Relative Abundance of *Mytilus edulis* (invasive) and *Mytilus trossulus* (native) in Differing CO_2 Concentrations of Vancouver Coastal Waters

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I. Abstract

The relative abundance of the invasive blue mussel species, *Mytilus edulis,* to the native species, *Mytilus trossulus,* was recorded at Acadia Beach and Sunset Beach Park on Vancouver's coast. Carbon dioxide measurements of the water at each location were also compared. *M. edulis* is of interest as it can potentially outcompete the native mussel species, affecting British Columbia's marine ecosystem. Nineteen mussel samples were taken at both locations, as well as ten water samples, which were used to measure CO_2 concentration. DNA was isolated from the mantle tissue of the mussel, and used for polymerase chain reaction (PCR) and gel electrophoresis analysis to identify the phenotypically-identical species. A t-test was performed on the mean water CO_2 measurements, resulting in a statistically significant difference between the two locations (p-value < 0.001). The relative abundance of *M. trossulus* to *M. edulis* (n=19) at the site of lower CO_2 concentration (Acadia Beach, 3.27 ppm) was 15.8%, while at the site of higher CO_2 concentration (Sunset Beach Park, 6.74 ppm) the relative abundance (n=15) was 20.0%. A Fisher's test resulted in a statistically insignificant difference (p = 1.000) between the relative abundance of the two mussel species at the two sites. Findings suggest that the relative abundance of *M. trossulus* and *M. edulis* is unaffected by water CO_2 concentration of the observed range. Consequently, native and invasive blue mussel species' relative distributions are likely to remain unaffected by a predicted increase in carbon dioxide level.

II. Introduction

Anthropogenic elevation of pCO_2 in the atmosphere is resulting in an increase in aquatic CO_2 concentrations and promoting ocean acidification (Caldeira & Wickett, 2005). This causes a shift in the ocean bicarbonate buffering system such that the production of bicarbonate and net dissolution of calcium carbonate minerals is favoured (Figure 1). The outcome poses an issue for calcifiers as they depend on calcium carbonate to create their shells. Small-scale discrepancies in aqueous CO_2 concentrations can be explained by variations in water turbulence, vegetation, and biological activity. The latter is mainly driven by nutrient availability. As Sunset Beach Park is located deeper in the estuary than Acadia Beach, which likely receives more nutrient runoff contributing to elevated biological activity, we predicted a difference in CO_2 concentrations between waters at the two locations.

$$\mathbf{H_2O\ (l) + CO_2\ (aq) + CaCO_3}\ (s) \rightarrow \mathbf{Ca^{2+}\ (aq) + 2HCO_3^-\ (aq)}$$

Figure 1. Carbonate buffering system in the oceans. Increased carbon dioxide results in dissolution of calcium carbonate minerals.

Vancouver's waters are home to four blue mussel species: the native species *M. trossulus* (bay mussel) and *M. californianus* (California mussel), as well as two non-native species *M. edulis* (blue mussel) and *M. galloprovincialis* (Mediterranean mussel). *M. galloprovincialis* and *M. californianus* were not of particular interest in this study as their abundance is known to be quite low in the area (Gurney-Smith, Wade & Abbott, 2017).

The shells of blue mussels play an important role in aquatic ecosystems serving as an attachment site for other organisms (Sadler, Lemasson & Knights, 2018). Blue mussels are often co-farmed with salmon in integrated farming systems. Their coexistence has shown to increase

blue mussel growth rates (Handå *et al*., 2012). The fecal matter excreted by the salmon contributes to total, dissolved, organic matter of the water, which is then filtered by the mussels (Lander *et al*., 2012). A study conducted by Reid *et al*. (2010) in New Brunswick, Canada investigated the relationship between both *M. trossulus* and *M. edulis* and Atlantic salmon. They concluded that both mussel species were able to absorb the salmon waste-matter with high efficiency, resulting in increased mussel growth (Reid *et al*., 2010). In such ways, blue mussels are an integral part of the local marine ecosystem.

With predictions of the continuous rise of atmospheric pCO_2, blue mussels continue to be at risk (Caldeira & Wickett, 2005). As CO_2 concentrations increase, the surface area and thickness of the shells have been shown decrease (Sadler *et al.,* 2018). Melzner *et al*. (2011) studied the effects of increased pCO_2 and limited food sources on the internal shells of *M. edulis.* They found that the blue mussels experienced net shell dissolution when exposed to waters with high CO_2 and low nutrients. Blue mussels become more susceptible to predation when their shell dimensions become compromised (Sadler *et al.,* 2018).

While less is known about the effects of increasing CO_2 on *M. trossulus*, due to their physiological similarities, one could expect the two species to experience similar shell-growth impediments. However, effects on whole populations may be different and are not extensively studied. With the recent success of *M. edulis* on British Columbian shores, the relative abundance of the two species in relation to each other was hypothesized to vary at the two locations of differing CO_2 concentrations.

III. Methods

Two locations, 8 km apart, along Vancouver's coast were used for data collection: Acadia Beach and Sunset Beach Park. Acadia Beach, site A, is located north-east of the University of British Columbia Vancouver campus. Sunset Beach Park, site B, is located on the south-west side of downtown Vancouver, on the right bank of False Creek (Figure 2). At each site, water samples were collected at ten points along a 20 m transect line, chosen using a random number generator smartphone application. The transect line was laid in an easily accessible yet generally undisturbed area with evident mussel habitat. The temperature, salinity, and CO_2 concentration of each water sample was measured using a thermometer, salinity refractometer, and a field CO_2 titration kit, respectively. A two-tailed unpaired t-test was performed on the mean water CO_2 concentrations to determine statistical difference. Salinity and temperature were not statistically analyzed.

Nineteen mussels were collected from each site, at the same points along the 20 m transect line that were used for the collection of water samples. At the first nine points, two mussels were taken from separate rocks, and one mussel was taken from the last point. The mussels were placed in two sealed plastic bags and frozen for three days in a conventional household freezer (Figure 3). In the lab, each mussel was thawed and a small piece (about 10 mm^2) of the mantle tissue was cut out and placed in a sterile Eppendorf tube for DNA isolation.

Figure 2. Partial map of Vancouver showing the sites of data collection indicated by red circles: Site A (Acadia Beach) on the left at 49°16'48"N 123°14'32"W and Site B (Sunset Beach Park) on the right at 49°16'39"N 123°8'10"W. North is upwards.

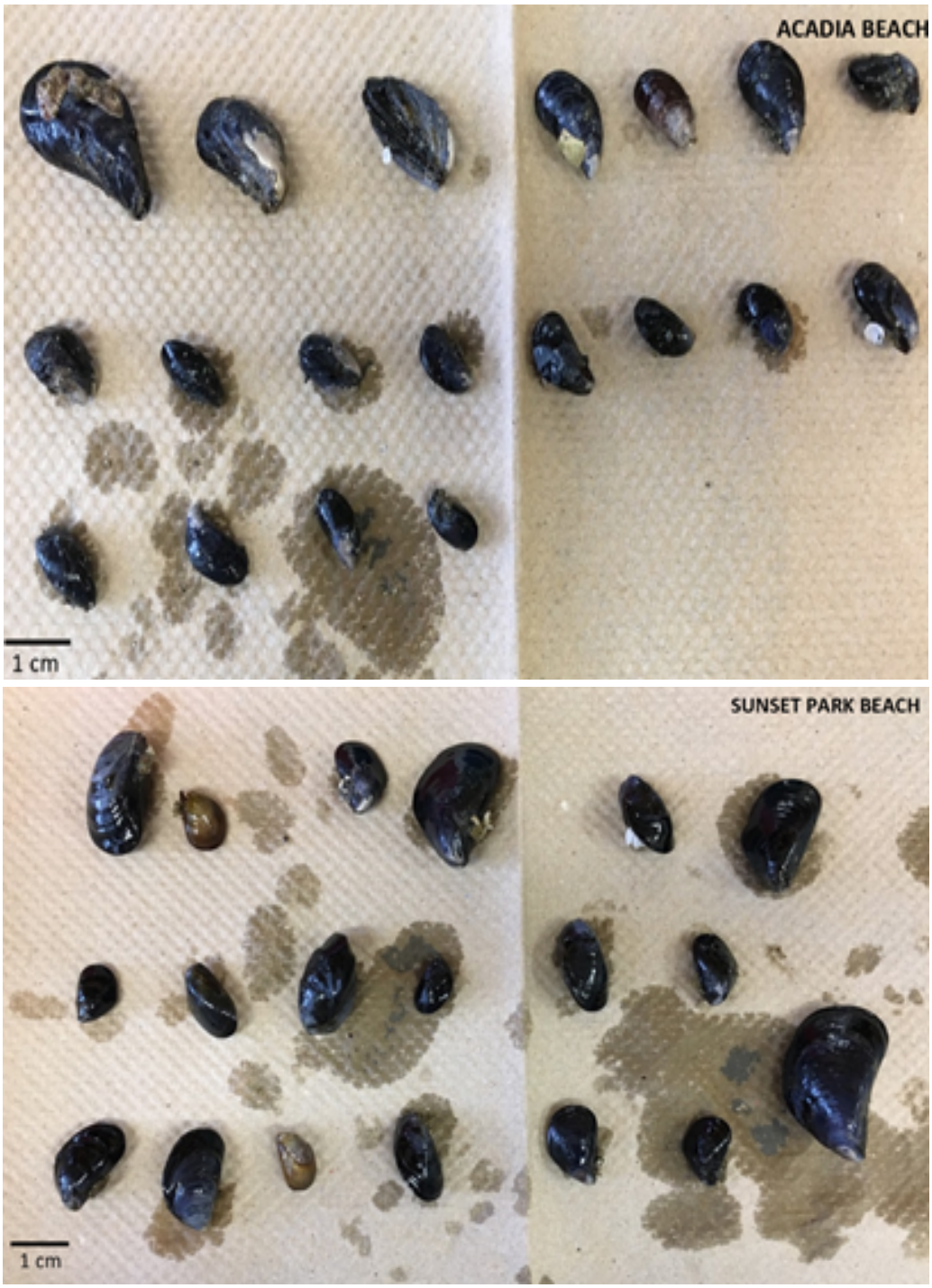

Figure 3. Mussel samples collected from Acadia Beach and Sunset Beach Park on October 26th 2018, frozen for three days, and thawed in the laboratory prior to analysis. Ten randomly chosen points along a 20-meter transect line were used for mussel collection: two mussels from separate rocks at the first nine chosen points and one mussel from the tenth point.

To isolate DNA, 300 µL of "Cell Lysis Solution with Proteinase K" was added to each Eppendorf tube, followed by incubation at 65°C and vortexing at five minute intervals for 15 minutes total. Afterwards, each sample was placed on ice for five minutes. Next, 150 µL of "Protein Precipitation Reagent" was added to each tube, vortexed, and centrifuged at maximum speed (16.1 rcf) for ten minutes. The supernatant of each sample was transferred into 38 new sterile Eppendorf tubes and 500 µL of ice cold isopropanol was added to each supernatant, inverting the tubes 30 - 40 times to mix. Each sample was then centrifuged at 16.1 rcf for ten minutes before pouring off the isopropanol. Similarly, each sample was rinsed twice with 500 µL of cold ethanol and left open for two days to dry at room temperature.

Once the DNA pellets had fully dried, 30 µL of TE buffer was added to each sample, pipetting up and down to resuspend the DNA. To prepare the Master Mix (MM), the components indicated in Table 1 were combined into a sterile Eppendorf tube, in the indicated amounts. The distilled water was added first and the Taq polymerase was added last. The resulting volume of the MM was enough for all 38 samples as well as 7 extras. All components were kept on ice at all times. Next, 23 µL of the MM and 2 µL of each of the DNA samples were added to 38 sterile 1.5 mL Eppendorf tubes. The samples were loaded into the thermocycler machine with the PCR cycle outlined in Table 2. Once complete, the samples were left in a 4°C freezer for three days.

Component	Amount per Sample (µL)	Total Amount for 45 Samples (µL)
Sterile distilled water	11.5	517.5
50% glycerol	5.0	225.0
10 µM forward primer (Me15)	1.0	45.0
10 µM reverse primer (Me 16)	1.0	45.0
10X PCR buffer	2.5	112.5
10 mM dNTP	0.5	22.5
25 mM MgCl2	2.0	90
Taq polymerase	0.5	22.5

Table 1. Ingredient list for the Master Mix (MM) solution. Order of addition of each component not specific, except for distilled water, which was added first, and Taq polymerase, which was added last. The final volume of the MM was 1080 µL.

Temperature	Time	
95˚C	2 minutes	
95˚C	30 seconds	X 35 times
54˚C	40 seconds	
72˚C	90 seconds	
72˚C	5 minutes	

Table 2. PCR machine cycle settings used for all mussel DNA samples.

Gel electrophoresis was performed by adding 4.0 µL of 6X Loading Dye to each of the 38 PCR samples, which were all pipetted up and down to mix. Then, 12 µL of each sample was loaded into a 3% agarose gel and the gel was run at 120 V for 150 minutes, before decreasing to 50 V for an additional two hours.

After obtaining the finished gel electrophoresis of the DNA samples, the banding patterns were examined based on fragment lengths to determine the species of each sample: *M. trossulus* (native) if banding at 168 bp, *M. edulis* (invasive) if banding at 180 bp, *M. galloprovincialis* (invasive) if banding at 126 bp, and hybrids if a combination of the listed banding patterns were displayed. The percent abundance was calculated in relation to the sum of the *M. trossulus* and *M. edulis* samples. A Fisher's statistical test was used to analyze the species proportions at both sites. The results from the Fisher's test and the t-test used for water CO_2 concentrations were used to draw conclusions about species relative abundance in association with water CO_2.

IV. Results

The water CO_2 concentration of each location was determined by averaging the measurements of the ten samples. The mean water CO_2 concentration was 3.27 ppm at Acadia Beach and 6.74 ppm at Sunset Beach Park (Figure 4). A two-tailed unpaired t-test was performed on these values, resulting in a p-value of less than 0.0001. Therefore, the null hypothesis was rejected, indicating a statistically significant difference between the mean aqueous CO_2 concentrations for the two locations. Mean water temperature and salinity were 12.1°C and 27.2‰ at Acadia Beach, and 11.7°C and 28.9‰ at Sunset Beach Park, respectively. However, no statistical tests were used to analyze these two variables.

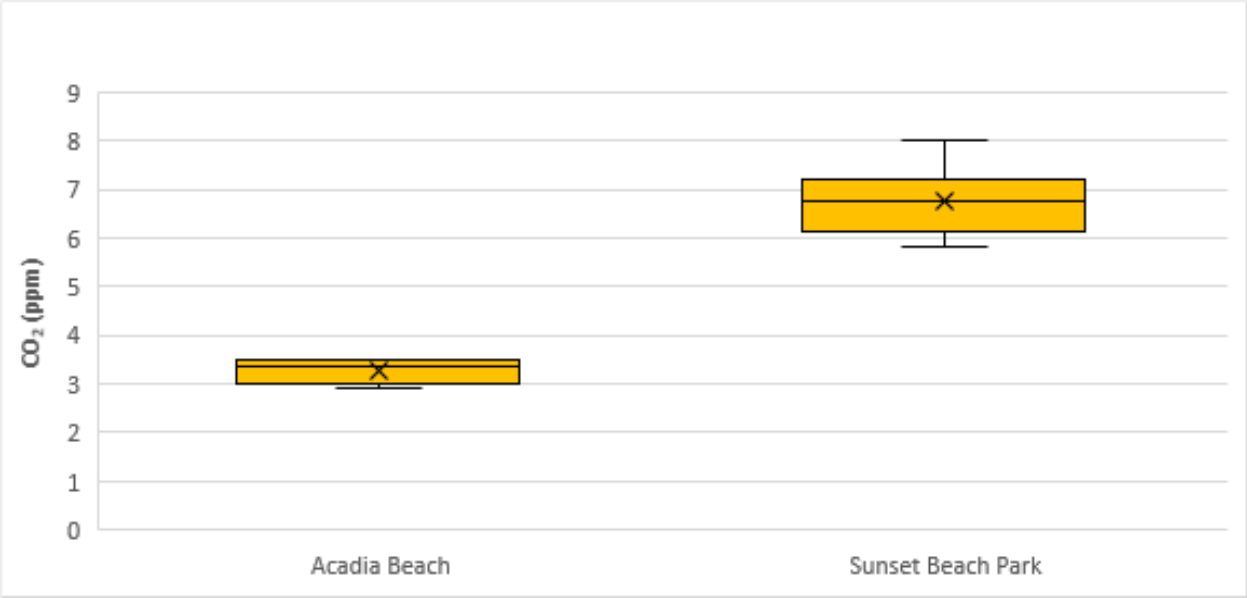

Figure 4. Analysis of mean water CO_2 concentration, including IQR, from Acadia Beach (mean = 3.270 ppm, s = ±0.254ppm, n = 10) and Sunset Beach Park (mean = 6.740 ppm, s = ±0.665, n = 10) using an unpaired t-test (p-value < 0.0001, t-value = 15.4074, df = 18). The X in the boxes represent the means. The line in the boxes are the medians for Acadia Beach (3.35 CO_2 ppm) and Sunset Beach Park (6.75 CO_2 ppm).

Gel electrophoresis was used to identify the species of each mussel based on fragment size (Figure 5). Of the 19 samples from Acadia Beach, 16 were identified as *M. edulis* and 3 as *M. trossulus*. Of the 19 samples from Sunset Beach Park, 12 were identified as *M. edulis,* 3 as *M. trossulus,* and 2 as *M. galloprovincialis*. Two samples from Sunset Beach Park did not display any banding, indicating the absence of DNA.

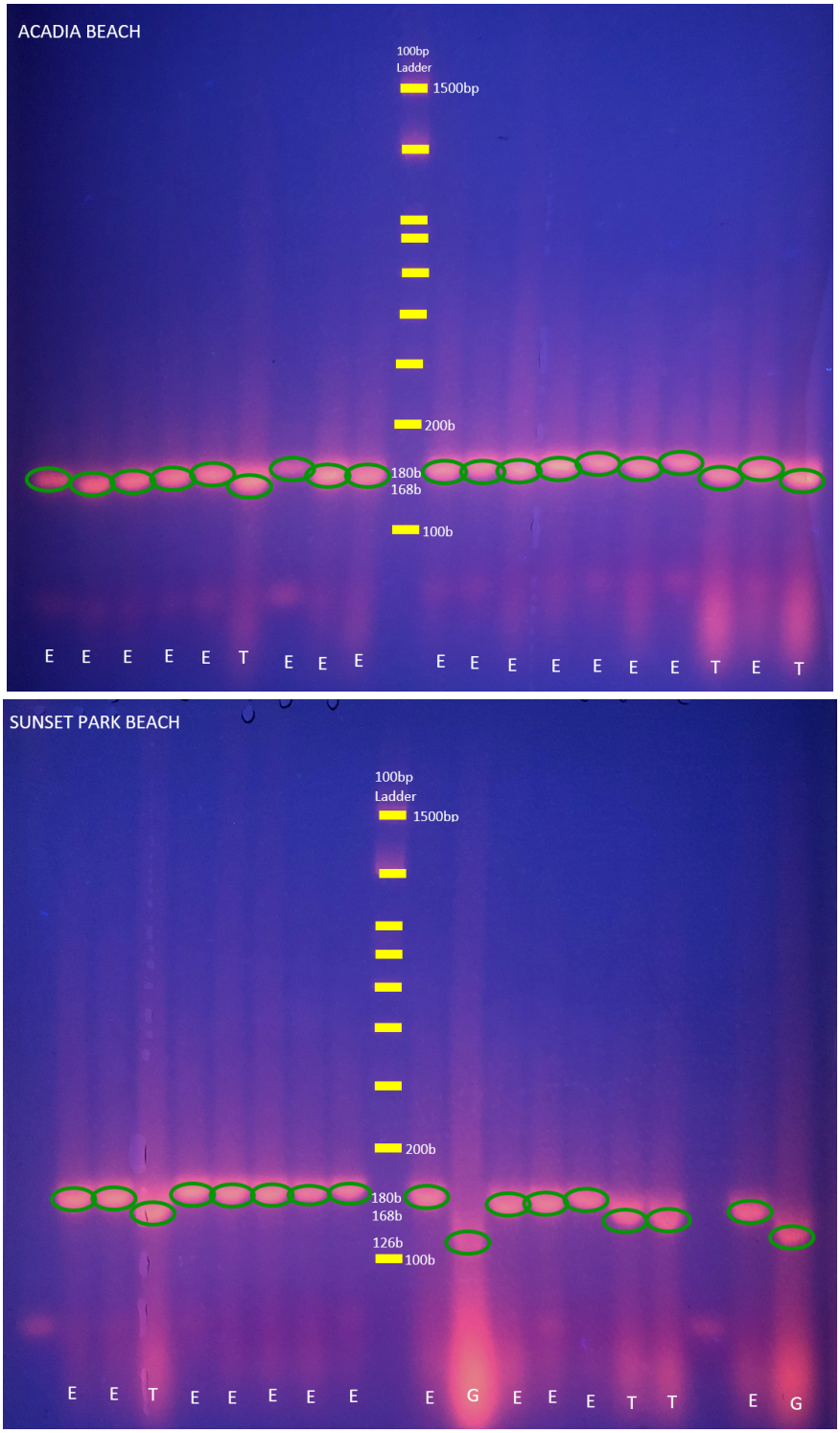

Figure 5. Electrophoresis gel of the 38 mussel DNA samples collected at Acadia Beach (site A) and Sunset Beach Park (site B). At Acadia Beach, 16 *M. edulis* (180 bp) and 3 *M. trossulus* (168 bp) samples were identified, labelled by letters E and T, respectively. At Sunset Beach Park, 12 *M. edulis*, 3 *M. trossulus*, and 2 *M. galloprovincialis* (126 bp) samples were identified, labelled by the letters E, T, and G respectively. Two samples at Site B did not present any banding.

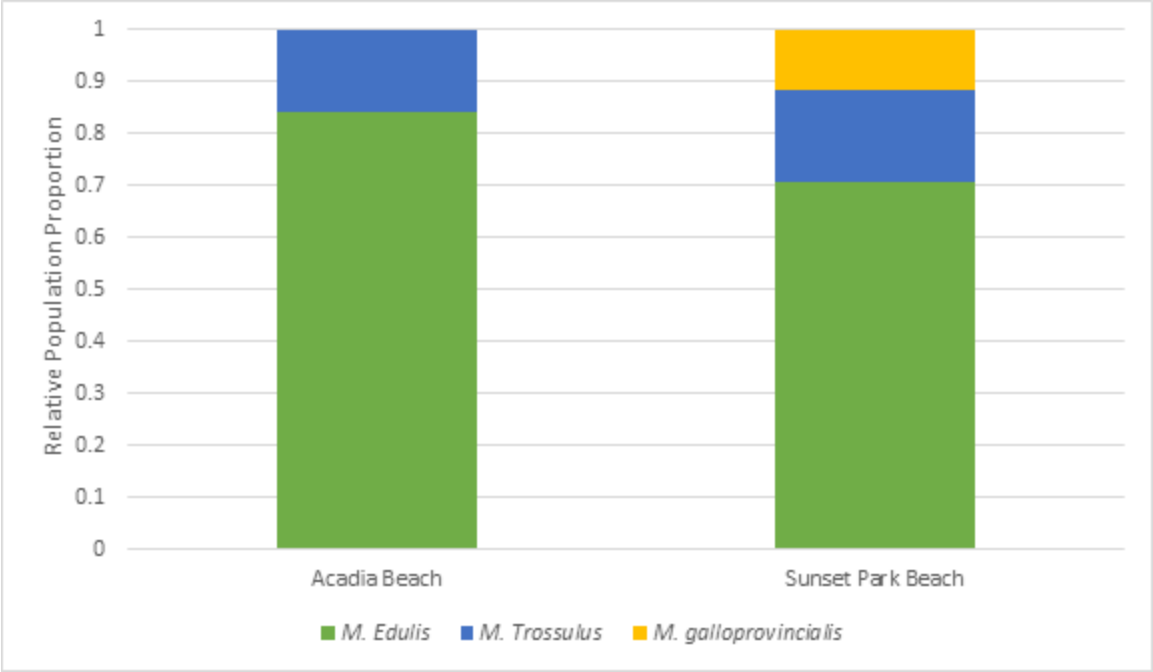

Figure 6. Sample proportions of blue mussel species collected at two location (n = 36). Mussels from Acadia Beach include 16 *M. edulis* samples and 3 *M. trossulus* samples. Mussels from Sunset Beach Park include 12 *M. edulis* samples, 3 *M. trossulus* samples, and *2 M. galloprovincialis* samples. Species identification was done through gel electrophoresis using mussel mantle tissue. Two samples from Sunset Beach Park failed to display banding.

The proportional population distributions of all the present species is graphed in Figure 6. The invasive *M. edulis* was the most abundant out of all of the blue mussel species collected at both Acadia Beach (84.2%) and at Sunset Beach Park (70.6%). The relative abundance of *M. trossulus* to *M. edulis* was also compared (Figure 7). The native *M. trossulus* made up 15.8% and 20.0% of their combined total at Acadia Beach and Sunset Beach Park, respectively. Fisher's exact test was performed on the two species' proportions resulting in a p-value of 1.000. Thus, we failed to reject the null hypothesis, meaning that there was no statistically significant difference between the proportions of *M. trossulus* (native) to *M. edulis* (invasive) at the two locations.

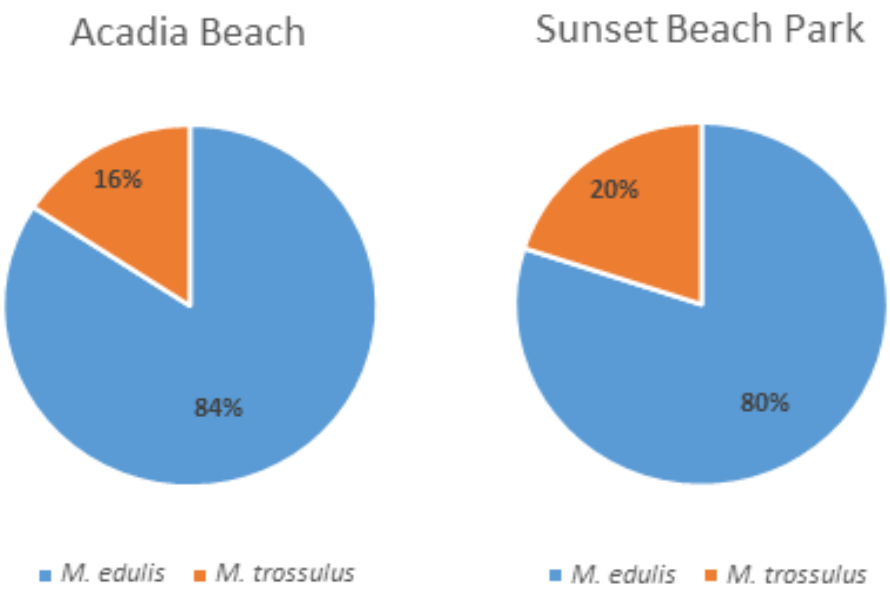

Figure 7. Relative abundance of two species of blue mussels at two locations with statistically different water CO_2 concentrations (Acadia Beach = 3.27 ppm; Sunset Beach Park = 6.74 ppm). Percentages were calculated against the total number of the two species, *M. trossulus* and *M. edulis,* at each location. At lower CO_2 concentration, *M. trossulus* made up 15.8% of the total (n=19); at higher CO_2 concentration, *M. trossulus* made up 20% of the total (n = 15). Fisher's statistical test resulted in a p-value = 1.000.

V. Discussion

We hypothesized that the relative abundance of *M. edulis* (invasive) to *M. trossulus* (native) blue mussels would differ due to a predicted difference in water CO_2 concentrations between two locations: Acadia Beach and Sunset Beach Park. Based on our results, there exists a statistically significant difference in CO_2 water concentrations between the two locations. However, we failed to reject the null hypothesis that there was no difference in the relative abundance of native and invasive mussels between the two sites. Thus, our findings suggest that

the difference in water CO_2 does not significantly impact the relative population abundances of *M. edulis* to *M. trossulus* blue mussel species.

In addition to CO_2 concentrations, we collected data on salinity and temperature, which was not statistically analyzed. The mean salinity and temperature were similar between Sunset Beach Park (28.9‰, 11.7°) and Acadia Beach (27.2‰, 12.1°), allowing us to treat these factors as controlled variables. A possible explanation as to why the CO_2 concentration at Sunset Beach Park was double that at Acadia Beach, despite the close proximity of the two locations, is the difference in biological activity in the water. Higher biological activity in the waters at Sunset Beach Park, in comparison to that at Acadia Beach, would result in more CO_2 being added to the water column through respiration and the decay of living organisms. Biological activity can be influenced by many factors, including sunlight, nutrient, and trace metal availability. It is possible that the waters at Sunset Beach Park are higher in nutrient availability than at Acadia Beach due to its location on the bank of False Creek, which could be carrying nutrients from further inland.

Physical water mixing caused by winds could also be used to rationalize the difference in water CO_2 concentrations between the two locations. Stronger winds and water turbulence at Sunset Beach Park could help dissolve more atmospheric CO_2 into the water. The presence of boats and ferries near Sunset Beach Park due to its proximity to the Burrard Civic Marina and the Aquatic Centre Ferry Dock could also introduce additional CO_2 into the water from boat exhaust. Similarly, Acadia Beach is surrounded by extensive vegetation due to the nearby Pacific Spirit Regional Park, which could have influenced more CO_2 being drawn away from the surrounding air and water by plants.

After thorough banding pattern analysis and statistical testing, we were unable to conclude that the relative proportions of the native and invasive blue mussel species differ between the two locations. Our results suggest that fluctuations in water CO_2 concentrations in the observed range do not significantly impact the relative abundance of each of the mussel species. This implies that both *M. edulis* and *M. trossulus* populations are affected to a similar extent by the difference in water CO_2 concentrations. While prolonged exposure to high aqueous CO_2 concentrations has harmful effects on mollusks (Kurihara, 2008; Caldeira & Wickett, 2005), the proportions between the two species can be expected to remain generally consistent, even if their respective populations decline.

Previous studies suggest that increased aqueous CO_2 concentrations have negative effects on *M. edulis* shells, growth, and survival (Melzner *et al*, 2011; Beesley *et al*., 2008). However, little data exists of such impacts on *M. trossulus*. Our findings suggest that *M. trossulus* populations would experience similar effects to water CO_2 as *M. edulis*. Considering the presence of both species at the sampled locations, the observed levels of CO_2 are likely within a tolerable range of both mussels.

The two absent DNA gel bands from Sunset Beach Park could be attributed to improper resuspending of the DNA pellets during PCR preparation. If properly analyzed, the two samples would have contributed to the statistical significance of our study. Furthermore, the collected mussels were quite small, making the mantle extraction process rather difficult. Potential contamination from other parts of the mussel tissue may have affected the DNA isolation process resulting in a lack of banding in the two unknown mussel samples. In addition, the difference in

CO_2 may be attributed to the fact that titrations for the water samples from Acadia beach were done immediately, whereas the Sunset Beach Park samples were done two days later.

Our predictions and implications are only supported for a small range of water CO_2 concentrations. The effects of water CO_2 outside of this range on the proportions of the two species requires further investigation. Testing additional areas with varying CO_2 concentrations allows for statistical correlation of water CO_2 to mussel relative abundance. In addition, other variables must be considered, such as differences in radiance, trace element availability, salinity, and predator interaction. A controlled laboratory setting could be used to further investigate CO_2 effects on blue mussel populations. Further implications of the two mussel species' distribution on other organisms in the ecosystem, such as salmon, should also be investigated.

VI. Conclusion

In conclusion, *M. edulis* (invasive blue mussel) was found to be more abundant than *M. trossulus* (native blue mussel) at both locations of significantly different water CO_2 concentrations. Our findings suggest that the relative abundance of *M. edulis* to *M. trossulus* mussels is unaffected by the observed water CO_2 concentrations. The observed range is likely tolerable by both species. Further analysis is required to predict species population proportions outside of this range, especially as atmospheric pCO_2 is predicted to rise.

VII. Acknowledgements

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IX. Appendix

	Acadia Beach	Sunset Beach Park	Marginal Row Totals
M. trossulus	3	3	6
M. edulis	16	12	28
Marginal Column Totals	19	15	34 (Grand Total)

Table 3. 2x2 contingency table used for the Fisher's test to evaluate *M. edulis* and *M. trossulus* species proportions differences. At Acadia Beach, 3 *M. trossulus* and 16 *M. edulis* were collected for a total of 19 samples. At Sunset Beach Park, 3 *M. trossulus* and 12 *M. edulis* were collected for a total of 15 samples.

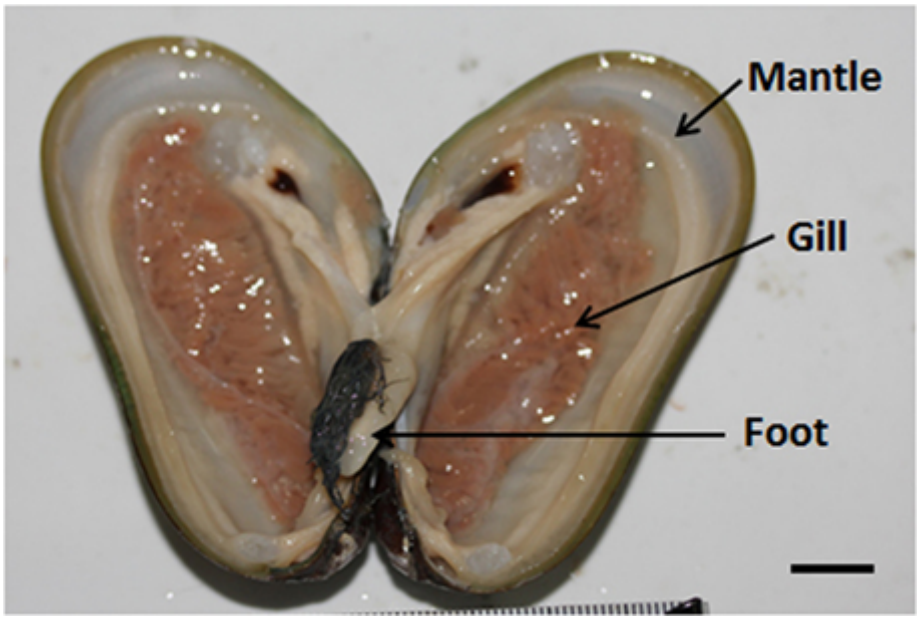

Figure 7. *Bathymodiolus platifrons*. Scale bar is 1 cm (Wong *et al*., 2015).