Effect of intertidal terrain surface aspect on *Mytilus* Species Complex distribution after the 2021 Pacific Northwest heat dome (PNWHD)

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Abstract

In June 2021, the Pacific Northwest heat dome (PNWHD) impacted British Columbia (BC), leading to unprecedented heat-induced mass mortality of blue mussels (*Mytilus* spp.). The invasive species, *Mytilus galloprovincialis* (Mg) and *Mytilus edulis* (Me), may threaten the native species, *Mytilus trossulus* (Mt) because Mt has a lower heat tolerance - resulting in a shift in BC's marine ecosystem dynamics. In this observational study, we examined four cardinal aspects (north, south, east, and west) of Pasley Island, B.C., to understand the distribution of *Mytilus* species' post-disturbance recruitment, as each aspect reflects different solar radiation levels. We expect to see Mt being the worst competitor of the species complex at the south and west aspects, where mussel beds were more exposed to solar radiation at daylight low tides during the PNWHD. We collected 40 mussels (10 from each aspect) from Pasley Island. To identify the *Mytilus* species, DNA isolation, Polymerase Chain Reaction (PCR), and gel-electrophoresis were performed. Our observational data showed higher counts of non-native mussels (Mg and Me) than native juvenile mussels (Mt) in all aspects, with higher differences in the south and west aspects. Me was found only in south and west sample locations. Our findings suggest that Mg has the highest tolerance to direct solar radiation relative to Me and Mt.

Introduction

As a result of climate change, marine heatwaves (MHWs) are predicted to increase in both severity and frequency in recent decades (Hobday et al., 2016; Seuront et al., 2019). A community on the edge of thermal extremes, the rocky intertidal is one of the most stressful environments on the planet (Seuront, 2019). This makes organisms such as blue mussels (*Mytilus* spp.) particularly vulnerable to extreme heat events evidenced by the mass mortality of *Mytilus* spp. during MHWs (Seuront, 2019; White et al., 2021).

MHWs can drastically alter intertidal communities (Hobday, 2016). A mass mortality event can, for example, reconfigures the distribution patterns of the *Mytilus* species complex: *Mytilus trossulus* Gould (Mt) which is indigenous to the Pacific Northwest (PNW), and *Mytilus galloprovincialis* Lamark, (Mg) and *Mytilus edulis* Linnaeus (Me) which are both invasive to the PNW (Crego-Prieto, 2015; Bakhmet, 2022; Schneider, 2007). Mass mortality

induced by MHWs represents competitive opportunities for invasive mussels Mg and Me which are faster-growing and more heat-adapted blue mussel species compared to Mt (Stachowicz, 2002; Vasquez, 2022). Mg is one of the world's worst invasive species and given its relatively high heat tolerance and growth rate, this species has been demonstrated to outcompete and smother the native Mt during recruitment (Schneider, 2007; Shinen, 2009). Mass die-offs may disproportionately impact less heat-tolerant native species, leaving higher numbers of invasive spawners after disturbances (Amstutz, 2021), and higher numbers of fast-growing invasive recruits which outcompete indigenous juvenile Mt recruits (Shinen, 2009). Increases of invasive mussels in the PNW can lead to declines and the extirpation of the indigenous Mt, so predicting the distribution of invasive Mg and Me is important for the conservation of Mt (Seuront, 2019).

Physiological limits determine species community membership and are important factors in predicting future range shifts under climate change (Bowman, 2021; Helmuth, 2010). Recent studies have highlighted the importance of measuring microhabitat variation along with species physiology to generate 'physiological landscapes' to predict species response to climate change (Barbosa, 2022; Harley, 2008; Li, 2021). Because of this, many studies have focused on air and water temperature physiological limitations in *Mytilus* spp. (Braby, 2006; Sarver, 1993; Suchanek, 1997). However, more recent studies have found direct solar radiation to be the most important factor influencing internal body temperature in ectothermic poikilotherms (Amstutz, 2021; Helmuth, 2010; Lathlean, 2014; Seuront, 2019). For instance, Seuront (2019) found that Me body temperature can be significantly greater than air temperature during emersion when exposed to direct solar radiation.

The level of solar radiation a mussel receives in the intertidal zone during emersion is a function of the aspect of that mussel's position in the intertidal zone, with midday south-facing aspects receiving the most solar radiation followed by west, and decreasing with east and then north aspects (Amstutz, 2021; Bennie et al., 2017; Figure 1). Because of this, spatial patterns of aspects in the intertidal can influence how the *Mytilus* species complex is distributed (Amstutz, 2021; Bennie et al., 2017; Schneider, 2007). Overall range shifts in *Mytilus* distribution may be driven by microhabitat selection pressures (solar radiation) at the microhabitat scale (Schneider, 2007).

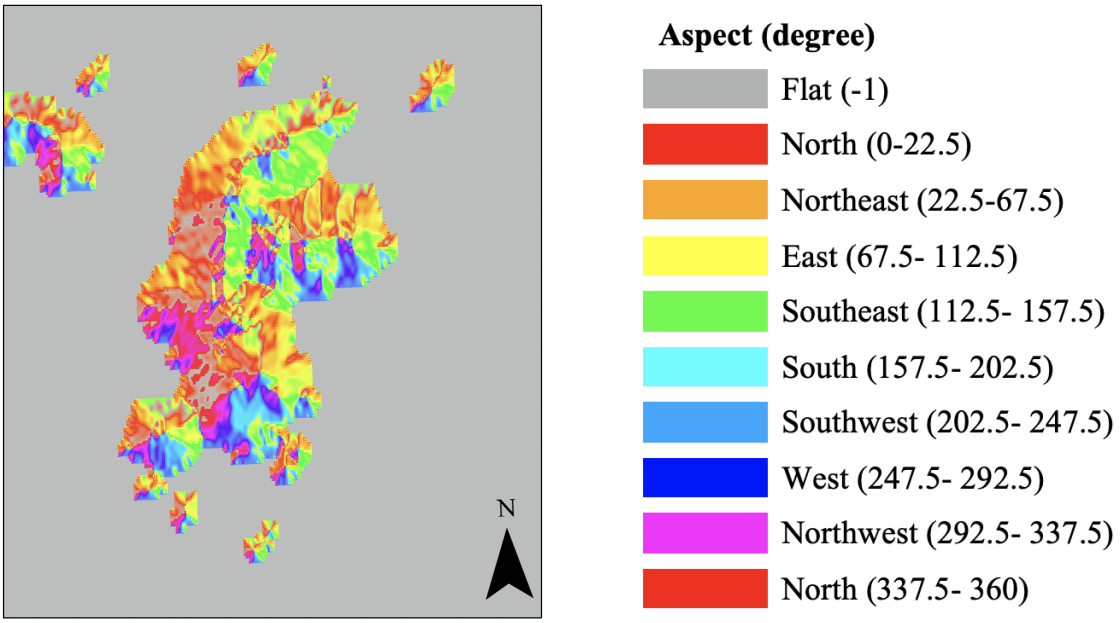

Figure 1. Map of terrain surface aspect of Pasley Island, British Columbia representing different microhabitats due to variations in levels of solar radiation. The colours correspond to the compass direction (degree) that the terrain surface faces (Terenzek, 2022).

During June 2021 to July 2021, the PNW was hit with an unprecedented MHW which led to air and sea surface temperatures 16-20°C above normal and low tides coinciding with daytime high temperatures, particularly for south-west facing aspects (White et al., 2021). This resulted in ~70% mortality of Mt with > 1 million mussels deaths within 100m of the

shoreline (Figure 2; White et al., 2021). Given that Mt are less heat tolerant than Me and Mg, and Me is less heat tolerant than Mg; here we observationally investigate the distribution of *Mytilus* species complex recruits based on the four cardinal aspects (north, east, south, west) at Pasley Island, British Columbia (Braby, 2006; Han, 2020; Tomanek, 2010; Vasquez, 2022). We predict that the distribution of blue mussels at different aspects (N,W, E,S) differs at Pasley Island because it reflects the different levels of solar radiation and environmental change that different blue mussels species could tolerate and survive during the heat dome.

Figure 2. Images from Pasley Island, British Columbia in August 2021 after the 2021 Pacific Northwest Heat Dome. (A) open shells of *Mytilus* that died due to thermal stress during PNWHD, (B) Bare rock surfaces apparent in the intertidal zone after PNWHD, (C and D) *Mytilus* shell accumulations from mass *Mytilus* mortality after PNWHD. (photographs by Kyla Terenzek).

Methods

Collection

To investigate the effect patterns of solar radiation on *Mytilus* spp. distribution, we examined recruits to the intertidal zone 16 months after the 2021 PNWHD disturbance. Peak settlement of *Mytilus* larva occurs from June-July in the PNW (Kagley, 2013). As a result, two main cohorts colonizing disturbed areas after the PNWHD were expected from June/July 2021 recruits and June/July 2022 recruits. To avoid including adult Mt which could be the same size as 2022 juvenile invasive recruits, we define recruitment as "colonization after the most recent settlement" and consider 'recruits' as mussels <6 months old. In this case, we found recruit sizes would be: Mg <19.5mm, Me <25mm, and Mt >5mm, and collected mussels in the 5mm-25mm range (Jacobs, 2014; Martel, 2000; Prieto, 2020).

A pool of *n*=20 sample locations with qualitatively similar: wave exposure (protected to semi-exposed), intertidal substrate (boulder platform), dominant mussel predators (*Pisaster ochraceus*), slope (20-40 degrees), and level of human activity (moderate) were used to randomly select one sample location for each cardinal direction (N,S,E,W) (Figure 3). Samples were collected October 28-31, 2022 during ~0.5m low tides. The mussel band width was measured, and a 30 m transect line was placed at the mid-point of the mussel band, horizontal to the water line - accounting for predator effects that control the lower limit of the mussel band, and abiotic effects that control the upper limit (Barbosa, 2022).

Samples were collected using randomised locations along the transect line. Digital callipers were used to measure sample length and mussels that did not meet the 5mm-25mm length criteria were carefully returned, and a new random sample location was generated. The aspect and location of the sample point was recorded with a Garmin GPSMAP 78 gps unit

(Garmin, n.d.) and aspect with a Suunto A-10 NH compass (Suunto, n.d.). A total of 10 samples were collected from each of the four locations producing *n*=40 samples for the study. The samples were labelled, placed in a cooler <1 hour, then frozen intact until transported to the lab.

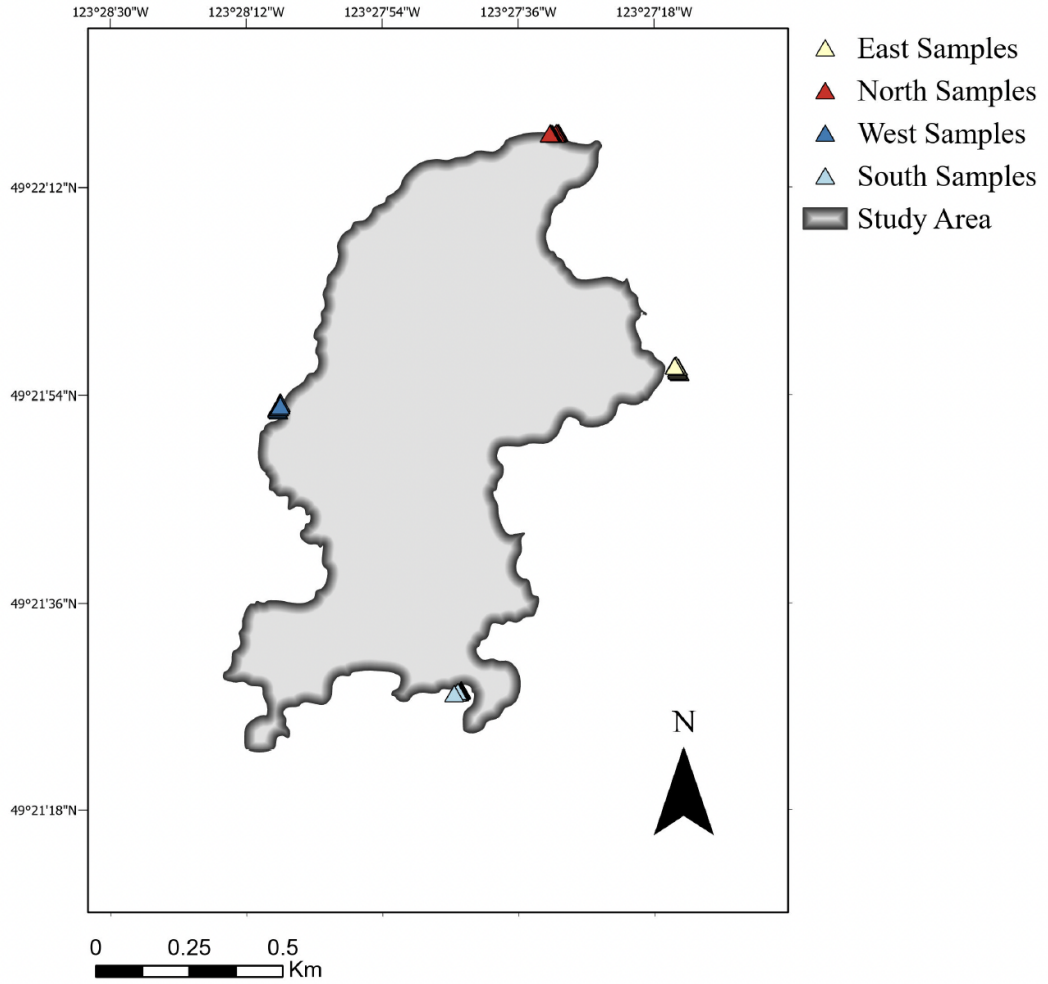

Figure 3. Map of four sample locations on Pasley Island, British Columbia to reflect the four cardinal directions, north, south, east and west (Terenzek, 2022).

DNA Isolation and PCR

40 mussels were pried open with gloves and scalpels to extract whole mussel tissue to be masticated with sterile toothpicks in the respective labelled 1.5 mL Eppendorf tubes. Next, 300 uL of Cell Lysis Solution with Proteinase K was added to each mussel sample, incubated at 65ºC for 15 minutes, and vortexed every 5 minutes until the solution looked somewhat

cloudy. After cooling in ice, 150 uL of Protein Precipitate Reagent was added and vortexed for 10 seconds. Subsequently, each mussel sample was then spun in a balanced centrifuge for 10 minutes at maximum speed. Each sample's supernatant was transferred into its own newly labelled 1.5 mL Eppendorf tube via a pipette with fresh tip each time. 500 uL of ice-cold isopropanol was added and inverted 30-40 times to be centrifuged again for 10 minutes at maximum speed. Lastly, two rounds of ethanol rinses were performed and were left to air dry overnight. Before the PCR stage, 30 uL of TE buffer was added to dry each DNA pellet created during DNA isolations. Master mix (MM) was prepared by combining the following:

Component	Amount per sample (uL)	Amount for 50 samples (uL)
10X PCR buffer	2.5	125.0
10 mM dNTPs	0.5	25.0
25 mM MgCl2	1.0	50.0
10 μM forward (5') primer (Me15)	1.0	50.0
10 μM reverse (3') primer (Me16)	1.0	50.0
Taq polymerase	0.5	25.0
50% glycerol	5.0	250.0
Sterile distilled water	11.5	575.0

Table 1. Basic Recipe for Master Mix preparation. Specific components required along with their amount per sample and the total amount for 50 samples in uL.

The MM was made for 10 extra samples in case of spills or pipetting errors, and water control was used. 23 uL of MM was pipetted to each newly labelled PCR tube, and then 2 uL of DNA (or sterile distilled water for water control) from each mussel sample was also added. Thereafter, tubes were put in the PCR machine and set to the temperature cycle required for the repeated DNA replication flanked by primers. This is shown below:

Temperature (˚C)	Time	
95	2 min	
95	30 sec	X 35
54	40 sec	
72	90 sec	
72	5 min	
4	Overnight	

Table 2. PCR Cycle. Various temperatures (˚C) set to given times required for DNA replication flanked by primers. 35 repeated rounds of DNA replication performed for 95˚C set to 30 seconds, 54˚C to 40 seconds, and 72˚C to 90 seconds.

Gel-Electrophoresis

In each PCR sample and the one water control sample, 5 uL of 6X loading buffer was directly added and mixed by pipetting the solution up and down. Consequently, 15 uL of each sample was loaded into the 3% agarose gel well. Initially, the gel ran at 50V till all samples left the well. Then, it ran on 120V for 150 minutes until the loading buffer ran off the gel. Afterwards, samples were classified based on their DNA base pairs (bp). The categorization was as follows: Mt (180 bp), Mg (168 bp), and Me (126 bp) (Inoue et al., 1995). Bands that were hard to read were categorised as "unidentified"(Figure 4). To visualise the relative abundance of identified *Mytilus* spp at different aspects, a bar graph was generated respectively in Rstudio (version : 2022.7.2.576) with R (version : 4.2.1) using the Dplyr and Ggplot2 packages (Figure 5).

Results

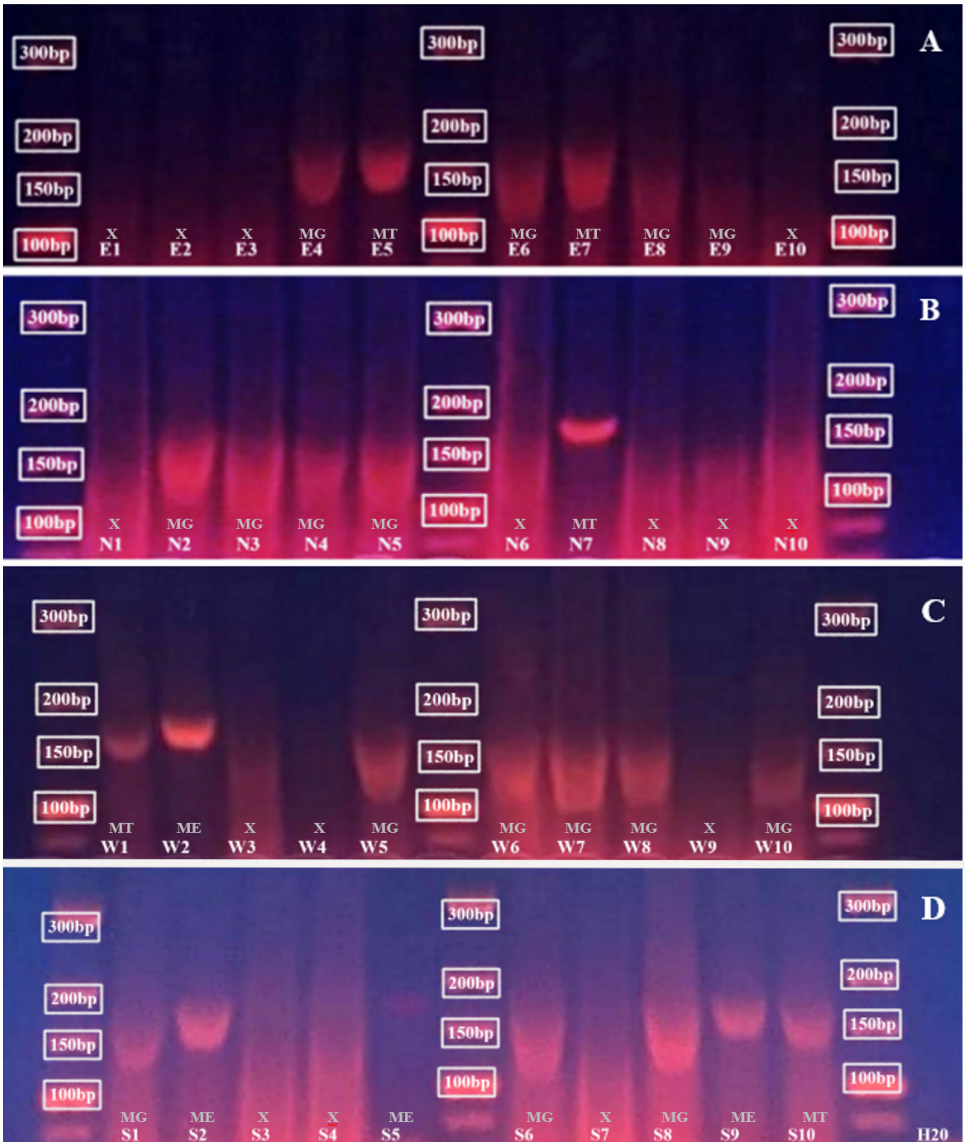

Figure 4. Gel-electrophoresis results of the 40 *Mytilus* DNA samples collected at different aspects of Pasley Island, British Columbia, Canada. Specifically, at the (A) east, (B) north, (C) west, and (D) south intertidal zone of the Island. All samples are labelled based on their sample number respectively. H_2O in panel D represents distilled water (control). MT, MG, ME, and X respectively represent *M. trossulus*, *M. galloprovincialis*, *M. edulis* and samples that were difficult to identify. Ladders are labelled based on the number of base pairs.

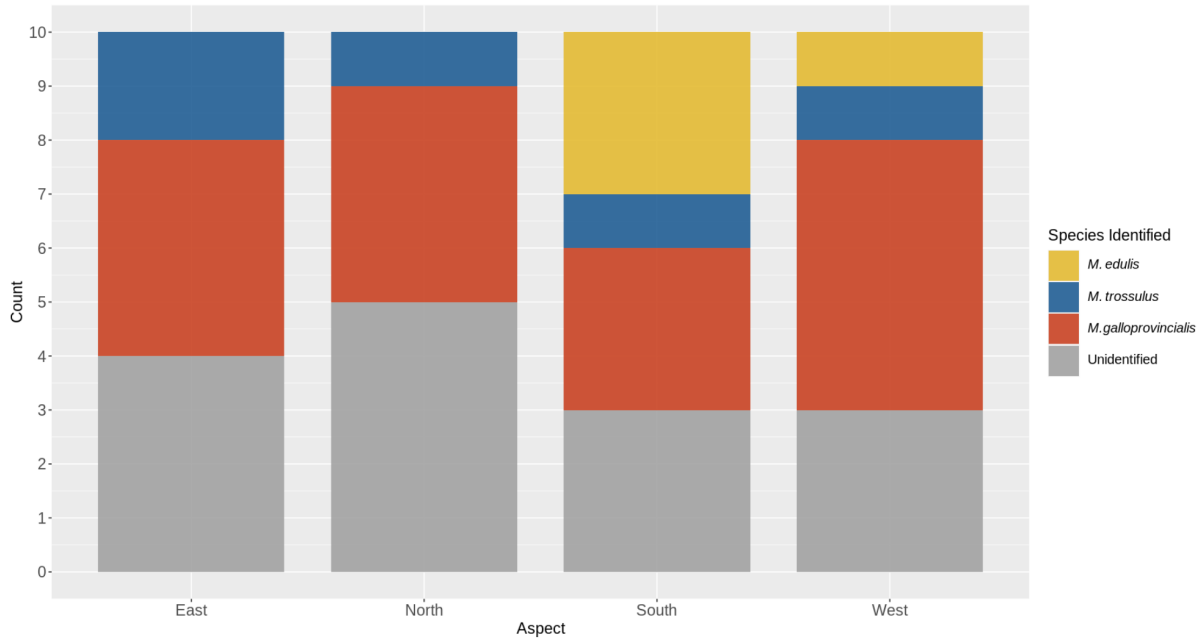

Figure 5. Count of *Mytilus* species collected at four aspects (east, north, south, west intertidal zones) of Pasley Island, British Columbia, Canada (total n = 40, with each aspect n = 10). Species were identified based on gel-electrophoresis, and "unidentified" represent samples that were difficult to identify through gel-electrophoresis.

The *Mytilus* spp. was determined by the gel-electrophoresis result in Figure 4. Our distilled water control sample showed no contaminated result. Figure 5 displays a count comparison of different Mytilus species between the 4 aspects; they are as follows: mussels identified at the east intertidal zone include 2 Mt, 4 Mg, and 4 unidentified. Mussels identified at the north intertidal zone include 1 Mt, 4 Mg, and 5 unidentified. Mussels identified at the south intertidal zone include 3 Me, 1 Mt, 3 Mg, and 3 unidentified. Mussels identified at the west intertidal zone include 1 Me, 1 Mt, 5 Mg, and 3 unidentified.

Discussion

In this study, we expected that where mussel beds were exposed to greater solar radiation (south and west facing aspects) during daylight low tides during the PNWHD, the native Mt would be the worst competitor of the species complex: Mg> Me > Mt. Our findings indicate a high variation of blue mussel species in the different aspects of Pasley island. We did not identify any hybrids (eg. Mt x Me) on gels as found in previous studies conducted in British Columbia (Gurney-Smith et al., 2017). Bp in gel-electrophoresis shown in Figure 4 were matched to known DNA lengths for classifying blue mussel species. Based on Figure 4, all 3 species were found in the south and west facing aspects of Pasley Island. Since both Mg and Me had a higher count (with overall highest counts of Mg) than the native Mt in the south facing aspect, our prediction was supported. Mg and Me indeed have a higher thermal tolerance compared to Mt since they are able to survive in the south and west facing intertidal zones which are exposed to greater solar radiation (Buckley & Huey, 2016).

Our findings suggest that Mg has the highest thermal tolerance compared to Me and Mt. Additionally, east and north aspects both present more Mg than Mt. This portrays the impacts of the PNWHD. Although the east and north aspects are exposed to less solar radiation compared to the south and west aspects, the solar radiation during the PNWHD would have been significantly higher than before, consequently affecting the mussel beds in the east and north as well. Furthermore, the ratio of native mussels (Mt) to non-native mussels (Mg and Me) is higher in the south (1:6) and west (1:6) aspects compared to the east (1:2) and north (1:4) aspects, suggesting that although the PNWHD affected all the mussel beds in all aspects, the greatest impact was in the south and west aspects. The Me species was only found in the south and the west aspects. We assumed mean salinity is homogenous in all sample locations, however any differences in salinity may affect distribution. The presence

of all the three species in the west and south aspects may also reflect the intermediate disturbance hypothesis, which states that the highest biodiversity is observed when ecological disturbance is not too frequent or too scarce (Dial & Roughgarden, 1998). Although intermediate disturbance may increase diversity in the short term, increasing MHW disturbance over time may lead to the local extinction of Mt. The intermediate disturbance hypothesis could explain the greater diversity in south and west aspects after the PNWHD disturbance and can be further studied in the future.

Valuable knowledge was obtained in this experimental study on the distribution of blue mussel species (*Mytilus* spp.). We discovered the presence of invasive species in all aspects of Pasley island. As noted by Bonham et al. (2017), Mg is one invasive species found with quick rates of reproduction and the ability to outcompete native mussels (e.g. Mt). Thus, an increased invasion rate of the species can potentially cause a shift in ecosystem dynamics, as well as reduce the biodiversity of the island by displacing the native species and monopolising food resources. By better understanding the distribution of the mussels with respect to aspect, we can evaluate the impact of invasive mussel species on the ecology of Pasley Island.

We suspect that during DNA isolation, protein contamination could have resulted from our whole mussel samples, sterilization, and pipetting errors. A possible source of error in this experiment is the sterilization of the scalpels and tweezers while prying open the mussels. Some were flamed only, some with ethanol only, and some with ethanol and then flamed - all can lead to issues with contamination. This would have affected the results because solely flaming or using ethanol does not remove the DNA of the mussels completely, which would have resulted in cross-contamination of the mussel DNA. This error

significantly affects our data since the species of the mussels were determined by their DNA. To eliminate this error in the future, the tools should be sterilized by both dipping and flaming tools with alcohol.

During the DNA isolation process, two possible sources of errors have occurred. There were pipetting errors when adding the Protein Precipitate Reagent during the DNA isolation process. Additionally, the centrifuge machine was not spun for sufficient lengths. These errors would have resulted in poor DNA isolation, which would consequently affect the PCR and gel-electrophoresis and result in the 'unidentified' species.

Further studies can be done to investigate the ocean conditions that affect the distribution and abundance of blue mussels. As mentioned above, the potential interactions of salinity and the intermediate disturbance hypothesis may be areas of interest for future research. Furthermore, precise investigation on how heat affects the *Mytilus* species complex is crucial.

Conclusion

Taken together, our result showed higher counts of non-native mussels (Mg and Me) than native mussels (Mt) in all aspects, with higher differences in the south and west aspects. This is consistent with our prediction and reflects Mg has the highest tolerance to direct solar radiation (which increases internal body temperature) than Me and Mt. Further research can be done on the variables (eg. salinity) that affect the distribution of *Mytilus* species.

Acknowledgments

We acknowledge that the land on which we gathered and collected this data is the traditional, ancestral and unceded territory of the Skwxwú7mesh (Squamish) Nations. We would like to express our deep gratitude to the land itself, the blue mussels, and the People who have and who continue to steward them. We would like to graciously thank Pasley Island for the opportunity to study *Mytilus* surrounding the island. We are very grateful to Professor Celeste Leander for encouraging us to study the *Mytilu*s species complex, with conceptual guidance, and scientific expertise. We would like to express our appreciation and thanks for Tessa Blanchard's thoughtful assistance with equipment and technical expertise. We would further express our gratitude to Mindy Chow and Jarnail Mehroke for the experimental setup and guidance. Finally, we would like to thank the University of British Columbia for the opportunity to gain hands-on field and lab experience, and to learn about and study *Mytilus* species.

References

Amstutz, A., Firth, L. B., Spicer, J. I., & Hanley, M. E. (2021). Facing up to climate change: Community composition varies with aspect and surface temperature in the rocky intertidal. Marine Environmental Research, 172, 105482-105482. https://doi.org/10.1016/j.marenvres.2021.105482

Bakhmet, I., Aristov, D., Marchenko, J., & Nikolaev, K. (2022). Handling the heat: Changes in the heart rate of two congeneric blue mussel species and their hybrids in response to water temperature. Journal of Sea Research, 185, 102218. https://doi.org/10.1016/j.seares.2022.102218

Barbosa, R. V., Jaud, M., Bacher, C., Kerjean, Y., Jean, F., Ammann, J., & Thomas, Y. (2022). High-resolution drone images show that the distribution of mussels depends on microhabitat features of intertidal rocky shores. Remote Sensing (Basel, Switzerland), 14(21), 5441. https://doi.org/10.3390/rs14215441

Bennie, J., Huntley, B., Wiltshire, A., Hill, M. O., & Baxter, R. (2008). Slope, aspect and climate: Spatially explicit and implicit models of topographic microclimate in chalk grassland. Ecological Modelling, 216(1), 47-59. https://doi.org/10.1016/j.ecolmodel.2008.04.010

Bonham, V., Shields, J., & Riginos, C. (2017). *Mytilus galloprovincialis* (Mediterranean mussel). CABI Compendium, CABI Compendium. https://doi.org/10.1079/cabicompendium.73756

Bowman, W. D., & Hacker, S. D. (2021). *ecology* (Fifth ed.). Sinauer Associates.

Braby, C. E., & Somero, G. N. (2006). Ecological gradients and relative abundance of native (*mytilus trossulus*) and invasive (*mytilus galloprovincialis*) blue mussels in the california hybrid zone. Marine Biology, 148(6), 1249-1262. https://doi.org/10.1007/s00227-005-0177-0

Buckley, L. B., & Huey, R. B. (2016). How extreme temperatures impact organisms and the evolution of their thermal tolerance. Integrative and Comparative Biology, 56(1), 98–109. https://doi.org/10.1093/icb/icw004

Crego-Prieto, V., Ardura, A., Juanes, F., Roca, A., Taylor, J. S., & Garcia-Vazquez, E. (2015). Aquaculture and the spread of introduced mussel genes in british columbia.Biological Invasions, 17(7), 2011-2026. https://doi.org/10.1007/s10530-015-0853-z

Dial, R., & Roughgarden, J. (1998). Theory of marine communities: The intermediate disturbance hypothesis. Ecology, 79(4), 1412–1424. https://doi.org/10.1890/0012-9658(1998)079[1412:tomcti]2.0.co;2

Garmin (n.d.). GPSMAP78. [Apparatus]. https://www.garmin.com/en-CA/p/63601

Gurney-Smith, H. J., Wade, A. J., & Abbott, C. L. (2017). Species composition and genetic diversity of farmed mussels in British Columbia, Canada. Aquaculture, 466, 33–40. https://doi.org/10.1016/j.aquaculture.2016.08.038

Han, G., & Dong, Y. (2020). Rapid climate-driven evolution of the invasive species *mytilus galloprovincialis* over the past century. Anthropocene Coasts, 3(1), 14-29. https://doi.org/10.1139/anc-2019-0012

Harley, C. (2008). Tidal dynamics, topographic orientation, and temperature-mediated mass mortalities on rocky shores. Marine Ecology. Progress Series (Halstenbek), 371, 37-46. https://doi.org/10.3354/meps07711

Helmuth, B., Broitman, B. R., Yamane, L., Gilman, S. E., Mach, K., Mislan, K. A. S., & Denny, M. W. (2010). Organismal climatology: Analyzing environmental variability at scales relevant to physiological stress. Journal of Experimental Biology, 213(6), 995-1003. https://doi.org/10.1242/jeb.038463

Hobday, A. J., Alexander, L. V., Perkins, S. E., Smale, D. A., Straub, S. C., Oliver, E. C. J., Benthuysen, J. A., Burrows, M. T., Donat, M. G., Feng, M., Holbrook, N. J., Moore, P. J., Scannell, H. A., Sen Gupta, A., & Wernberg, T. (2016). A hierarchical approach to defining marine heatwaves. Progress in Oceanography, 141, 227-238. https://doi.org/10.1016/j.pocean.2015.12.014

Inoue, K., Waite, J.H., Matsuoka, M., Odo, S., Harayama, S. (1995). Interspecific variations in adhesive protein sequences of *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus*. The Biological bulletin, 189(3), 370-375. doi: 10.2307/1542155

Jacobs, P., Beauchemin, C., & Riegman, R. (2014). Growth of juvenile blue mussels (*mytilus edulis*) on suspended collectors in the dutch wadden sea. *Journal of Sea Research, 85*, 365-371. https://doi.org/10.1016/j.seares.2013.07.006

Kagley, A. N., Snider, R. G., Krishnakumar, P. K., & Casillas, E. (2003). Assessment of seasonal variability of cytochemical responses to contaminant exposure in the blue mussel *mytilus edulis* (complex). *Archives of Environmental Contamination and Toxicology, 44*(1), 43-52. https://doi.org/10.1007/s00244-002-1303-3

Lathlean, J. A., Ayre, D. J., Minchinton, T. E., & McGeoch, M. (2014). Estimating latitudinal variability in extreme heat stress on rocky intertidal shores. Journal of Biogeography, 41(8), 1478-1491. https://doi.org/10.1111/jbi.12311

Martel, A. L., Robles, C., Beckenbach, K., & Smith, M. J. (1999). Distinguishing early juveniles of eastern pacific mussels (mytilus spp.) using morphology and genomic DNA. *Invertebrate Biology, 118*(2), 149-164. https://doi.org/10.2307/3227056

Prieto, D., Tamayo, D., Urrutxurtu, I., Navarro, E., Ibarrola, I., & Urrutia, M. B. (2020). Nature more than nurture affects the growth rate of mussels. *Scientific Reports, 10*(1), 3539-3539. https://doi.org/10.1038/s41598-020-60312-y

Schneider, K., & Helmuth, B. (2007). Spatial variability in habitat temperature may drive patterns of selection between an invasive and native mussel species. Marine Ecology. Progress Series (Halstenbek), 339, 157-167. https://doi.org/10.3354/meps339157

Seuront, L., Nicastro, K. R., Zardi, G. I., & Goberville, E. (2019). Decreased thermal tolerance under recurrent heat stress conditions explains summer mass mortality of the blue mussel *mytilus edulis*. Scientific Reports, 9(1), 17498-14. https://doi.org/10.1038/s41598-019-53580-w

Shinen, J., & Morgan, S. (2009). Mechanisms of invasion resistance: Competition among intertidal mussels promotes establishment of invasive species and displacement of native species. *Marine Ecology. Progress Series (Halstenbek), 383*, 187-197. https://doi.org/10.3354/meps07982

Stachowicz, J. J., Terwin, J. R., Whitlatch, R. B., & Osman, R. W. (2002). Linking climate change and biological invasions: Ocean warming facilitates nonindigenous species

invasions. Proceedings of the National Academy of Sciences - PNAS, 99(24), 15497-15500. https://doi.org/10.1073/pnas.242437499

Suunto (n.d.). NH Compass (model A-10). [Apparatus]. https://www.suunto.com/en-ca/Products/Compasses/Suunto-A-10/Suunto-A-10-NH-Compass/

Terenzek, K. (2022). Microhabitats of Pasley Island [map]. Scale Not Given. "World Topographic Map". December 8, 2022. http://www.arcgis.com/home/item.html?id=30e5fe3149c34df1ba922e6f5bbf808f. (May 25, 2017). Using: *ArcGIS* Pro [GIS software]. Version 3.0.2. Redlands, CA: Environmental Systems Research Institute, Inc., 2022.

Terenzek, K. (2022). Sample Locations [map]. Scale Not Given. "World Topographic Map". December 8, 2022. http://www.arcgis.com/home/item.html?id=30e5fe3149c34df1ba922e6f5bbf808f. (May 25, 2017). Using: *ArcGIS* Pro [GIS software]. Version 3.0.2. Redlands, CA: Environmental Systems Research Institute, Inc., 2022.

Tomanek, L., Zuzow, M. J., Hitt, L., Serafini, L., & Valenzuela, J. J. (2012). Proteomics of hyposaline stress in blue mussel congeners (genus mytilus): Implications for biogeographic range limits in response to climate change. Journal of Experimental Biology, 215(Pt 22), 3905-3916. https://doi.org/10.1242/jeb.076448

Vasquez, M. C., Houston, C. T., Alcantar, C. Y., Milshteyn, L., Brazil, C. A., & Zepeda, O. G. (2022). Interactive effects of multiple stressors on the physiological performance of

the invasive mussel *mytilus galloprovincialis*. *Marine Environmental Research, 178*, 105665-105665. https://doi.org/10.1016/j.marenvres.2022.105665

White, R., Anderson, S., & Booth, J. (2021). The Unprecedented Pacific Northwest Heatwave of June 2021. Research Square. https://doi.org/10.21203/rs.3.rs-1520351/v1