC: three vendors from Richmond and two vendors from Vancouver.

To evaluate the distribution of mussels, we screened for a genetic barcode that corresponds to different variations in one of their adhesive protein genes. The test consists of DNA isolation, polymerase chain reaction and gel electrophoresis. Our PCR test is based on the variation in the adhesive protein gene sequences, using the Me15 and Me16 primers (Inoue *et al*., 1995). The 5' (Me15) and 3' (Me16) are used to amplify fragments of the non-repetitive region of the sequence. These amplified fragments vary in length and can therefore be used to distinguish the mussel species: 126, 168 and 180 bp amplicons correspond with *M. galloprovincialis*, *M. trossulus* and *M. edulis*, respectively (Inoue *et al*., 1995).

Farming was first attempted with *M. trossulus* which came with many challenges such as post-spawning mortalities (Gurney-Smith *et al.*, 2017). This led to *M. edulis* being intentionally introduced to British Columbia in the 1980s as an alternate solution to aquaculture of *M. trossulus* (Gurney-Smith *et al.*, 2017). It is also argued that invasive species such as *M. galloprovincialis* may have been introduced to BC through hull fouling, ballast water, or were always present but never properly identified (Heath *et al.*, 1995).

The most commonly farmed mussels in BC nowadays are *M. edulis* and *M. galloprovincialis*, both invasive species (Canadian Aquaculture, 2015). Farmed mussels in BC are harvested by procuring the mussels from hatcheries and suspending mussel socks along a line of ropes until they've grown to market size (Fisheries and Oceans Canada, 2017). This can take anywhere from 1.5 to 3 years (Canadian Aquaculture, 2015).

Methods

To gather our samples, we purchased mussels from five different vendors around Greater Vancouver. All the mussels were collected one day prior to genotyping, and stored in 4°C

overnight. The test started by sampling three mussels from each vendor, making for a total of 15 mussel samples. The mussel samples were randomly picked by our biology lab course teaching assistant.

Figure 1. The mussels collected in Vendor1.

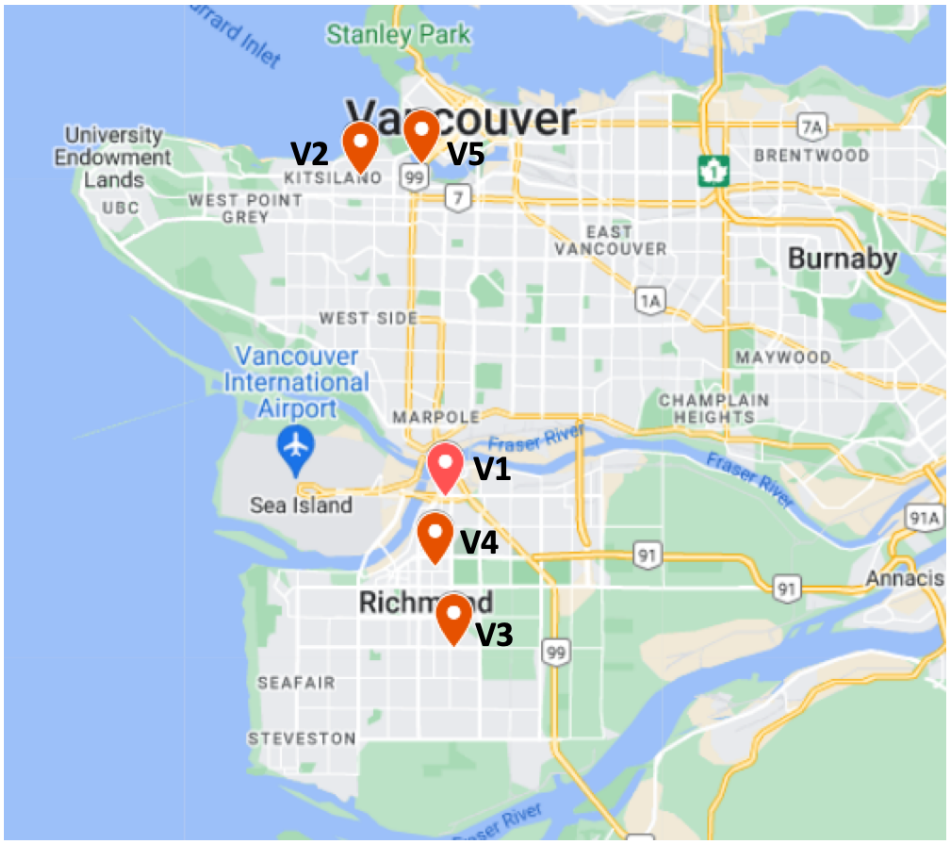

Figure 2. The locations of five vendors in Greater Vancouver.

The first step was DNA isolation. Fifteen 1.5 mL sterile Eppendorf tubes were appropriately labeled. The mussels were opened by tweezers and 100mg of mussel gills were cut using scissors. The samples were transferred to the labeled tubes and 300 µl of "Cell Lysis Solution with Proteinase K" were added to each tube before homogenizing the samples using toothpicks. Samples were incubated at 65°C for 15 minutes. The samples were vortexed for 10 seconds every 5 minutes until the solution looked cloudy and then placed on ice. This was followed by 150 µl of "Protein Precipitate Reagent" being added to each tube and vortexed for 10 seconds. After that, the samples were centrifuged by an Eppendorf 5415D centrifuge at 13200 rpm for 10 minutes at room temperature. Following this, the supernatants were transferred to 1.5 mL sterile Eppendorf tubes, and then 500 µl ice cold isopropanol was added. All the samples were carefully inverted in the tube 30 - 40 times. Later, centrifuged the samples at 13200 rpm for 10 minutes at room temperature. The isopropanol was carefully decanted, and the pellets were washed twice with 500 µl of 70% ethanol. The tubes were placed at room temperature for one day to evaporate any residual ethanol.

Location	**Sample1**	**Sample2**	**Sample3**
Vendor 1	MA1	MA2	MA3
Vendor 2	MB1	MB2	MB3
Vendor 3	MC1	MC2	MC3
Vendor 4	MD1	MD2	MD3
Vendor 5	ME1	ME2	ME3
Control	NC		

Table 1. Sample labeling of PCR reactions.

After completing DNA isolation, the next process was to perform polymerase chain reaction (PCR). All components were kept on ice for the whole process. 30 µl of TE buffer were

added to redissolve each pellet. The Master Mix was prepared in an 1.5 ml Eppendorf tube. The primer sequences used for our PCR reactions were: **Me15: 5'-CCA GTA TAC AAA CCT GTG AAG ACA-3'** and **Me16: 5'-TGT TGT CTT AAT AGG TTT GTA AGA-3'**. 23 µl of Master Mix were pipetted into 16 labeled PCR tubes. Then, 2 µl of mussel DNA was added into each PCR tube, and 2 µl distilled water was added into 1 PCR tube for negative control. The PCR tubes were placed in the thermocycler, and then the PCR program was started.

Component	**Amount**
Sterile distilled water	11.5 µl
50% Glycerol	5.0 µl
10X PCR buffer	2.5 µl
25mM MgCl2	1.0 µl
5' Primer 10uM (Me15)	1.0 µl
3' Primer 10uM (Me16)	1.0 µl
10 mM dNTPs	0.5 µl
Taq Polymerase	0.5 µl
Total	23.0 µl

Table 2. PCR Master Mix contents.

Temperature	**Time**	**Cycles**
95°C Initial denaturation	2min	1
95°C Denaturation	30s	35
54°C Annealing	40s	
72°C Extension	90s	
4°C Storage	Overnight	N/A

Table 3. PCR program conditions.

Once PCR was completed, our next step was gel electrophoresis. A 4% agarose gel in TAE with GelRed was prepared by the teaching assistant of our lab. 2.7 µl of 10X loading dye were added to each PCR tube. The solution was pipetted up and down several times. 20 µl of each sample was added into the gel wells, two DNA ladders (Ultra Low Range DNA Ladder by Invitrogen) were also placed for comparison. The gels ran for 2 hours and 15 minutes at 150V.

Results

Figure 3. Agarose gel under UV light.

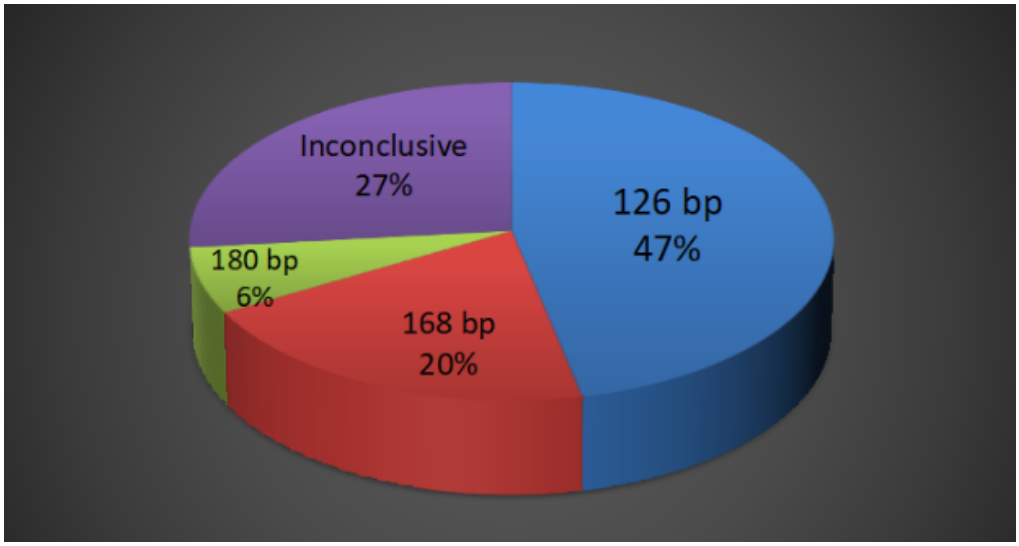

Figure 4. Distribution of amplicons of PCR results.

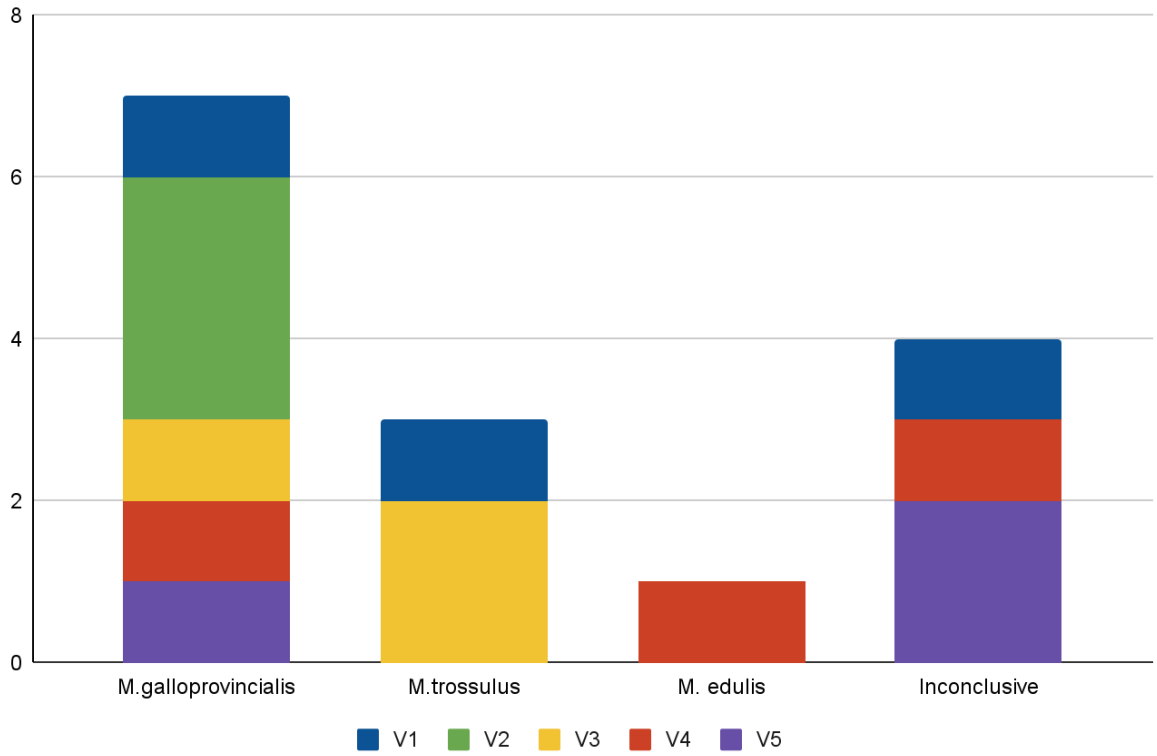

Figure 5. Bar graph of the species distribution of mussels. Colors indicate different vendors across Greater Vancouver.

Based on the previous research, banding at 180 bp indicates the tested mussels are *M. edulis*, 168 bp bands are *M. trossulus* and 126 bp bands are *M. galloprovincialis* (Inoue *et al.*, 1995).

After the gel electrophoresis, the results were collected. Vendor 1 mussel samples gave rise to one 126 bp amplicon, one 168 bp amplicon and one inconclusive sample. Vendor 2 samples gave rise to three 126 bp amplicons. Vendor 3 samples gave rise to one 126 bp amplicon and two 168 bp amplicons. Vendor 4 samples gave rise to one 126 bp amplicon, one 180 bp amplicon and one inconclusive sample. Vendor 5 samples gave rise to one 126 bp amplicon and two inconclusive samples.

Discussion

Our paper aimed to distinguish the different species of mussels labeled as "Gallo mussels", "Fresh mussels", "Live mussels" and "Blue mussels" commonly found in markets around Greater Vancouver. Out of 15 original samples, 11 gave successful PCR products of which we were able to identify and assign a species denomination according to the protocol established by Inoue *et al*. (1995).

For our MA series purchased from Vendor 1 in Richmond, we successfully identified 2 out of 3 samples. 1 sample was confirmed to be the invasive species *M. galloprovincialis* by the detection of a PCR product of 126bp in sample MA1. A second sample was identified as *M. trosullus*, which is an endemic species by detection of a 168 bp amplicon from mussel genomic DNA. The third PCR test was inconclusive.

Our MB series from Vendor 2 in Vancouver was successfully identified as *M. galloprovincialis* since they all showed a 126 bp amplicon as a product of our PCR reactions. This also makes the Vendor 2 series the most homogenous sample given the fact that all three individuals tested for the same species.

As for our MC series from Vendor 3 in Richmond, all samples were successfully identified, and similarly 2/3 were confirmed to be *M. trosullus* and 1/3 M.galloprovincialis.

The MD series from Vendor 4 in Richmond successfully identified 2/3 of the samples. MD1 was confirmed to belong to the invasive species *M. edulis* by the presence of a 180 bp amplicon. MD2 was confirmed as *M. galloprovincialis* as per previous methods.

Finally, in our ME series from Vendor 5 in Granville Island, only 1 sample was successfully identified as *M. galloprovincialis* while the other two tests were inconclusive.

We believe that our approach is good for determining the identity of an individual mussel given the fact that all of these species are very similar in both morphology and habitat. This potentially leads to hybridization and can result in mislabeling. However, there are limitations to our study that do not allow us to further investigate the source of these products.

From the 15 samples collected across the lower mainland, only 11 were successfully assigned to a living species of mussel giving a 73.3% rate of success in identifying mussels by genotyping with our set of primers. Given the small sample size, we could expect a larger population study to provide a more robust analysis. Even though 8 out of the 15 samples were identified as invasive, we believe that this number could be an underestimate given the size of our study, and *M. edulis* may be underrepresented.

With that being said, we observe significant protein contamination in our PCR products, mostly observable through the smearing of DNA in most samples as seen on the gel electrophoresis results. This indicates that the genomic DNA extracted from the mussels was not pure and this may have interfered with some of the PCR reactions. We were not able to assess the purity of the extracted DNA and devise a standard procedure for our PCR reaction based on known DNA concentration values. As a possible solution, we suggest that future studies improve

the quality of the DNA extraction by using spin DNA columns and checking for protein contamination through a spectrophotometer before using the genomic DNA in PCR to ensure the best possible rate of success. We would also recommend increasing the sample size per location to better assess the genetic makeup of a given population. Another possibility would be to introduce a second set of primers to evaluate the same population with a different molecular marker, allowing more data to be collected thus further increasing the accuracy of our genotyping experiment as initially performed by Inoue *et al*. (1995).

Overall, we observe that this method proved to be useful in rejecting our null hypothesis that all of the mussels were from a given species, and was also useful in identifying the three possible species found in the lower mainland. These results are also consistent with data presented by Dimtriou *et al.*(2022) where two of the three possible species are the most abundant being *M. trosullus* and *M. galloprovincialis* more likely to be found than *M. edulis*.

Conclusion

We conclude that our method proved to be useful in determining the species of a mussel obtained from a local vendor by PCR and allowed us to present evidence that supports our hypothesis while confirming our prediction that all three species were going to be found. PCR is a useful tool in determining the origin of mussels and can be used to appropriately label mussel products according to their species of origin.

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