**The Instance of Mussel Mislabeling Among Richmond and Vancouver Markets of Native *M. trossulus,* Invasive *M.galloprovincialis* and *M.edulis*, and their Hybrids.**

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**Abstract**

Seafood fraud is a prevalent issue globally, and Canada is no exception with one of the highest rates of mislabelling in the world. This poses significant challenges to traceability, sustainability, and consumer protection. Among the commonly consumed types of mussels, the Blue Mussel (genus *Mytilus*) is particularly susceptible to mislabeling as a result of its morphological indistinguishability between multiple species, as well as its frequent hybridization. In this study, nine mussel samples, all believed to be *M.galloprovincialis,* were collected from three seafood markets in the Vancouver and Richmond area and were identified using PCR and gel electrophoresis. The banding patterns were compared to the expected banding patterns for each species of *Mytilus* to determine if the samples were labeled correctly or not. The results revealed that 11% of the mussel samples were mislabeled, or 1 out of the 9 mussels collected.

**Introduction**

The mislabelling of seafood products presents a serious problem, as it has the potential to cause economic damage, diminish consumer trust, pose health risks (depending on the type of seafood mislabelled), and decrease support for sustainable fisheries (Hu et al., 2018). Among the most commonly mislabeled seafoods are sea bass and red snapper, with mislabelling rates of 55 and 47 percent in the U.S, respectively (Oceana, 2019). Mislabeling can occur intentionally, by substituting higher quality fish for less expensive types, or by selling seafood from an unregulated supplier. Alternatively, it can occur unintentionally, due to incorrect species identification or vague labeling regulations (Hu et al., 2018).

Another type of seafood that is commonly mislabeled are mussels – a popular food source with a global production of 2.2 million tonnes in 2018 (FAO, 2019). In addition to leading to distrust in the seafood industry and having negative impacts on the sustainability of fisheries, the mislabeling of mussels can also have nutritional implications. Different types of mussels have varying nutritional values and flavor profiles, and they can also carry different levels of contaminants. For example, *Mytilus galloprovincialis*, commonly known as the Mediterranean mussel, was found to accumulate higher levels of Cadmium, Mercury, and Zinc compared to other mussels of the *Mytilus* genus (Sussarellu, 2022).

The mislabeling of *Mytilus* mussels may be attributed to the fact that they are morphologically indistinguishable without molecular testing (White et al., 2014). However, in places where there are more stringent protocols this does not pose an issue. For example, in the EU, mussel samples were found to have a 0% mislabeling rate in a study done in 2022 (Giusti et al., 2022). In their study, Giusti et al. (2022) tested mussel species that are also found along the B.C coast, including the native *M.trossulus* (bay mussel), the invasive species *M.edulis* (common blue mussel), and *M.galloprovincialis* (the Mediterranean mussel), as well as their hybrids (especially those of *M.galloprovincialis* and *M.edulis*). However, the rate of mussel mislabeling in B.C specifically has yet to be elucidated.

Canada has one of the highest rates of seafood mislabeling globally, yet little progress has been made to address this issue. Despite the Canadian government's promise to implement a boat-to-plate traceability system in 2019, 46% of seafood was found to be mislabeled in 2021, a mere 1% decrease from the mislabeling rate observed between 2017 and 2019 (Oceana, 2021). Our study aims to evaluate the adequacy of current mussel labeling regulations in Canada by quantifying the incidence of mislabeling in B.C. We collected three mussel samples labeled as *M. galloprovincialis* from three vendors in the Richmond and Vancouver area for genetic testing. Although our sample size is small, comparing the rates of *Mytilus* mislabeling in

Canada to other areas such as the EU may shed light on the inadequacy of Canada's current seafood labeling regulations.

**Methods**

To investigate the instances of seafood fraud among marketed *Mytilus* species in the B.C area, three mussels were randomly selected based on availability at three fresh seafood markets in Vancouver and Richmond. The mussels were all marketed as the same species, *M.galloprovinicialis*. Mussels were collected from the vendors one hour before arriving at the lab. Samples four through six were kept in a plastic bag at room temperature, and samples one through three, as well as seven through nine, were stored in plastic bags with ice. Our experiment was divided between 3 days; Monday, February 27th, Tuesday, February 28th, and Monday, March 6th. On day 1, each mussel was cut open by inserting sterilized scissors between the right and left shell and cutting around the mussel. Once open, samples the size of a pencil eraser of either, or both, of the anterior and posterior adductor muscle were extracted using sterilized scissors and tweezers (Figure 1). These samples were then placed into labeled Eppendorf tubes.

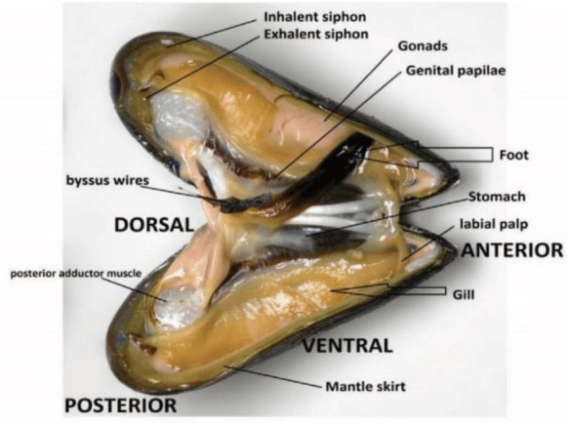


Figure 1. General anatomy of a mussel (Atasaral et al., 2020)

A separate toothpick was then used to mashed each sample for 1 minute. For each of the 9 tubes, a fresh pipette tip was used to add 300uL of Protein Lysis Solution with Proteinase K before incubation in a hot (65º C) water bath for 30 minutes. Each tube was vortexed every 5 minutes until the solution became cloudy before it was placed back in the water bath until the end of the 15 minute incubation period. Following incubation, the samples were placed on ice for 5 minutes. Using fresh pipette tips for each sample, 150uL of Protein Precipitation Reagent was added to each tube and vortexed for 10 seconds. Then, the centrifuge was used at maximum speed for 10 minutes. The supernatant of each tube was then transferred using a pipette to 9 new labeled Eppendorf tubes and the old pellets were discarded. To each new tube, a fresh pipette tip was used to deliver 500uL of ice cold isopropanol and the tubes were inverted slowly 40 times. The centrifuge was again used at maximum speed for an additional 10 minutes before the isopropanol was slowly poured out of each. Each pellet was rinsed twice with 20ul of ethanol using fresh pipette tips. All tubes were left uncovered on their sides on a layer of paper towels until the following day.

On day 2, all components were kept on ice for the duration of the day. To each tube, 60uL of TE buffer was added using a fresh pipette tip, although 30uL would have been sufficient. In a fresh Eppendorf tube, enough master mix was made for 11 samples (10 PCR tubes and 1 extra). We added the component needed in the largest quantity, dH2O, first followed by the second-largest quantity (50% glycerol) until all components were added (Table 1).

Table 1. Components of master mix for 11 PCR samples.

| **Component** | **Amount** | **Master Miix (x11)** |
|---|---|---|
| dH20 | 11.5 uL | 126.5 uL |

| | | |
|---|---|---|
| 50% glycerol | 5.0 uL | 55.0 uL |
| 10uM forward primer | 1.0 uL | 11.0 uL |
| 10uM reverse primer | 1.0 uL | 11.0 uL |
| 10x PCR buffer | 2.5 uL | 27.5 uL |
| 10mM dNTP | 0.5 uL | 5.5 uL |
| 25mM $MgCl_2$ | 1.0 uL | 11.0 uL |
| Taq polymerase | 0.5 uL | 5.5 uL |
| **Final Volume** | **23.0 uL** | **253.0 uL** |

The master mix was thoroughly mixed using a pipette after the addition of Taq. Ten new PCR tubes were labeled as samples 1-9 and a negative control, and 23uL of master mix was delivered to each tube using a pipette. Finally, 2uL of DNA from samples 1-9 was added to the corresponding PCR tube and 2uL of dH2O was added to the control PCR tube before all samples were placed in the PCR machine set to the appropriate cycle settings (Table 2). Samples were then stored in the freezer until the following week.

Table 2. Thermocycler procedure for PCR.

| Temperature | Time | |
|---|---|---|
| 95 °C | 2 min. | |
| 95 °C- > 54 °C -> 72°C -> 72°C | 30 sec. -> 40 sec. -> 90 sec. -> 5 min. | repeat x35 |
| 4 °C | overnight | |

On day 3, 1uL of 6X loading dye was added directly into each PCR tube using a fresh pipette tip for each before mixing the tubes thoroughly. The dye was then loaded onto a 2% agarose gel using a fresh tip for each. The location of each sample was recorded. Ladders were added between every 4 samples. Samples were run at 80V for 10 minutes (until every sample

was out of the wells) and at 150V for 50 minutes. The banding pattern produced for each sample was then observed and compared to previously identified banding patterns for *M.galloprovincialis*, and other *Mytilus* species (Figure 2).

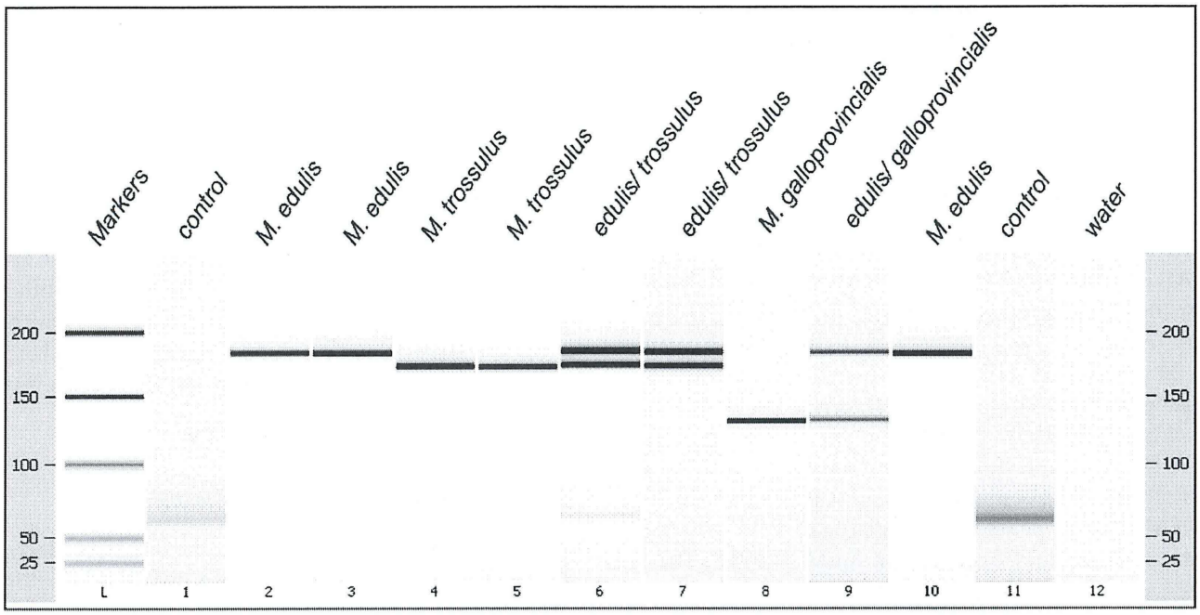


Figure 2. Previously identified banding patterns using gel electrophoresis on genus *Mytilus* (Brooks and Farmen, 2013)

**Results**

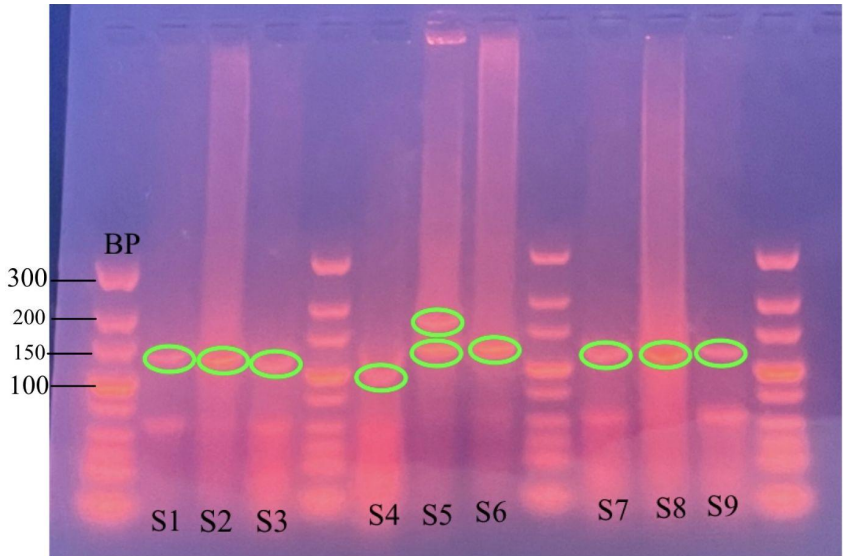

Figure 3. Banding pattern. Ladder is between every 4 samples. Samples are measured in the number of DNA base pairs.

PCR analysis showed 1 band at 126bp for samples 1-3, and 5-9, which is indicative of *M.galloprovincialis*. Disregarding the lower band, this indicates that these samples were *M. galloprovincialis*. Although the band for sample 4 seems to be lower than samples 1-3 and 5-9, since no mussel species corresponds to a band under 126bp, we are confident that sample 4 can also be identified as *M.galloprovincialis*. Sample 5 shows 2 bands, one at 126bp and one at 180bp, which suggests that this sample was an *M. galloprovincialis* and *M. edulis* hybrid.

**Discussion**

Our study found that eight out of nine mussel samples were accurately labeled as *M. galloprovincialis*, with one sample identified as a hybrid of *M. galloprovincialis* and *M. edulis*, resulting in an 11% mislabeling rate. Although our sample size was small, our results suggest that while the Mytilus mislabeling rate in Canada is lower than the overall seafood mislabeling rate of 46%, it is still higher than the 0% mislabelling rate observed in the EU. This present a cause for concern, especially considering the nutritional implications of mussel mislabelling (Sussarellu, 2022) in addition to other potential health risks and negative impacts on consumer

trust and sustainable fisheries associated with seafood mislabelling in general. As such, our findings indicate that further labeling regulations for blue mussels are likely necessary in Canada. To address the issue of mislabeling, we propose that genetic testing methods, similar to those used in this project, could be used as a feasible option to decrease mislabeling. Even occasional testing with small sample sizes can reveal inconsistencies and possible sources of error.

In our study, we found a hybrid mussel species, which was identified as a cross between *M. galloprovincialis* and *M. edulis*, both of which are non-native species that have been introduced to British Columbia, primarily for aquaculture purposes (White et al., 2014). The non-native mussels are known to have a longer lifespan than the native species, *M. trossulus*, which makes them more suitable for commercial aquaculture. However, it is crucial to preserve the native species to maintain the balance of the ecosystem in B.C.

*M. galloprovincialis*, in particular, is known to outcompete the native mussel species, which can lead to their displacement. The spread of this non-native species to different parts of the world is mainly attributed to its transportation through ballast water and use in aquaculture. Its invasiveness is so severe that it is listed as one of the world's worst invaders by the Global Invasive Species Database (2023). Therefore, it is essential to take necessary measures to conserve the native mussel species, especially *M. trossulus,* in B.C. to avoid any adverse impacts on the marine ecosystem.

Future studies should consider obtaining multiple samples from the same mussel to control for possible contamination and other testing errors. It would also be beneficial to include samples from various fish shops and farms to obtain a more representative depiction of the Mytilus mislabeling rate. Studies such as ours are important for increasing testability and accuracy in the seafood industry - within shellfish as a whole, genetic testing needs to be developed further; genetic methods are less developed in shellfish (depending on taxa) than fish due to a lack of information on DNA markers (Bernatchez et al., 2017). Overall, our findings

underscore the importance of accurate labeling in the seafood industry and highlight the need for continued efforts to improve seafood traceability and labeling practices in Canada.

## Conclusion

Mussel mislabelling, as well as seafood mislabelling as a whole, can be harmful to consumers and fisheries alike, but by continuing to research and work to find easy and accessible ways to reduce mislabelling, we can significantly reduce its prevalence. In our study, which used only 9 mussel samples, we were able to identify 1 sample as a mislabeled hybrid through the use of genetic testing. We suspect that future studies that utilize a larger sample size will be able to better elucidate the true proportion of mislabelled blue mussels in B.C. This information is vital in order to determine whether Canada's current mussel labeling regulations are sufficient, and if not, take steps towards ensuring that they are.

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