Identifying Blue Mussel Species in store bought and locally collected Blue Mussels

Brendan Lam, Kai Kim

2024.12.07

Special thanks to Professor Celeste Leander, lab assistant Mindy Chow, TA Miriam Fenniri and TA Josh Yang for their helping hands with lab setup, aiding lab procedures, equipment and material procurement, and overall support.

Abstract

With the farming of blue mussel species *Mytilus edulis* (Common Blue Mussel) and *Mytilus galloprovincialis* (Mediterranean Mussel) being prevalent in BC, it poses a threat to the native species *Mytilus Trossulus* (Pacific Blue Mussel) as these species compete for resources. With the aim to determine the impact of these invasive species, samples from two large local grocers, an independent seller, and samples collected from False Creek are DNA isolated, then run through a PCR cycle, and finally electrophoresed in a 3% agarose gel to determine each sample's species. Most samples were inconclusive but two of the locally collected samples were found to be *M. galloprovincialis*, one of two invasive species.

1. Introduction

When it comes to global trade, Canada is a leading exporter of mussels, ranking second in overall quantity exported in 2022 (WITS, 2022). In British Columbia, blue mussels hold the 24th

position in the province's seafood export revenue (BC Ministry of Agriculture, Food, and Fisheries, 2020). Notably, the primary species farmed for commercial use in BC are *Mytilus edulis* (Common Blue Mussel) and *Mytilus galloprovincialis* (Mediterranean Mussel). These species are native to the North Atlantic and Europe, respectively.

The introduction and extensive farming of these non-native species, particularly with Mediterranean Mussel farming near native habitats, has led to significant ecological impacts on BC's native mussel species, *Mytilus Trossulus* (Pacific Blue Mussel). Hybridization between *M. trossulus* and the introduced species is becoming increasingly common, threatening the genetic integrity of the native population (Health et al., 1995). Additionally, the Common Blue Mussel and Mediterranean Mussel pose competitive threats, outcompeting the Pacific Blue Mussel for resources and exhibiting superior resilience to harsh weather and predation (Braby & Somero, 2006; Rius et al., 2008).

To better understand these invasive species' ecological implications and interactions with M. trossulus, it is essential to determine their presence and distribution in the Vancouver area. Because the external morphology of these mussels cannot reliably differentiate between species, molecular tools such as polymerase chain reaction (PCR) are required to identify them accurately (McDonalid et al., 1991). In this study, we aim to assess the occurrence of *M. edulis*, *M. gallovincialis,* and *M. trossulus* in the region by analyzing samples collected from three local markets and one nearby beach. By doing so, we hope to provide insight into the prevalence of invasive mussels and their potential impact on BC's native marine ecosystems.

2. Methodology

In order to identify the species of the mussels, each sample is DNA isolated, then a polymerase chain reaction (PCR) is run on each sample. Finally, to determine the species, electrophoresis is run on each sample.

Mussel samples were either purchased from two large local grocers: Grocer T and Grocer L, an independent shellfish retailer, or collected along False Creek in Vancouver during late October in 2024. The mussels purchased were fresh while the mussels collected from False Creek were frozen overnight.

2.1 DNA Isolation

For DNA isolation, a tissue sample is extracted from the mantle of each mussel. Each tissue sample is then placed in a labeled 1.5mL microcentrifuge tube where 300 uL of cell lysis solution with proteinase K is added. The test tubes are then placed in a 65°C incubation bath for 15 minutes, vortexing each tube every 5 minutes until the solution appears cloudy. After incubation, the samples are placed on ice for 5 minutes. After letting the samples sit in ice, 150 uL of mussel protein precipitate reagent is pipetted into each sample, vortexing for 10 seconds after adding. The samples are then centrifuged at maximum speed for 10 minutes with as much of the supernatant being transferred into new, labeled, 1.5mL microcentrifuge tubes. Only the supernatant is kept moving forward, discarding any remainers of the centrifuged samples. 500 uL of ice cold isopropanol is then added to the supernatant after which each test tube is inverted gently approximately 40 times. The new test tube is then centrifuged once again for 10 minutes at maximum speed, concentrating the DNA into a pellet at the bottom of each test tube. After the

cycle is complete, the remaining isopropanol is poured out, trying not to disrupt the DNA pellet at the bottom of the test tubes. The DNA pellets are then rinsed with 500 uL of ethanol twice, gently pouring out the ethanol each time. The test tubes containing the remaining DNA pellet are then stored at room temperature, on its side and with the lid open. This is done to allow any remaining ethanol to evaporate.

2.2 Polymerase Chain Reaction (PCR)

Before starting PCR, 30 uL of TE buffer is added to each DNA pellet, letting it sit for at least an hour. When adding the buffer, ensure that the DNA pellet is well suspended in the solution. A Mastermix of reagents, enough for 15 samples, is then made. The extra amount will be used for a negative control as well as accounting for potential loss during pipetting. The Mastermix is kept on ice the entire time.

Mussel Mastermix for 15 samples	
10X PCR buffer	2.5 ul x 15 = 37.5 ul
10 mM dNTPs	0.5 ul x 15 = 7.5 ul
25 mM MgCL2	1 ul x 15 = 15 ul
5’ Primer 10 uM (Me15)	1 ul x 15 = 15 ul

3’ Primer 10 uM (Me16)	1 ul x 15 = 15 ul
Taq polymerase	0.5 ul x 15 = 7.5 ul
50% Glycerol	5.0 ul x 15 = 75 ul
dH2O	11.5 ul x 15 = 172.5 ul
Total	**345 ul**

Figure 1: Mussel Mastermix formula for 15 samples with volume in ul.
This figure shows the volume of each solution, primer, and buffer needed for each sample. These volumes are then multiplied by 15 to make a mixture enough for 15 samples. The final volume is divided by 15 to determine how much of the mixture is combine with each sample.

To prepare for the PCR test, add 23 uL of ice cold Mastermix to each, labeled, PCR tube, keeping them on ice. 2 uL of each DNA sample is then added to the corresponding tube. A negative control is done as well, with 2 uL of sterile distilled water being added instead of DNA.

The test tubes are then placed into the PCR machine, running the following cycle:

PCR Cycle for Mussels	
1. 95°C	2 minutes
2. 95°C	30 seconds

3. 54°C	40 seconds
4. 72°C	90 seconds
repeat 2 - 4	**x 35**
5. 72°C	5 minutes
6. 4°C	overnight

Figure 2: PCR cycle for Mussels

The figure shows the temperatures and time that each sample is run at during the PCR process.

Once complete, the samples are stored overnight in the freezer.

2.3 Electrophoresis

For electrophoresis, a pre-prepared, 3% agarose gel with 20 wells is used; 12 wells, one for each sample, as well as two wells for ladders and 1 well for negative control. Before pipetting into the gel, 5 uL of 6X gel loading buffer is directly added to each PCR sample, including the negative control, and mixed with a clean pipette. Before adding the samples, 10 uL of an ultra low ladder is added to the second well with 15 uL of each sample being pipetted into the subsequent wells, being sure that there is no overflow and that a diagram is labeled with the name of each sample. The gel is then run at 50-80V until the samples are out of the wells, before running at 150V for two hours. The ladder rungs from top to bottom are as follows: 300bp, 200

bp, 150bp, 100 bp, 75bp, 50bp, 35bp, 20bp, 10bp. The brightest point of the ladder indicates 100bp.

2.4 Species Identification

Each mussel sample is then identified by examining the bands in the gel with a transilluminator with *Mytilus edulis* being at 180bp, *Mytilus trossulus* at 168bp, and *Mytilus galloprovincialis* at 126 bp.

3. Results

The mussels obtained from both large grocery chains were labelled simply as “Blue Mussels”. According to the banding, sample 2 from Grocer T, sample 3 from Grocer L, and samples 2 and 3 from the independent seller are between 20bp and 35bp while samples 2 and 3 from False Creek are very clearly at around 125bp. Some hybrids species were observed as well such as sample 3 from Grocer T, sample 2 from Grocer L, and sample 1 from False Creek. This is seen by the two clear bands at approximately 60bp and 30bp in lane 5 for Grocer T, 35bp and 10bp in lane 7 for Grocer L, and at 35bp and 10bp in lane 12 for the independent seller. Independent seller sample 1 in lane 11 is possibly a hybrid as well but there is heavy smearing found in the lane so the result is inconclusive. Sample 1 from Grocer T and sample 1 from Grocer L are unintelligible.

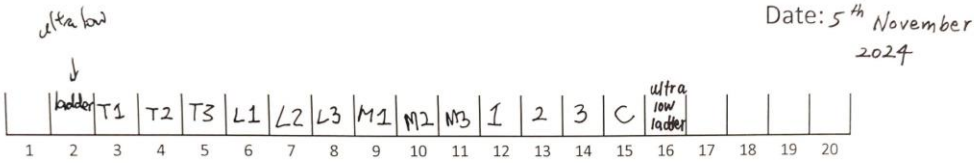

Figure 4: Lane Map for Gel Electrophoresis

This schematic shows the layout of gel electrophoresis lanes. Lane 2 and Lane 16 contain ultra-low molecular weight DNA ladders. Lanes 3-5 represent samples from T&T Supermarket (T1, T2, T3); Lanes 6-8 are samples from Loblaws (L1, L2, L3); Lanes 9-11 are samples from The Lobster Man (M1, M2, M3); and Lanes 12-14 are samples from UBC beach (1, 2, 3). Lane 15 contains the negative control (C).

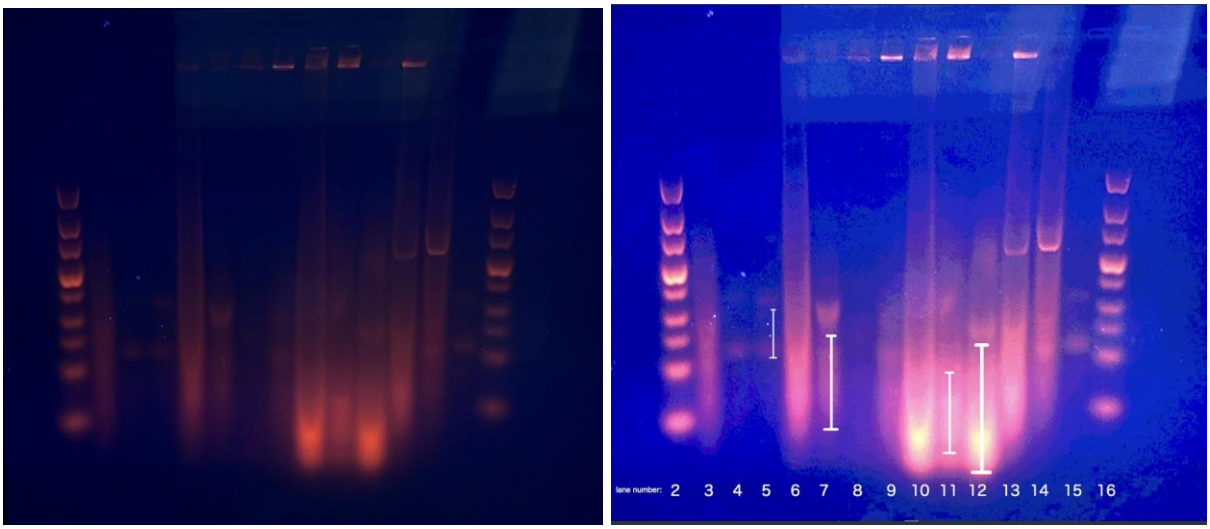

Figure 3: Gel Electrophoresis Result for Blue Mussel PCR Identification

This figure shows the electrophoresis results of DNA samples extracted from mussels collected from three markets (T&T, Loblaws, The Lobster Man) and a beach nearby UBC. Lane 2 and Lane 16 contain ultra-low molecular weight ladders, with bands that correspond to known DNA sizes. Lanes 3–15 represent PCR-amplified DNA from mussel samples. Lane 15 serves as the negative control (no amplification).

4. Discussion

As shown in the results, the bands for most of the samples are very unclear and do not correspond to the expected bps of *Mytilus edulis* (180bp), *Mytilus trossulus* (168bp), and *Mytilus galloprovincialis* (126 bp). The two exceptions are samples 2 and 3 from False Creek, found in lanes 13 and 14, where they are at an appropriate band at around 125 bp, close to the 126 bp of *M. galloprovincialis*. This is concerning as these samples were found naturally by False Creek and are clearly identified as *M. Galloprovincialis.* This species is a farmed blue mussel species in BC and is considered invasive to *M. trossulus,* the native species. It is noted that the mussels from False Creek were borrowed from another group as some of our initial samples were lost. The borrowed mussels were much smaller than the ones purchased.

There were a few samples that were poorly separated during the DNA isolation process. Namely the second sample from Grocer T and the third sample from Grocer L. This however, still produced visible, although barely, bands. It was found that after incubation, the samples from False Creek were the least cloudy. Samples 1 and 3 from Grocer T as well as sample 1 from the independent seller were very cloudy after adding the ice-cold isopropanol. After the first centrifuge cycle, it was found that the first sample from the independent seller had poor separation. After the second centrifuge cycle, the supernatant from sample 1 Grocer L was very snotty, causing it to be very challenging to pipette out. This could explain the immense smearing for that particular sample. As for the PCR step, the 50% glycerol used in the Mastermix was replaced with the same volume of dH2O due to the lack of glycerol in the lab. This substitution should not have affected the experiment. After adding the samples to the Mastermix and mixing well, samples 2 and 3 from Grocer T, sample 1 from Grocer L, sample 3 from the independent

seller, and samples 1 and 2 from False Creek were very snotty potentially causing smearing for those samples. The thick consistency also caused inconsistent amounts of solution in each PCR tube after swirling with a pipette. After PCR, samples 1 and 3 from Grocer T remained snotty. It is unsure as to why the electrophoresis bands do not correspond to what is expected for all samples except samples 2 and 3 from False Creek. The possible hybrids in lanes 5, 7, and 12 also have bands that do not correspond to the expected bps therefore it is unsure if those samples are actually hybrids. Lab assistant Mindy Chow suggested that the bands shown are actually primer dimers, a potential by-product that can form during PCR and cause a false positive after electrophoresis is run. It's possible that the electrophoresis was run for far too long at far too high of a voltage. Some of the smearing could also be due to the mucus-like consistency of the mussels, creating a source of error there. A combination of these errors as well as the formation of primer dimers may have caused the uncertainty found in the results.

5. Conclusion

This study aimed to assess the presence and distribution of blue mussel species (*Mythilus trossulus, Mytilus edulis,* and *Mytilus galloprovincialis*) in the Vancouver area using PCR-based identification methods. By analyzing mussel samples from local markets and a nearby beach, we observed clear banding patterns that allowed for the differentiation of species based on DNA fragment sizes. Despite limitations such as small sample size, lost samples, and inconsistencies during the DNA isolation and electrophoresis process, key findings emerged.

We observed that *M. galloprovincialis* is prevalent in our beach samples, while market samples exhibited greater variability, including potential hybrids. The presence of hybrids highlights the potential for genetic mixing between native and invasive species, which could pose ecological risks to the native *M. trossulus* population. However, challenges such as sample contamination, smearing, and unexpected banding patterns indicate that procedural refinements are needed for more robust data.

In conclusion, while the findings are preliminary, they emphasize the ecological impact of invasive mussels and underscore the importance of further research into their distribution and genetic interactions. Future studies with larger sample sizes and optimized protocols will be essential to confirm these observations and support conservation efforts for BC's native mussel populations.

Index

Mussel Mastermix for 15 samples	
10X PCR buffer	2.5 ul x 15 = 37.5 ul
10 mM dNTPs	0.5 ul x 15 = 7.5 ul
25 mM MgCL2	1 ul x 15 = 15 ul
5' Primer 10 uM (Me15)	1 ul x 15 = 15 ul
3' Primer 10 uM (Me16)	1 ul x 15 = 15 ul
Taq polymerase	0.5 ul x 15 = 7.5 ul
50% Glycerol	5.0 ul x 15 = 75 ul
dH2O	11.5 ul x 15 = 172.5 ul
Total	**345 ul**

Figure 1: Mussel Mastermix formula for 15 samples with volume in ul.
This figure shows the volume of each solution, primer, and buffer needed for each sample. These volumes are then multiplied by 15 to make a mixture enough for 15 samples. The final volume is divided by 15 to determine how much of the mixture is combine with each sample.

PCR Cycle for Mussels	
1.95°C	2 minutes

2.95°C	30 seconds
3.54°C	40 seconds
4.72°C	90 seconds
repeat 2 - 4	**x 35**
5.72°C	5 minutes
6.4°C	overnight

Figure 2: PCR cycle for Mussels

The figure shows the temperatures and time that each sample is run at during the PCR process.

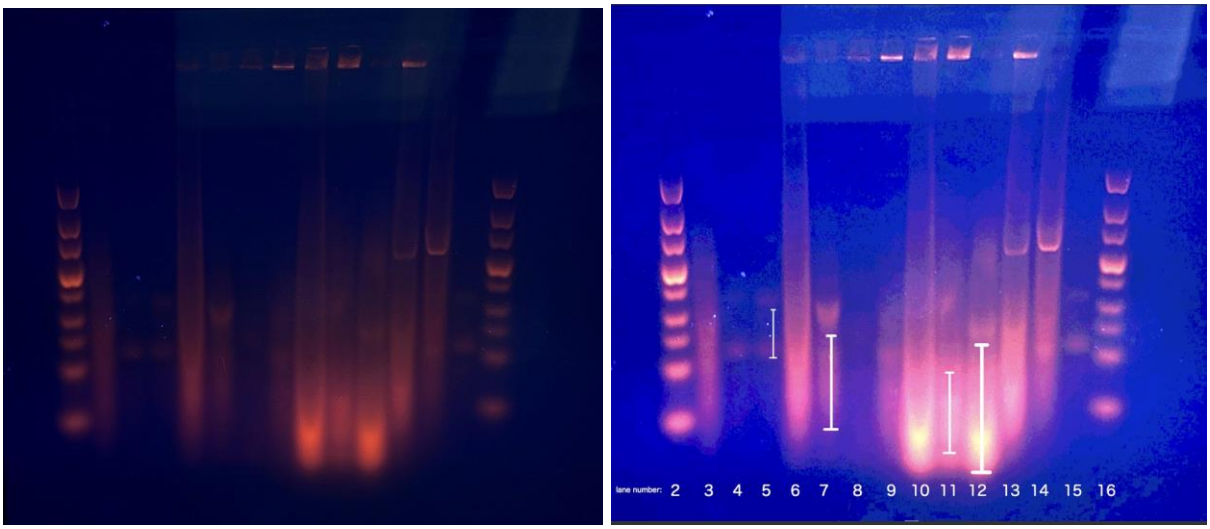

Figure 3: Gel Electrophoresis Result for Blue Mussel PCR Identification

This figure shows the electrophoresis results of DNA samples extracted from mussels collected from three markets (T&T, Loblaws, The Lobster Man) and a beach nearby UBC. Lane 2 and Lane 16 contain ultra-low molecular weight ladders, with bands that correspond to known DNA sizes. Lanes 3–15 represent PCR-amplified DNA from mussel samples. Lane 15 serves as the negative control (no amplification).

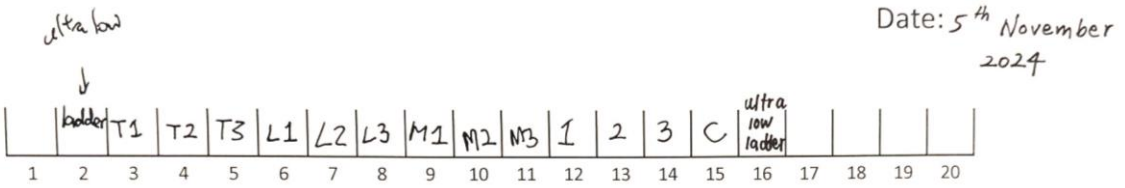

Figure 4: Lane Map for Gel Electrophoresis

This schematic shows the layout of gel electrophoresis lanes. Lane 2 and Lane 16 contain ultra-low molecular weight DNA ladders. Lanes 3-5 represent samples from T&T Supermarket (T1, T2, T3); Lanes 6-8 are samples from Loblaws (L1, L2, L3); Lanes 9-11 are samples from The Lobster Man (M1, M2, M3); and Lanes 12-14 are samples from UBC beach (1, 2, 3). Lane 15 contains the negative control (C).

Funding

None

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this report

Authors' Contributions

Methodology, results, discussion: BL.

Introduction, conclusion, index: KK.

References

BC Ministry of Agriculture, Food, and Fisheries. (2020). *Agriculture, seafood, food and beverage report*. Retrieved from [https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/agriculture-and-seaf ood/statistics/market-analysis-and-trade-statistics/2020_bc_agrifood_and_seafood_export_highli ghts.pdf].

Biological Bulletin, vol. 193, no. 2, 1997, pp. 187–94. JSTOR. https://doi.org/10.2307/1542764. Accessed 23 Nov. 2023.

Braby, C. E., & Somero, G. N. (2006). Ecological and evolutionary consequences of thermal tolerance limits in mussels (*Mytilus* spp.). *Marine Biology*, 149(5), 1251-1260.

Heath, D. D., Rawson, P. D., & Hilbish, T. J. (1995). PCR-based nuclear markers identify alien mussel (*Mytilus galloprovincialis*) on the west coast of Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 52(12), 2621-2627.

Kim, So-Hee, et al. "Diverse methods of reducing and confirming false-positive results of loop-mediated isothermal amplification assays: A Review." *Analytica Chimica Acta*, vol. 1280, Nov. 2023, p. 341693, https://doi.org/10.1016/j.aca.2023.341693.

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.

Michalek, Kati, et al. "*mytilus trossulus* introgression and consequences for shell traits in longline cultivated mussels." *Evolutionary Applications*, vol. 14, no. 7, 10 May 2021, pp. 1830–1843, https://doi.org/10.1111/eva.13245.

Rius, M., Pineda, M. C., & García-Castro, B. (2008). The competitive interactions of invasive *Mytilus galloprovincialis* with native species: Density-dependent effects. *Aquatic Invasions*, 3(3), 259-264.

Suchanek, T. H., et al. "Zoogeographic Distributions of the Sibling Species Mytilus

Galloprovincialis and M. Trossulus (Bivalvia: Mytilidae) and Their Hybrids in the North Pacific."

Vendrami, David L., et al. "Genome-wide insights into introgression and its consequences for genome-wide heterozygosity in the *mytilus* species complex across Europe." *Evolutionary Applications*, vol. 13, no. 8, 24 Apr. 2020, pp. 2130–2142, https://doi.org/10.1111/eva.12974.
"What We Farm." *BC Shellfish Growers Association*, 13 May 2024, bcsga.ca/what-we-farm/.

WITS (World Integrated Trade Solution). (2022). *Global trade in mussels*. Retrieved from [https://wits.worldbank.org/trade/comtrade/en/country/ALL/year/2022/tradeflow/Exports/partner

/WLD/product/030739#:~:text=In%202022%2C%20Top%20exporters%20of,1%2C379.59K%20

0%2C%2024%2C070%20Kg].