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Cover Image Description

This is the Bubble Nebula and it happens to be the birthplace of a future star. The Bubble Nebula is an emission nebula, meaning this beautiful gaseous structure emits its own light. The predominantly red colour of this interstellar cloud is due to the ionization and light emission of hydrogen gas, the most common element in the universe. Emission nebulae, like the Bubble Nebula, are areas of star formation and can be found in the spiral arms of most galaxies, such as the Milky Way. The Bubble Nebula is located in the constellation Cassiopeia approximately 7,000 light years away from Earth and is estimated to be 300,000 years old. The diameter of the sphere in the centre is 7 light years. Almost four of our Solar Systems, up to and including the Oort Cloud, can be lined up end to end within that little sphere. The diameter of the sphere is also almost twice the distance from our Solar System to the nearest star, Alpha Centauri. It's hard to grasp the enormity of the Bubble Nebula when it's unfathomably far away.

This photo was captured and processed by Sarah A. Savić Kallesøe, an undergraduate student at Simon Fraser University in Burnaby, Canada on September 29, 2016. The total exposure for this image is approximately 3.5 hours, using hydrogen alpha narrowband, red, green, blue, and luminance filters. Each frame had an exposure of 120 seconds. The luminance frames were captured with 2×2 binning and the colours and hydrogen alpha were taken with 4×4 binning. A total of 60 images were selected to create this image.

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"Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something, and that this thing, at whatever cost, must be attained."

- Marie Skłodowska Curie

Foreword

Dear Reader,

Since its inaugural year, the central vision of the SFU Science Undergraduate Research Journal was to connect the undergraduate research community and create a platform to recognize and distribute scholarly, peer reviewed research by undergraduates. The first issue of the journal was published in 2016 and since then, we have published 19 research and review articles, worked with 79 different faculty and graduate student reviewers, and showcased the work of 52 undergraduate research authors.

As researchers ourselves, we know firsthand the many hours of physical and mental work which go into the creation of a manuscript. The quality of research and intellect that we see in our submissions each year is astounding. It is with great joy that we work with authors, editors, and reviewers in the peer review process to prepare papers for potential publication. When we look back at all we have accomplished, we are truly thankful. We would not be able to assemble such an issue without the work from our editorial team and peer reviewers.

This year, we have continued to broaden our online presence with our continuing blog series featuring undergraduate researchers and have expanded it to include graduate students and Faculty. We are also hard at work organizing the second annual science undergraduate research poster competition. We look forward to seeing SFU SURJ grow in the years to come, and we invite you to come along for the ride. Without further delay, we present to you, the third edition of the SFU Science Undergraduate Research Journal.

Sincerely,

Siobhan Ennis, Emily Leung, Anish Verma, and Christine Wang

Executive Editors

Table of Contents

Preface

Acknowledgements iii
Board of Editors v
Foreword vii

Research Articles

1 Evidence of a Divergent Growth Response in the Pacific Oyster, *Magallana Gigas*, When Exposed to Native and Invasive Crab Chemical Cues
1.1 Introduction ... 1
1.2 Materials and Methods ... 3
1.3 Results ... 5
1.4 Discussion ... 6
1.5 Conclusion ... 7
1.6 Acknowledgments ... 7
References ... 8
1.A Appendix ... 12

2 Phototactic Responses of *Drosophila Melanogaster* to UVA, UVB, White Light, and Dark Environments
2.1 Introduction ... 14
2.2 Materials and Methods ... 15
2.3 Results ... 17
2.4 Discussion ... 18
2.5 Conclusion ... 20
2.6 Acknowledgments ... 21
References ... 21

3 The Influence of Surface Scratches on Copper's Antibacterial Activity
3.1 Introduction ... 23
3.2 Materials and Methods ... 24
3.3 Results ... 27
3.4 Discussion ... 28
3.5 Conclusion ... 29
3.6 Acknowledgments ... 30
References ... 30

4 The Interactive Effects of Colour and Odour on *Drosophila melanogaster* Trap Design for Pest Control Applications
4.1 Introduction ... 34
4.2 Materials and Methods ... 35
4.3 Results ... 39

4.4 Discussion 41
4.5 Acknowledgments 44
References 44

5 The Effects of Pipeline Clearings on Plant Communities, Soil Microbe Density, and Invertebrate Morphogenera Richness in a Second-growth Temperate Rainforest
5.1 Introduction 47
5.2 Materials and Methods 49
5.3 Results 51
5.4 Discussion 52
5.5 Conclusion 55
5.6 Acknowledgments 56
References 56
5.A Appendix 63

SFU SURJ | SIMON FRASER UNIVERSITY SCIENCE UNDERGRADUATE RESEARCH JOURNAL
Vol. 3 (2017-18)

Evidence of a Divergent Growth Response in the Pacific Oyster, *Magallana Gigas*, When Exposed to Native and Invasive Crab Chemical Cues

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Abstract

Marine invertebrates exhibit a variety of plastic morphological defenses in response to predator chemical cues. Typically, bivalves increase shell mass and strength in order to mitigate predation risk. However, invasive species may give off unfamiliar chemical cues, rendering native prey unable to detect and respond to foreign predators. Nevertheless, some native prey may adapt to recognize foreign predators over relatively short time scales (i.e. decades). The Pacific oyster, *Magallana gigas*, was introduced into Barkley Sound in 1937 and has experienced predation from native predators for nearly 80 years. Little is known about its defence capabilities and how it responds to the invasive European green crab, *Carcinus maenas*, with which it has coexisted for less than a decade. This offers a unique opportunity to study short-term evolution of defence mechanisms in response to predators over multiple time scales. We conducted a laboratory experiment to test if shell growth of juvenile *M. gigas* would be influenced by chemical effluent from native red rock crabs, *Cancer productus*, and invasive European green crabs, *Carcinus maenas*. Our results suggest that oysters grown in the presence of red rock crabs produce heavier shells than oysters grown in the presence of green crabs and controls, although this was not statistically significant. Shell weight increase in response to red rock crabs suggests that Pacific oysters may have had time to evolve a response to red rock crabs, but not yet for invasive European green crabs.

Keywords — Phenotypic Plasticity, Predation Cues, Morphological Defenses, Invasive Species

1. Introduction

Intertidal and sublittoral predators drive an immense variety of defence responses in marine prey populations [1]. In the presence of predators, prey can modulate their behavior, life history or morphology in order to mitigate predation risk [2, 3, 4]. However, these defense mechanisms are energetically costly, and often trade-off with reductions in growth and fecundity. [2, 5, 6]. Consequently, many prey species reduce these costs by exhibiting plastic responses that are only upregulated in the presence of a predator [6].

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Sessile or slow-moving organisms like some bivalve species are widely known to alter their morphology in order to alleviate predation risk [3, 6, 7, 8]. Some prey species can detect chemical cues given off by their predators' physiological functions, such as waste excretion, [9], allowing them to adapt their phenotype to decrease the risk of predation [10]. For example, Côté [11] concluded that bivalve mussels increase byssal thread production in the presence of a predator in order to increase dislodgement force, and therefore predator handling time. Additionally, Freeman and Byers [12] found that mussels increase their shell thickness in the presence of a native predator.

Introduction of invasive species can alter predator composition, and has therefore been found to shift community dynamics [13]. Antipredatory responses of native prey to invasive species tend to vary with geographical location and the length of time that the prey and predator have coexisted [13]. Increases in predator composition also increase the variation in which prey plastically alter their morphology in response to predation risk [12]. For example, Freeman and Byers [12] found that the recent crab invader, *Hemigrapsus sanguineus*, did not induce shell thickening in naïve mussel populations from the West Atlantic. However, populations of mussels that had already coexisted with *H. sanguineus* for more than 15 years were able to demonstrate a shell thickening response [12]. In contrast, both the naïve and familiarized mussel populations thickened their shells in the presence of *Carcinus maenas*, a well-established invader [12]. This suggests that prey species are capable of evolving their plastic responses over relatively short time-scales (i.e. decades) to defend against invasive predators.

The European green crab (*Carcinus maenas*) was first observed on the West Coast of North America in San Francisco Bay, CA, in 1989 [14], and has invaded British Columbia waters over the past 15 years. Because of their recent introduction, *C. maenas* may not be recognized by native prey species [15]. This decapod crustacean feeds on a variety of prey, including many bivalve species [16]. *C. maenas* are extremely tolerant to fluctuating temperature, as they can survive in water temperatures ranging from 0°C to above 30°C, [17, 18], and are euryhaline to 4 ppt as adults, and up to 17 ppt as larvae [19]. *C. maenas* thrive in open sand, mudflats, shell, cobble, and algae beds [20]. The physiological tolerance of *C. maenas* facilitates their ability to successfully invade diverse environments, subsequently altering ecological structure at several levels. [21]. For example, *C. maenas* invasion into Maine, USA, coincided with an extensive decline in soft shell clam beds [21]. Since C. maenas influence the abundance and dynamics of bivalve communities in the Atlantic [22], we would like to investigate if bivalves can adapt and respond defensively to the recently invasive *C. maenas* in Eastern Pacific communities.

The Pacific oyster (*Magallana gigas*) is native to the western Pacific, ranging from Russia to China, and into Southeast Asia [23, 24] but has been widely introduced, for commercial use, across the globe [25]. *M. gigas* was first introduced to Puget Sound in 1902, and Barkley Sound (the site of our study) in 1937, [25], and is now Canada's second most valuable commercial shellfish species (after mussels). *M. gigas* represented 59% of total oyster production in British Columbia in 2013, with an estimated economic contribution of $ 12.4 million [26]. On the West Coast of North America, *Metacarcinus magister* (Dungeness crab), *Metacarcinus gracilis* (graceful rock crab), and *Cancer productus* (red rock crab) are the primary predators of *M. gigas* [25]. As such, *M. gigas* has had

a longer evolutionary history of exposure (80 years) to native crab predators than the recently invasive *C. maenas*. Despite the importance of crab predation on *M. gigas*, little is known about its defence capabilities and responses to *C. maenas*.

Recent studies on the eastern oyster, *Crassostrea virginica*, have found that oysters increase shell thickness, mass and strength but produce less soft tissue in the presence of predators [7, 27, 28]. Furthermore, Robinson et al, [6], found that recently settled eastern oysters raised in the presence of crab predators produced thicker, wider and stronger shells and were less susceptible to predation by crabs. These findings suggest that oyster prey have the ability to appropriately allocate energy stores in order to successfully defend against more familiar predators.

Continual invasion of *C. maenas* into British Columbia prompts our investigation of plastic defence responses by *M. gigas*. In this study, we examine how growth in juvenile *M. gigas* is impacted when raised in the presence of two crab predators: the native *C. productus*, and the invasive *C. maenas*. We predict that *M. gigas* will increase shell weight when exposed to the red rock crab *C. productus*, to mitigate predation risk from a more familiar predator. We predict no such changes when oysters are exposed to the invasive predator, *C. maenas*, relative to controls. Results from this study could aid in management of wild and farmed oyster beds in the face of permanently settling invasive *C. maenas*. Furthermore, understanding the timescale associated with predator response evolution has far reaching implications in invasion ecology, beyond the organisms studied here.

2. Materials and Methods

2.1. Experimental Organisms

We collected male *C. maenas* by hand and crab trap from Effingham Inlet (N49°5′45.449", W125°11′54.882") on October 17, 2015. We collected male *C. productus* by crab trap from Bamfield Inlet (N48°49′2.15", W125°8′42.843") and Grappler Inlet (N48°49′53.667", W125°7′5.41") on October 25, 2015, and November 8, 2015, respectively. All collection sites are situated within Barkley Sound, British Columbia (Figure 1). We obtained three-month-old hatchery raised *M. gigas* from NOVA Harvest Ltd. (an aquaculture facility). Oysters ranged from 0.001 to 0.029 g in shell weight (0.896 - 13.94 mm shell length), which is within the limits of green crab predation (up to 60mm) and red rock crab predation (who eat seed and juvenile oysters) [29, 30]. We housed all organisms at the Bamfield Marine Sciences Centre where laboratory experiments were conducted.

We fed juvenile *M. gigas* 1 g of oyster spat formula (Innovative Aquaculture Products Ltd) per 200 mL of seawater. Spat formula contained the microalgae *Phaeodactylum tricornutum*, *Chaetocerus-B*, and *Nannochloropsis oculata*. We fed oysters every morning and evening throughout the experiment. We fed crabs chopped *Mytilus californianus* every three days until satiation. We also provided control aquaria with chopped mussel to control for any effects of mussel effluent on oyster growth. Lastly, we cleaned all aquaria, including controls, every fourth day after substantial feeding time. Oyster growth is most efficient in water ranging from 15-18°C (JP Hastey, NOVA Harvest aquaculture. Email. 10 Nov 2015. pers. comm.). Therefore, in each sea table, we used an 11.5cm deep heated water bath around all experimental aquaria. We warmed water

using 100 watt Theo Hydor aquarium heaters set to 18°C. We limited water flow to a twice daily flush of 13 minutes each, as incoming seawater was approximately 12°C, and would not be able to sustain maximal oyster growth. We recorded temperature in aquaria twice daily before seawater flushing in the morning (08:00hrs), and after seawater flushing in the evening (19:00hrs) to ensure temperature did not act as a confounding variable.

2.2. Experimental Design

Our experiment consisted of two treatments and a control designed to test the effects of, 1, *C. maenas* chemical effluent cues on *M. gigas* growth, and, 2, *C. productus* chemical effluent cues on *M. gigas* growth. We designed our control to assess juvenile oyster growth in the absence of any crab. We placed a small, perforated container housing 10 *M. gigas* in a 7 L aquarium that contained an individual *C. maenas*, or *C. productus* (treatments), or an aquarium with no crab (control).

We arranged aquaria in a complete randomized block design within sea tables. Each block consisted of four replicates of each predator treatment and four controls (no predator), totaling to 12 containers per sea table. We used three sea tables, for a total of 3 blocks, containing 4 sets of each treatment per block.

We weighed crabs with a Yamato Accu-Weigh SPC-5005 electronic scale to ensure all individuals were similar in weight. *C. productus* individuals ranged from 92 - 182 grams in weight, and *C. maenas* individuals ranged from 90 - 142 grams in weight. We acclimated crabs to aquaria for 24 hours before trials began.

We selected three hundred and sixty juvenile oysters for the experimental trial. We measured oyster length using Mastercraft 58-6800-4 digital calipers (accuracy = 0.01 mm). We measured length from the umbo (hinge line) of the oyster to the tip of the longest frill. We measured oyster wet weight and shell weight to the nearest 1 mg using the Mettler BasBal BB240 electronic scale, as adapted from Palmer [31]. This non-destructive technique allowed us to obtain initial and final measurements of wet weights and shell weights of the same oysters. The scale rested on a wooden scaffold that allowed for suspension of a weighing dish from underside of the scale. The weighing dish was immersed in a container of seawater and tared to compensate for the weight of the dish. This allowed us to measure oysters while they were immersed in seawater. We placed an individual oyster in the dish; assuming the density of seawater is equal to the density of the oyster tissue [31], and the scale measured the weight of the shell alone. First, we took wet weight measurements using the standard platform on the scale. Next, we measured the immersed weight of the same oyster to obtain the shell weight. We calculated tissue weight by subtracting shell weight from the total wet weight of each oyster. Afterwards, we placed ten oysters in one of thirty-six perforated containers. These containers allowed for effluent flow, but deterred direct crab predation. Lastly, we placed containers in aquaria attached to an air stone.

We ran the experimental trial for 22 days. At the end of the exposure, we removed juvenile oysters from aquaria and measured them using the same methods as described above for their final length, wet weight, shell weight, and tissue weight. One *C. productus* moulted, and one *C. maenas* escaped and was promptly replaced with a new individual during our experiment. We do not believe this affected the amount of chemical cues

oysters experienced and these replicates were included in our analysis.

2.3. Statistical Analysis

We considered the mean of each group of ten oysters a replicate. For each treatment, we calculated mean shell length, shell weight, wet weight and tissue weight. We took the differences by subtracting initial values from final values, and calculated unitless proportions (i.e. growth change) by subtracting mean initial weight from mean final weight and dividing by mean final weight.

Many of the differences we calculated for total wet weight, tissue weight, and shell length were negative, suggesting that growth decreased. We suspect that wet weight measurements were confounded by water weight between frills of the oyster shells, so we omitted wet weight data in our analysis. We also omitted length measurements from our analysis, as we postulate that negative values are indicative of human error. Shell weight of oysters was the most accurate measurement in our study (Dr. R. Palmer, Conversation. 29 Nov 2015. pers. comm.), so we used only shell weight data in our analysis.

We completed graphing and analysis in 'RStudio' version 0.98.1087 (R version 3.0.2 GUI 1.62 Snow Leopard build) using the 'nlme' package (Pinheiro et al., 2015). We analyzed mean proportional growth of shell weight for each treatment using a mixed effects analysis of variance (ANOVA), blocked by sea table as a random effect to account for within sea table growth variation. We performed this analysis using a linear mixed effects model (lme) fit by restricted maximum likelihood (REML). Finally, we performed an ANOVA on temperature from the three sea tables used in our experiment, and found that temperature did not significantly differ between sea tables over the course of the experimental exposure time (ANOVA; DF=1, SS=0.82, MS=0.82, f-value=1.02, p=0.317).

3. Results

Over the 22-day experimental predator exposure, shell weights in both treatments and the control increased significantly relative to initial values (DF=1, denDF=21, f-value=39.08, $p<0.0001$)

Results indicated that *M. gigas* grown in the presence of *C. productus* had a higher mean proportional shell weight increase (0.525 ± 0.094) relative to *C. maenas* (0.354 ± 0.092) and controls (0.444 ± 0.088). However, we found no statistically significant differences in *M. gigas* growth between treatments (Blocked ANOVA; DF=2, denDF=21, f-value=1.66, p=0.215, Figure 2).

In all treatments, large *M. gigas* had similar proportional shell growth relative to initial size, whereas small *M. gigas* appeared to have greater differences between treatments, indicated by the converging treatment lines in Figure 3. Smaller *M. gigas* had a higher mean proportional shell weight growth in the *C. productus* treatment, than in the *C. maenas* treatment, or control (Figure 3). However, smaller *M. gigas* in the *C. maenas* treatment had a lower mean proportional shell weight growth than the control (Figure 3).

4. Discussion

4.1. Effect of Treatment

The purpose of our study was to determine whether or not *M. gigas* could recognize novel predator cues from the invasive *C. maenas*, compared to that of *C. productus*, which has co-existed with *M. gigas* for nearly 70 more years [25]. Although we did not find a significantly different effect between native and invasive crab treatments, the heavier shell mass produced by oysters grown in the presence of *C. productus* suggests a biologically relevant trend. Below, we consider our results in light of our predictions and their implications for responses of sessile organisms to familiar and unfamiliar chemical cues.

Our results suggest that juvenile oysters may be able to increase shell weight depending on the crab predator the oyster is exposed to. Robinson et al [6] found that juvenile (<5mm) eastern oysters, *Crassostrea virginica*, increased shell mass and diameter significantly more when grown in the presence of native blue crab predators, compared to controls, providing evidence that *C. virginica* can plastically adopt morphological defences that protect themselves from crab predators. Our results compliment those of Robinson et al [6] demonstrating that juvenile *M. gigas* may adopt plastic defenses depending on predator familiarity. This may suggest that continual coexistence of the invasive *C. maenas* and *M. gigas* could lead to the evolution of predator cue recognition in decades to come.

Studies from the East Coast of North America have demonstrated that predator cue recognition to *C. maenas* has already evolved in areas where the crab first invaded. For example, Large and Smee [13] found that populations of dogwhelks in the Gulf of Maine produced heavier shells in areas where *C. maenas* were well established. Conversely, they found that dogwhelk populations unfamiliar with *C. maenas* did not produce heavier shells compared to controls [13]. Similarly, the whelk Nucella lapillus was found to increase shell thickness when familiar with *C. maenas*, although populations both familiar and unfamiliar to the invasive crab responded by reduced foraging [32]. These studies indicate that species may quickly evolve predator cue recognition and upregulate plastic morphological defence mechanisms in response to foreign predators given sufficient time to coexist. Our study sheds light on prey responses during early *C. maenas* invasion in the Eastern Pacific, suggesting that *M. gigas* has not yet evolved the required predator cue recognition to defend itself.

Numerous studies have found plastic defence responses in bivalves, including other oysters [8, 13, 33]. We therefore suggest that juvenile *M. gigas* does produce heavier shells in the presence of a familiar predator, but that our study failed to significantly detect these differences. Our study had a limited sample size and effect size, which led to higher variance and lower power between treatments. In addition, our study exposed oysters to crab predators for 3 weeks, whereas most studies on morphological plasticity in molluscs range from approximately 6-8 weeks [28, 34]. Therefore, our study may not have had sufficient power to resolve differences between treatments.

Furthermore, we suspect that increased fouling by *C. maenas* on *M. gigas* (due to waste excretion) and unfamiliar predator cues could have led to smaller proportional growth. In contrast, control *M. gigas* were able to grow in an environment free of fouling,

potentially explaining the smaller proportional growth in the *C. maenas* treatment compared to controls.

4.2. Effect of Size

Smaller *M. gigas* grown in the presence of *C. productus* had higher mean proportional increases in shell weight, although this was not significantly different between treatments. Bivalve prey size can affect their vulnerability to crab predators [35]. Crabs will prey more heavily on smaller bivalves because they require less energy to open [28], and have a lower probability of causing claw injury [16]. Our findings suggest that juvenile *M. gigas* compensate for this by growing significantly faster when they are smaller in size. Johnson and Smee [28] found that smaller oysters grew significantly less tissue and more shell weight in the presence of a crab predator. Our findings prompt further investigation as to whether or not juvenile oysters are able to perceive differences in predator species.

Future studies should examine if plastic defence strategies in bivalves have consequences on survival rates [6, 33, 36]. Robinson et al [6] found that juvenile Eastern oysters grown in the presence of crab predators required a 30-50% increase in shell crushing force, and therefore higher survival rates. To further expand on our study, measuring shell crushing force would provide insight into the mechanism by which oysters increase their fitness given the crab predator encountered, as this has not yet been demonstrated in *M. gigas*. Furthermore, predation assays would determine if observed changes in shell mass have biologically relevant consequences on survival of juvenile *M. gigas*. Lastly, future studies should also assess whether the plastic growth morphology of bivalves could not only be governed by predator effluent, but also by effluent from conspecifics being preyed upon.

5. Conclusion

By testing the effects of chemical cues from invasive and native crab predators on a commercially important bivalve, we were able to assess short-term evolution of defence mechanisms in response to familiar and unfamiliar predators at known time intervals.

Bivalve aquaculture operations in the Eastern Pacific are commonly run in open environments where the organisms reared are exposed to predators [25]. Predation is of great concern to bivalve farmers [37], where *C. maenas* has been reported to predate upon juvenile oyster spat [38]. In the coming decades, *C. maenas* may be a significant pest for farmed and wild oyster beds. However, over a longer time scale, *M. gigas* may evolve predator cue recognition for *C. maenas* to increase fitness and survival.

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References

[1] Lee B. Kats and Lawrence M. Dill. The scent of death: Chemosensory assessment of predation risk by prey animals. *Écoscience*, 5(3):361–394, 1998. doi:10.1080/11956860.1998.11682468.

[2] Todd A. Crowl and Alan P. Covich. Predator-induced life-history shifts in a freshwater snail. *Science*, 247(4945):949–951, 1990. ISSN 0036-8075. doi:10.1126/science.247.4945.949.

[3] Geoffrey C. Trussell and L. David Smith. Induced defenses in response to an invading crab predator: An explanation of historical and geographic phenotypic change. *Proceedings of the National Academy of Sciences*, 97(5):2123–2127, 2000. ISSN 0027-8424. doi:10.1073/pnas.040423397.

[4] Delbert L. Smee and Marc J. Weissburg. Hard clams (mercenaria mercenaria) evaluate predation risk using chemical signals from predators and injured conspecifics. *Journal of Chemical Ecology*, 32(3):605–619, Mar 2006. ISSN 1573-1561. doi:10.1007/s10886-005-9021-8.

[5] Large SI, Torres P, and Smee DL. Behavior and morphology of nucella lapillus influenced by predator type and predator diet. *Aquatic Biology*, 16(2):189–196, 2012. doi:10.3354/ab00452.

[6] Robinson EM, Lunt J, Marshall CD, and Smee DL. Eastern oysters crassostrea virginica deter crab predators by altering their morphology in response to crab cues. *Aquatic Biology*, 20(2):111–118, 2014. doi:10.3354/ab00549.

[7] Joshua P. Lord and Robert B. Whitlatch. Inducible defenses in the eastern oyster crassostrea virginica gmelin in response to the presence of the predatory oyster drill urosalpinx cinerea say in long island sound. *Marine Biology*, 159(6):1177–1182, Jun 2012. ISSN 1432-1793. doi:10.1007/s00227-012-1896-7.

[8] Masahiro Nakaoka. Nonlethal effects of predators on prey populations: Predator-mediated change in bivalve growth. *Ecology*, 81(4):1031–1045. doi:10.1890/0012-9658(2000)081[1031:NEOPOP]2.0.CO;2.

[9] Grant E. Brown, Jessica A. Paige, and Jean-Guy J. Godin. Chemically mediated predator inspection behaviour in the absence of predator visual cues by a characin fish. *Animal Behaviour*, 60(3):315 – 321, 2000. ISSN 0003-3472. doi:10.1006/anbe.2000.1496.

[10] Timothy C. Edgell and Christopher J. Neufeld. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. *Biol Lett*, 4(4):385–387, Aug 2008. ISSN 1744-9561. doi:10.1098/rsbl.2008.0204.

[11] Isabelle M. Côté. Effects of predatory crab effluent on byssus production in mussels. *Journal of Experimental Marine Biology and Ecology*, 188(2):233 – 241, 1995. ISSN 0022-0981. doi:10.1016/0022-0981(94)00197-L.

[12] Aaren S. Freeman and James E. Byers. Divergent induced responses to an invasive predator in marine mussel populations. *Science*, 313(5788):831–833, 2006. ISSN 0036-8075. doi:10.1126/science.1125485.

[13] Scott I. Large and Delbert L. Smee. Biogeographic variation in behavioral and morphological responses to predation risk. *Oecologia*, 171(4):961–969, Apr 2013. ISSN 1432-1939. doi:10.1007/s00442-012-2450-5.

[14] G.S. Jamieson, E.D. Grosholz, D.A. Armstrong, and R.W. Elner. Potential ecological implications from the introduction of the european green crab, carcinus maenas (linneaus), to british columbia, canada, and washington, usa. *Journal of Natural History*, 32(10-11):1587–1598, 1998. doi:10.1080/00222939800771121.

[15] GE Gillespie, AC Phillips, DL Paltzat, TW Therriault, Department of Fisheries, and BC(Canada). Science Branch Oceans, Nanaimo. Status of the european green crab, carcinus maenas, in british columbia- 2006. Technical report, Citeseer, 2007.

[16] Francis Juanes. Why do decapod crustaceans prefer small-sized molluscan prey? *Marine Ecology Progress Series*, 87(3):239–249, 1992. ISSN 01718630, 16161599.

[17] J.J. Beukema. The abundance of shore crabs carcinus maenas (l.) on a tidal flat in the wadden sea after cold and mild winters. *Journal of Experimental Marine Biology and Ecology*, 153(1):97 – 113, 1991. ISSN 0022-0981. doi:10.1016/S0022-0981(05)80009-7.

[18] M. Cuculescu, D. Hyde, and K. Bowler. Thermal tolerance of two species of marine crab, cancer pagurus and carcinus maenas. *Journal of Thermal Biology*, 23(2):107 – 110, 1998. ISSN 0306-4565. doi:10.1016/S0306-4565(98)00008-4.

[19] Erik Rasmussen. Systematics and ecology of the isefjord marine fauna (denmark). *Ophelia*, 11(1):1–507, 1973. doi:10.1080/00785326.1973.10430115.

[20] M. Thiel and T. Dernedde. Recruitment of shore crabscarcinus maenas on tidal flats: Mussel clumps as an important refuge for juveniles. *Helgoländer Meeresuntersuchungen*, 48(2):321–332, Jun 1994. ISSN 1438-3888. doi:10.1007/BF02367044.

[21] John B. Glude. The effects of temperature and predators on the abundance of the soft-shell clam, mya arenaria, in new england. *Transactions of the American Fisheries Society*, 84(1):13–26, 1955. doi:10.1577/1548-8659(1954)84[13:TEOTAP]2.0.CO;2.

[22] F. J. Ebling, J. A. Kitching, Louise Muntz, and C. Mary Taylor. The ecology of lough ine. *Journal of Animal Ecology*, 33(1):73–82, 1964. ISSN 00218790, 13652656. doi:10.2307/2349.

[23] Kohman Y. Arakawa. Commercially important species of oysters in the world. *Marine Behaviour and Physiology*, 17(1):1–13, 1990. doi:10.1080/10236249009378756.

[24] EV Coan, PV Scott, and FR Bernard. Bivalve seashells of western north america, santa barbara museum of natural history monographs number 2. *Studies in Biodiversity*, (2), 2000.

[25] Graham E Gillespie, Susan M Bower, Kerry L Marcus, and Dorothee Kieser. Biological synopsises for three exotic molluscs, manila clam (venerupis philippinarum), pacific oyster (crassostrea gigas) and japanese scallop (mizuhopecten yessoensis) licensed for aquaculture in british columbia. *Can Sci Advis Sec Res Doc*, 13, 2012.

[26] Fisheries and Communications Branch Oceans Canada. Farmed oysters, Mar 2017. URL http://www.dfo-mpo.gc.ca/aquaculture/sector-secteur/species-especes/oysters-huitres-eng.htm.

[27] Roger I. E. Newell, Victor S. Kennedy, and Kristi S. Shaw. Comparative vulnerability to predators, and induced defense responses, of eastern oysters crassostrea virginica and non-native crassostrea ariakensis oysters in chesapeake bay. *Marine Biology*, 152(2):449–460, Aug 2007. ISSN 1432-1793. doi:10.1007/s00227-007-0706-0.

[28] Johnson KD and Smee DL. Size matters for risk assessment and resource allocation in bivalves. *Marine Ecology Progress Series*, 462:103–110, 2012. doi:10.3354/meps09804.

[29] European green crab (carcinus maenas) - aquatic invasive species | washington department of fish & wildlife. URL https://wdfw.wa.gov/ais/carcinus_maenas/.

[30] European green crab (carcinus maenas) - aquatic invasive species | washington department of fish & wildlife. URL https://wdfw.wa.gov/ais/carcinus_maenas/.

[31] A Richard Palmer. Growth in marine gastropods. a non-destructive technique for independently measuring shell and body weight. *Malacologia*, 23(1):63–74, 1982.

[32] Aaren S. Freeman, Emily Dernbach, Candice Marcos, and Elise Koob. Biogeographic contrast of nucella lapillus responses to carcinus maenas. *Journal of Experimental Marine Biology and Ecology*, 452:1 – 8, 2014. ISSN 0022-0981. doi:10.1016/j.jembe.2013.11.010.

[33] A.M. Flynn and D.L. Smee. Behavioral plasticity of the soft-shell clam, mya arenaria (l.), in the presence of predators increases survival in the field. *Journal of Experimental Marine Biology and Ecology*, 383(1):32 – 38, 2010. ISSN 0022-0981. doi:10.1016/j.jembe.2009.10.017.

[34] Keith D. Johnson and Delbert L. Smee. Predators influence the tidal distribution of oysters (crassostrea virginica). *Marine Biology*, 161(7):1557–1564, Jul 2014. ISSN 1432-1793. doi:10.1007/s00227-014-2440-8.

[35] Wai Hing Wong, Shawn Gerstenberger, Wen Baldwin, and Bryan Moore. Settlement and growth of quagga mussels (dreissenia rostriformis bugensis andrusov, 1897) in lake mead, nevada-arizona, usa. *Aquatic Invasions*, 7(1):7, 2011.

[36] Delbert L Smee and Marc J Weissburg. Clamming up: environmental forces diminish the perceptive ability of bivalve prey. *Ecology*, 87(6):1587–1598, 2006.

[37] John A. Lindsay and Neil B. Savage. Northern new england's threatened soft-shell clam populations. *Environmental Management*, 2(5):443–452, Sep 1978. ISSN 1432-1009. doi:10.1007/BF01872919.

[38] Peter John Dare, G Davies, and DB Edwards. *Predation on juvenile Pacific oysters (Crassostrea gigas Thunberg) and mussels (Mytilus edulis L.) by shore crabs (Carcinus maenas L.)*. Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research, 1983.

A. Figures

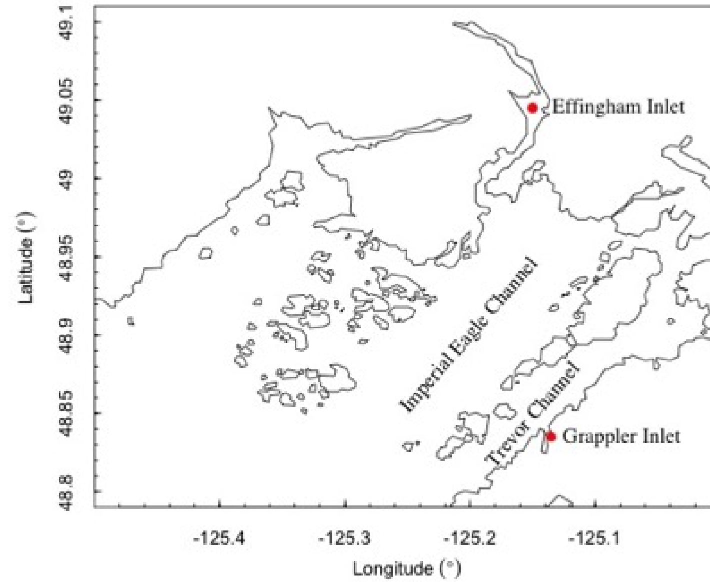

Figure 1: *Sites of green crab collection (Effingham Inlet - N49°5′45.449″, W125°11′54.882″), and red rock crab collection (Grappler Inlet - N48°49′53.667″, W125°7′5.41″), in Barkley Sound, British Columbia. Green crabs were collected from Effingham Inlet on October 17, 2015. Red rock crabs were collected from Grappler Inlet on October 25, 2015.*

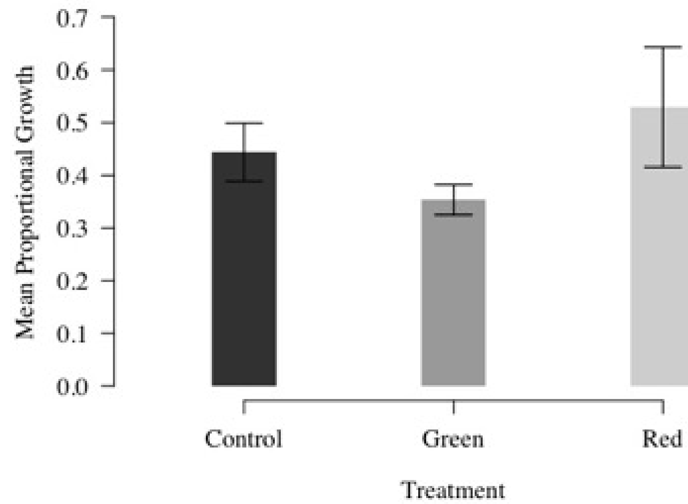

Figure 2: *Mean proportional growth of oysters exposed to green crabs* ($0.354 \pm 0.092; n = 12$)*, red rock crabs* ($0.525 \pm 0.094; n = 12$)*, and no crab predators* ($0.444 \pm 0.088; n = 12$)*. Data was collected on December 2 and 3, 2015.*

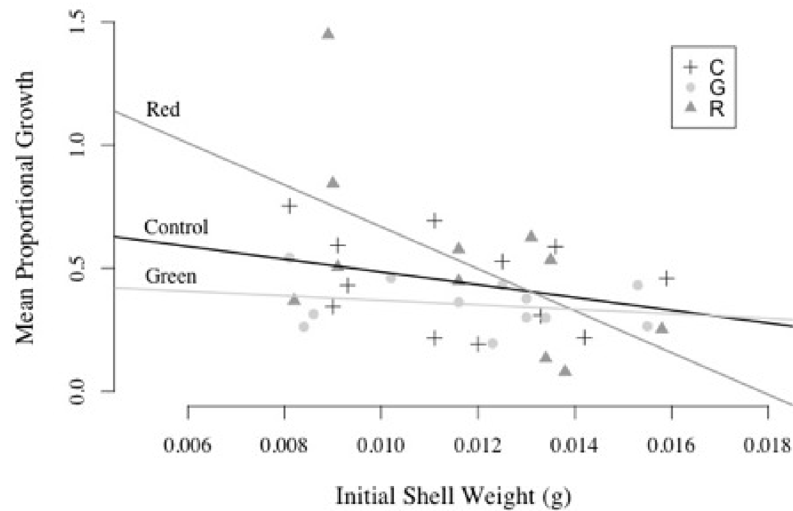

Figure 3: *Mean proportional growth in weight versus initial weight. Regression lines were fit using means of each treatment* ($n = 12$ *for each*)*. Equations of the lines were calculated to be* $y = -85.215x + 1.521; (R^2 = 0.32)$ *for native crab treatments,* $y = -9.149x + 0.462; (R^2 = 0.05)$ *for invasive crab treatments, and* $y = -25.949x + 0.745; (R^2 = 0.11)$ *for controls.*

Phototactic Responses of *Drosophila Melanogaster* to UVA, UVB, White Light, and Dark Environments

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Abstract

Ultraviolet A (UVA) radiation comprises a large portion of the solar radiation found on Earth. UVA exposure has been shown to induce both harmful and beneficial biological effects on insects. The objective of this study was to observe if *Drosophila melanogaster*, a relative of the North American common fruit fly, are attracted to or repelled by UV light when alternative light conditions are present. We performed three experiments that gave the flies a choice between (1) UVA or UVB light areas, (2) UVA light or a dark space, (3) a white light only area or a UVA in combination with white light space. In all three cases, we found significantly more flies in the UVA exposed areas ($\geq$ 80%). This suggests that fruit flies exhibit positive phototaxis toward UVA light. Our data provide insight into the behavioral preferences of fruit flies, and show potential for a UVA component to be involved in a successful pest-trapping device.

Keywords — Phototaxis, Ultraviolet, Fruit Fly, Two-Choice Bioassay, Pest Management, Light Trap

1. Introduction

Ultraviolet (UV) radiation is visible to insects [1]. Three types of UV are emitted from the sun: UVA (400-315 nm), UVB (315-240 nm), and UVC (240-100 nm). UVC and most UVB radiation are largely absorbed by the ozone layer, which is beneficial as they are both harmful to living organisms [2]. This leaves UVA as the primary component (> 90%) of UV that reaches the Earth's surface, causing it to be the main type of radiation interaction with ecosystems and organisms [3].

The fly genus *Drosophila* are common pests that are present on almost every continent on Earth [4]. *Drosophila* possess photoreceptive cells which have specific spectral sensitivities, allowing them to detect differences between light [1]. One type of response that comes from these cells is phototaxis, which can be either positive or negative [1]. A positive phototactic response provides potential for pest trapping uses, however it has been found that effective intensities and wavelengths vary greatly between insects

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[1]. A negative phototactic response can be used to protect cultivation areas by displaying light at an intensity or wavelength that repels a particular species [1]. This study explores how such innate reactions to light can be better implemented into pest management.

Our ultimate goal with this research was to observe if UVA light serves as a repellant or attractant for *Drosophila melanogaster*, a relative of the North American common fruit fly, when alternative light options are present [1]. For *D. melanogaster*, UVA exposure is disadvantageous to the F1 generation's fitness, and causes an increase in the duration of time from egg to hatching of the first filial generation 5. There seems to be a gap in existing research involving more long-term studies. Although UVA has been found to be primarily damaging, it has also been noted that UVA radiation can stimulate beneficial photorepair in some organisms [2]. Developing ways to attract these flies to UVA can be utilized in traps to help manage infestations.

The purpose of our first experiment was to determine if different wavelengths of UV light were more attractive to the flies than others. Our second experiment's goal was to confirm the previous finding that the flies would be found in a UVA light environment as opposed to a dark environment [1]. Our final experiment explored fruit flies' phototactic response to UVA light when white light was also present, as a way to mimic an indoor setting.

We hypothesized that, because it is often evolutionarily beneficial to avoid harmful conditions, *D. melanogaster* would (1) be found more often in the UVA light environment than the UVB environment, (2) be found less often in the UVA light environment than the dark environment, and (3) be found less often in the UVA light environment than a white light only environment.

2. Materials and Methods

2.1. Experimental Insects

The *D. melanogaster* used in this experiment are of the Oregon-Roseburg wild-type strain, sourced from Simon Fraser University's Department of Biological Sciences. The flies received a standard fruit fly diet composed of distilled water, yeast, agar, cornmeal, molasses, tetracyclin and tegosept [5].

2.2. General Experimental Setup: Two Choice Bioassay

We used two-choice bioassays to compare which light environment captured more flies, modelling a previous study [6]. For our experiment, we used a T-tube adapted from black construction paper to connect two test tubes (Ultident Scientific Borosilicate Disposable GlassCulture Tubes) together (Figure 1). This setup allowed the fruit flies to move towards one test tube or the other. Any fly that did not move toward either of the environments, and instead remained in the T-tube, was identified as a "non-responder". The T-tube served as a way to insert fruit flies into the bioassay apparatus and expose them to the two environments at the same time.

To control for external variables, we used cardboard boxes as chambers to surround the test tubes and enclose the different lighting conditions. We hot glued heavyweight

black construction paper to the inside of both boxes. We inserted one of the test tubes 3.0 cm up from the bottom of the first box, so that approximately 0.7 cm of the open end of the test tube remained outside the box. This was repeated for the second test tube, which was set up identically in the adjacent box. Each cardboard box was exposed to one of two lighting conditions for each experiment. In a preliminary test (not shown), we randomly alternated the UV bulbs and the sides of the T-maze, concluding that these caused no significant impact on our results.

A vial of fruit flies contained 25-137 flies. For each trial, we inserted fruit flies from a vial into the T-tube using a funnel which we then closed off using a cotton ball. When the flies were being inserted, the open end of the T-tube was facing upward. As soon as the flies were inserted, we rotated the T-tube apparatus 90 degrees to prevent the flies from being encouraged to crawl back upwards and to avoid a third-choice environment. We started a timer for two minutes, giving the fruit flies enough time to explore both lighting options and move towards one test tube or the other, without temperature acting as a confounding variable [7]. We then separated the test tubes from the T-tube using slips of paper, capturing the flies in their respective environments. The flies still in the T-tube (non-responders) were then transferred to a third test tube. All test tubes for each trial were placed into a freezer to sacrifice the flies, after which we counted and identified them by sex.

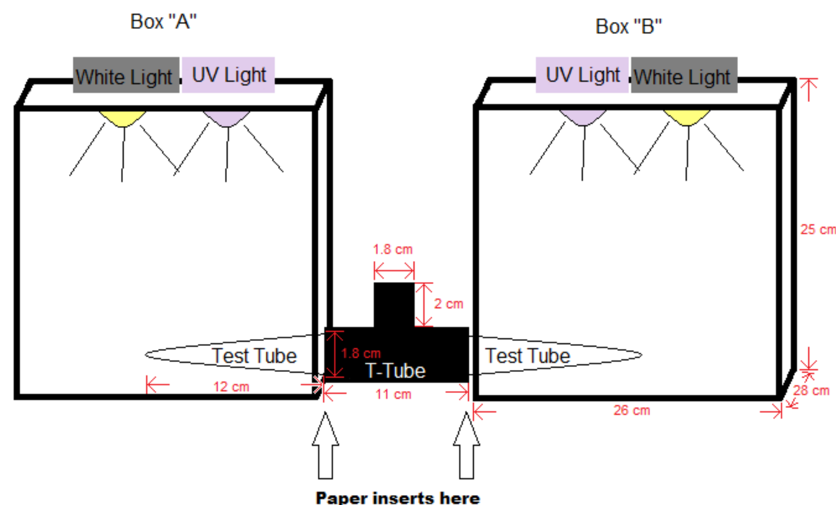

Figure 1: ***General Setup and Dimensions for Two-Choice Bioassay*** *Flies were deposited into the top of the T-Tube. At the end of the experiment, paper slips were inserted to trap the flies present in each condition. Any flies remaining in the T-Tube after the experiment ended were classified as "non-responders". The specific lighting conditions varied for each experiment.*

For each experiment, the bioassays were replicated 10 times. For every replication, new T-tubes and test tubes were used to avoid contaminating equipment with *D. melanogaster* stress hormones or other signals [8].

2.3. Experimental Light Conditions

The three experiments conducted had specific light conditions (Table 1). A Mineralight® lamp (Model UVGL - 58, 115 V) and a Voltax® LED Work Light (160 lm) were used as the sources for UV and white light, respectively. The Mineralight® lamp had two wavelength settings: long (365 nm, 1200 $\mu W\,cm^{-2}$) and short (254 nm, 1350 $\mu W\,cm^{-2}$). In Experiments 2 and 3, a UV light was turned on but covered by black construction paper to control for any noise, temperature, vibration, or other confounding factors that might have arisen from the UV lamp itself.

Table 1: *Light Conditions for Experiments.*

Experiment	Box A		Box B	
	Mineralight	Voltax Flashlight	Mineralight	Voltax Flashlight
1	UVA (365 nm)	-	UVB (254 nm)	-
2	UVA	-	UVA, covered	-
3	UVA	On	UVA, covered	On

2.4. Statistical Analysis

We converted the fly-count data to percentages of the total number of flies in each vial. We then graphed the mean percentages (with 95% confidence intervals) of the total number of flies, including non-responders. We performed chi-squared tests of goodness-of-fit ($\alpha = 0.05$) on the two responding groups of flies for all 3 experiments, to determine if the proportions differed significantly from 50%, our null hypothesis. This would indicate that our observed values were significantly different between the two environments. A Two Way ANOVA ($\alpha = 0.05$) was used to examine if the phototactic responses of female and male flies were statistically different for each experiment.

3. Results

3.1. Experiment 1: UVA vs. UVB Light

The UVA light environment captured significantly more flies that the UVB light environment (Figure 2; $\chi^2(2, N = 10) = 188.16, p < 0.0001$).

3.2. Experiment 2: UVA Light vs. Darkness

The UVA light environment captured significantly more flies than the dark environment (Figure 3; $\chi^2(2, N = 10) = 360.08, p < 0.0001$).

3.3. Experiment 3: UVA And White vs. Only White Light

The UVA and white light environment captured significantly more flies than the "white light only" environment (Figure 4; $\chi^2(2, N = 10) = 206.35, p < 0.0001$).

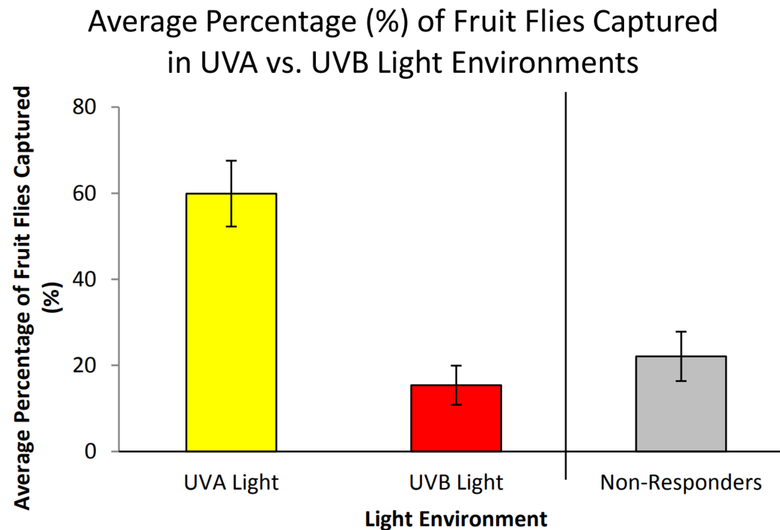

Figure 2: ***Average Percentage (%) of Fruit Flies Captured in UVA vs. UVB Light Conditions*** *This graph displays the mean percentages of the fruit flies that were captured by UVA light, UVB light, and also the percentage of fruit flies that remained in the T-tube (non-responders). Error bars represent 95% confidence intervals.*

We found that there was no significant difference in the average percentage of male and female flies captured in the UVA environment in experiments 1 through 3 (graph not shown; $F(59) = 2.17, p = 0.124$).

4. Discussion

The results from the first experiment suggest that more fruit flies would be captured by the UVA light environment than UVB (Figure 2). While UVB radiation is exclusively harmful for organisms, UVA wavelengths display a variety of detrimental and beneficial effects [2]. Some beneficial effects of UVA involve the substantial inhibition of growth or survival in bacteria that are harmful to the flies [9]. Both UVA and UVB have been found to cause mortality and pyrimidine dimers, however, UVA causes damage to a much lesser degree [2, 10]. The flies may have avoided the UVB due to the damage associated to its higher intensity, or they may simply be more attracted to the specific wavelength of UVA.

More fruit flies were found in the UVA light environment when given the alternative of the dark environment (Figure 3). The observed phototaxic behaviour of the adults is opposite than that seen in larvae [11].To promote survival, larvae prefer to reside and feed in dark environments, such as rotting fruit, to simultaneously avoid predators while obtaining a source of food [12]. Therefore, from an evolutionary view, it would be beneficial for the larvae to seek dark conditions. Once the flies reach maturity and leave these dark spaces, their perception of a safe and opportunistic environment may change. A possible explanation of our results, and adult insect attraction to UVA light,

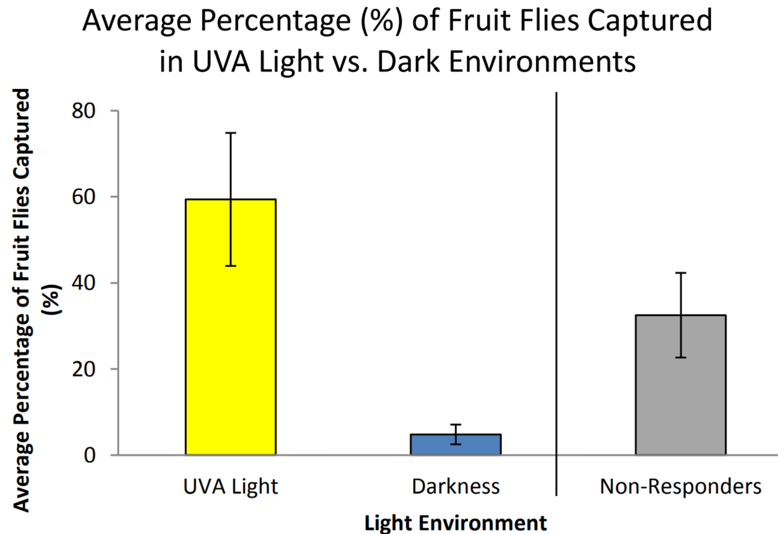

Figure 3: ***Average Percentage (%) of Fruit Flies Captured in UVA Light vs. Dark Environments*** *This graph displays the mean percentages of the fruit flies that were captured by UVA light, Darkness, and also the percentage of fruit flies that remained in the T-tube (non-responders). Error bars represent 95% confidence intervals.*

is that UVA light is associated with the recognition of open sky, in which food and other essentials may be found [13].

Our final experiment suggests that UVA is still a superior attractant even when a white LED light is present (Figure 4). Williamson et al. [2] discovered that UVA radiation, in combination with visible light, stimulates photoenzymatic repair - a light-mediated process to repair UVB-damaged DNA. This could be a potential underlying reason for positive phototaxis to UVA light. The results from these experiments point toward the conclusion that a UVA trap may be effective for adults in both dark and light conditions during the day.

One aspect that may have added variability to the data was the possibility of social interactions influencing the observed response. Due to the large variation in sample size per trial, in some cases flies may have attempted to avoid crowded conditions in some trials, which may have impacted their location. This large variation, as well as the brief time limit given, may be responsible for the number of flies classified as "non-responders".

Regarding the investigation of the differences in behaviour between sexes, we used a one-way ANOVA and found that there was no statistically significant difference in the movement of male and female flies. The absence of a sex-dependent phototactic response suggests that a UVA light trap could be equally effective against male and female flies. This adds to the potential effectiveness of an indoor trap because UVA light has also been shown to lower fecundity in fruit flies [14]. Thus, overtime, it may reduce population growth as well.

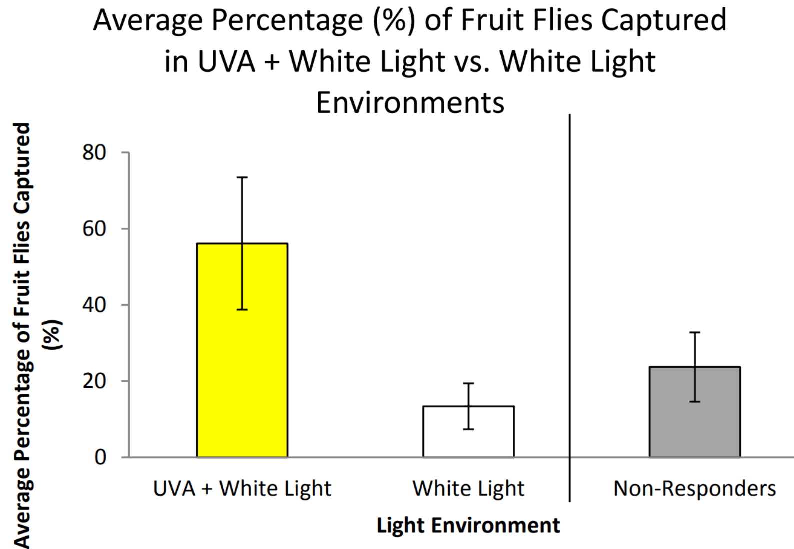

Figure 4: ***Average percentages (%) of Responding Flies Captured in UVA + White Light vs. Only White Light Environments*** *This graph displays the mean percentages of the fruit flies that were captured by UVA light, white light, and also the percentage of fruit flies that remained in the T-tube (non-responders). Error bars represent 95% confidence intervals.*

5. Conclusion

Our results suggest that *Drosophila* are more attracted to UVA light than UVB. The results also suggest that fruit fly photoreceptors are sensitive to different wavelengths of light, and that certain wavelengths may be more attractive than others. A trap utilizing UVA radiation could be combined with olfactory stimuli to increase its effectiveness in attracting the flies [15]. A follow up experiment could be to determine which olfactory stimuli, when combined with UVA, is the most effective in attracting and trapping fruit flies.

A challenge in research with adult *Drosophila* is that their phototactic responses may be modulated by their circadian clock[16]. Due to the diurnal nature of fruit flies, it is possible that their phototactic response to UVA light can oscillate throughout the day [14, 17]. An additional future experiment could be to conduct similar bioassay trials at night and examine if there are any observable changes in the flies' phototactic behaviour.

A major practical application of our study's results is to use the attractive properties of UVA as bait to capture the disruptive populations of these flies in various environments. Our data offers potential for UVA radiation to be applied as part of a pest-control mechanism. This type of mechanism, which would involve light rather than scent, would be beneficial in terms of avoiding an unpleasant odor. The effectiveness of such a trap can be further explored by studying the behavioral and physiological effects of UVA exposure on *D. melanogaster*, particularly on their reproductive processes. These studies are needed to ascertain if a sanitation technique would be needed in a trap that includes UVA, or if UVA exposure alone is enough to impede population

growth.

In conclusion, *Drosophila melanogaster* appear to be able to distinguish these different types of light from one another, and demonstrate positive phototactic responses to UVA light in both light and dark conditions. The difference in phototactic response between male and female fruit flies toward UVA light does not appear to be significantly different, which indicates that a UVA light trap could be effective for both sexes.

6. Acknowledgments

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References

[1] Masami Shimoda and Ken-ichiro Honda. Insect reactions to light and its applications to pest management. *Applied Entomology and Zoology*, 48(4):413–421, Nov 2013. ISSN 1347-605X. doi:10.1007/s13355-013-0219-x.

[2] Craig E. Williamson, Patrick J. Neale, Gabriella Grad, Hendrika J. De Lange, and Bruce R. Hargreaves. Beneficial and detrimental effects of uv on aquatic organisms: Implications of spectral variation. *Ecological Applications*, 11(6):1843–1857. doi:10.1890/1051-0761(2001)011[1843:BADEOU]2.0.CO;2.

[3] *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans: Solar and Ultraviolet Radiation*. International Agency for Research on Cancer World Health Organization, 1992.

[4] Jean R. David and Pierre Capy. Genetic variation of drosophila melanogaster natural populations. *Trends in Genetics*, 4(4):106 – 111, 1988. ISSN 0168-9525. doi:10.1016/0168-9525(88)90098-4.

[5] Sandra R. Schulze, Donald A. R. Sinclair, Kathleen A. Fitzpatrick, and Barry M. Honda. A genetic and molecular characterization of two proximal heterochromatic genes on chromosome 3 of drosophila melanogaster. *Genetics*, 169(4):2165–2177, 2005. ISSN 0016-6731. doi:10.1534/genetics.103.023341.

[6] Robert W. AU Fernandez, Marat AU Nurilov, Omar AU Feliciano, Ian S. AU McDonald, and Anne F. AU Simon. Straightforward assay for quantification of social avoidance in drosophila melanogaster. *JoVE*, (94):e52011, 2014. ISSN 1940-087X. doi:10.3791/52011.

[7] Therese Ann Markow. Phototactic behavior of drosophila species at different temperatures. *The American Naturalist*, 114(6):884–892, 1979. doi:10.1086/283535.

[8] Greg S. B. Suh, Allan M. Wong, Anne C. Hergarden, Jing W. Wang, Anne F. Simon, Seymour Benzer, Richard Axel, and David J. Anderson. A single population of olfactory sensory neurons mediates an innate avoidance behaviour in drosophila. *Nature*, 431:854 EP –, Sep 2004. doi:10.1038/nature02980.

[9] R. Sommaruga, I. Obernosterer, G. J. Herndl, and R. Psenner. Inhibitory effect of solar radiation on thymidine and leucine incorporation by freshwater and marine bacterioplankton. *Appl Environ Microbiol*, 63(11):4178–4184, Nov 1997. ISSN 0099-2240. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1389280/. 16535724[pmid].

[10] James E. Trosko and Kathy Wilder. Repair of uv-induced pyrimidine dimers in drosophila melanogaster cells in vitro. *Genetics*, 73(2):297–302, Feb 1973. ISSN 0016-6731. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1212892/. 4633159[pmid].

[11] Tim-Henning Humberg and Simon G. Sprecher. Age- and wavelength-dependency of drosophila larval phototaxis and behavioral responses to natural lighting conditions. *Frontiers in Behavioral Neuroscience*, 11:66, 2017. ISSN 1662-5153. doi:10.3389/fnbeh.2017.00066.

[12] Raúl Godoy-Herrera. Selection for digging behavior indrosophila melanogaster larvae. *Behavior Genetics*, 8(5):475–479, Sep 1978. ISSN 1573-3297. doi:10.1007/BF01067940.

[13] Yoshiaki Tokushima, Takuya Uehara, Terumi Yamaguchi, Kentaro Arikawa, Yooichi Kainoh, and Masami Shimoda. Broadband photoreceptors are involved in violet light preference in the parasitoid fly exorista japonica. *PLOS ONE*, 11(8):1–16, 08 2016. doi:10.1371/journal.pone.0160441.

[14] Lijun Zhou, Junli Zheng, Zhenxing Liu, Weihua Ma, and Chaoliang Lei. Effects of uva radiation on the performance of adults and the first filial generation of the fruit fly drosophila melanogaster meigen, 1830 (diptera: Drosophilidae). *Proceedings of the Entomological Society of Washington*, 118(3):456–465, 2016. doi:10.4289/0013-8797.118.3.456.

[15] Andrei V. Alyokhin, Russell H. Messing, and Jian J. Duan. Visual and olfactory stimuli and fruit maturity affect trap captures of oriental fruit flies (diptera: Tephritidae). *Journal of Economic Entomology*, 93(3):644–649, 2000. doi:10.1603/0022-0493-93.3.644.

[16] Balaji Krishnan, Stuart E. Dryer, and Paul E. Hardin. Circadian rhythms in olfactory responses of drosophila melanogaster. *Nature*, 400:375 EP –, Jul 1999. doi:10.1038/22566.

[17] B. Lu, W Liu, F Guo, and A Guo. Circadian modulation of light-induced locomotion responses in drosophila melanogaster. *Genes, Brain and Behavior*, 7(7):730–739. doi:10.1111/j.1601-183X.2008.00411.x.

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The Influence of Surface Scratches on Copper's Antibacterial Activity

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Abstract

Copper metal is capable of killing more than 99.9% of bacteria upon contact, and has been researched as an alternative material to hospital surfaces with aim to prevent hospital-acquired infections. Because copper is a malleable metal, it is more likely to acquire surface scratches than other common materials in high-traffic health care facilities. To investigate the effects these scratches may have on copper's antibacterial activity, we inoculated scratched and unscratched copper C110 with either *Escherichia coli* ATCC 11303 or *Staphylococcus carnosus* 51365 and quantified the bacteria present at various time intervals. We found no significant difference in the antibacterial activity of copper between the two surface textures, with 99.9% of *E. coli* and the majority of *S. carnosus* bacteria killed within 10 minutes. This suggest that copper surfaces implemented in health care facilities would not require extensive maintenance of smooth surfaces to retain their bactericidal effects. Additionally, we found a significantly greater concentration of bacteria present on stainless steel compared to copper, supporting the claims that copper surfaces could help decrease the transmission of hospital-acquired infections.

Keywords — Hospital Acquired Infections, Surface Texture, Copper C110, Staphylococcus Carnosus 51365, Escherichia Coli ATCC 11303, Infection, Stainless Steel

1. Introduction

Health care facilities are the source of many infections [1, 2, 3], as bacteria can be deposited by a touch, sneeze, or cough from an infected patient [4]. Despite the various infection protocols implemented every year [1, 5, 6], hospital acquired infections remain prevalent today [3, 7, 8]. The Center for Disease Control estimates that 1 in every 25 patients in American acute care hospitals acquires an infection during their stay [9], with approximately 75 000 of these infections resulting in fatality in 2011 [9].

Frequently touched surfaces within health care facilities such as door knobs, handrails and light switches are often made from materials on which bacteria are able to survive and grow, such as aluminum or stainless steel [10, 11, 12]. Copper is classified by the Environmental Protection Agency as a solid antimicrobial material, and is capable

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of killing viruses, yeasts and bacteria upon contact [10]. Because of its well-established antimicrobial properties [10, 13], copper metal and its alloys have been researched as an alternative material for these frequently touched surfaces [10, 14, 15, 16, 17, 18], with aim to decrease infection transmission. Although the mechanism by which copper kills bacteria is not fully understood, studies that replaced a variety of frequently touched surfaces with copper alloys saw a significant decrease in pathogens present on these surfaces [15, 19, 20].

Despite these promising results, copper is more malleable than aluminum or stainless steel, and therefore more susceptible to surface damage [13]. Because hospitals are high traffic facilities, copper surfaces are likely to obtain scratches and abrasions. If bacteria-metal contact is needed for efficient bacterial killing, we expect to see a decrease in the efficiency of copper's antimicrobial activity on scratched surfaces, as bacteria may become suspended in solution within these crevices. Our research aims to investigate the effects that surface scratches may have on copper's antibacterial property, which could indicate whether hospitals that replace frequently touched surfaces with copper should be vigilant in maintaining smooth surfaces.

2. Materials and Methods

2.1. Preparation of Bacterial Broths

We prepared a broth of each bacterial strain being tested (Table 1). Broth inoculation was done inside a biosafety class IIA/B3 Biological safety cabinet. First, we incubated each strain on Mueller Hinton agar (Forma Scientific IN2-MIC E-63 incubator; 37°C for 22-48 hours). Then, we isolated one bacterial colony on the agar plate and inoculated it in 10 ml Mueller Hinton broth (BBLTM Mueller Hinton Broth; 22 g broth powder per litre), and incubated each sample (Max Q 4000 Barnsteed Lab-Line incubator). All broths in this experiment were prepared with these methods, with incubation parameters depicted in Table 1.

Table 1: *Naming system for bacterial broths used in this experiment.*

Bacterial Strain	Incubation Parameters	Name
E. coli strain ATCC 11303	190 rpm; 37 °C; 16 hours	Broth A
S. carnosus strain 51365	155 rpm; 37 °C; 19 hours	Broth B

2.2. Preparation of the Copper

We obtained 32 copper pieces (3 cm × cm C110 copper (99.9% pure copper); 0.064 inch thickness, Metal Supermarket).

Scratching the Surfaces of Copper Coupons

We scratched 12 parallel lines onto half of the copper pieces to introduce the variable of surface texture. To ensure all scratches were of comparable depth, we built a lever

(Figure 1) and placed a lead block (1.4515 kg, length: 10.1 cm, width: 4.9 cm, height: 2.4 cm) 2.54 cm from one edge of the board, and taped a piece of copper to be scratched 2.54 cm from the opposite edge. Using the corner of a razor blade (Personna 94-120-71 stainless steel razor blades), we applied pressure to the left upper corner of the copper piece until the wooden board was level to the ground. Then, we slid the blade towards the bottom edge of the copper piece, keeping pressure constant and the board level. We repeated this procedure 12 times from left to right for each copper piece, using a new blade for each square. Approximately 2% of the surface of the copper coupon was scratched.

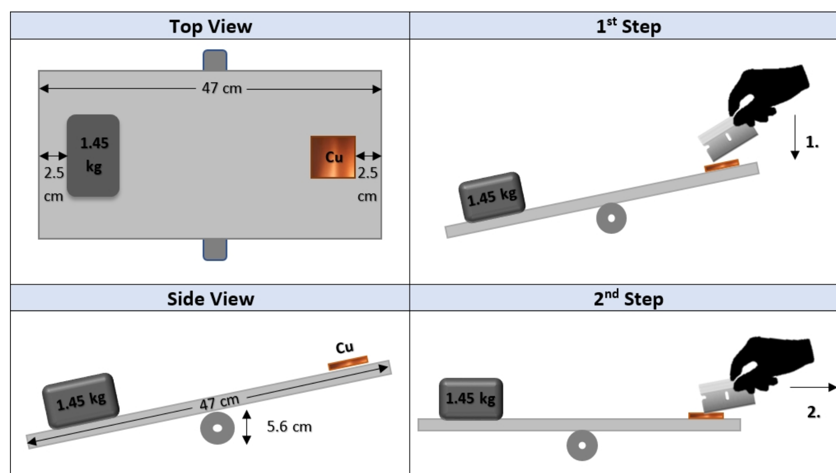

Figure 1: *Overview of copper metal scratching mechanism implemented in this experiment. Pictures not drawn to scale.*

Acetic Acid Wash and Sterilization

The scratches we introduced onto our copper pieces exposed copper that had not yet had contact with open air, resulting in variability in the oxidation levels of each copper piece. Previous studies have shown that oxide layers on copper surfaces affect copper's antibacterial activity [18, 21]. To account for this confounding variable, we performed an acid wash to ensure all copper being tested underwent the same level of oxidation.

We rinsed all copper pieces with distilled water, then 70% ethanol to remove dirt and debris. We immersed each copper square in glacial acetic acid for 15 seconds, as glacial acetic acid removes CuO layers without damaging the copper metal underneath [22]. Immersion time was based on results of a previous study [22]. After 15 seconds, we removed the copper pieces from the glacial acetic acid and let them air-dry. We placed 1 scratched and unscratched piece side by side in each glass petri dish to organize our copper such that the scratched and unscratched piece for each time interval were in the same environment. We autoclaved all the petri dishes with copper inside, then para-filmed each dish to prevent constant oxygen flow that could increase the rate of oxide layer formation [23].

2.3. Measuring the Rate of Bacterial Death on Scratched and Unscratched Copper

S. Carnosus

We removed the parafilm on each petri dish immediately before using them to keep oxygen exposure consistent for each copper piece. For each petri dish, we pipetted 10 µL of Broth B of *S. carnosus* onto the centre of the scratched and unscratched copper piece at the same time, using one plastic inoculating loop (VWR North American. Cat. No. 12000-810) per copper piece to spread the broth evenly over the copper surface. We used a cotton swab (Puritan Cotton Tipped Applicators 806-WC) and swabbed both copper pieces at either 1, 30, 60, or 90 minutes after bacteria was introduced then placed each swab into 1 mL Mueller Hinton broth. We performed 3 serial 10-fold dilutions of this broth, then plated 100 µL of each dilution on Mueller Hinton agar and incubated each agar plate (Forma Scientific IN2-MIC E-63 incubator; 37°C; 21 hours). (Figure 2) Data were collected by counting the number of visible colonies present on each plate. Colony counts greater than zero that followed a colony count of zero earlier in the dilution series were considered contamination and therefore excluded.

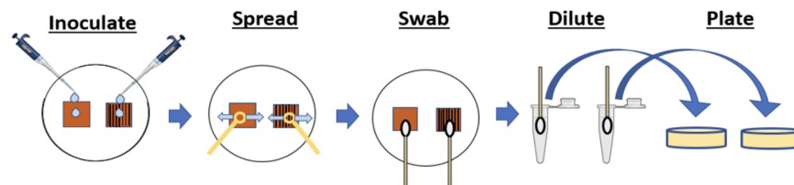

Figure 2: ***Overview of inoculation, spreading and plating of bacterial broth.*** *Pictures not drawn to scale.*

E. Coli

The same methods as used for *S. carnosus using Broth B were repeated* using Broth A of *E. coli* (Figure 2), following the time intervals and replicates depicted in Table 2. Preliminary results were more variable for *S. carnosus* than for *E. coli*, so the remainder of our time and resources were allocated to more replicates and an additional 10-minute time interval for *E. coli*. We performed a negative control (5 replicates) on stainless steel using these methods, swabbing only at 30 minutes to ensure the decrease in bacteria concentration was not a result of our copper preparation methods.

Table 2: *Number of replicates performed per time interval of E. Coli on C110 copper.*

Times swabbed (minutes after introduction of bacteria)	1	10	30	60	90
Number of replicates	5	5	5	2	2

2.4. Statistical Analysis

For each of our agar plates, we converted the number of visible colonies into colony forming units per millilitre (CFU/mL) to account for our dilutions. We then graphed the log of mean colony forming units per millilitre (with 95% confidence intervals) of both bacterial strains present on both scratched and unscratched copper pieces at all time intervals. We used unpaired t tests ($\alpha = 0.05$) with two-tailed p tests to look for differences in bacterial concentration between both bacterial strains on copper pieces of both surface textures. Statistical tests were performed on original, non-logarithmic data.

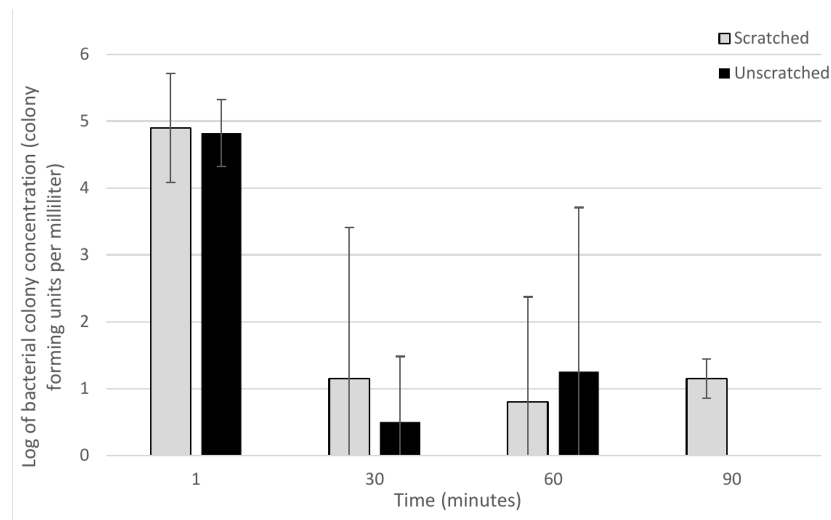

Figure 3: ***Mean S. carnosus strain 51365 colonies present on scratched and unscratched copper 1, 30, 60 and 90 minutes after introduction of bacteria.*** *Swab times are presented in minutes ± 15 seconds. Error bars represent 95% confidence intervals.*

3. Results

The antibacterial effectiveness of scratched and unscratched copper against the *S. carnosus* broth was determined by comparing the average logs of the bacterial colony forming units per millilitre on both types of copper. There was no statistically significant differences observed in bacterial concentrations between scratched and unscratched copper for any time interval tested (Figure 3; $t(2) = 0.42, p = 0.7181; t(2) = 0.95, p = 0.4408; t(2) = 0.87, p = 0.4755; t(2) = 2.8, p = 0.1074$). Non-logarithmic data for the 90-minute time interval showed no significant difference in bacterial concentrations on scratched and unscratched copper ($t(2) = 2.8, p = 0.1074$).

The effectiveness of scratched and unscratched copper against the *E. coli* broth was also determined by comparing the average logs of the bacterial colony forming units per millilitre on both types of copper. There was no statistically significant difference in the mean *E. coli* colony concentration on scratched versus unscratched copper at any time interval tested after introduction of bacteria onto the surfaces (Figure 4; $t(8) = 1.23, p = 0.2527; t(8) = 0.98, p = 0.3537; t(8) = 1.41, p = 0.1950$).

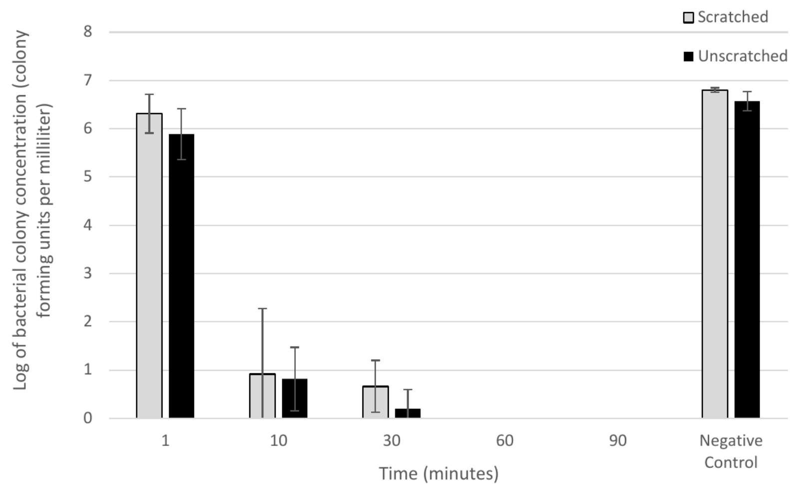

Figure 4: ***Mean E. coli strain ATCC 11303 colonies present on scratched and unscratched copper at times 1, 10, 30, 60 and 90 minutes after introduction of bacteria.*** *Negative control represents experiment done on stainless steel with swab taken 30 minutes after introduction of bacteria. Swab times are presented in minutes ± 15 seconds. Error bars represent 95% confidence intervals.*

There was a significant decrease of bacterial concentration on the copper surfaces over time between 1 minute and 10 minute intervals (Figure 4; $t(8) = 2.88, p = 0.0206$), however there were no statistically significant changes in bacterial concentration on the copper surfaces between 10 and 30 minutes (Figure 4; $t(8) = 0.99, p = 0.3500$). The bacterial concentration on copper surfaces decreased to 0 by 60 minutes. The stainless steel negative control had no significant difference between bacterial concentration present on scratched and unscratched surfaces after 30 minutes of contact (Figure 4; $t(5) = 1.08, p = 0.3293$), however there were significant differences in bacterial concentrations between stainless steel and copper at 30 minutes, for both scratched and unscratched surfaces (Figure 4; $t(5) = 33.56, p < 0.0001; t(8) = 3.71, p = 0.0060$). There were no significant differences in bacterial concentrations on stainless steel at 30 minutes and copper at 1 minute, for scratched or unscratched copper (Figure 4; $t(5) = 1.97, p = 0.1064; t(8) = 2.12, p = 0.0673$).

4. Discussion

Many mechanisms have been proposed to explain copper's antibacterial properties; some theorize that direct contact is required between bacteria and copper for effective bactericidal action, [17, 24] whereas others suggest that copper ions in solution may play a more important role [24, 25]. Our results support the latter, as there was a significant decrease in *E. coli* concentration after only 10 minutes of exposure to copper C110, yet liquid broth was still present on the copper surfaces at this time. Because liquid was still present on the copper surfaces when the majority of bacteria had been killed, it is possible the liquid facilitated the movement of Cu^{2+}, allowing bacteria to be killed without direct contact between the *E. coli* and the solid copper surface.

In previous studies working with *E. coli* strain O157 on copper alloys, it was found that a similar procedure resulted in little to no bacteria present on copper's surface after 75 minutes of exposure [17], a longer kill-time than recorded in this experiment. This time difference could be due to the volume of broth used and copper alloy composition. Seventy five minute kill-times were obtained using 20 µL of broth per 1×1cm 95% copper square, [17] whereas our data were collected using 10 µL of broth per 3×3cm 99% copper square, a much lower volume of liquid per unit surface area of copper. Further research is needed to investigate the surface area of copper needed per unit of volume of bacteria in liquid medium. Moreover, it is possible that *E. coli* strain ATCC 11303 is more susceptible to copper's antibacterial action than *E. coli* strain O157. *E. coli* O157 is a pathogenic strain [26], whereas strain ATCC 11303 used in this study is not. Various pathogenic microorganisms have been shown to secrete siderophores [27], which are small chelators used to bind iron molecules necessary to for cellular functions [28, 29]. Uropathogenic *E. coli* has been found to produce a siderophore able to bind copper, and protect cells against copper toxicity [30]. Additional research is needed to determine if this protective mechanism is a contributor to the longer exposure time needed to kill *E. coli* O157, and the extent to which this mechanism can protect pathogenic *E. coli* against coppers antibacterial activity.

Due to limited resources, we were only able to perform additional replicates for *E. coli*, which could be the reason for the larger error bars observed in the *S. carnosus* data. During future research into this topic, increasing the number of replicates may also increase the accuracy of the 95% confidence interval bars for the *S. carnosus* data. This may reveal new trends in the *S. carnosus* data not observed in this study.

For both *E. coli* and *S. carnosus*, there were no statistically significant differences in the concentration of bacteria on scratched versus unscratched copper at any time interval tested (Figure 3, Figure 4). This suggests that hospital surfaces replaced with copper metal may not require extensive maintenance of their smooth surfaces, provided that the oxidative effects of acquired scratches do not have a significantly negative effect. Further research investigating the relationship between surface scratches and the oxidation of copper surfaces, and the effects they may have on copper's antibacterial activity could help assess the benefits of implementing copper surfaces into hospitals in a real-world, uncontrolled environment.

5. Conclusion

Overall, our results suggest that copper surfaces implemented in health care facilities are an effective antibacterial measure whether visible surface scratches are present or not, as we observed no difference in copper's antibacterial activity when scratched at this magnitude. Additionally, as we saw significantly less bacteria present on copper when compared to stainless steel at the same time interval (Figure 3). Our results support the claims that the replacement of frequently touched hospital surfaces with copper metal could be beneficial in decreasing the transmission of hospital acquired infections.

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References

[1] Denise Gravel, Michael Gardam, Geoffrey Taylor, Mark Miller, Andrew Simor, Allison McGeer, James Hutchinson, Dorothy Moore, Sharon Kelly, and Michael Mulvey. Infection control practices related to clostridium difficile infection in acute care hospitals in canada. *American Journal of Infection Control*, 37 (1):9–14, Feb 2009. ISSN 0196-6553. doi:10.1016/j.ajic.2008.07.012.

[2] Parienti J, Thibon P, Heller R, and et al. Hand-rubbing with an aqueous alcoholic solution vs traditional surgical hand-scrubbing and 30-day surgical site infection rates: A randomized equivalence study. *JAMA*, 288(6):722–727, 2002. doi:10.1001/jama.288.6.722.

[3] Monica Harrington. Neutralizing hospital-acquired infections. *Lab Animal*, 40:288 EP –, Sep 2011. doi:10.1038/laban1011-288b.

[4] S. J. Dancer. How do we assess hospital cleaning?: A proposal for microbiological standards for surface hygiene in hospitals. *Journal of Hospital Infection*, 56(1):10–15, Jan 2004. ISSN 0195-6701. doi:10.1016/j.jhin.2003.09.017.

[5] Cherie Fox, Teresa Wavra, Diane Ash Drake, Debbie Mulligan, Yvonne Pacheco Bennett, Carla Nelson, Peggy Kirkwood, Louise Jones, and Mary Kay Bader. Use of a patient hand hygiene protocol to reduce hospital-acquired infections and improve nursesâĂŹ hand washing. *American Journal of Critical Care*, 24(3):216–224, 2015. doi:10.4037/ajcc2015898.

[6] Brian S Koll, Rafael E Ruiz, David P Calfee, Hillary S Jalon, Rachel L Stricof, Audrey Adams, Barbara A Smith, Gina Shin, Kathleen Gase, Maria K Woods, et al. Prevention of hospital-onset clostridium difficile infection in the new york metropolitan region using a collaborative intervention model. *Journal for Healthcare Quality*, 36(3):35–45, 2014.

[7] Maria Luisa Moro, Nicola Petrosillo, Claudia Gandin, and Antonino Bella. Infection control programs in italian hospitals. *Infection Control & Hospital Epidemiology*, 25 (1):36–40, 2004. doi:10.1086/502289.

[8] Gorana Ćosić, Jelena Đekić, M Petrović, Gordana Krtinić, Tatjana Karać, Jasmina Jandrić-Kočić, and Ljiljana Marković-Denić. The most frequent hospital-acquired infections related to medical interventions in hospitals in vojvodina province. *Archives of Biological Sciences*, 66(2):523–535, 2014. doi:10.2298/ABS1402523C.

[9] Shelley S. Magill, Jonathan R. Edwards, Wendy Bamberg, Zintars G. Beldavs, Ghinwa Dumyati, Marion A. Kainer, Ruth Lynfield, Meghan Maloney, Laura McAllister-Hollod, Joelle Nadle, Susan M. Ray, Deborah L. Thompson, Lucy E. Wilson, and Scott K. Fridkin. Multistate point-prevalence survey of health careâĂŞassociated infections. *New England Journal of Medicine*, 370(13):1198–1208, 2014. doi:10.1056/NEJMoa1306801. PMID: 24670166.

[10] Gregor Grass, Christopher Rensing, and Marc Solioz. Metallic copper as an antimicrobial surface. *Applied and Environmental Microbiology*, 77(5):1541–1547, 2011. ISSN 0099-2240. doi:10.1128/AEM.02766-10.

[11] Lawrence B. Hall and Margaret J. Hartnett. Measurement of the bacterial contamination on surfaces in hospitals. 79(11):1021–1024, 1964. ISSN 00946214. doi:10.2307/4592309.

[12] Phyllis J Kuhn. Doorknobs: a source of nosocomial infection. *Diagnostic Medicine*, 6(8):62–63, 1983.

[13] Donald G. Barceloux and Dr. Donald Barceloux. Copper. *Journal of Toxicology: Clinical Toxicology*, 37(2):217–230, 1999. doi:10.1081/CLT-100102421. PMID: 10382557.

[14] J. O. Noyce, H. Michels, and C. W. Keevil. Potential use of copper surfaces to reduce survival of epidemic meticillin-resistant *Staphylococcus aureus* in the healthcare environment. *Journal of Hospital Infection*, 63(3):289–297, Jul 2006. ISSN 0195-6701. doi:10.1016/j.jhin.2005.12.008.

[15] Nanako Niiyama, Takeshi Sasahara, Hiroshi Mase, Michiko Abe, Haruo Saito, and Kensei Katsuoka. Use of copper alloy for preventing transmission of methicillin-resistant staphylococcus aureus contamination in the dermatology ward. *Acta dermato-venereologica*, 93(3):294–300, 2013. doi:10.2340/00015555-1472.

[16] Christophe Espírito Santo, Paula Vasconcelos Morais, and Gregor Grass. Isolation and characterization of bacteria resistant to metallic copper surfaces. *Applied and Environmental Microbiology*, 76(5):1341–1348, 2010. ISSN 0099-2240. doi:10.1128/AEM.01952-09.

[17] J. O. Noyce, H. Michels, and C. W. Keevil. Use of copper cast alloys to control escherichia coli o157 cross-contamination during food processing. *Applied and Environmental Microbiology*, 72(6):4239–4244, 2006. ISSN 0099-2240. doi:10.1128/AEM.02532-05.

[18] Iman A. Hassan, Ivan P. Parkin, Sean P. Nair, and Claire J. Carmalt. Antimicrobial activity of copper and copper(i) oxide thin films deposited via aerosol-assisted cvd. *J. Mater. Chem. B*, 2:2855–2860, 2014. doi:10.1039/C4TB00196F.

[19] Michael G. Schmidt, Hubert H. Attaway, Peter A. Sharpe, Joseph John, Kent A. Sepkowitz, Andrew Morgan, Sarah E. Fairey, Susan Singh, Lisa L. Steed, J. Robert Cantey, Katherine D. Freeman, Harold T. Michels, and Cassandra D. Salgado. Sustained reduction of microbial burden on common hospital surfaces through

introduction of copper. *Journal of Clinical Microbiology*, 50(7):2217–2223, 2012. ISSN 0095-1137. doi:10.1128/JCM.01032-12.

[20] André Mikolay, Susanne Huggett, Ladji Tikana, Gregor Grass, Jörg Braun, and Dietrich H. Nies. Survival of bacteria on metallic copper surfaces in a hospital trial. *Applied Microbiology and Biotechnology*, 87(5):1875–1879, Aug 2010. ISSN 1432-0614. doi:10.1007/s00253-010-2640-1.

[21] Michael Hans, Andreas Erbe, Salima Mathews, Ying Chen, Marc Solioz, and Frank MÃijcklich. Role of copper oxides in contact killing of bacteria. *Langmuir*, 29(52): 16160–16166, 2013. doi:10.1021/la404091z.

[22] KL Chavez and DW Hess. A novel method of etching copper oxide using acetic acid. *Journal of The Electrochemical Society*, 148(11):G640–G643, 2001. doi:10.1149/1.1409400.

[23] Jian Li, J. W. Mayer, and E. G. Colgan. Oxidation and protection in copper and copper alloy thin films. *Journal of Applied Physics*, 70(5):2820–2827, 1991. doi:10.1063/1.349344.

[24] Salima Mathews, Michael Hans, Frank Mücklich, and Marc Solioz. Contact killing of bacteria on copper is suppressed if bacterial-metal contact is prevented and is induced on iron by copper ions. *Applied and Environmental Microbiology*, 79(8): 2605–2611, 2013. ISSN 0099-2240. doi:10.1128/AEM.03608-12.

[25] Marco Zeiger, Marc Solioz, Hervais EdonguÃľ, Eduard Arzt, and Andreas S. Schneider. Surface structure influences contact killing of bacteria by copper. *MicrobiologyOpen*, 3(3):327–332. doi:10.1002/mbo3.170.

[26] Nicole T. Perna, Guy Plunkett III, Valerie Burland, Bob Mau, Jeremy D. Glasner, Debra J. Rose, George F. Mayhew, Peter S. Evans, Jason Gregor, Heather A. Kirkpatrick, György Pósfai, Jeremiah Hackett, Sara Klink, Adam Boutin, Ying Shao, Leslie Miller, Erik J. Grotbeck, N. Wayne Davis, Alex Lim, Eileen T. Dimalanta, Konstantinos D. Potamousis, Jennifer Apodaca, Thomas S. Anantharaman, Jieyi Lin, Galex Yen, David C. Schwartz, Rodney A. Welch, and Frederick R. Blattner. Genome sequence of enterohaemorrhagic escherichia coli o157:h7. *Nature*, 409:529 EP –, Jan 2001. doi:10.1038/35054089.

[27] Marcus Miethke and Mohamed A. Marahiel. Siderophore-based iron acquisition and pathogen control. *Microbiology and Molecular Biology Reviews*, 71(3):413–451, 2007. ISSN 1092-2172. doi:10.1128/MMBR.00012-07.

[28] JB Neilands. Siderophores: structure and function of microbial iron transport compounds. *Journal of Biological Chemistry*, 270(45):26723–26726, 1995. doi:10.1074/jbc.270.45.26723.

[29] Claudia Andreini, Ivano Bertini, Gabriele Cavallaro, Gemma L. Holliday, and Janet M. Thornton. Metal ions in biological catalysis: from enzyme databases to general principles. *JBIC Journal of Biological Inorganic Chemistry*, 13(8):1205–1218, Nov 2008. ISSN 1432-1327. doi:10.1007/s00775-008-0404-5.

[30] Eun-Ik Koh and Jeffrey P. Henderson. Microbial copper-binding siderophores at the host-pathogen interface. *Journal of Biological Chemistry*, 290(31):18967–18974, 2015. doi:10.1074/jbc.R115.644328.

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The Interactive Effects of Colour and Odour on *Drosophila melanogaster* Trap Design for Pest Control Applications

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Abstract

Drosophila species cause widespread agricultural damage, and designing effective traps for pest control requires an understanding of multimodal cues. Prior studies have looked at the effects of colour and odour on trap efficacy separately, but few have looked at the combined effects of these two types of stimuli. We tested the effect of multimodal cues on *Drosophila melanogaster* trap efficacy by setting up colour versus white two-choice tests for both banana-baited traps and odourless traps. While colour had no significant impact on banana-baited trap efficacy, red odourless traps were significantly more effective than white odourless traps at capturing *D. melanogaster* females. This suggests that colour only affects choice of female *D. melanogaster* in the absence of an attractive odorant. Additionally, both odorous and odourless traps were more effective at capturing females than males, which indicates that these traps do not necessarily target males and females equally, and that pest control strategies need to be designed accordingly.

Keywords — Drosophila Melanogaster, Trap Design, Two-Choice Test, Multimodal Cues, Sex Differences

1. Introduction

Drosophila species, commonly known as vinegar flies, are common domestic and agricultural pests that infest fermenting fruit [1]. *Drosophila melanogaster* is a particular problem in China, where cherry cultivars, which represent a growing agricultural sector, suffer 35%-80% crop loss due to the species [2]. Furthermore, *D. melanogaster* acts as a vector for *E. coli*, transmitting it from infected to uninfected fruits [3]. Additionally, the invasive species *Drosophila suzukii* has presented a new problem, as it has the unique ability to oviposit in fresh soft-skinned fruit [4, 5], causing crop losses of up to 80% in Europe. [6] Given the high economic damage that *Drosophila* species cause, it is valuable to study effective trapping mechanisms that offer non-insecticidal approaches for controlling these pests [7].

Previous studies have looked at colour and odour separately when exploring trap design. Two-choice tests with a white control found red, purple, and checkered disks to be most effective at trapping *D. suzukii* [8] Traps baited with overripe mango [9] and

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banana [1] have also shown to be effective attractants for *Drosophila* species. However, few studies have tested odour and colour in combination. One study of *Drosophila repleta* showed that red traps were the most effective in colour-only tests, while there was no differential response to different colours when colour was combined with an attractive red wine lure [7]. Many insects recognize and select host fruits for feeding and ovipositing based on the integration of olfactory and visual cues [10], so it is important to examine how these multimodal cues interact and how they can be combined to optimize trap designs.

Our study examined the effect of trap colour on the effectiveness of odourous and odourless *D. melanogaster* traps. We used banana-baited traps as our odourous traps, based on previous studies that indicate it is an effective attractant [1, 11]. We set up colour versus white two-choice tests, and hypothesized that traps with colours that resemble oviposition sites, such as red, yellow, and green, would be more effective than white traps [12]. Conversely, we hypothesized that blue, an ecologically unimportant colour for many insects [13], would decrease trap efficacy compared to white traps. We also hypothesized that there would be a larger difference in effectiveness between coloured and white traps in the absence of an odour [7].

2. Materials and Methods

2.1. Test Insects

We used lab-reared adult *D. melanogaster* fruit flies of wildtype strain Oregon-R, from a colony maintained at Simon Fraser University. The colony was raised on a solid food diet of agar, yeast, and cornmeal. The food was checked daily and replaced when low, and the flies had the opportunity to feed until they were put in the cages (i.e., there was no period of starvation prior to the test). Males and females were inserted into two-choice cages simultaneously.

2.2. Coloured Cardstock

We made coloured cones and disks out of non-fluorescent cardstock (Michael's Craft Store, Recollections ®; grey : # 267824; white: # 267815; green: # 470525; red: # 26776; yellow: # 267780; blue: # 470530). For Experiment 1 and the control experiment, we made each cone out of an 8cm $\times$ 8cm square of cardstock, rolled and glued using a glue gun (Mastercraft General Purpose Glue Sticks). Each cone had a $\sim$ 3cm top and a $\sim$ 0.4cm hole cut out of the bottom through which the flies could pass. For Experiment 2, we made 2.7cm diameter flat circular disks.

2.3. General Two-Choice Cage Setup

We used Oak Stump Farms©Yellow Jacket Traps (12.5 cm high, 11.0 cm diameter; white with a black lid) as cages, plugging the holes in the sides of the cages with cotton balls. We taped two traps to the bottom of each cage (Figure 1). We added one vial of fruit flies into each cage by lightly anesthetizing the fruit flies with CO_2 for $\sim$30 seconds, then transferring them to the bottom center of the cage, ensuring that no flies fell into

either trap. The number of flies per cage varied (means are provided in 3). We screwed the lids on the cages tightly and stored them in a room with both artificial light from above and natural light from a window; both traps in each cage were equidistant from the window (Figure 1). For all tests, cages received ∼8 hours of natural lighting. The duration of time in which artificial light was available varied between the tests, but for all tests the cages received at least 4 hours of artificial light. After ∼24 hours, we transferred the cages to a freezer for at least 24 hours in order to kill all fruit flies. We counted the number of flies in each trap and number of flies in neither trap for each cage, sorting flies by sex according to the Carolina Drosophila manual [14].

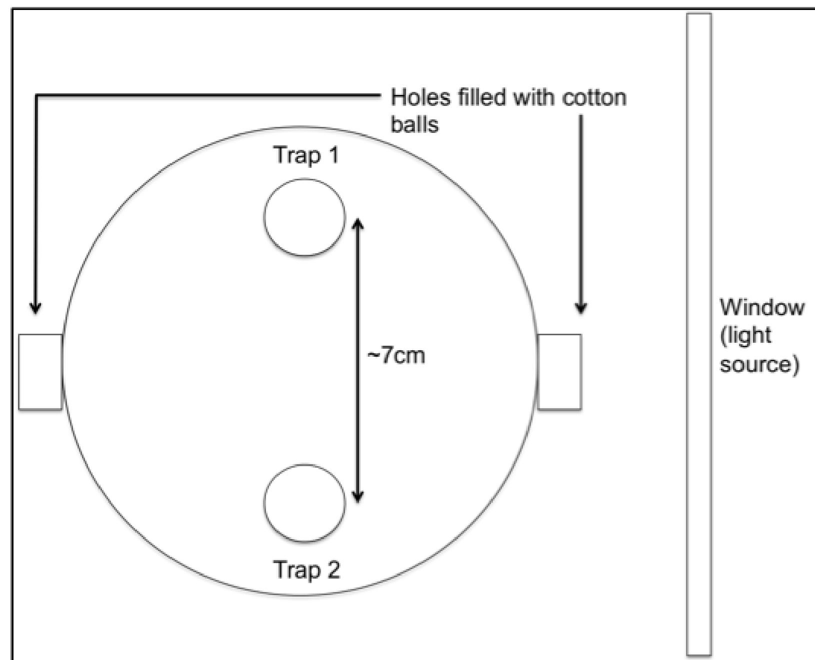

Figure 1: *Cage Setup for two-choise tests. In Experiment 1 and the control experiment, each trap consisted of a vial filled with mashed banana, and a cardstock cone inserted into the vial. In Experiment 2, each trap consisted of a petri dish with a cardstock circle taped to its bottom outer side, and filled with soapy solution (Table 1).*

All two-choice tests compared a coloured trap to a white trap, and were conducted as listed in Table 1.

2.4. Control Experiment: Grey vs. White Two-Choice Test

We conducted a control experiment, adapted from Arnold et al. [13], to ensure that white cardstock cones were appropriate to use as controls. To ensure that the response by *D. melanogaster* to the coloured cones was a response to the chromaticity of the cardstock rather than the fact that the coloured cardstock was darker in comparison to white cardstock, we set up a grey vs. white two-choice experiment. Any differences in response observed would be due to difference in the darkness of the cardstock, since there is no distinguishable difference in chromaticity between grey and white cardstock [13].

Table 1: *Summary of Two-Choice Experiments*

Experiment	Two-Choice Test Colour	Two-Choice Test Control	Trap Type (Odour/NoOdour)	Replicates
Control	Grey	White	Odour (Mashed Banana)	10
Experiment 1	Green Red Yellow Blue	White	Odour (Mashed Banana)	10 10 10 10
Experiment 2	Red Blue	White	No Odour (Soapy Water Mixture)	10 10

2.5. Experiment 1: Two-Choice Tests with Green, Red, Yellow, and Blue-Coned Traps

We used 20 mL glass vials, with coloured cardstock cones inserted into them, as traps, in a similar design to Hottel et al. [7]. We added $\sim$ 7g mashed ripe bananas (Chiquita®organic bananas, mashed to a paste with a mortar and pestle) to each glass vial, which filled about one third of the vial, and inserted the tip of a cone into the opening of each vial (Figure 2).

2.6. Experiment 2: Two-Choice Experiment with Traps of Various Colours Without an Attractive Odour

We set up an experiment analogous to Experiment 1 but removed the attractive banana odour. We used STARSTEDT petri dishes (35 mm diameter $\times$ 10 mm height) as traps. We taped a 27 mm circle to the bottom outer side of each petri dish (Figure 3). We then created a soapy water solution (170 mL distilled water and 0.85 g Sparkleen Fisherbrand detergent) and added 3.5 mL of the solution to each trap. The purpose of this soapy water mixture was to break surface tension and drown trapped flies [15].

We chose to change the trap design for Experiment 2 to better assess attraction to colour. The cone traps were designed such that the flies would be lured down the cone towards the attractive odour. These traps were appropriate in two-choice tests where both traps had the same attractive odour. In the absence of such attractive odour, the flies may have been attracted to the colour of the cones and may have approached them, but likely would not have gone down the cones and gotten trapped. We used petri dishes with soapy water because flies that were attracted to the colour and approached it would drown in the solution.

Due to time constraints, for Experiment 2, we were only able to set up two-choice tests for two colours of interest (red vs. white and blue vs. white).

2.7. Statistical Analyses

For all of our two-choice tests, we converted the counts to percentages for each cage (number of flies caught by coloured trap/number of flies caught in total). We plotted

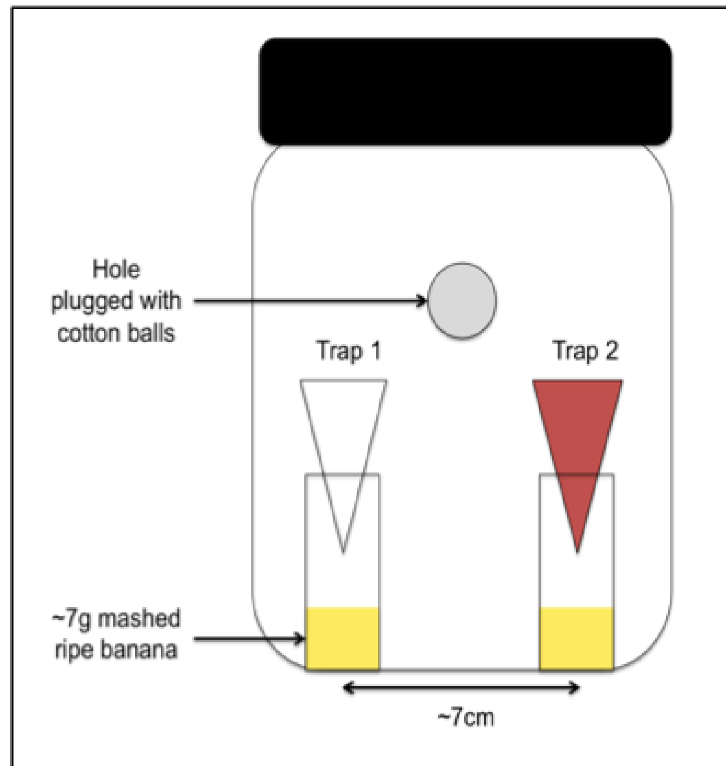

Figure 2: *Set up for Control Experiment and Experiment 1. Trap 1 had a white cone. For the Control Experiment, Trap 2 had a grey cone. For Experiment 1, Trap 2 had either a green, red, yellow, or blue cone (Table 1).*

mean percentages with 95% confidence intervals and used a one-sample t test ($\alpha = 0.05$) with a null hypothetical mean of 50% to test for significance. This test determined whether the percentage of flies captured by the coloured differed significantly from 50% , assuming that if the coloured and white traps were equally effective, then 50% of captured flies would be caught by each. We excluded non-responders from the above-mentioned analysis and analyzed them separately: we converted counts to percentages (# of non-responders/total # of flies in cage) for each cage and then calculated the mean percentages with 95% confidence intervals.

We used an unpaired two-sample t-test ($\alpha = 0.05$) to look for differences between male and female non-responders. For all statistical tests, values of $p < 0.1$ were considered marginally significant.

Both paired and unpaired t-tests were conducted using the GraphPad t-test calculator [16].

We chose to use a t-test rather than a chi-squared test because a t-test allowed each replicate cage to be treated as an individual experimental unit ($n = 10$ as there were 10 replicates for each two-choice assay). A chi-squared test would require combining the data of all 10 replicates into a single percentage for each assay, which would result in lost information.

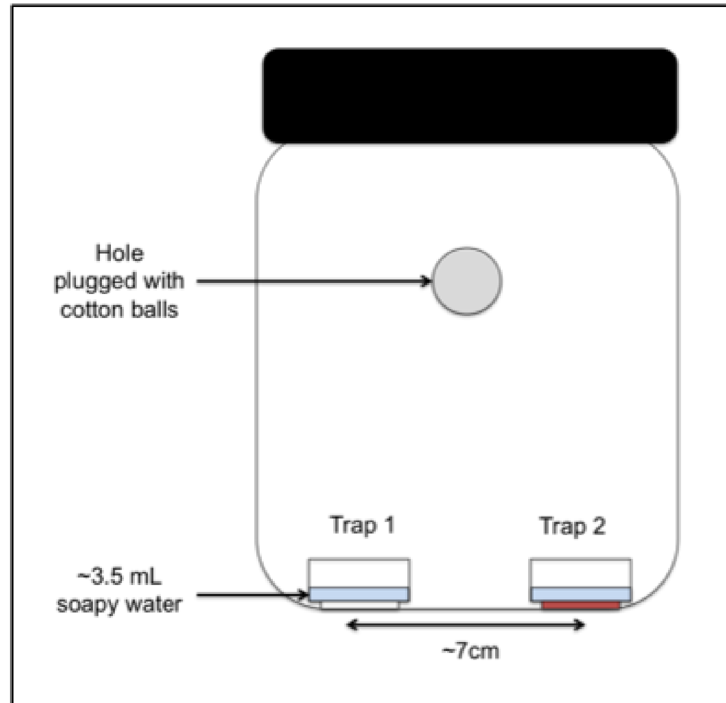

Figure 3: *Set up for Experiment 2. Trap 1 had a white circle stuck to the bottom outer side of the trap, while Trap 2 had either a red or blue circle.*

3. Results

3.1. Control Experiment: Grey vs. White Two-Choice Test

Percentage of captured flies caught by grey traps was not significantly different from 50% for males ($t(9) = 0.3556, p = 0.7303$) or females ($t(9) = 0.1017, p = 0.9212$) (Figure 1). There was a mean of 29.9 males and 25.7 females in each cage.

3.2. Experiment 1: Two-Choice Tests with Green, Red, Yellow, and Blue-Coned Traps

In the red vs. white two-choice test, the percentage of captured female flies caught by the red trap differed marginally significantly from 50% ($M = 60.43\% \pm 10.26\%; t(9) = 1.9923, p = 0.0775$). For all other two-choice tests, the percentage of captured flies caught by the coloured trap was not significantly different from 50% . There was a mean of 30.9 males and 33.6 females in each cage.

3.3. Experiment 2: Two-Choice Experiment with Traps of Various Colours Without an Attractive Odour

In the red vs. white two-choice test, the percentage of captured female flies caught by the red trap differed significantly from 50% ($M = 70.23\% \pm 7.72\%; t(9) = 5.136, p = 0.0006$). In the blue vs. white two-choice test, the percentage of captured male flies caught by

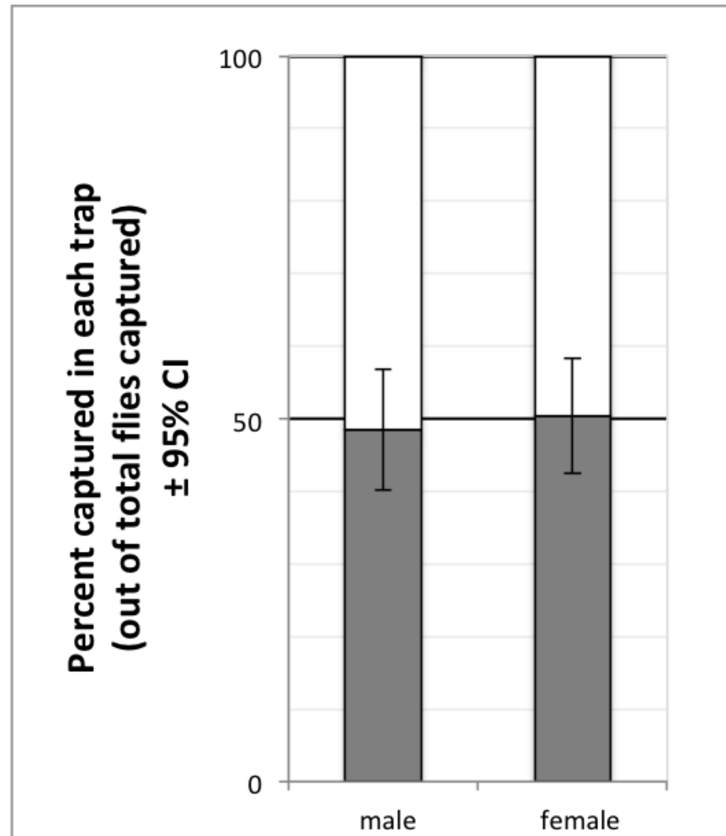

Figure 4: *Control Test Examining the Effect of Cone Darkness on Banana-Baited Trap Capture. 100% stacked bar chart with the grey segment representing mean percentage of captured flies caught by grey traps, and the white segment representing mean percentage of captured flies caught by white traps. Non-responders are excluded from this data.*

the blue trap differed marginally significantly from 50% ($M = 70.64\% \pm 20.64\%; t(9) = 1.9605, p = 0.0816$). For all other two-choice tests, the percentage of captured flies caught by the coloured trap was not significantly different from 50%. There was a mean of 6.0 males and 13.3 females in each cage, which was fairly low compared to the previous two experiments.

3.4. Non-Responder Data

In all two-choice tests across all 3 experiments, there was a significantly larger percentage of male non-responders than female non-responders (Table 2; Figure 7). In Experiment 1 (Figure 7b), there was also a significantly larger percentage of male non-responders in the red vs. white two-choice test compared to the blue vs. white two-choice test ($t(9) = 2.5806, p = 0.0189$). There were very low percentages of female non-responders in Experiment 2.

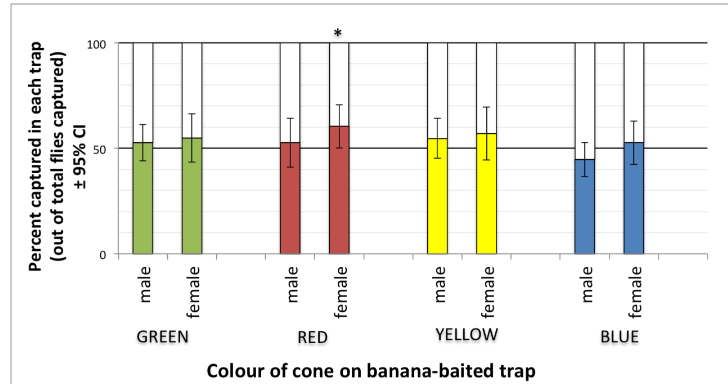

Figure 5: *Effect of Colour on Banana-Baited Trap Capture. 100% stacked bar chart with the coloured segment representing mean percentage of captured flies caught by coloured traps, and the white segment representing mean percentage of captured flies caught by white traps. Marginal significance (∗) is shown ($p < 0.1$). Non-responders are excluded from this data.*

Table 2: *Non-responders: Male vs. female unpaired two-sample t-test.*

	Two-Choice Test (Colour vs. White)	p-Value	t-Value	df
(a) Control Experiment	Grey	0.0075	3.0133	18
(b) Experiment 1	Green	0.0088	2.9368	18
	Red	0.0053	3.169	18
	Yellow	0.0119	2.7963	18
	Blue	0.0117	2.8036	18
(c) Experiment 2	Red	0.001	3.919	18
	Blue	0.0025	3.5137	18

4. Discussion

The results from the control study suggest that white and grey banana-baited traps are equally effective (Figure 4) and that *D. melanogaster* are not differentially attracted to darker or lighter colours, such that any significant results observed in Experiment 1 should be due to chromaticity rather than colour shade. However, we observed no significant differences in capture between coloured and white traps for any colour in Experiment 1 (Figure 5), though these banana-baited traps captured a large percentage of total flies (Figure 7b), confirming that banana is an attractive odour and an effective lure.

Results from the colour-only Experiment 2 tests (Figure 6) suggest that there is a preferential attraction to red by *D. melanogaster* females. A field study on *D. repleta* also showed a preferential attraction to red, though a sex difference was not examined [7]. Interestingly, this preference for red is not evident in the presence of an attractive odour. In red vs. white two-choice tests, the percentage of captured flies caught by red traps was both larger (roughly 70% vs. 60%) and more significant in the absence of an attractive banana odour (Figure 6) than in the presence of the odour (Figure 5). Similar

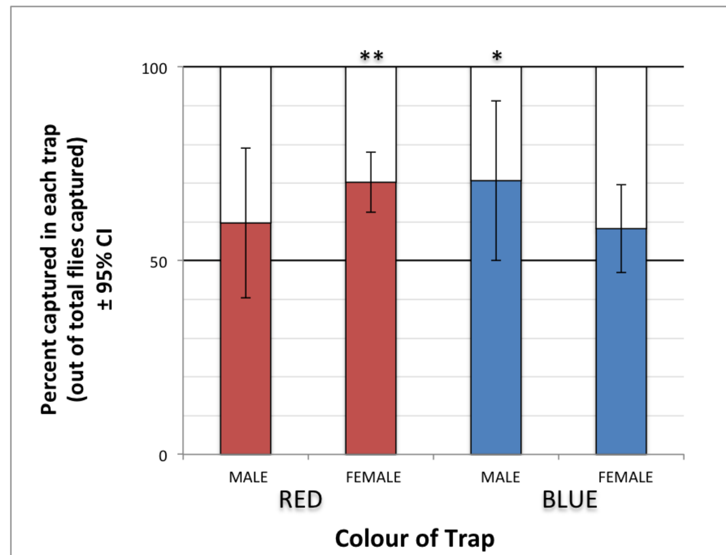

Figure 6: *Effect of Colour on Odourless Trap Capture. 100% stacked bar chart with the coloured segment representing mean percentage of captured flies caught by coloured traps, and the white segment representing mean percentage of captured flies caught by white traps. Significance ($**, p < 0.05$) and marginal significance ($*, p < 0.1$) are shown. Non-responders are excluded.*

effects were seen with *D. repleta*; a colour preference for red was seen when testing odourless coloured traps, but no colour preference was observed when colour was combined with an attractive red wine lure [7]. These results, therefore, suggest that in the presence of an attractive odour, colour is less important in *Drosophila* choice-making. *D. melanogaster* are highly sensitive to olfactory cues, able to navigate by detecting differences in odour concentration [17], and can use plant-specific olfactory cues to identify suitable hosts from a distance [10]; thus odour is evidently a primary stimulus in *D. melanogaster* navigation.

Although these results suggest that olfactory cues are more important than chromatic cues in this navigation process, it is possible that, comparing Experiment 1 and 2, the difference in type of trap (petri dish vs. cone) contributed to the differences in trap capture observed.

Both odourous and odourless traps seem to be more effective at trapping females than males (Figure 7). A similar female bias in baited trap catch was observed in blowflies, which was explained by the postulation that lures represent both feeding and egg-laying sites for females [18]. Likewise, our results support the hypothesis that colours may be preferentially attractive to *Drosophila* females because they resemble oviposition host sites [12]. A study on *D. suzukii* showed that males and females respond similarly to various colours of odourless traps, and thus traps can be designed to target them simultaneously [8]. This does not seem to be the case for *D. melanogaster*, and this differential response to colour must be considered when designing traps.

Notably, the design of Experiment 1 may have affected non-respondent results, as the cones were not tightly fixed to vials, so flies may have been able to escape

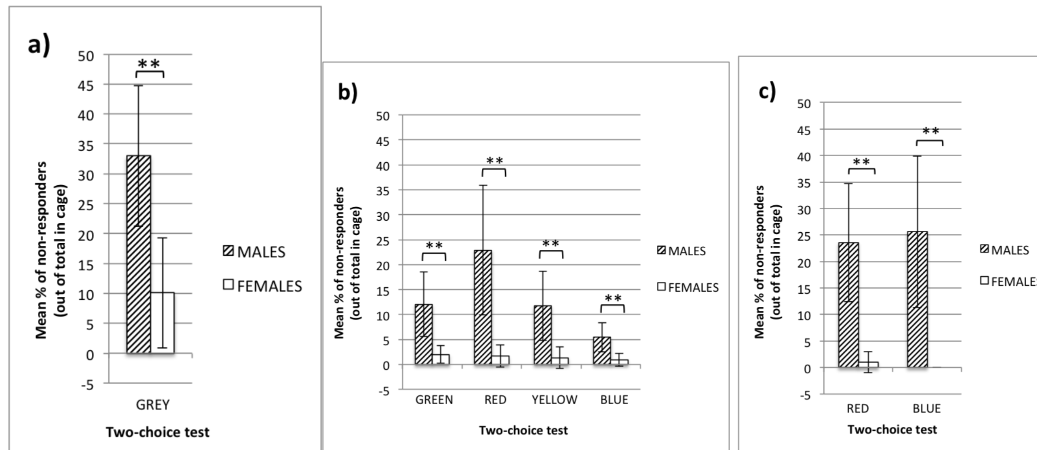

Figure 7: *Non-responders (flies caught by neither trap) compared by sex for each a) the control experiment; b) Experiment 1; c) Experiment 2. Significant differences between males and females are shown (∗∗).*

from the sides. As males are smaller and may escape more easily than females, this design flaw could potentially account for the significantly high proportion of male non-responders in Experiment 1 (Figure 7b). However, since a similar sex difference in non-responders was seen in Experiment 2 (Figure 7c), in which the trap design does not favour the escape of one sex over the other, we can infer that the sex difference seen in non-responders is truly reflective of the differential attractiveness of the traps to males and females.

Yeast-inoculated banana-baits are usually used for *Drosophila* traps [1], and previous studies comparing yeast-inoculated and non-inoculated banana baits showed that non-inoculated baits attracted very few flies [19]. However, our non-inoculated banana-baited traps had fairly low non-response rates for females, generally $< 10\%$ (Figure 7a, 7b). When yeast metabolizes fruit, it produces the phenolic compounds that are believed to be the primary attractants for *D. melanogaster* [20]. However, no yeast was added to our traps, representing a novel result.

The possibility that the traps were naturally exposed to yeast spores could explain this result. Assuming that any yeast spores in the lab are similar to those that would occur in natural settings, this result would show that active yeast-inoculation of banana baits is not necessary to produce effective lures. However, experimental evidence of this assumption is lacking, limiting this conclusion.

One limitation of this study is that we did not focus on insects of a particular age. We used the colonies as they were renewed. It is possible that the age of the flies may affect how attractive they find potential nutrition sources and how actively they are looking for oviposition sites. Additionally, in Experiment 2, a lower number of *D. melanogaster* was present in the cages (mean of 6.0 males and 13.3 females in each cage). It is possible this change may have affected the differences seen between Experiments 1 and 2.

These results offer insight into multimodal trap design, and should be further applied to field studies to test for variation in results [7]. Red colour-only water-based

traps that were effective at trapping females could offer a simple, non-insecticidal option for pest control of *D. melanogaster*, and a possible solution to the agricultural damage that they cause. Females are the main threat to agricultural crops because they look for a suitable location with food resources to lay their eggs. By capturing and eliminating female *Drosophila*, the population would be controlled and less damage to crops would be caused.

5. Acknowledgments

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References

[1] Anna L. Birmingham, Ervin Kovacs, Jean Pierre Lafontaine, Norman Avelino, John H. Borden, Isak S. Andreller, and Gerhard Gries. A new trap and lure for drosophila melanogaster (diptera: Drosophilidae). *Journal of Economic Entomology*, 104(3):1018–1023, 2011. doi:10.1603/EC10432.

[2] Qing-Cai Lin, Yi-Fan Zhai, Cheng-Gang Zhou, Li-Li Li, Qian-Ying Zhuang, Xiao-Yan Zhang, Frank G. Zalom, and Yi Yu. Behavioral rhythms of drosophila suzukii and drosophila melanogaster. *Florida Entomologist*, 97(4):1424–1433, Dec 2014. ISSN 0015-4040. doi:10.1653/024.097.0417.

[3] W. J. Janisiewicz, W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. Fate of escherichia coli o157:h7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Appl Environ Microbiol*, 65(1):1–5, Jan 1999. ISSN 0099-2240. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC90974/`. 0671[PII].

[4] Santosh Revadi, Silvia Vitagliano, Marco V. Rossi Stacconi, Sukanya Ramasamy, Suzan Mansourian, Silvia Carlin, Urska Vrhovsek, Paul G. Becher, Valerio Mazzoni, Omar Rota-Stabelli, Sergio Angeli, Teun Dekker, and Gianfranco Anfora. Olfactory responses of drosophila suzukii females to host plant volatiles. *Physiological Entomology*, 40(1):54–64. doi:10.1111/phen.12088.

[5] Omar Rota-Stabelli, Mark Blaxter, and Gianfranco Anfora. Drosophila suzukii. *Current Biology*, 23(1):R8–R9, 2013. doi:10.1016/j.cub.2012.11.021.

[6] K Steffen, F Grousset, G Schrader, F Petter, and M Suffert. Identification of pests and pathogens recorded in europe with relation to fruit imports. *EPPO Bulletin*, 45(2):223–239, 2015. doi:10.1111/epp.12215.

[7] B. A. Hottel, J. L. Spencer, and S. T. Ratcliffe. Trapping drosophila repleta (diptera: Drosophilidae) using color and volatiles. *Florida Entomologist*, 98(1):272–275, Mar 2015. ISSN 0015-4040. doi:10.1653/024.098.0144.

[8] D. M. Kirkpatrick, P. S. McGhee, S. L. Hermann, L. J. Gut, and J. R. Miller. Alightment of spotted wing drosophila (diptera: Drosophilidae) on odorless disks varying in color. *Environmental Entomology*, 45(1):185–191, 2016. doi:10.1093/ee/nvv155.

[9] Junwei Zhu, Kye-Chung Park, and Thomas C. Baker. Identification of odors from overripe mango that attract vinegar flies, drosophila melanogaster. *Journal of Chemical Ecology*, 29(4):899–909, Apr 2003. ISSN 1573-1561. doi:10.1023/A:1022931816351.

[10] Toby J. A. Bruce, Lester J. Wadhams, and Christine M. Woodcock. Insect host location: a volatile situation. *Trends in Plant Science*, 10(6):269–274, Jun 2005. ISSN 1360-1385. doi:10.1016/j.tplants.2005.04.003.

[11] Melia R. Reed. The olfactory reactions of drosophila melanogaster meigen to the products of fermenting banana. *Physiological Zoology*, 11(3):317–325, 1938. doi:10.1086/physzool.11.3.30151465.

[12] P Ravikumar and Shashidhar Viraktamath. Attraction of fruit flies to different colours of methyl eugenol traps in guava and mango orchards. *Karnataka Journal of Agricultural Sciences*, 20(4):749, 2007.

[13] S.E.J. Arnold, P.C. Stevenson, and S.R. Belmain. Responses to colour and host odour cues in three cereal pest species, in the context of ecology and control. *Bulletin of Entomological Research*, 105(4):417–425, 2015. doi:10.1017/S0007485315000346.

[14] Raymond O. Flagg. *Carolina drosophila manual*. Carolina Biological Supply Co., Burlington, NC, 1981.

[15] Karla M. Addesso, Jason B. Oliver, and Paul A. O'Neal. Survey for spotted-wing drosophila (diptera: Drosophilidae) in the five-county nursery production region of middle tennessee, usa. *Florida Entomologist*, 98(4):1050–1055, Dec 2015. ISSN 0015-4040. doi:10.1653/024.098.0407.

[16] Graphpas quickcalcs: t test calculator. URL `https://www.graphpad.com/quickcalcs/ttest1.cfm.`

[17] Quentin Gaudry, Katherine I Nagel, and Rachel I Wilson. Smelling on the fly: sensory cues and strategies for olfactory navigation in drosophila. *Current Opinion in Neurobiology*, 22(2):216 – 222, 2012. ISSN 0959-4388. doi:10.1016/j.conb.2011.12.010.

[18] Anders Aak and Geir K. Knudsen. Sex differences in olfaction-mediated visual acuity in blowflies and its consequences for gender-specific trapping. *Entomologia Experimentalis et Applicata*, 139(1):25–34. doi:10.1111/j.1570-7458.2011.01103.x.

[19] Marcos R.D. Batista, Fabiana Uno, Rafael D. Chaves, Rosana Tidon, Carlos A. Rosa, and Louis B. Klaczko. Differential attraction of drosophilids to banana baits inoculated with *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* within a neotropical forest remnant. *PeerJ*, 5:e3063, March 2017. ISSN 2167-8359. doi:10.7717/peerj.3063.

[20] Geraldine A Wright. Olfaction: Smells like fly food. *Current Biology*, 25(4):R144–R146, Feb 2015. ISSN 0960-9822. doi:10.1016/j.cub.2014.12.052.

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The Effects of Pipeline Clearings on Plant Communities, Soil Microbe Density, and Invertebrate Morphogenera Richness in a Second-growth Temperate Rainforest

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Abstract

Pipeline clearings require frequent maintenance which disturb the forests they are present in. We studied native and non-native plant species richness, overall plant species richness, density of culturable soil bacteria, and invertebrate morphogenera richness in a portion of a second-growth temperate rainforest in the coastal western hemlock zone that is intersected by the Kinder Morgan Trans Mountain Pipeline. We found a significant increase in the number of non-native plant species in the pipeline clearing along with a significant decrease in the number of native species. Within 50 meters of the pipeline we saw no significant differences in overall plant species richness. We also found significantly greater soil bacteria density at the edge of the pipeline clearing and no significant differences in invertebrate richness. The abundance of non-native plant species raises concerns for what implications this may have on the diversity and abundance of native plant species in this section of the forest ecosystem.

Keywords — Species Richness, Non-Native Plants, Soil Bacteria, Environmental Impact, Oil Pipeline

1. Introduction

Continual maintenance and disturbance of linear forest clearings, such as the ones created during pipeline and transmission line installation, can have various effects on forest ecosystems [1]. For example, the heightened colonization opportunities which follow continual disturbances [2] can facilitate entry for non-native plant species into the native plant community [2, 3, 4]. These non-native species can include invasive plants, which are described as plants that can rapidly establish new areas and cause harm to ecosystems [5]. Invasive plants cause harm by creating changes in composition of the native plant community [6, 7, 8, 9], soil microbial communities [10], invertebrate communities [11], ecosystem nutrient cycles [12] and trophic interactions [11]. These changes can ultimately result in the alteration or reduction of the overall

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diversity of forest ecosystems [12, 13, 14]. Forests which boast higher diversity and species richness have been shown to support higher levels of ecosystem functioning [15, 16] and services [17]. Such findings highlight the possible effects linear forest clearings may have not only on forest ecosystems, but on humans as well.

Continual maintenance of linear forest clearings can also directly impact forest soil communities and functions. Linear clearings have been associated with soils with lower organic material [18] and decreased microbial activity [19]. Soil microbial communities have major influences on many ecosystem processes [20], such as plant diversity and productivity [20]. Soil bacteria, for example, are sources of the majority of nitrogen fixation for vegetation in temperate forests [20]. Due to the importance of soil microbial communities on ecosystem functioning and services [21], any changes resulting from continual linear forest clearing can have negative consequences on forest ecosystems.

Invertebrate communities can also be directly impacted by continual linear forest clearings. Removal of vegetation may cause loss of habitat and food sources for invertebrates and alter invertebrate abundance in the clearing as compared to intact sections of forest [22, 23]. As aforementioned, continual forest clearing can result in changes in plant species composition [2, 3, 4], which can lead to changes in compositions of invertebrate communities [11, 24, 25]. However, maintenance of linear forest clearings can also be beneficial to some pollinating invertebrates due to the maintenance of a stable open canopy habitat [26]. The role terrestrial invertebrates play in nutrient cycling for forest ecosystems has been well documented [27]; therefore, it is important to continue documenting the effects continual linear clearing may have on invertebrate communities within forest ecosystems.

One such linear clearing can be found in the Burnaby Mountain Conservation Area located in Burnaby, British Columbia, Canada. It exists in this secondary growth temperate rainforest due to the construction of the Kinder Morgan Trans Mountain Pipeline which was built in 1952-53. This pipeline is 1,150-km long and transports crude oil from Edmonton, Alberta to Burnaby, British Columbia, and cuts through the Burnaby Mountain Conservation Area on Burnaby Mountain. Due to the possibility of tree roots and other vegetation damaging the pipeline or preventing maintenance access, clearings on and around the pipeline are created and maintained at Kinder Morgan's discretion, ultimately resulting in the linear forest clearing [28].

Taking into consideration the previously mentioned potential consequences the maintenance of pipeline clearings may have on surrounding forest ecosystems, and the current conflict surrounding the expansion of the new Kinder Morgan Trans Mountain Pipeline, we are presented with a unique opportunity to investigate the potential environmental impacts of the pre-existing Kinder Morgan Pipeline clearing. As such, we have conducted a brief environmental study of the Kinder Morgan Trans Mountain Pipeline in the Burnaby Mountain Conservation Area to research the possible effects this pipeline clearing may have on the surrounding environment. In our study we attempted to quantify any effects by looking at i) native, non-native and overall plant species richness, ii) concentrations of culturable bacteria in soil, and iii) invertebrate morphogenera richness, at different distances from the pipeline.

2. Materials and Methods

2.1. Study Site

Our study site was centered on a 70-meter section of the Kinder Morgan Trans Mountain Pipeline, buried at the base of Burnaby Mountain within the Burnaby Mountain Conservation Area (49°16'17.4"N by 122°56'14.4"W). In our area of examination, the Trans Mountain Pipeline runs east to west for a length of approximately 200 meter through this part of the conservation area (Figure 1). The studied section is bordered by the Kinder Morgan Canada facility 50 meters to the east, and by a residential street 100 meters to the west. This section was chosen in hopes that effects of any confounding factors from city streets, neighbourhoods and general human activity would be reduced, as suggested by previous reports [29].

Monthly precipitation means in the region range between 312 mm in November and 65 mm in July. Monthly temperature averages range between 17°C in August and 3°C in December [30]. The conservation area consists of a secondary-growth temperate rainforest with patches of old-growth due to past logging which ended in the 1940s [31]. The forest overstory was mostly dominated by Western Redcedar (*Thuja plicata*), Bigleaf Maple (*Acer macrophyllum*) and Red Alder (*Alnus rubra*). The forest understory was dominated by Salmonberry (*Rubus spectabilis*), English Ivy (*Hedera helix*), Indian Plum (*Oemleria cerasiformis*) and Vine Maple (*Acer circinatum*). A complete species list can be found in Appendix A.

The area around the pipeline is regularly cleared of vegetation to allow for easy access for maintenance by Kinder Morgan. The clearing is between 17 and 20 meters wide and the pipeline is marked closer to the northern edge of the clearing, making the distance to the northern forested area between 5-7 meters. In contrast, the distance from the pipeline markers to the southern forested area is 10-12 meters. All vegetation in the clearing is kept at a height of less than 1 meter through mechanical control or the use of herbicides if necessary. Trees that have roots growing towards the pipeline pose a threat to the pipeline's integrity and are removed.

We conducted our survey from June 16 to July 20, 2017. Burnaby Mountain experienced an average of 0.30 mm of precipitation per day during this stretch [32, 33]. This may have affected activity and sampling of invertebrates [34] and bacteria [35, 36]. Vegetation was leafy and many species were in bloom or bearing fruit, aiding with accurate morphospecies identification.

2.2. Site Marking

We measured 8, 50 m long transects running perpendicular (north and south) to the pipeline to prevent other disturbances surrounding the forest from affecting results. Similar distances have also been used in similar studies [3, 37, 38]. The transects were spaced 10 m apart and contained 6 plots also separated in 10 m intervals (Figure 1). We flipped a coin to determine the direction of the first transect and alternated directions of subsequent transects to ensure no human bias interfered with our results.

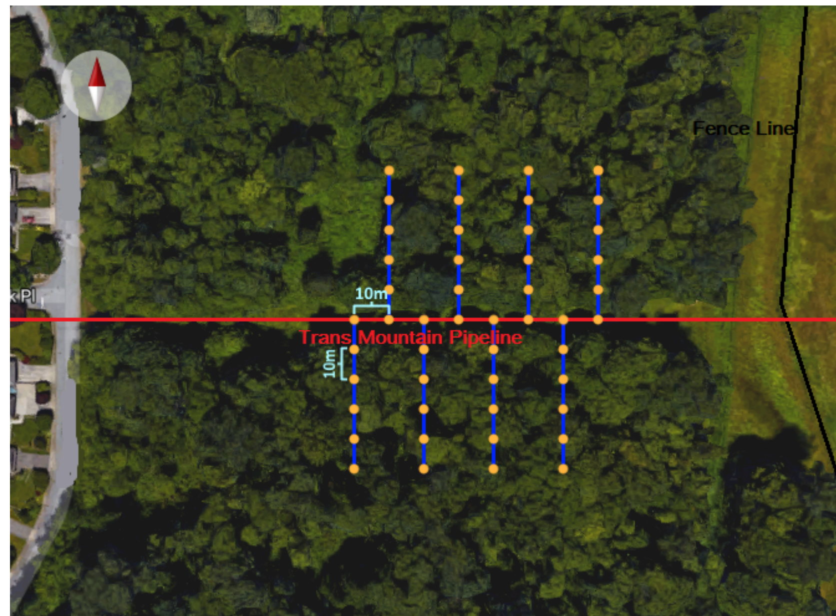

Figure 1: ***Study Site.*** *Red line represents the pipeline. Blue lines represent our transects (10m apart) while orange dots represent experimental plots.*

2.3. Vegetation Surveys

At each plot we counted the number of moss, fern, forb and shrub morphospecies within a 2 m radius. We also counted the number of tree morphospecies within a 4 m radius due to their larger stem size. We identified plant morphospecies using dichotomous keys and plant identification literature [39, 40, 41]. All moss and one shrub species were distinguished from other species but not identified. Unidentified plant species were only included in overall plant species richness data. Identified species were categorized as native, invasive, noxious or exotic using online resources [42]. Plant abundance was not measured.

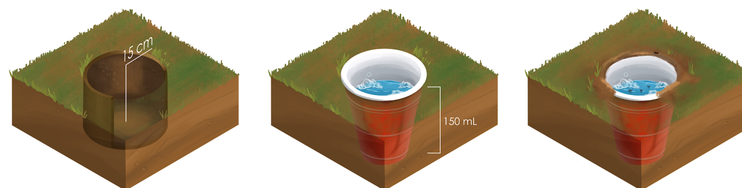

Figure 2: ***Insect Pitfall Traps.*** *Representation of pitfall trap setup.*

2.4. Soil Microbe Sampling and Plating

We collected a 10 mL sample of soil at each plot location. To avoid sampling microbes from plant litter, we took the samples from a depth of 15 cm below the ground surface. Tools used to collect samples were cleaned and sterilized between plots using ethanol. We mixed the collected soil with 10 mL of distilled water in a 50 mL centrifuge tube and agitated it by vigorously shaking the tube and solution for 30 seconds. We diluted our solutions 1000 times in order to be able to accurately distinguish between bacterial colonies. We plated the diluted mixtures using inoculation loops, onto Mueller-Hinton *II* agar plates [43]. Each sample was plated on two different plates. We sealed and stored the plates in a dark drawer at approximately 23°C. We counted colonies 48 hours after completion of the bioassay as it allowed enough time for slower growing culturable bacteria to appear while preventing overgrowth, and thus further ensuring colonies were still distinguishable from one another.

2.5. Invertebrate Surveys

We dug a 15 cm deep hole in the center of each plot and fit 473 mL red solo cups (Solo Cup Company, Lake Forest, Illinois) for pitfall traps into each hole (Figure 2). The top of each cup sat flush with the ground. A solution containing 150 mL of distilled water, and approximately 1 tablespoon of both Fisherbrand™Sparkleen™1 detergent (Thermo Fisher Scientific, Waltham, Massachusetts) and iodized table salt was used as our killing agent [44] and added to the traps to capture insects and other invertebrates. Forty-eight hours after the traps were set, we collected and preserved the caught invertebrates in 70% ethanol, after which they were identified to morphogenera. Invertebrates smaller than ant-size were excluded from our final counts due to difficulties in their identification.

2.6. Statistical Analysis

To control for variation from confounding factors (such as elevation or distance from the residential road) among transects, we normalized all plant and bacteria data from each transect by dividing each variable measurement by its corresponding transect mean. We plotted the mean of each variable against distance away from the pipeline with 95% confidence intervals. To determine whether there were any differences in the normalized variable means among the 6 distances, we did one-way ANOVA tests. If a one-way ANOVA had $p < 0.05$, we did a Tukey HSD ($\alpha = 0.05$).

3. Results

We did not find any non-native tree nor fern species. The mean normalized number of non-native plant species at 0 meters was significantly greater than the means found at all other distances (Figure 3; $F_{5,42} = 11.55, P < 0.0001$). The mean normalized number of native plant species at 0 meters was significantly less than the means found at 20 or more meters (Figure 4; $F_{5,42} = 5.88, P = 0.000175$). There were no significant differences among the mean normalized total number of plant morphospecies for different distances from the pipeline (Figure 5; $F_{5,42} = 1.21, P = 0.437$).

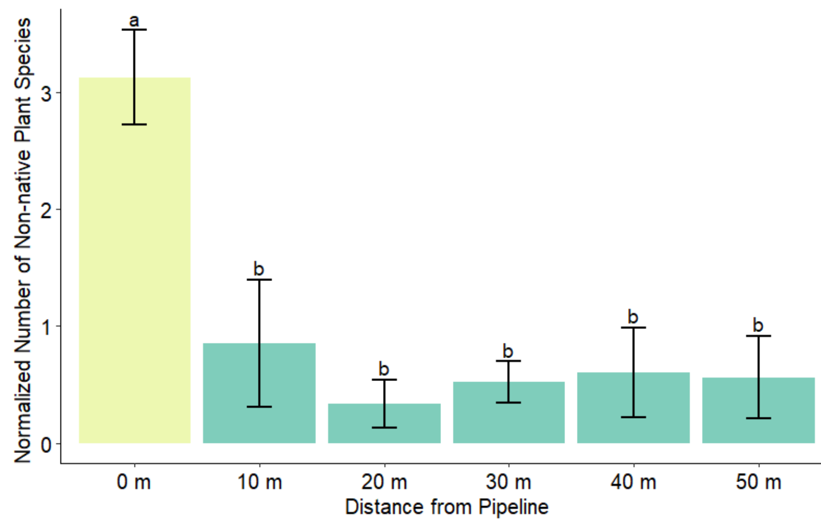

Figure 3: ***Normalized number of non-native plant species at each distance from the pipeline.*** *Bars represent means with 8 replicates. The error bars represent 95% confidence intervals. Bars with the same letters and colours are not significantly different (Tukey HSD, $P > 0.05$).*

The mean normalized number of culturable bacteria colonies from soil samples taken at 10 meters away from the pipeline was significantly greater than the means from 30 or more meters away (Figure 6; $F_{5,95} = 5.08, P = 0.0004$).

We found that mean normalized invertebrate morphogenera richness did not differ significantly among different pipeline distances (Figure 7; $F_{5,42} = 0.908, P = 0.152$).

4. Discussion

4.1. Plant Diversity

The significant increase in non-native plant species richness (Figure 3) and the significant decrease in native plant species richness (Figure 4) in the pipeline clearing is likely due to disturbance of the ecosystem during pipeline construction and continual maintenance of its clearing [45]. This result agrees with previous research on the effects of pipeline clearings [46], in which the dominant plant community found in the pipeline clearing was non-native annuals and was dissimilar to surrounding native communities. A similar result is also seen in continually maintained powerline clearings [47], in which a greater species richness of non-native vegetation was seen in the clearing as compared to the adjacent intact forest. This result could also explain the lack of significant difference seen in overall plant species richness (Figure 6). The significantly lower native species richness in the pipeline clearing (Figure 4) is offset by the high overall species richness (Figure 3), resulting in a change in species composition but no change in overall species richness. This result is also reflected by previous studies [3, 48, 49].

The continual disturbance associated with removal of vegetation in the pipeline clearing facilitates entry for non-native plant species [45, 50]. Of these non-native species, some may be harmful species such as invasives and noxious weeds. Such is

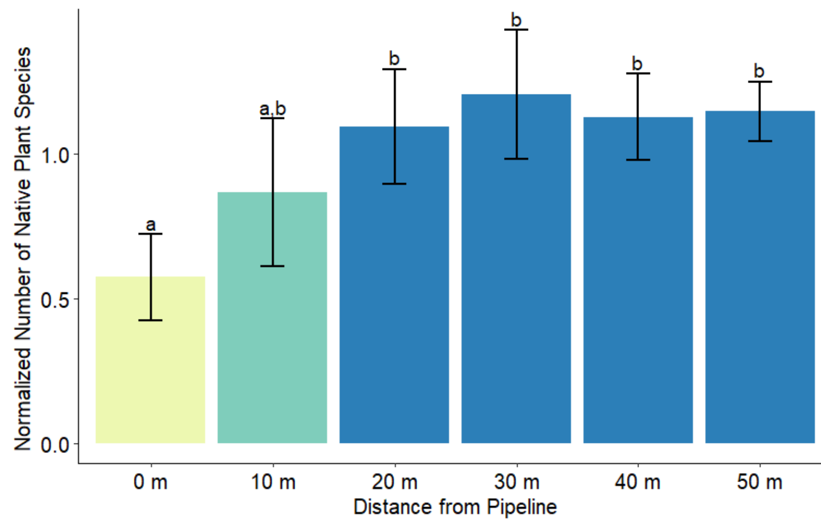

Figure 4: ***Normalized number of native plant species at each distance from the pipeline.*** *Bars represent means with 8 replicates. The error bars represent 95% confidence intervals. Bars with the same letters are not significantly different (Tukey HSD, $P > 0.05$). Each bar colour indicates a different letter combination.*

the case in our experimental pipeline clearing (Appendix A). The harm invasive plant species can have on ecosystems has been well documented [6, 7, 8, 9, 10, 11, 12, 13, 14] and is cause for concern for the survival of native plant species, animal life, and ecosystem functioning of this stretch of the Burnaby Mountain Conservation Area. An example of one such species we have identified in our experimental pipeline clearing is Himalayan Balsam (*Impatiens glandulifera*) (Appendix A). It has been shown to negatively affect invertebrate and detritivore communities by reducing species diversity and population abundance in European riparian forests [51]. Another invasive prevalent in our site was Himalayan Blackberry (*Rubus armeniacus*), a species that has been correlated with decreased bird biodiversity when overgrown into a monoculture [52]. Because no environmental assessments were performed throughout the sixty years of the pipeline's presence, we were unable to determine the extent of colonization by non-native species in our site, compared to other parts of the Burnaby Mountain Conservation Area. With no baseline data available to work with, it was difficult to ascertain the proportion of invasives within the site, and whether an equilibrium had been reached over the last sixty years.

While our results depict that the introduction of non-native plants into this pipeline clearing has negligible effects on overall plant species richness (Figure 6), the potential harm of non-native invasive plants on ecosystem functioning and services overrides it. Maintaining the presence of native species in the Burnaby Mountain Conservation area could be considered a crucial factor for the homeostasis and biodiversity of this ecosystem, which have been identified as important contributors to productive ecosystem services such as soil nutrient storage [15], and biomass production [17]. With this in mind, further research should be done to enhance the current procedures used to clear vegetation in the pipeline clearing. Vegetation clearance methods should be

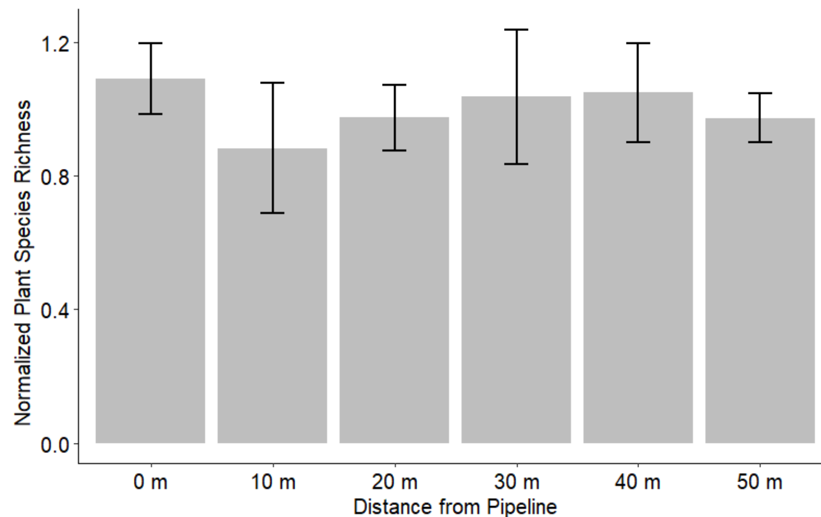

Figure 5: ***Normalized overall plant species richness at each distance from the pipeline.*** *Bars represent means with 8 replicates. The error bars represent 95% confidence intervals.*

done in a way that minimizes the introduction of non-native species into a site, and developing an industry standard may be helpful in achieving this goal.

4.2. Soil Bacteria Density

There were more culturable bacteria in soil samples 10 meters away from the pipeline compared to samples from 30, 40, and 50 meters away (Figure 6). Our results are similar to those of a study in a coniferous forest in Taiwan, which found more soil bacteria in disturbed forest regions than in secondary-growth regions [53]. The results in Figure 6 may be explained by the presence of two major factors contributing to an increase in soil bacteria density. The increased exposure to sunlight in the clearing at 0 m, and the forest-clearing edge at 10 m, could contribute to raising the soil temperature [54], which would increase the activity and growth rate of soil bacteria originating from forests [55, 56]. The second factor limiting bacterial density in the clearing at 0 m from being significantly greater than the forest interior at 30, 40, and 50 m (Figure 6), could be forest bacteria typically being observed to have greater soil densities than bacteria from grasslands or forest edges, as found in previous scientific literature [57]. The overlap of these two factors at the forest-clearing edge could explain the significant increase in soil bacteria density at 10 m from the clearing as opposed to 0 m from the clearing. The abundance of bacteria can influence nutrient availability [20], plant community composition [20], and may have facilitated the establishment of non-native plant species within the pipeline clearing [21, 58, 59].

4.3. Invertebrate Morphogenera Richness

Invertebrate morphogenera richness does not vary with distance from the pipeline clearing (Figure 7). It is well documented that plant species richness has significant impact on invertebrate communities [24, 25]. Due to our lack of significant difference

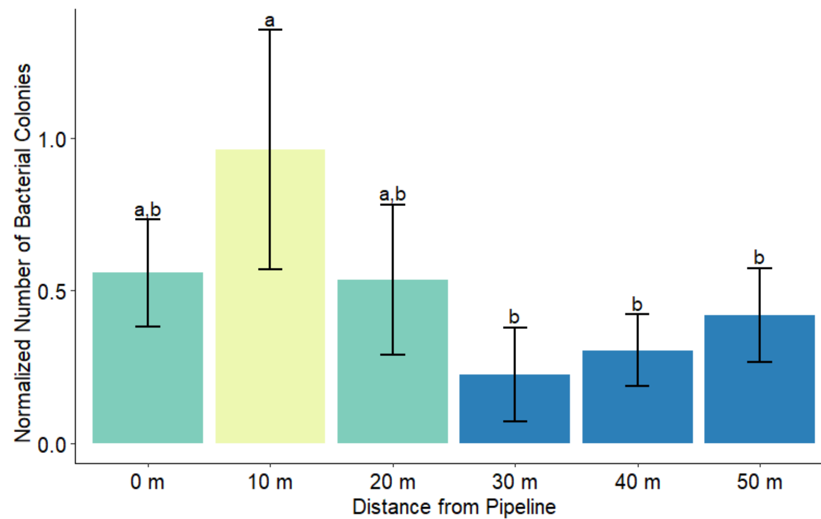

Figure 6: ***Normalized number of culturable bacterial colonies at each distance from the pipeline.*** *Bars represent means with 8 replicates. The error bars represent 95% confidence intervals. Bars with the same letters are not significantly different (Tukey HSD, $P > 0.05$). Each bar colour indicates a different letter combination.*

observed in plant species richness in and around the pipeline clearing (Figure 5), our observed lack of change in invertebrate morphogenera richness is not surprising. The presence of non-native plant species in the clearing (Figure 3) may also play a role in the observed lack of change in invertebrate morphogenera richness. These non-native plant species could shelter non-native species of invertebrates and reduce the presence of species native to the Burnaby Mountain Conservation Area [11]. Thus, changing invertebrate composition rather than richness.

Invertebrate communities with a high morphogenera richness are important contributors to nutrient cycling and plant production [27, 60]. Therefore, it is important to continue to observe how invertebrate species composition and richness is impacted by the composition of non-native and native plant species in the Burnaby Mountain Conservation Area due to the pipeline's presence and its maintained clearing. We subjectively observed a greater abundance of Arachnida and Formicidae families in the clearing. A possible caveat of the invertebrate survey could be features such as decaying nurse logs or anthills as they may have affected the composition of invertebrates trapped resulting in an inaccurate representation of that entire site. Further research on invertebrate diversity should consider using more traps per plot to get a better representation of the experimental plot.

5. Conclusion

Our results showed a significant increase in non-native vegetation, and a significant decrease in native vegetation in the pipeline clearing, suggesting that the clearing introduces a habitat that is able to support populations of non-native plant species. This trend likely contributes to the lack of significant differences found in overall species

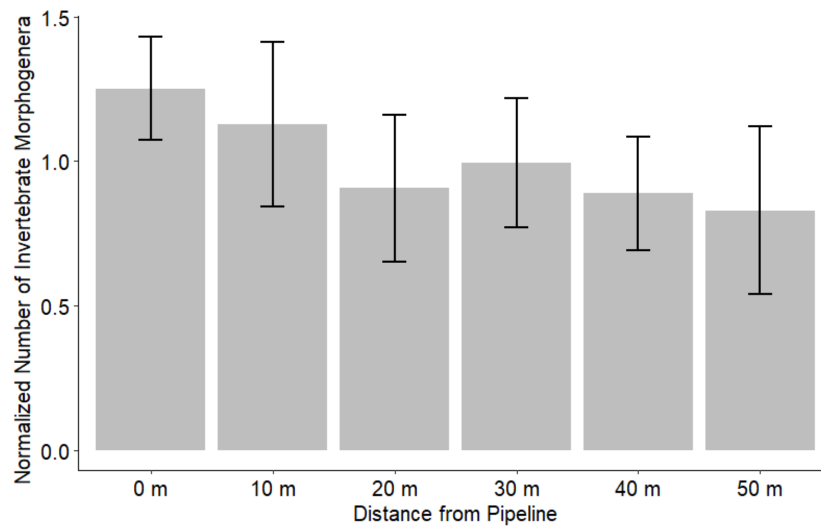

Figure 7: ***Normalized Invertebrate Morphogenera Richness at each distance from the pipeline.*** *Bars represent means with 8 replicates. Errors bars represent 95% confidence intervals.*

richness of vegetation. Culturable soil bacteria density close to the pipeline clearing showed an increase compared to further distances, which could further facilitate the success of the non-native vegetation in the clearing. This non-native vegetation could also alter invertebrate population composition.

Our study shows that the Kinder Morgan pipeline clearing impacts multiple aspects of the Burnaby Mountain Conservation ecosystem in which it was installed. The most prominent is the introduction of non-native vegetation and the subsequent reduction of native vegetation in the clearing. As such, our study can be used as a starting point for further research into the impact pipeline clearings have on their surroundings, and for the monitoring of non-native vegetation in this area.

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References

[1] Joern Fischer and David B. Lindenmayer. Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography*, 16(3):265–280. doi:10.1111/j.1466-8238.2007.00287.x.

[2] Jane A. Catford, Curtis C. Daehler, Helen T. Murphy, Andy W. Sheppard, Britta D.

Hardesty, David A. Westcott, Marcel Rejmánek, Peter J. Bellingham, Jan Pergl, Carol C. Horvitz, and Philip E. Hulme. The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. *Perspectives in Plant Ecology, Evolution and Systematics*, 14(3):231 – 241, 2012. ISSN 1433-8319. doi:10.1016/j.ppees.2011.12.002.

[3] E. R. Olson and J. M. Doherty. The legacy of pipeline installation on the soil and vegetation of southeast wisconsin wetlands. *Ecological Engineering*, 39:53–62, 2012.

[4] Aníbal Pauchard and Paul B. Alaback. Edge type defines alien plant species invasions along pinus contorta burned, highway and clearcut forest edges. *Forest Ecology and Management*, 223(1):327 – 335, 2006. ISSN 0378-1127. doi:10.1016/j.foreco.2005.11.020.

[5] Ministry of Forests Lands and Natural Resource Operations. Invasive species, 2016. URL https://www2.gov.bc.ca/gov/content/environment/plants-animals-ecosystems/invasive-species.

[6] Martin Hejda, Petr Pyšek, and Vojtěch Jarošík. Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology*, 97(3):393–403. doi:10.1111/j.1365-2745.2009.01480.x.

[7] S. Luke Flory and Keith Clay. Non-native grass invasion alters native plant composition in experimental communities. *Biological Invasions*, 12(5):1285–1294, May 2010. ISSN 1573-1464. doi:10.1007/s10530-009-9546-9.

[8] Stuart H. M. Butchart, Matt Walpole, Ben Collen, Arco van Strien, Jörn P. W. Scharlemann, Rosamunde E. A. Almond, Jonathan E. M. Baillie, Bastian Bomhard, Claire Brown, John Bruno, Kent E. Carpenter, Geneviève M. Carr, Janice Chanson, Anna M. Chenery, Jorge Csirke, Nick C. Davidson, Frank Dentener, Matt Foster, Alessandro Galli, James N. Galloway, Piero Genovesi, Richard D. Gregory, Marc Hockings, Valerie Kapos, Jean-Francois Lamarque, Fiona Leverington, Jonathan Loh, Melodie A. McGeoch, Louise McRae, Anahit Minasyan, Monica Hernández Morcillo, Thomasina E. E. Oldfield, Daniel Pauly, Suhel Quader, Carmen Revenga, John R. Sauer, Benjamin Skolnik, Dian Spear, Damon Stanwell-Smith, Simon N. Stuart, Andy Symes, Megan Tierney, Tristan D. Tyrrell, Jean-Christophe Vié, and Reg Watson. Global biodiversity: Indicators of recent declines. *Science*, 328(5982): 1164–1168, 2010. ISSN 0036-8075. doi:10.1126/science.1187512.

[9] Maria Dornelas, Nicholas J. Gotelli, Brian McGill, Hideyasu Shimadzu, Faye Moyes, Caya Sievers, and Anne E. Magurran. Assemblage time series reveal biodiversity change but not systematic loss. *Science*, 344(6181):296–299, 2014. ISSN 0036-8075. doi:10.1126/science.1248484.

[10] The invasive plant impatiens glandulifera affects soil fungal diversity and the bacterial community in forests. *Applied Soil Ecology*, 124:335 – 343, 2018. ISSN 0929-1393. doi:10.1016/j.apsoil.2017.11.021.

[11] M. Carolina M. Simao, S. Luke Flory, and Jennifer A. Rudgers. Experimental plant invasion reduces arthropod abundance and richness across multiple trophic levels. *Oikos*, 119(10):1553–1562. doi:10.1111/j.1600-0706.2010.18382.x.

[12] Richard N. Mack, Daniel Simberloff, W. Mark Lonsdale, Harry Evans, Michael Clout, and Fakhri A. Bazzaz. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, 10(3):689–710. doi:10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2.

[13] Liba Pejchar and Harold A. Mooney. Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution*, 24(9):497–504, Sep 2009. ISSN 0169-5347. doi:10.1016/j.tree.2009.03.016.

[14] Bradley J. Cardinale, J. Emmett Duffy, Andrew Gonzalez, David U. Hooper, Charles Perrings, Patrick Venail, Anita Narwani, Georgina M. Mace, David Tilman, David A. Wardle, Ann P. Kinzig, Gretchen C. Daily, Michel Loreau, James B. Grace, Anne Larigauderie, Diane S. Srivastava, and Shahid Naeem. Biodiversity loss and its impact on humanity. *Nature*, 486:59 EP –, Jun 2012. doi:10.1038/nature11148.

[15] Robert Costanza, Brendan Fisher, Kenneth Mulder, Shuang Liu, and Treg Christopher. Biodiversity and ecosystem services: A multi-scale empirical study of the relationship between species richness and net primary production. *Ecological Economics*, 61(2):478 – 491, 2007. ISSN 0921-8009. doi:10.1016/j.ecolecon.2006.03.021.

[16] D. U. Hooper, F. S. Chapin III, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. SetSlS, A. J. Symstad, J. Vandermeer, and D. A. Wardle. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. 75(1):3–35, 2005. doi:10.1890/04-0922.

[17] Lars Gamfeldt, Tord Snäll, Robert Bagchi, Micael Jonsson, Lena Gustafsson, Petter Kjellander, María C. Ruiz-Jaen, Mats Fröberg, Johan Stendahl, Christopher D. Philipson, Grzegorz Mikusinski, Erik Andersson, Bertil Westerlund, Henrik Andrén, Fredrik Moberg, Jon Moen, and Jan Bengtsson. Higher levels of multiple ecosystem services are found in forests with more tree species. *Nature Communications*, 4:1340 EP –, Jan 2013. doi:10.1038/ncomms2328. Article.

[18] Y. K. Soon, M. A. Arshad, W. A. Rice, and P. Mills. Recovery of chemical and physical properties of boreal plain soils impacted by pipeline burial. *Canadian Journal of Soil Science*, 80(3):489–497, 2000. doi:10.4141/S99-097.

[19] Peng Shi, Jun Xiao, Ya-Feng Wang, and Li-Ding Chen. The effects of pipeline construction disturbance on soil properties and restoration cycle. *Environmental Monitoring and Assessment*, 186(3):1825–1835, Mar 2014. ISSN 1573-2959. doi:10.1007/s10661-013-3496-5.

[20] Marcel G. A. Van Der Heijden, Richard D. Bardgett, and Nico M. Van Straalen. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11(3):296–310. doi:10.1111/j.1461-0248.2007.01139.x.

[21] Thomas Bell, Jonathan A. Newman, Bernard W. Silverman, Sarah L. Turner, and Andrew K. Lilley. The contribution of species richness and composition to bacterial services. *Nature*, 436:1157 EP –, Aug 2005. doi:10.1038/nature03891.

[22] Allan D. Watt, Nigel E. Stork, Colin McBeath, and Gerald L. Lawson. Impact of forest management on insect abundance and damage in a lowland tropical forest in southern cameroon. *Journal of Applied Ecology*, 34(4):985–998, 1997. ISSN 00218901, 13652664. doi:10.2307/2405288.

[23] Bert C. Klein. Effects of forest fragmentation on dung and carrion beetle communities in central amazonia. *Ecology*, 70(6):1715–1725, 1989. ISSN 00129658, 19399170. doi:10.2307/1938106.

[24] Kai Zhang, Siliang Lin, Yinqiu Ji, Chenxue Yang, Xiaoyang Wang, Chunyan Yang, Hesheng Wang, Haisheng Jiang, Rhett D. Harrison, and Douglas W. Yu. Plant diversity accurately predicts insect diversity in two tropical landscapes. *Molecular Ecology*, 25(17):4407–4419. doi:10.1111/mec.13770.

[25] Evan Siemann, David Tilman, John Haarstad, and Mark Ritchie. Experimental tests of the dependence of arthropod diversity on plant diversity. *The American Naturalist*, 152(5):738–750, 1998. ISSN 00030147, 15375323. doi:10.1086/286204.

[26] Katrine Eldegard, ø rjan Totland, and Stein R. Moe. Edge effects on plant communities along power line clearings. *Journal of Applied Ecology*, 52(4):871–880. doi:10.1111/1365-2664.12460.

[27] E. Siemann and W. W. Weisser. *Testing the Role of Insects in Ecosystem Functioning*, pages 383–401. Springer Berlin Heidelberg, Berlin, Heidelberg, 2008. ISBN 978-3-540-74004-9. doi:10.1007/978-3-540-74004-9_18.

[28] Kinder Morgan Canada. Integrated vegetation management plan for kinder morgan canada pipeline systems, 2016. URL `https://www.kindermorgan.com/content/docs/kmc_row_ivmp.pdf`.

[29] Karen Wuyts, An De Schrijver, Jeroen Staelens, and Kris Verheyen. Edge effects on soil acidification in forests on sandy soils under high deposition load. *Water, Air, & Soil Pollution*, 224(6):1545, May 2013. ISSN 1573-2932. doi:10.1007/s11270-013-1545-x.

[30] Environment and Climate Change Canada. Canadian climate normals 1981-2010 station data, . URL `http://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?searchType=stnProx&txtRadius=25&selCity=&selPark=&optProxType=custom&txtCentralLatDeg=49&txtCentralLatMin=16&txtCentralLatSec=17&txtCentralLongDeg=122&txtCentralLongMin=56&txtCentralLon`.

[31] Phillips Farevaag Smallenberg Inc. Management plan for burnaby mountain conservation area, 1999.

[32] Environment and Climate Change Canada. Daily data report for july 2017, . URL http://climate.weather.gc.ca/climate_data/daily_data_e.html?hlyRange=%7C&dlyRange=1965-02-01%7C2017-12-21&mlyRange=1965-01-01%7C2007-02-01&StationID=731&Prov=BC&urlExtension=_e.html&searchType=stnProv&optLimit=specDate&StartYear=1840&EndYear=2018&selRowPerPage=25&Line=25&Month=7&Day=1&lstProvince=BC&timeframe=2&Year=2017.

[33] Environment and Climate Change Canada. Daily data report for june 2017, . URL http://climate.weather.gc.ca/climate_data/daily_data_e.html?hlyRange=%7C&dlyRange=1965-02-01%7C2017-12-21&mlyRange=1965-01-01%7C2007-02-01&StationID=731&Prov=BC&urlExtension=_e.html&searchType=stnProv&optLimit=specDate&StartYear=1840&EndYear=2018&selRowPerPage=25&Line=25&Month=6&Day=1&lstProvince=BC&timeframe=2&Year=2017.

[34] Madhav P. Thakur, Peter B. Reich, Sarah E. Hobbie, Artur Stefanski, Roy Rich, Karen E. Rice, William C. Eddy, and Nico Eisenhauer. Reduced feeding activity of soil detritivores under warmer and drier conditions. *Nature Climate Change*, 8(1): 75–78, 2018. ISSN 1758-6798. doi:10.1038/s41558-017-0032-6.

[35] Chengjie Ren, Ji Chen, Xingjie Lu, Russell Doughty, Fazhu Zhao, Zekun Zhong, Xinhui Han, Gaihe Yang, Yongzhong Feng, and Guangxin Ren. Responses of soil total microbial biomass and community compositions to rainfall reductions. *Soil Biology and Biochemistry*, 116:4 – 10, 2018. ISSN 0038-0717. doi:10.1016/j.soilbio.2017.09.028.

[36] Johan Schnürer, Marianne Clarholm, Sven Boström, and Thomas Rosswall. Effects of moisture on soil microorganisms and nematodes: A field experiment. *Microbial Ecology*, 12(2):217–230, Jun 1986. ISSN 1432-184X. doi:10.1007/BF02011206.

[37] Peng Shi, Jun Xiao, Ya-Feng Wang, and Li-Ding Chen. The effects of pipeline construction disturbance on soil properties and restoration cycle. *Environmental Monitoring and Assessment*, 186(3):1825–1835, Mar 2014. ISSN 1573-2959. doi:10.1007/s10661-013-3496-5.

[38] David L. Wagner, Kenneth J. Metzler, Stacey A. Leicht-Young, and Glenn Motzkin. Vegetation composition along a new england transmission line corridor and its implications for other trophic levels. *Forest Ecology and Management*, 327:231 – 239, 2014. ISSN 0378-1127. doi:10.1016/j.foreco.2014.04.026.

[39] E. Elle. Dichotomous key for plant id.

[40] Jim Pojar, Andrew MacKinnon, Paul Alaback, Joe Antos, Trevor Goward, and Rosamund Pojar. *Plants of coastal British Columbia including Washington, Oregon & Alaska*. Forest Service British Columbia, 2016.

[41] *Keys to the common shrubs and herbs of the Prince George Forest Region*. Ministry of Forests, Forest Science Program, 2003.

[42] B. Klinkenberg. Noxious, and problem plants of british columbia, 2012.

[43] J. Howard Mueller and Jane Hinton. A protein-free medium for primary isolation of the gonococcus and meningococcus. *Proceedings of the Society for Experimental Biology and Medicine*, 48(1):330–333, 1941. doi:10.3181/00379727-48-13311.

[44] Curtis A Laub, Roger Ray Youngman, Kenner Love, Timothy Mize, et al. Using pitfall traps to monitor insect activity. 2009.

[45] Environment and Climate Change Canada. How alien species get into canada, . URL `https://www.ec.gc.ca/eee-ias/default.asp?lang=En&n=B28651E6-1`.

[46] Thomas A. Zink, Michael F. Allen, BÃd'rbel Heindl-Tenhunen, and Edith B. Allen. The effect of a disturbance corridor on an ecological reserve. *Restoration Ecology*, 3 (4):304–310. doi:10.1111/j.1526-100X.1995.tb00098.x.

[47] Donald S. Cameron, Donald J. Leopold, and Dudley J. Raynal. Effect of landscape position on plant diversity and richness on electric transmission rights-of-way in new york state. *Canadian Journal of Botany*, 75(2):242–251, 1997. doi:10.1139/b97-026.

[48] Pablo V. Prieto, JerÃťnimo B.B. Sansevero, MÃąrio L. Garbin, JoÃčo M.A. Braga, and Pablo J.F.P. Rodrigues. Edge effects of linear canopy openings on understorey communities in a lowland atlantic tropical forest. *Applied Vegetation Science*, 17(1): 121–128. doi:10.1111/avsc.12043.

[49] Matthew L. Richardson, Benjamin A. Wilson, Daniel A. S. Aiuto, Jonquil E. Crosby, Alfonso Alonso, Francisco Dallmeier, and G. Karen Golinski. A review of the impact of pipelines and power lines on biodiversity and strategies for mitigation. *Biodiversity and Conservation*, 26(8):1801–1815, Jul 2017. ISSN 1572-9710. doi:10.1007/s10531-017-1341-9.

[50] Richard J. Hobbs and Laura F. Huenneke. Disturbance, diversity, and invasion: Implications for conservation. *Conservation Biology*, 6(3):324–337, 1992. ISSN 08888892, 15231739. doi:10.2307/2386033.

[51] Robert A. Tanner, Sonal Varia, RenÃľ Eschen, Suzy Wood, Sean T. Murphy, and Alan C. Gange. Impacts of an invasive non-native annual weed, impatiens glandulifera, on above- and below-ground invertebrate communities in the united kingdom. *PLOS ONE*, 8(6):1–13, 06 2013. doi:10.1371/journal.pone.0067271.

[52] Caroline Astley. How does himalayan blackberry (rubus armeniacus) impact breeding bird diversity?: a case study of the lower mainland of british columbia. 2010.

[53] Ed-Haun Chang, Guanglong Tian, and Chih-Yu Chiu. Soil microbial communities in natural and managed cloud montane forests. *Forests*, 8(2), 2017.

[54] Anna Dabros, H.E. James Hammond, Jaime Pinzon, Brad Pinno, and David Langor. Edge influence of low-impact seismic lines for oil exploration on upland forest vegetation in northern alberta (canada). *Forest Ecology and Management*, 400:278 – 288, 2017. ISSN 0378-1127. doi:10.1016/j.foreco.2017.06.030.

[55] Man Lang, Zucong Cai, and Scott X. Chang. Effects of land use type and incubation temperature on greenhouse gas emissions from chinese and canadian soils. *Journal of Soils and Sediments*, 11(1):15–24, Jan 2011. ISSN 1614-7480. doi:10.1007/s11368-010-0260-0.

[56] F.C.S. Vieira and E. Nahas. Comparison of microbial numbers in soils by using various culture media and temperatures. *Microbiological Research*, 160(2):197 – 202, 2005. ISSN 0944-5013. doi:10.1016/j.micres.2005.01.004.

[57] Shiping Chen, Wantong Wang, Wenting Xu, Yang Wang, Hongwei Wan, Dima Chen, Zhiyao Tang, Xuli Tang, Guoyi Zhou, Zongqiang Xie, Daowei Zhou, Zhouping Shangguan, Jianhui Huang, Jin-Sheng He, Yanfen Wang, Jiandong Sheng, Lisong Tang, Xinrong Li, Ming Dong, Yan Wu, Qiufeng Wang, Zhiheng Wang, Jianguo Wu, F. Stuart Chapin, and Yongfei Bai. Plant diversity enhances productivity and soil carbon storage. *Proceedings of the National Academy of Sciences*, 2018. ISSN 0027-8424. doi:10.1073/pnas.1700298114.

[58] Kurt O. Reinhart and Ragan M. Callaway. Soil biota and invasive plants. *New Phytologist*, 170(3):445–457. doi:10.1111/j.1469-8137.2006.01715.x.

[59] Benjamin E. Wolfe and John N. Klironomos. Breaking new ground: Soil communities and exotic plant invasion. *BioScience*, 55(6):477–487, 2005. doi:10.1641/0006-3568(2005)055[0477:BNGSCA]2.0.CO;2.

[60] José G.B. Derraik, Gerard P. Closs, Katharine J.M. Dickinson, Barbara I.P. Barratt, and Phil J. Sirvid. Invertebrate species richness and density in relation to size of the new zealand shrub olearia bullata. *Journal of the Royal Society of New Zealand*, 32(4):571–585, 2002. doi:10.1080/03014223.2002.9517710.

A. Plant Species Identification

For all tables in A, a "1" denotes the species was present in the corresponding plot number, a "-" denotes it was not present.

Table 1: *Native plant species identified by plot number, meters is the distance from the pipeline.*

Acer circinatum, Vine Maple								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	1	-	-	-	-	-	-
20 m	1	-	-	1	-	1	-	1
30 m	1	-	1	1	1	-	-	-
40 m	-	1	1	-	-	1	1	1
50 m	1	-	1	-	-	1	-	1
Acer macrophyllum, Bigleaf Maple								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	1	1	1	-	-	1
20 m	1	-	1	-	-	-	-	1
30 m	-	1	-	1	-	-	1	-
40 m	1	1	-	1	-	-	-	-
50 m	1	1	-	-	-	-	1	-
Adenocaulon bicolor, Pathfinder								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	1	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-
Alnus rubra, Red Alder								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	1	-	-	-	1	-	-	-
30 m	-	1	-	1	1	-	-	-
40 m	1	-	-	1	1	-	1	-
50 m	-	-	-	-	1	-	-	-

Athyrium filix-femina, Lady Fern								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	1	1	1	1	-	1	1	-
30 m	1	1	1	1	1	1	-	1
40 m	1	-	1	1	1	1	-	1
50 m	1	1	1	1	1	1	-	1

Corylus cornuta, Beaked Hazelnut								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	1	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	1	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Dicentra formosa, Pacific Bleeding Heart								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	1	-	-	-	1	-	-
10 m	-	1	-	1	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Mahonia aquifolium, Oregon Grape								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	1	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Oemleria cerasiformis, Indian Plum

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	1	-	-	-	-	-	-	-
10 m	-	1	-	1	-	-	1	-
20 m	-	1	-	-	-	1	1	1
30 m	1	-	1	-	1	-	-	-
40 m	1	-	1	-	1	-	1	-
50 m	1	1	1	-	1	-	1	-

Poa palustris, Fowl Bluegrass

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	1	1	1	1	1	1	-	-
10 m	-	1	-	-	-	1	-	1
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Polystichum munitum, Sword Fern

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	-	-	-	-	-	1	-	-
10 m	-	-	1	1	-	-	-	-
20 m	1	1	-	1	1	1	1	1
30 m	1	1	1	-	1	1	-	1
40 m	1	1	1	-	-	1	1	1
50 m	1	-	1	1	1	1	1	1

Pseudotsuga menziesii, Douglas Fir

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	1	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Pteridium aquilinum, Bracken Fern

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	1	1	-	-	-	-	-	-
10 m	-	1	-	-	-	-	-	-
20 m	-	1	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Rubus parvifolus, Thimbleberry

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	1	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Rubus spectabilis, Salmonberry

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	1	1	1	1	1	1	1	-
10 m	1	1	-	1	-	1	1	1
20 m	1	1	-	1	-	-	1	-
30 m	-	1	1	1	1	1	1	-
40 m	1	1	1	1	1	1	1	1
50 m	1	1	1	1	1	1	-	-

Rubus ursinus, Trailing Blackberry

Metres from Pipeline	Transect							
	A	B	C	D	E	F	G	H
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	1	-	-	-	-	-	1
50 m	1	-	-	-	-	1	-	1

Stachys cooleyae, Cooley's Hedge-Nettle

Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	1	1
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Thuja plicata, Western Red Cedar

Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	1	1	1	-	-	1
20 m	1	-	1	-	1	1	1	1
30 m	1	1	-	-	1	1	1	1
40 m	-	1	-	-	-	-	1	-
50 m	-	1	-	1	-	-	1	-

Tsuga heterophylla, Western Hemlock

Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	1	-	-	-	-
20 m	-	-	-	1	-	-	-	1
30 m	1	-	1	-	-	-	-	1
40 m	-	-	-	-	-	-	-	-
50 m	-	1	-	-	-	-	1	-

Vaccinium parvifolium, Red Huckleberry

Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	1	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	1	-	-	-	-	-	-

Table 2: *Non-native plant species identified by plot number, metres is the distance from the pipeline. Species are designated invasive, noxious or exotic with their species and common name.*

Bromus inermis, Smooth Bromegrass - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	1	-	-	-	1	-	-	1
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Convolvulus arvensis, Field Bindweed - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	1	1	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	1	-	-	-	-	-

Galium aparine, Cleavers - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	1	1	-	1	-	1	1	-
10 m	-	-	-	-	-	-	-	1
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Hedera helix, English Ivy - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	1	-	-	-	-	-	-
10 m	1	1	-	-	-	-	-	-
20 m	1	1	1	1	1	-	-	-
30 m	1	1	1	-	1	-	1	-
40 m	1	-	1	-	1	-	-	-
50 m	1	-	1	1	1	-	1	-

Ilex aquifolium, English Holly - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	1	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	1	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Impatiens glandulifera, Himalayan Balsam - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	1	1	1	1	1	1	1
10 m	-	1	-	1	-	1	-	1
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Phalaris arundinacea, Reed Canary Grass - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	1	-	1	1	-	1	1	1
10 m	-	1	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Ranunculus repens, Creeping Buttercup - Invasive

Metres from Pipeline	Transect							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	1	1	1	1	1	1	1	1
10 m	-	1	-	-	-	-	-	1
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Rubus armeniacus, Himalayan Blackberry - Invasive								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	1	-	1	1	1	1	1	1
10 m	-	1	-	-	-	-	1	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	1	-	-	-	-	-	1	-
50 m	-	-	-	-	-	-	-	-

Sorbus aucuparia, Rowan/Mountain-Ash - Invasive								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	1	-	-	-	-	-	-	1
50 m	1	-	-	1	-	-	-	-

Cyperus esculentus, Yellow Nutsedge - Noxious								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	1	1	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Erodium cicutarium, Common Stork's-Bill - Invasive								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	1	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-

Holcus lanatus, Velvet Grass - Exotic								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	1	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-
Lactuca muralis, Wall Lettuce - Invasive								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	-	-	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	1
40 m	-	-	-	-	1	-	-	1
50 m	-	-	-	-	-	-	-	-

Table 3: *Unidentified plant species by plot number, metres is the distance from the pipeline. The number of disparate mosses per plot are listed under "unidentified mosses" as 1, 2, or 3 distinct mosses present at each plot.*

Unidentified Mosses								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	1	1	2	-	1	-	2	-
20 m	1	2	2	1	1	2	1	3
30 m	1	2	-	1	2	2	-	2
40 m	2	1	2	2	-	2	2	1
50 m	1	2	1	-	-	2	-	2
Unidentified Pipeline								
Metres from Pipeline	**Transect**							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
0 m	-	-	-	-	-	-	-	-
10 m	-	-	-	-	-	-	1	-
20 m	-	-	-	-	-	-	-	-
30 m	-	-	-	-	-	-	-	-
40 m	-	-	-	-	-	-	-	-
50 m	-	-	-	-	-	-	-	-