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Dear Reader,

The humble publication that you are about to delve into began with quite a simple goal in mind: to promote and celebrate undergraduate research. Reflecting on the six compelling articles we now present you with, we would put forward that we have accomplished that goal, or at least made a very good start. But going beyond the pages of this journal, we also hoped to foster a more integrated science community at Simon Fraser University. This is more challenging to quantify, but we will do our best now. Over the past year, we worked with a team of 14 hardworking, passionate undergraduate editors from six different departments. We had over 65 graduate students, professors, and researchers from nine different institutions agree to be peer reviewers. Our blog, X & Y, featured thoughtful posts from a wide variety of students on a wide variety of topics.

So yes, we are featuring undergraduate research, and giving these students the opportunity to experience a peer review process. We also hope that we have offered our editors the opportunity to learn the ins and outs of the journal publication process alongside us, and graduate students the opportunity to conduct a peer review.

This project has been met with excitement and support, fueling us as we navigated the adventure of publishing our first issue, and driving us forwards into our second year with energy to improve and build upon what has been started.

It is with pleasure that we introduce you to the first edition of the SFU Science Undergraduate Research Journal.

Sincerely,

Emma Atkinson, Tomas Rapaport, and Kiera Warren

Executive Editors
Fast and furious: shorter handling times reveal a foraging advantage of the invasive Carcinus maenas over its native competitor Metacarcinus gracilis

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2University of Alberta, Department of Biological Sciences
3University of British Columbia, Department of Biology

Abstract

Invasive species have been shown to decrease the fitness of native species and reduce biodiversity. The European green crab (Carcinus maenas) is a prolific invasive crab species responsible for habitat degradation and biodiversity reduction across the globe, and has been present on Canada’s southwestern coast since the late 1990’s. Using feeding rates as a proxy for individual fitness, we examined how the rate of prey handling and consumption in C. maenas compares to that of the native graceful rock crab (Metacarcinus gracilis). The time required by C. maenas and M. gracilis to handle and consume a mussel (handling time) was compared at two flume velocities (10 and 19cm/s) to quantify feeding rates under differing flow conditions. These measurements have possible implications for how C. maenas populations may affect M. gracilis demography in coming years. At both flume speeds, C. maenas had a lower handling time than M. gracilis. At 10cm/s flow, C. maenas fed an average of 2 minutes faster than M. gracilis, whereas at 19cm/s they fed 5.2 minutes faster on average. These data suggest C. maenas is capable of more efficient foraging at a variety of current speeds. Handling time is widely recognized as an important influence on invasion success, and our findings thus suggest that C. maenas has a competitive advantage over M. gracilis across its native range. These results have implications for modeling the predicted spread of C. maenas along the coastal East Pacific.

Keywords — European green crab, competition, invasive species, resource use, feeding rate, handling time

1. Introduction

The introduction of non-native species can negatively impact the fitness of native species, lower biodiversity, disrupt ecosystem function, and threaten human enterprise [1]. Studying species traits that have a direct impact on individual fitness is useful for understanding the competitive interactions between invasive and native species. Since a successful invasion is partially dependent on how efficiently

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an invasive species can compete with native species for resources \[2, 3\] and because
invasive predators can reshape the community structure they inhabit depending on their
ability to catch and consume prey items \[4\], studying the rate of resource consumption
between an invader and a native competitor is helpful for predicting impacts. Handling
time, the amount of time it takes a predator to handle its prey from initial contact to the
time the prey item is eaten, can be used as a proxy for feeding rates \[5\]. Furthermore,
handling time is a vital component of optimal foraging theory, which is a model that
predicts how organisms search, compete, and consume resources \[6\]. According to
optimal foraging theory, predators with shorter handling times are able to coexist with,
or may even out-compete other predators \[7\]. These interactions have implications for
a predator’s ability to exploit prey items and are thereby important to shaping the
ecosystem in which predators can reside \[7\].

The European green crab (\textit{Carcinus maenas}), a native of Europe’s Baltic Sea and
English Channel, is a well-established invasive species of North America’s east and
west coasts, as well as areas of South America, southern Africa and Australia \[8\]. The
origin of \textit{C. maenas} on the west coast of Vancouver Island, located on the south coast
of British Columbia, Canada, can be traced to a strong El Niño event in 1997/1998,
when larvae were transported from established invasive populations in California \[9\].
\textit{C. maenas} directly competes with other crustacean populations and is an aggressive
predator of other benthic invertebrate species \[10\]. They exert significant top-down
control on coastal marine food web, i.e. the structure of invaded communities becomes
determined by the severity of \textit{C. maenas} predation, not by the amount of nutrients or
habitat available \[11\]. Despite earlier predictions that populations would eventually die
out, \textit{C. maenas} has persisted on Vancouver Island’s west coast \[9\]. Studies suggest that
\textit{C. maenas} persistence seems to be partly dependent on the presence of relatively warm
and sheltered water, where they feed at rates 2-3 times higher than exposed habitats
\[12, 8, 13\].

The foraging time of \textit{C. maenas} is increased with increasing flow velocity, while
its feeding rate is decreased \[14\]. Consequently, \textit{C. maenas} populations are highest
in sheltered habitats with lower flow velocities, such as bays, and decline in higher
velocity, exposed habitats \[15\]. A native competitor, the graceful crab (\textit{Metacarcinus
gracilis}) and \textit{C. maenas} share habitats of sheltered, muddy or sandy bottoms with high
eel grass coverage. However, \textit{M. gracilis} also inhabit more exposed rocky shores \[16\].
Both populations of \textit{M. gracilis} and \textit{C. maenas} exist in the Pacific Northeast coastal
regions and are present in Barkley Sound, an ocean inlet located in southwestern
Vancouver Island, where \textit{C. maenas} was first reported in Canada in 1999 \[8, 16\].

To assess the risk of \textit{C. maenas} outcompeting \textit{M. gracilis} in shared habitat, we
compared the handling times of \textit{C. maenas} and \textit{M. gracilis} by measuring each species’
handling times at two different flow rates. We hypothesized that at low levels of flow,
\textit{C. maenas} will have a similar or lower total mean handling time than \textit{M. gracilis} since
\textit{C. maenas} populations have been well established in sheltered areas along the west
coast \[9\]. At faster flows we expect the total handling time of \textit{C. maenas} to increase at
a greater rate than that of \textit{M. gracilis}, and will therefore have a longer total handling
time than \textit{M. gracilis} at greater flows. These findings are important for predicting the
future implications of \textit{C. maenas} presence on \textit{M. gracilis} populations and other native
crab species in the North-eastern Pacific. Furthermore, our description of *M. gracilis’* handling times is among the first information available in the literature describing its feeding behaviour.

2. **Results**

At a flow rate of 10cm/s, mean handling time for *C. maenas* (302.9 ± 31.2s) was 1.5 times faster than that of *M. gracilis* (461.6 ± 43.8s) (**Figure 2**), although the difference was only marginally significant (**Table 1**; Linear Regression, *p* = 0.077, *df* = 14). Feeding rates of *M. gracilis* had a larger variance than those of *C. maenas*, with a difference of 312 seconds between the maximum and minimum feeding rates versus a difference of 290 seconds for *C. maenas*. Mussel size, crab size, and flume temperature were found to have no significant effect on the handling times of either crab species at the flow speed of 10cm/s, and were removed through the stepwise AIC process. However, the effect of water temperature varied depending on the species (**Table 1**; Linear Regression, *p* = 0.058, *df* = 14). Water temperature had a greater effect on *M. gracilis* compared to *C. maenas* (**Figure 3**).

**Table 1:** Linear regression table of parameter estimates, *t*-values, and their respective *p*-values for the handling times of *C. maenas* and *M. gracilis* with a flume speed of 10cm/s.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Std. Error</th>
<th><em>t</em>-value</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2439.86</td>
<td>2391.00</td>
<td>1.020</td>
<td>0.32</td>
</tr>
<tr>
<td>Species</td>
<td>-2842.5</td>
<td>1486.84</td>
<td>-1.912</td>
<td>0.076</td>
</tr>
<tr>
<td>Flume Temp</td>
<td>-155.68</td>
<td>156.62</td>
<td>0.994</td>
<td>0.337</td>
</tr>
<tr>
<td>Species: Flume Temp</td>
<td>201.71</td>
<td>98.08</td>
<td>2.057</td>
<td>0.059</td>
</tr>
</tbody>
</table>

At the higher flow rate of 19cm/s the difference in total mean handling between the two species was significant (**Figure 4** (**Table 2**; Linear Regression, *p* < 0.001, *df* = 21). The average handling time for *C. maenas* (270.6s ± 25.6s) was 2.2 times faster than *M. gracilis* (582.5s ± 57.9s) at the higher flume speed (**Figure 4**). Feeding rate variance for the *M. gracilis* was also shown to be higher than that of the *C. maenas* at the 19cm/s flume speed, with a difference of 565 seconds between the maximum and minimum feeding rates (**Figure 4**). Conversely, *C. maenas* feeding rates only differed by 299 seconds between the maximum and minimum handling times (**Figure 4**).

We found that at a high flow speed of 19cm/s, both mussel size and crab size were correlated with handling time (**Figure 5**). A significant positive correlation between crab size and total handling time was found across both species (**Table 2**, Linear Regression, *p* = 0.027, *df* = 21), with an increase in crab size associated with an increase in total handling time (**Figure 5a**). The same was found for mussel size (**Figure 5b**), with an increase handling time significantly correlated with an increase in mussel size (**Table 2**, Linear Regression, *p* = 0.0057, *df* = 21).
Table 2: Linear regression table of parameter estimates, t-values, and their respective p-values for the handling times of *C. maenas* and *M. gracilis* with a flume speed of 19cm/s.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1298.51</td>
<td>530.61</td>
<td>-2.447</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Species</td>
<td>480.54</td>
<td>97.73</td>
<td>4.917</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Flume Temp</td>
<td>46.69</td>
<td>28.09</td>
<td>1.662</td>
<td>0.11</td>
</tr>
<tr>
<td>Mussel Size</td>
<td>396.42</td>
<td>125.37</td>
<td>3.082</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Crab Size</td>
<td>-87.53</td>
<td>36.77</td>
<td>-2.381</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

3. Discussion

The primary purpose of our study was to determine differences in handling time in varying flow conditions between *M. gracilis* and *C. maenas*. In our feeding trials, we were unable to detect statistical difference for total mean handling time at a flume speed of 10cm/s between the two species; however, we did observe a trend for *C. maenas* to have a faster handling time of ~1.5 minutes compared to *M. gracilis*. At higher flume speeds (19cm/s), *M. gracilis* had a much longer total mean handling time than that of *C. maenas*; *M. gracilis* fed an average of ~5.2 minutes slower than *C. maenas*. Mussel size and crab size were correlated with handling time at a flow speed of 19cm/s, which may explain the larger difference in handling time observed at 19cm/s compared to 10cm/s. At higher flow-speeds, dynamic pressure differences between the upstream side and downstream side are greater, thus the force of drag is greater than that of lower flow-speeds. As an organism increases in size, the difference in dynamic pressure also increases [17]; the much larger *M. gracilis* is therefore subject to greater drag forces, which should negatively affect their ability to handle food.

Rovero et al. [18] stated that for *C. maenas* the amount of time spent handling prey, and not energy expended, better represents the cost of prey-handling behaviour; it is reasonable to believe the same holds true for other shore crabs, including *M. gracilis*. Our results show that *C. maenas* feeding behaviour is more profitable at faster flow rates, suggesting the ability to obtain energy more efficiently may give *C. maenas* a fitness advantage over *M. gracilis* where distributions overlap in high flow environments [18]. While the unidirectional nature of flow inside the flume used in this study is rare in coastal marine systems, flow velocities upwards of 19cm/s in crab habitats are typical and likely highly directional in inlets and narrow channels. For example, in the many high flow inlets characteristic of the Barkley Sound region, our results suggest the efficient feeding behaviour of *C. maenas*’ may prove detrimental for local *M. gracilis* populations. Extirpation of the *M. gracilis* due to competition for food [7] and subsequent changes to community composition [19] are realistic outcomes of prolonged *C. maenas* presence in Barkley Sound.

Although the reduced efficiency of *M. gracilis* foraging could contribute to a competitive disadvantage that may threaten their existence in areas where *C. maenas* is present [7], additional competitive factors could moderate these species interactions. Direct confrontations between crustaceans are often decided in favour of the largest individual.
[10], which, based on our sizing data, is likely to be the *M. gracilis*. Instead, the faster mean handling time suggests that *C. maenas* are more likely to consume resources at a faster rate than *M. gracilis* and as such, out-compete them in an indirect manner by diminishing food patches at a rate greater than *M. gracilis* can exploit them. This theoretical fitness advantage of *C. maenas* is dependent on whether they are equally capable as *M. gracilis* at finding patches of food. It is possible that *M. gracilis* are more sensitive to the olfactory cues given by local prey species than the recently-introduced *C. maenas*.

In our study both species of crab often did not react to the placement of a crushed mussel in the flume. It is possible that the size of the container did not allow for a demonstration of typical behaviours. Future studies should incorporate a larger flume to allow the crabs to behave more naturally. Another possibility is the unidirectional flow produced by the flume caused crabs to huddle down and become unresponsive. Wave exposure usually occurs in a back-and-forth motion and rarely exists as unidirectional flow in nature. Crabs of both species were more likely to become unresponsive at a higher flow velocity (pers. obsv.). As suggested by Robinson et al. [14], unresponsiveness to food may be due to increased mixing of the odour plume or increased dissipation of odour molecules at higher velocities. However, because of the small flume, effluent concentrations are more likely to have remained sufficiently high to initiate a response. Alternatively, it is possible that crabs are less likely to risk any movement at high unidirectional flows due to the perceived risk of being swept away [20]. Future studies should record the number of feeding trials that conclude with no response. These data may reveal the comparative vulnerability of each species to hydrodynamic forces.

Although we observed differences in handling times between the two species studied, there are several factors that may limit the interpretation of our findings. For example, we were not able to change flume water after every trial. This may have confounded our data due to a build-up of both crab feeding effluent and prey death effluent in the water, causing a decrease in handling times. Future studies, if unable to change the flume water after every trial, could include the trial number (1st after water change, 2nd, etc.) as a covariate to help standardize the data. Also, we did not standardize our design for starvation throughout experimental trials, which can have impacts on feeding rates. Future protocols should incorporate standardization of starvation periods by use of randomization of flume speeds, species used, as well as randomization of crab individuals for each trial. Lastly, although *C. maenas* and *M. gracilis* were subject to the same surface flow velocities, the actual flow velocity experienced by the crab would have differed slightly depending on height. As the distance from the bottom of the flume increases, so does the velocity of the water, owing to the no-slip condition, i.e. the zero-velocity condition of fluids adjacent to a solid boundary, and the subsequent gradient of increasing velocities as distance from the solid boundary increases [17]. With this in mind, the squat *C. maenas* would have been exposed to a slightly slower flow velocity compared to the more bulbous *M. gracilis*; this would have reduced drag and may have contributed to their comparatively quick handling times.

By determining how food handling times compare between a native crab and an
invasive crab species, this study elucidates an important piece of the puzzle in the prediction of how community structure may change due to the *C. maenas* invasion. Hampton and Griffiths [21] demonstrated that *C. maenas* is unlikely to out-compete local competitors in South African wave-swept shorelines owing to its lack of morphological adaptations suited to high-energy areas. An analysis of *M. gracilis*’ morphological features could allow a similar comparison to be made for the Barkley Sound region. While our results imply the *C. maenas* is a better competitor in a fast, unidirectional-flowing environment, an in-depth comparative analysis of graceful and other crab morphologies would be useful in determining the relative structural advantages of each species, thereby helping to model the projected spread of the *C. maenas* across the Canadian Pacific. This information can then be used to assess which prey and competitor species are most vulnerable to the *C. maenas* invasion, and allow for the appropriate conservation measures to be taken.

4. Methods

4.1. Experimental Design & Protocols

Thirty *C. maenas* with a carapace width of 6-7 cm were collected from Hillier Island (Figure 1) in Barkley Sound in July 2014 using Fukui crab traps. Only males were collected due to the fact that *C. maenas* is highly invasive and there are restrictions to their collection. Crabs were acclimated to a 12:12 hour light-dark cycle and fed with blue mussels (*Mytilus trossulus*) measuring 2-3 cm, collected by hand from Bamfield Inlet in Barkley Sound for 4 weeks before feeding trials. Thirty *M. gracilis* crabs with a carapace width of 6-10 cm were collected throughout the month of October 2014 from Burlo Island (Figure 1) in Barkley Sound using folding Fukui crab traps and were held in an identical manner to the *C. maenas* for 2 weeks before feeding trials began. Again, only males were collected to provide an appropriate comparison to the male *C. maenas*. Size measurements for crabs were taken using digital calipers at the widest point of the carapace. All crabs were held in sea tables with circulating seawater approximately 30 ppm and 14°C. To help initiate feeding behaviour during the trials, all crabs for the 10cm/s trials were deprived of food for 3.5 days and crabs for the 19cm/s trials for 4.5-5 days before feeding trials took place. This mismatch of starvation periods between 10cm/s trials and 19cm/s trials restricts the inferences that can be made; thus no comparisons were made between the two water velocities, only within.

A total of 46 feeding trials were conducted. Each feeding trial involved randomly selecting a flow-rate, species, and individual, placing the selected crab in a 75 cm long X 10 cm wide X 14 cm deep flume for 30 minutes. Each 30-minute feeding trial included a 7 minute acclimation period followed by a 3 minute effluent period, where a mussel was placed in the water but kept out of reach via a mesh gate. The length of each mussel was measured and mussels were cracked open with the palm of a hand against a flat surface to encourage feeding. Although cracking the shell is an important component of the handling time for mussel foraging, *C. maenas* scavenge a wide array of other food that does not require shell-cracking [22]; while the diet of *M. gracilis* is not well-described in the literature, given its morphology and life history typical of other shore crabs, it is reasonable to presume its diet is varied like other scavenging shore
Figure 1: Map of Barkley Sound on Vancouver Island, British Columbia with the collection sites of Carcinus maenas and Metacarcinus gracilis labeled as Hillier Is. and Burlo Is. respectively.

crabs. We therefore argue that our results remain a relevant and useful comparison of M. gracilis and C. maenas food handling times.

The 20-minute feeding period followed where the gate separating the mussel and crab was removed. If a crab did not feed on the mussel during the allocated 20-minute feeding trial the crab was placed back into the holding tank and another crab was randomly selected. Feeding trials were recorded using a Sony Handycam HDR-CX550V camcorder and the handling time (seconds from first touch of the mussel until completion of feeding) of each crab was quantified using a stopwatch.

The water in the flume was replaced after every three trials. For each trial flume depth and temperature were recorded. Ten feeding trials were conducted for each species at a flume velocity of 10cm/s, and 13 feeding trials tested at a flow rate of 19cm/s. No individual crabs were used more than once throughout the experiment. The chosen flume velocities were based on flow velocities of C. maenas habitat observed in other studies [16]. Flume velocities were calibrated by observing the average time it took a 1 cm-wide paper ball to float across a set distance. Water temperature in the flume increased a significant amount across the time span of 3 trials, and was therefore included as a test variable.
4.2. Data Analysis

Two separate generalized linear regression models were selected for each flume speed due to inconsistent starvation periods (see Table 1 and Table 2). Therefore, no comparisons of handling times were made across flume speeds. Models were selected using stepwise AIC selection through removal of insignificant terms with removal priority on interaction terms. The variables considered were crab species, crab size (carapace width in cm), mussel size (shell length in cm), and water temperature (°C). A Shapiro-Wilk test was used to test for normality and the residuals were analyzed to check that the data meet the assumptions of generalized linear models. All data analyses were performed using the R statistical program [23].

5. Conclusion

By determining how food handling times compare between a native crab and an invasive crab species, this study elucidates an important piece of the puzzle in the prediction of how community structure may change due to the C. maenas invasion.

Future studies should conduct observations throughout the year to better understand differing handling times due to seasonal changes in metabolism and should also examine the difference in response to olfactory cues, or a comparison of search times required to find food. This would help determine how relatively capable C. maenas is at tracking down food patches consisting of prey species local to the M. gracilis range. Better control of starvation periods and flume temperature will also allow for comparisons between water velocities, which would increase the inferences that can increase the inferences made from the statistical model.

6. Acknowledgements

This student report is dedicated to River Sidley. He was kind, fun-loving, goofy, and had a curiosity that could not be satiated. His dedication to this project was admirable and it was nothing but an honour to be able to work alongside him not only as a colleague, but as a lifelong friend. He will be sorely missed.

We would also like to thank Kylee Pawluk, Alex Clifford, Allan Roberts, Anna Smith and Brett Howard for all their insight when approached for help. Their patience and kindness were highly appreciated, especially in times of frustration. We would also like to thank the Bamfield Marine Sciences Centre for their contribution of resources and facilities.

We would like to extend a big "thank-you" to everyone in Fall Program 2014 that helped us with our specimen collection. This project would not have happened without your generosity. The time you sacrificed to collect crabs with us in the worst weather was unbelievable and your company was well appreciated.
References


**Figure 2:** Handling times of *C. maenas* (*N* = 10) and *M. gracilis* (*N* = 8) at a flume speed of 10cm/s with an overlaying scatterplot of the data points. Red, downward-facing triangles represent data points of *C. maenas* whereas blue, upward-facing triangles represent data for *M. gracilis*. Bold line represents the median, top and bottom of the box represent the location of the upper and lower quartile respectively. Whisker lines represent the maximum and minimum values, excluding outliers.
Figure 3: Handling times of both C. maenas and M. gracilis at a flume speed of 10cm/s as temperature increases. The dashed line represents the trend line for M. gracilis data \( (r^2 = 0.034, Y = 46.03x - 402.68) \), while the solid line represents the trend line for C. maenas data \( (r^2 = 0.87, Y = 247.7x - 3245.2) \).
Figure 4: Handling times of C. maenas ($N = 13$) M. gracilis ($N = 13$) at a flume speed of 19cm/s with an overlaying scatterplot of the data points. Red, downward-facing triangles represent data points of C. maenas whereas blue, upward-facing triangles represent data for M. gracilis. Bold line represents the median, top and bottom of the box represent the location of the upper and lower quartile respectively. Whisker lines represent the maximum and minimum values, excluding outliers.
Figure 5: a) Scatterplot of the handling times of both C. maenas and M. gracilis at a flume speed of 19cm/s with crab size. The line represents the line of best fit ($r^2 = 0.21$, $Y = 76.37x - 158.59$).

b) Scatterplot of the handling times of both C. maenas and M. gracilis at a flume speed of 19cm/s with mussel size. The line represents the line of best fit ($r^2 = 0.22$, $Y = 484.2x - 860.20$).
Neural networks and their application in the assessment of the healthiness of the human sitting posture

Ahmad Al Attar¹, Chingiz Jakubaliyev¹, Haitham Kaddoura¹, Mohammad Azarbar¹

¹Simon Fraser University, Department of Mechatronic Systems Engineering

Abstract

Sitting posture can have a significant effect on an individual's health. Machine learning can be used to assess the "healthiness" of a given sitting posture. A target equation was conceived and applied with input data into a neural network learning algorithm. The resulting trained neural network was successful in assessing the healthiness of the sitting postures of four hypothetical cases. The neural network design brings us one step closer to an artificially intelligent chair that can alert users if their sitting postures are unhealthy.

Keywords — Neural Networks, Machine Learning, Ergonomics, Sitting Posture, Unhealthiness Value

1. INTRODUCTION

Sitting for too long or in an improper way can lead to various health issues. Sitting posture is an important factor that can significantly affect the health of an individual [1]. Ergonomics is the study of human efficiency in the workplace. Several ergonomically-designed chairs exist that promise healthier seating during work. However, not all ergonomic chairs match everyone. Selecting the right chair can be difficult. It is important to find the chair that best suits one’s body and activity for a healthy work style [2]. Machine learning neural networks can be used to design and program an artificially intelligent system that learns from a set of previously collected data. The trained neural network is then able to assess the healthiness of a given sitting posture. In this research, sitting posture data was generated and target data was calculated from a conceived mathematical equation that describes the unhealthiness level of a given posture. Both data sets were used to train the neural network. The neural network was then tested on new data and assessment results were obtained.

2. THEORY

2.1. Machine Learning

Machine learning is a branch of artificial intelligence in which computers are programmed to modify and adapt their algorithms so that they become more accurate.
There are three main types of learning: supervised, unsupervised, and reinforcement learning. See Figure 1. Supervised learning is very similar to giving a student a problem set along with the solutions to study and prepare for a quiz. The premise of this approach is that the student generalizes the ideas contained in the problem set. Unsupervised learning is similar to giving the student the problem set without solutions and expect the student learns to generalize the ideas to prepare for the quiz. Lastly, reinforcement learning is somewhere in-between supervised and unsupervised learning. In reinforcement learning, the student gets told when the answer is wrong, but does not get told how to correct the mistakes [3].

**Figure 1:** The three types of machine learning.

### 2.2. Neural Networks

Neural networks are a branch of supervised machine learning and cognitive science that are inspired by the neural networks in the human nervous system and brain. There are a lot of benefits to learning about these models and their utility in intelligent systems. Since it is a form of supervised learning, the "problem set" along with the "solutions" must be provided to help the neural network generalize and prepare answers to new questions. The elementary unit that processes the computations within the nervous system of a human is the neuron. Similarly, the elementary unit that processes the computations within an artificial neural network is the perceptron. Before diving into
the details of these models, biological neurons, from which we build our model of the perceptron, will be briefly introduced. In the human brain, there are about $10^{11}$ neurons that can communicate with each other. Each neuron has hundreds or even thousands of connections to other neurons in the human brain \[4\]. See Figure 2 for a figure of the generic neuron along with some key terminologies.

For our interest, only two features provide the basis for artificial neural networks: dendrites and synapses. The dendrites serve as the input terminals of a neuron. Synapses are junctions at the output of a neuron which also serve as input to other neurons. It is important to note that in neural network theory, it is believed that synapses vary in strength. Connections between some neurons are enhanced while between others are inhibited \[5\]. In an over simplified example, let’s say a certain set of neurons contain the image of your aunt, and if your aunt gives you a banana every time you visit her, then the connections between the neurons that contain your aunt’s image and the neurons that contain the image of a banana grows stronger. Now, the next time you see your aunt, you will instantly think of bananas.

A neuron receives inputs and then it either fires a signal or not. This is neatly modelled by the McCulloch-Pitts perceptron. See Figure 3.

Figure 3: McCulloch-Pitts perceptron.

The inputs are multiplied by weights to account for the fact that their connection strengths vary. Whether a perceptron fires or not is determined by the weighted sum of all inputs. If the sum exceeds a certain threshold function, it fires, otherwise it does not emit any signal \[6\]. Instead of this 1 or 0 output caused by the choice of activation function, one can pick different activation functions. The most commonly used linear activation function generates a continuum of values. As shown in Figure 3, a single perceptron has many inputs and generates a single output. Just like a system of neurons build up a brain, a system of perceptrons build up a neural network. See Figure 4 for a simple neural network (the weights have been dropped out of the figure for clarity).

We see that all the inputs are fed into all the perceptrons and the number of outputs matches the number of perceptrons (which matches the number of input features). The column that has the inputs is usually called the input layer and the column that has the outputs is usually called the output layer. It is often useful to have a hidden layer of perceptrons in between the input and output layers that aid in performing more complex machine learning. See Figure 5.

So far, we have discussed the basic working principles of a neuron, introduced the computational analogy of a neuron, the perceptron, and looked at how neural networks
can be built from a system of perceptrons. Only one fundamental ingredient is left: the learning part of the model. How does a neural network learn? Or even starting simpler, how does a perceptron learn? Let us think of the four key features that make up a perceptron. The input comes from a set of previously collected data, the output either also comes from previously collected data (in case of training) or it is to be figured out (in case of testing), and the activation function is selected before running the algorithm. In other words, the input is set, the output is set, and the activation function is set. Thus, learning happens at the weights as shown in Figure 6.

![Simple neural network](image)

**Figure 4:** Simple neural network.

![Weights to be determined during training stage](image)

**Figure 6:** Weights to be determined during training stage.

In brief, the algorithm starts by setting the weights to small random numbers. During the training stage, it then finds the output using those weights and compares it to the target (solution) and then comes up with better weights to match the target more accurately. This training technique, called backpropagation, results in a set of weights that allows the neural network to produce the correct outputs to the inputs used in training. It is now ready to be tested on new inputs. The neural network provides an effective method to create intelligent systems that can learn.
2.3. Input Data

In our research, angles in a given sitting posture are randomly generated. These angles will then serve as the input set to our artificially intelligent algorithm. These featured angles are listed in Table 1 which leads to assumption 1, which is presented in the appendix.

Table 1: List of input features of interest.

<table>
<thead>
<tr>
<th>Input Features of Interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Knee Angle</td>
</tr>
<tr>
<td>2. Ankle Angle</td>
</tr>
<tr>
<td>3. Trunk Inclination Angle</td>
</tr>
<tr>
<td>4. Elbow Angle</td>
</tr>
<tr>
<td>5. Hip Angle</td>
</tr>
<tr>
<td>6. Leg Crossing</td>
</tr>
<tr>
<td>7. Upper Arm Angle</td>
</tr>
<tr>
<td>8. Lower Arm Angle</td>
</tr>
</tbody>
</table>

Each of these features have a healthy range which is measured by the Canadian Centre for Occupational Health and Safety. Figure 7 shows the configurations of the healthy ranges of the sitting posture features or feature angles listed Table 1. Table 2 summarizes the healthy ranges of the sitting posture features that are of interest.
Table 2: Healthy ranges for sitting posture features of interest.

<table>
<thead>
<tr>
<th>Sitting Posture Feature</th>
<th>Healthy Angle Ranges (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Angle</td>
<td>90–130</td>
</tr>
<tr>
<td>Ankle Angle</td>
<td>100–120</td>
</tr>
<tr>
<td>Trunk Inclination</td>
<td>0–30</td>
</tr>
<tr>
<td>Elbow Angle</td>
<td>90–120</td>
</tr>
<tr>
<td>Hip Angle</td>
<td>90–120</td>
</tr>
<tr>
<td>Forearm Angle</td>
<td>0–20</td>
</tr>
<tr>
<td>Upper Arm Inclination</td>
<td>0–30</td>
</tr>
</tbody>
</table>

Some people tend to cross their legs while sitting. Crossing legs has three types which are:

Ankle-to-Ankle  Ankle-to-Knee  Knee-to-Knee

Ankle-to-Ankle means resting an ankle on another ankle. Ankle-to-Knee is when a subject rests an ankle on a knee. Knee-to-Knee means resting a knee on the other knee [8]. For each feature, an “Unhealthiness Value” is to be assigned. The unhealthiness value or level is a number that suggests how unhealthy a given posture angle is, leading to the second assumption. The unhealthiness values of the leg crossing types were assumed to be those shown in Table 3.

Table 3: Unhealthiness values of leg crossing.

<table>
<thead>
<tr>
<th>Leg Crossing</th>
<th>Unhealthiness Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Crossing</td>
<td>0</td>
</tr>
<tr>
<td>Ankle-to-Ankle</td>
<td>10</td>
</tr>
<tr>
<td>Ankle-to-Knee</td>
<td>20</td>
</tr>
<tr>
<td>Knee-to-Knee</td>
<td>30</td>
</tr>
</tbody>
</table>

2.4. Target of Learning Stage

Since neural networks are a type of supervised learning, the targets (solutions) must be provided along with the inputs for the algorithm to learn to generalized and prepare itself to give answers to new inputs. The supervised nature of neural networks divides the assessment process in two stages: training and testing. In the training stage, the algorithm is trained by being shown the inputs and targets (the problems and solutions). In the testing stage, the algorithm is shown new inputs (problems only) from which it needs to intelligently provide targets (answers) to. The features mentioned in Table 1 serve as the inputs. The question we seek to answer is: in the training stage of the neural network, what are the targets that are fed into the algorithm along with the inputs?

We already know that an input is simply a collection (in fact, a vector) of feature angles (such as upper arm angle, lower arm angle, etc.). However, what is the target?
In other words, we have the problem set, what are the solutions so the algorithm can learn to generalize?

To get a good sense of the unhealthiness of a given sitting posture, we make use of the fact that within a given range of angles a certain feature (such as upper arm angle) is considered healthy. As we deviate from this healthy range the unhealthiness level increases. Figure 8 portrays this idea [leading to assumption 4].

![Unhealthiness Level](image)

**Figure 8:** Unhealthiness level of a particular sitting posture feature angle.

We notice that the unhealthiness level of an angle that is within the healthy range is zero. If there are \( N \) features, then there are \( N \) of such plots, each of which will give us an unhealthiness value for a given feature angle. A vector is constructed from a set of angles measured from an individual. This vector serves as the input set. Each angle in the vector is then converted to an unhealthiness value. The obtained values are mapped to a range from 0 to 10. The sum of these numbers can also be mapped to a range from 0 to 10, yielding the Overall Unhealthiness Value, \( R \). Thus, a given sitting posture in which all the features are within the healthy range will have \( R = 0 \). The mathematical equation that is used to generate the targets for a given input (both of which are used for the learning stage of the network) is presented in **Equation 1** [which leads to assumption 5].

\[
\sum_{i=1}^{n} y_i \rightarrow \{0 \leq y \leq 10\} = R = \text{Unhealthiness Value} \tag{1}
\]
where

\[-\tan \phi (\theta_1 - L_{1,1}) + \tan \phi (\theta_1 - L_{1,1}) u(\theta_1 - L_{1,1})
+ \tan \phi (\theta_1 - L_{1,2}) u(\theta_1 - L_{1,2}) \rightarrow \{0 \leq y \leq 10\} = y_1,\]

\[-\tan \phi (\theta_2 - L_{2,1}) + \tan \phi (\theta_2 - L_{2,1}) u(\theta_2 - L_{2,1})
+ \tan \phi (\theta_2 - L_{2,2}) u(\theta_2 - L_{2,2}) \rightarrow \{0 \leq y \leq 10\} = y_2,\]

\[\vdots\]

\[-\tan \phi (\theta_n - L_{n,1}) + \tan \phi (\theta_n - L_{n,1}) u(\theta_n - L_{n,1})
+ \tan \phi (\theta_n - L_{n,2}) u(\theta_n - L_{n,2}) \rightarrow \{0 \leq y \leq 10\} = y_n\]

where \(\theta_n\) is the \(n\)th input feather, \(\phi\) is the intensity, \(L_{n,1}\) and \(L_{n,2}\) are the limits of the healthy region, (from Figure 8, and \(u(\theta_n)\) is the unit step function [see assumption 6].

Since we are looking at only 7 features (see Table 1 or Table 2), \(n\) will be 7 (for leg crossing unhealthiness values, refer to Table 3). This target gives a good sense of how unhealthy a given sitting posture is. Now, we are able to train the neural network (given that we now have inputs and their corresponding targets that are the two essential components for the training stage of any supervised machine learning algorithm). Figure 9 presents a compact illustration of the training and testing stages of the algorithm.

![Figure 9: Training and testing stages of machine learning algorithm.](image)

Note that the input data was not collected from actual individuals, but was randomly generated instead in order to answer the question, "Can we use neural networks along with the target function conceived above to have the algorithm learn to assess new test cases?" within the timeframe available.

### 3. Method

The neural network was programmed in Matlab. The code took an input matrix and a sample matrix. The input matrix was made up of column vectors of sitting posture angles. Each vector had 8 instances. In other words, each column represents a single individual and each row is a feature – such as knee angle, lower arm angle, etc. The angles were randomly generated also in Matlab. The code uses this input matrix to
generate a target vector using the target function conceived (see Figure 8). Both the input matrix and target vector were used to train the neural network. The neural network consisted of 10 hidden layers. This neural network is illustrated in Figure 10.

Figure 10: Neural network with 10 hidden layers.

After training the neural network, test cases (test postures) were assessed to see whether or not the neural network has successfully generalized to the data and is able to assess the unhealthiness level of given sitting postures.

4. Results and Discussion

Input data were randomly generated that represent the sitting posture angles of 135 individuals in Matlab. The result is shown in Figure 11. The $x$-axis represents the 8 feature angles (in the order listed in Table 1).

Figure 11: Randomly generated input data that represent the 8 features of 135 individuals.

The input data was used to create the target vector (using the target function) that contained unhealthiness values of each individual in the input matrix. Both, the input matrix and the target vector, were inputted into the neural network constructed using
the Neural Network Toolbox in Matlab. The plots below in Figure 12 show that the slope of the curves are almost 1, which indicates that the algorithm has successfully learned from the input.

![Training: R=0.97251](image1)

![Validation: R=0.8727](image2)

![Test: R=0.87392](image3)

![All: R=0.93379](image4)

**Figure 12:** Neural network training results.

Next, we tested the algorithm using four test cases. The test set is tabulated in Table 4. The four columns represent four hypothetical subjects. The first and second column contain feature angles all within the healthy ranges. However, the third column represent an unhealthy (bad) case and the fourth column represents the worst of the four.

Inputting those values into the neural network and prompting for the Unhealthiness Values, we obtained 0, 0, 3, 10 for the four hypothetical subjects, respectively. These results matched the theory we developed. It indicated that the first two subjects are sitting in a healthy posture while the third is sitting in an unhealthy posture. The fourth subject is the unhealthiest with respect to the other three. The neural network successfully gave results that help in the assessment of the healthiness (or unhealthiness) of sitting postures.
Table 4: Neural network testing subjects.

<table>
<thead>
<tr>
<th>Individual</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Angle</td>
<td>100</td>
<td>120</td>
<td>140</td>
<td>20</td>
</tr>
<tr>
<td>Ankle Angle</td>
<td>120</td>
<td>115</td>
<td>130</td>
<td>20</td>
</tr>
<tr>
<td>Trunk Inclination Angle</td>
<td>20</td>
<td>10</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Elbow Angle</td>
<td>100</td>
<td>110</td>
<td>130</td>
<td>150</td>
</tr>
<tr>
<td>Hip Angle</td>
<td>100</td>
<td>110</td>
<td>135</td>
<td>150</td>
</tr>
<tr>
<td>Crossed Leg</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Upper Arm Angle</td>
<td>10</td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Lower Arm Angle</td>
<td>10</td>
<td>5</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

5. Conclusion

A neural network consisting of 10 hidden layers was used to assess the healthiness of an individual’s sitting posture. The Unhealthiness Value was used as a measure of how unhealthy an individual’s sitting posture is. A set of input data was randomly generated in Matlab. The targets were generated using Equation 1. Both the input data and the targets were input to the neural network. The algorithm was programmed in Matlab. Results showed that the neural network successfully learned from the input data and corresponding targets. The algorithm was then tested on four hypothetical cases of sitting postures. The neural network successfully assessed the unhealthiness level of the four cases. Thus, neural networks can be used to assess the healthiness of human sitting posture. The algorithm can be used to learn from real complex data and be integrated in smart chairs that might alert the user to sit in a healthy manner. It is important to note that the algorithm has limitations due to the fact that the healthiness of sitting postures depend on various aspects that were not considered in this research. These include age of the individual, duration of sitting, and physical injuries [7, 9, 10].

References


6. **Appendix**

6.1. **Assumptions**

1. Features that are vital to the healthiness of a sitting posture are assumed to be only the ones listed in Table 1.

2. Features are assumed to have a corresponding Unhealthiness Value or Level depending on their angle.

3. The unhealthiness values for leg crossing postures are assumed to be those listed in Table 3.

4. The unhealthiness value of any feature is assumed to follow Figure 8.

5. The target is assumed to follow Equation 1.

6. All features are assumed to have equal intensity $\phi$. 
Crab wars: testing the ideal free distribution with invasive *Carcinus maenas* and native *Hemigrapsus nudus*

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Abstract

Invasive species can cause changes in community composition, native species’ habitat acquisition and reduce abundance of native species. The Ideal Free Distribution (IFD) provides a conceptual framework for describing intraspecific distributions of individuals and can be modified for interspecific interactions to correct for differing competitive ability. We examined whether the IFD of *Hemigrapsus nudus* (*H. nudus*), native to the west coast of North America, varies with the introduction of invasive *Carcinus maenas* (*C. maenas*) with respect to food availability. We tested this experimentally by constructing artificial habitats with patches of high and low food availability and monitoring the distributions and feeding rates of *H. nudus* and *C. maenas* among these food patches. *C. maenas* was six times more competitive in acquiring food than *H. nudus*. Based on this foraging discrepancy, spatial distributions between food patches did not follow those predicted mathematically by the IFD. *H. nudus* did not distribute ideally in terms of food, while *C. maenas* did. Thus, the ability of *C. maenas* to ideally distribute combined with its high food acquisition rate, has the potential to affect *H. nudus* survival with the spread of *C. maenas* in the Pacific Northwest.

**Keywords** — *Carcinus maenas*, Ideal Free Distribution, *Hemigrapsus nudus*, interference model, marine invasions

1. Introduction

Human activities such as trade and transport have caused unprecedented rates of change in biotic systems in part by facilitating the spread of invasive species. Introduced species that become invasive can cause “invasional meltdowns” where invasive species can shift community composition and displace native species [1, 2, 3, 4]. One such mechanism by which these shifts may occur is by increased competitive pressure from invasive species [5]. In particular, invasive geckos have been shown to monopolize clumped resources thereby displacing native species [6]. As well, invasive species across taxa – plants, crustaceans, and vertebrates – often show superior competitive ability and use this ability to exclude native species from resources [7, 8, 9]. In these scenarios, unequal competitive ability is shown to be an important driver in predicting invasion success.

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Animals distribute themselves across landscapes as they attempt to optimize their access to spatially distributed resources [10]. These distributions are commonly described with models, such as the Ideal Free Distribution (IFD) [11], which describes how individuals distribute based on intraspecific competition between habitats of varying resource availability [12, 13]. The IFD assumes individuals move "ideally", such that they are optimizing for a resource (assuming complete environmental knowledge), and "freely", are not hindered in their movement by territoriality or physical barrier [11]. The model predicts that individuals are optimally distributed between good and poor habitats such that individuals have equal fitness when the number of individuals in a patch matches the resource abundance in said patch [11].

Traditionally, the IFD only predicts distributions based on intraspecific competition among individuals with equal competitive ability – often it is only this simpler model that is verified ecologically [10, 12, 14, 15]. However, the basic IFD can be expanded into an interference model where, in the absence of predatory interactions or physical interference, unequal competitors from different species can still distribute in an ideal free manner. In order to expand the model to include species of differing competitive ability, individuals are defined as competitive units (how likely an individual is to monopolize resources), where competitive units, rather than the number of individuals, are matched to resource abundance [16]. This expansion allows the IFD to be used as a predictive framework to model how invasive species with greater competitive ability may affect distributions of native species [17, 18].

One particularly successful invasive species in coastal marine communities is *Carcinus maenas* (European green crab), which has been introduced globally and is responsible for drastic changes in community composition among bivalves and crustaceans [3, 19, 20]. In their native range off the Atlantic coasts of Europe and North Africa, green crabs grow 6-10 cm across their carapace and may eat bivalves, gastropods, crustaceans, and seaweeds [21, 22, 23]. In their invasive range, *C. maenas* follow the IFD [24], colonize intertidal habitats with mud, sand, or rocky substrate [21] and often show higher competitive ability than native species [20]. As an invasive species, *C. maenas* contributed to the crash of the *Mya arenaria* (soft-shell clam) fishery [21] and McDonald et al. [3] found that increasing abundance of *C. maenas* corresponded to decreased recruitment and habitat displacement of the native *Metacarcinus magister* (Dungeness crab).

Due to the global expansion of *C. maenas*, researchers have examined their competitive ability and their impacts on native species. One study in Newfoundland experimentally examined *C. maenas* competition with the native *Cancer irroratus* and showed the presence of green crabs reduced *C. irroratus* foraging time, especially in competitive interactions between smaller individuals [25]. Other studies showed that juvenile lobsters spend significantly less time foraging in the presence of *C. maenas* [26] and that *C. maenas* have the potential to outcompete *Callinectes sapidus* (blue crabs) and *Hemigrapsus sanguineus* (Japanese shore crab) during competition experiments over food [3]. These studies, however, lack direct evaluations of *C. maenas*’ competitive advantage over native species under variable food availability and do not examine how competitive advantage of *C. maenas* affects distributions of native species across rich and poor habitats [27].
We tested how the IFD of native *Hemigrapsus nudus* Dana 1851 (purple shore crabs) change in the presence of *C. maenas*. *H. nudus* are native from Alaska to Baja California, inhabit rocky substrate in the mid to high intertidal [28]. They are dietary generalists that mainly feed on diatoms, green algae as well as scavenged prey [29, 30]. Although *C. maenas* and *H. nudus* ranges do not directly overlap at the present in British Columbia, as *C. maenas* continues to spread the two species will come into direct contact [31].

We predicted that *C. maenas* are more efficient at monopolizing resources than *H. nudus* and attempted to quantify this difference. We then used an interference IFD model to predict the most probable distribution of *C. maenas* and *H. nudus* between good and poor quality patches and tested the model experimentally in an artificial laboratory setting to see if empirical results matched theoretical IFD predictions. These experiments highlight the potential impacts of *C. maenas* on the ability of *H. nudus* to access resources and will provide further insight into the IFD’s effectiveness at predicting interspecific distributions.

2. Results

2.1. Crab Wars: Determining the relative competitiveness of *H. nudus* and *C. maenas*

We determined that *C. maenas* spent significantly more time eating than *H. nudus* (*p*-value < 0.001; Figure 1). On average *C. maenas* spent 27.1% of their time feeding, six times more than *H. nudus* (Figure 1). We confirmed this 6:1 ratio with observations in artificial habitats used in later experiments and got a comparable ratio of 5:1.

2.2. Interference Model

We used our results from determining the relative competitive ability to express *C. maenas* in terms of competitive units of *H. nudus*. We then constructed an IFD interference model predicting the most probable distribution of individuals between patches of high and low quality. With three *C. maenas* and six *H. nudus*, the model predicted the most probable distribution would be two *C. maenas* and six *H. nudus* in the good patch, and one *C. maenas* and no *H. nudus* in the poor patch (Table 1). Thus, with repeated trials, we expected this to be our average distribution. This is not what we saw, instead observing an average of one *C. maenas* and two *H. nudus* in the good patch and two *C. maenas* and four *H. nudus* in the poor patch (Table 1).

**Table 1:** Model predictions and experimental observations of *C. maenas* and *H. nudus* distributions among good and poor patches (*N* = 3, averages ±0.33 absolute error).

<table>
<thead>
<tr>
<th>Number of <em>C. maenas</em> in good patch</th>
<th>Number of <em>C. maenas</em> in poor patch</th>
<th>Number of <em>H. nudus</em> in good patch</th>
<th>Number of <em>H. nudus</em> in poor patch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted</td>
<td>Observed*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

*rounded to nearest whole number averages
2.3. Determining the distribution of *H. nudus* and *C. maenas* between resource patches

To verify the "free" assumption of the IFD, we examined the distribution of *C. maenas* and *H. nudus* in the good resource patches from our experimental results. We compared the relationship between the number of *C. maenas* and *H. nudus* in a given good patch (Figure 2) over the course of our experiments. As predicted by the IFD, there is no relationship between *C. maenas* and *H. nudus* distributions ($r^2 = 0.042$) and they do not distribute with respect to each other.

To determine if both species of crab distributed based on food availability, we compared the ratio of crabs in the good to poor patches (Figure 3). There was no significant difference in the ratio of *H. nudus* or *C. maenas* present in the good patches across the varying densities of *C. maenas* and constant levels of food ($p$-value = 0.41 and $p$-value = 0.256 respectively; Figure 3). The ratio of *H. nudus* in the good to poor patch was unchanging across trials and was about 1:1 – 50% of *H. nudus* were in the good patch and 50% were in the poor patch – implying they did not distribute based on food availability (Figure 3A). As well, note that this ratio matches that observed when there are zero *C. maenas* in the experimental system, which is our null model and mimics a natural system in which there are no *C. maenas*. This reaffirms that *H. nudus*
do not change their distribution based on the presence *C. maenas*. This was counter to our prediction that increasing the number of *C. maenas* would shift the IFD of *H. nudus* based on the relative competitive ability of the two species.

However, the ratio of *C. maenas* in the good versus poor patch was significantly lower in trials with excess food than with limiting food (*p*-value = 0.034; Figure 4). Since the amount of food was constant, the number of *C. maenas* in the system determined whether food was limiting (six, seven, or nine *C. maenas*) or in excess (three and nine *C. maenas*). In treatments with excess food, the ratio of *C. maenas* in the good to poor habitat is about 1:1. In treatments with limiting food, the ratio of *C. maenas* in the good to poor habitat is about 2.5:1, thus showing that, depending on food availability, *C. maenas* will distribute based on food as predicted by the model.

### 3. Discussion

As has been suggested by other research, *C. maenas* shows higher competitive ability than species in the ranges in which it invades [3, 20]. As we showed in our results, *C. maenas* also shows higher competitive ability than *H. nudus* and is better able to monopolize resources. This suggests that when both species are size-matched and are directly competing for the same food source *C. maenas* will be able to outcompete *H. nudus*. However, both *C. maenas* and *H. nudus* are dietary generalists [21, 22, 23, 30, 31]. This suggests that under high *C. maenas* density, if *H. nudus* is faced with direct competition from *C. maenas*, then *H. nudus* is likely to find alternate sources of food that are less preferable to *C. maenas*. This could have potential impacts on *H. nudus* populations if individuals are forced to spend more time foraging or consuming food of poorer nutrient quality.

We evaluated how the IFD of *H. nudus* changed under varying numbers of *C. maenas* and observed that *H. nudus* were freely distributed with respect to the number of *C. maenas* in a patch. We concluded this because increasing the number of *C. maenas* present in a trial did not effect *H. nudus* distribution: over all trials *H. nudus*
randomly assorted between the two good and poor quality patches. This implies that not only were *H. nudus* non-territorial to members of their own species, but also non-territorial with *C. maenas*. We are confident in our interpretation of this result, as we did not observe any defensive action or direct interaction between individuals within or between species that would have indicated territoriality.

While in our trials *H. nudus* did not distribute themselves based on the presence of *C. maenas*, behaviour of *H. nudus* might change with more exposure to *C. maenas* as a potential competitor and ultimately predator. Although we sized matched *H. nudus* and *C. maenas* in the experiments, adult *C. maenas* are more than 1.67 times as large as *H. nudus* [20] and are known to prey on juvenile *C. magister* and other crustaceans [21, 22, 23]. Thus, adult *C. maenas* could conceivably prey on *H. nudus*, but since these two species ranges to no yet overlap *H. nudus* has not developed a fear response. A predator response could shift the “free” nature of the IFD and cause *H. nudus* to distribute based on *C. maenas* avoidance. Further experiments should be conducted to evaluate this potential response and how it may limit *H. nudus* access to resources.

Although *H. nudus* distributed freely among patches, they were not distributed ideally: they did not distribute based on food availability and patch quality as predicted by the IFD. The interference IFD model did not accurately predict the distribution of *C. maenas* and *H. nudus* among good and poor patches because *H. nudus* did not distribute based on food availability, which skewed observed patterns away from the model’s theoretical predictions. In our experiments, *H. nudus* were evenly distributed among good and poor patches, thus suggesting *H. nudus* did not distribute based on food availability. During our experiments, we observed that *H. nudus* preferentially associated with corners and cover. Thus, our results indicate that *H. nudus* is sensitive
Figure 4: The ratio of C. maenas in good versus poor patches under scenarios with excess food (3 and 4 C. maenas) and limiting food (6, 7, and 9 C. maenas; t-test: \( t = -2.39, df = 11.742, \) p-value < 0.034, \( N = 6 \) for excess, \( N = 9 \) for limiting).

to stressful laboratory environments and optimizes shelter over food, thus creating an IFD based on shelter availability. Given that a variety of stressors, such as increased oceanic temperatures, decreased pH, noise pollution, and habitat loss, are expected to increase [32, 33, 34], further research should examine directly how these disturbances will change H. nudus distributions among various types of resources. As well, we only ran experiments at one density of H. nudus and with one size class of H. nudus and C. maenas, as such we suggest future research examine how general our results are across varying sizes of individuals and densities of H. nudus.

From previous studies, we know that C. maenas distributes according to the IFD [24]. Our experiments confirmed this result: C. maenas distributed based on food availability only in resource-limited conditions. This makes ecological sense; there are no fitness benefits to distributing based on relative patch quality if food is not limiting in any patch. This highlights a potential mechanism by which C. maenas is successful invader. Given that C. maenas was able to distribute according to the IFD in a potentially stressful lab environment as it does in its natural setting, we suggest that this implies C. maenas is less sensitive to changes in its environment. If this is true, this implies that C. maenas is able to be a successful invader over wide range of new habitats and environments. This
lack of sensitivity combined with a high competitive ability suggests that in situations where the environment changes, *C. maenas* will be able to outcompete more sensitive species, perhaps such as *H. nudus*.

4. Methods

4.1. Animal Care

All animals were collected in Barkley Sound, BC and experiments were conducted at Bamfield Marine Sciences Centre (BMSC). Thirty-four *C. maenas* were collected from the head of Pipestem Inlet, British Columbia (49°1’41”N 125°14’42”W), in an area sheltered from waves with muddy and sandy substrate. 37 *H. nudus* were collected from Dixon Island (48°51’ 10”N, 125°7’ 11”W) and 54 *H. nudus* from Seppings Island (48°50’ 24”N, 125°12’ 28”W). Both *H. nudus* collection sites consisted of large cobblestone beaches with sheltered wave exposure in the mid and high intertidal respectively. All individuals of both species were approximately 2-6cm long (carapace).

In the lab, three sea-tables (2.5 by 0.75m troughs filled with circulating sea-water) were used to create artificial habitats for each of our three crab populations. Cover was created by placing rocks and macroalgae (*Fucus sp*) within sea-tables. In order to ensure equal hunger levels, and thus motivation to eat, we starved crabs outside the experimental trials. Unfortunately, standardizing the length of starvation was not possible due to the limited time frame of this project and limited number of crabs. We used a mixture of *Mytilus sp*. collected from beneath the dock of the BMSC in Bamfield inlet as prey items during our experiments. We kept these in a separate sea table also filled with circulating seawater and crushed them immediately prior to use. Mussels were used within 24 hours of collection. Pieces, roughly one cm in diameter, were used as a single unit of food. All use and maintenance of crabs followed BMSC animal care guidelines and protocols (AUP# UP15-MBE-01).

4.2. Crab Wars: Determining the relative competitiveness of *H. nudus* and *C. maenas*

To determine the relative competitiveness of *H. nudus* versus *C. maenas*, we set up 30 small tanks each containing one size-matched individual from *C. maenas* and *H. nudus* [9]. We placed one mussel in each tank beneath a plastic enclosure with holes that prevented access to the mussel, but allowed the crabs to sense it. The crabs had 10 minutes to habituate, based our field observations and experiments conducted by MacDonald et al. (2001). After the habituation period, we removed the cover protecting the mussels and recorded which of the two species in each tank ate the mussel. We recorded this information at 1-minute intervals for 15 minutes. We used this information to estimate the percent of time that each individual of each species spent eating.
4.3. Building the IFD interference model

We used relative rates of consumption to express *C. maenas* in terms of *H. nudus* by constructing a conversion ratio based on food consumed by individuals of the two species. Given that *C. maenas* ate six times more than *H. nudus*, we defined one relative food unit for *C. maenas* as six pieces of mussel while one mussel piece was a food unit for *H. nudus*. This ratio allowed us to create a theoretical system in which a good patch (18 mussel pieces) had three times as many food units as a poor patch (six mussel pieces). Thus, there were enough food units in the poor patch to be sufficient for either one *C. maenas* or six *H. nudus*. The good patch had enough food units for three *C. maenas* or eighteen *H. nudus*.

We chose the 3:1 ratio between good and poor patch resource availability for both mathematical and practical purposes. Mathematically, any ratio between the good and poor patch could have been used to predict distributions. However, we excluded some possible ratios due to small effect size, or because they predicted randomness which we would not be able to distinguish from an insignificant result. We also excluded ratios that required more individuals than to which we had access in the lab. Thus the 3:1 ratio was the only ratio that was experimentally plausible as well as having a single outcome that was vastly more probable than other outcomes.

From this, we constructed a mathematical model to determine all the possible combinations of the distributions of three *C. maenas* and eighteen *H. nudus* among good and poor patches where each individual would have access to a single relative food unit. We used combinatorics to determine the number of ways all possible distributions could be constructed when individuals maintain access to one single relative food unit (six mussel pieces for *C. maenas*, one mussel pieces for *H. nudus*). Thus, the most probable outcome expected under the IFD is the one that had the most possible ways to be constructed.

4.4. Formula for determining the combination model:

\[ n(C. maenas \text{ total})C_n(C. maenas \text{ in good}) \cdot n(H. nudus \text{ total})C_n(H. nudus \text{ in good}) \]

4.5. Experimentally testing the IFD interference model

We designed and set up a lab system with three sea-tables (troughs filled with circulating sea-water) to test both the distributions predicted by the interference model and those theorized by the IFD. We created good and poor quality patches by placing crushed mussel (one piece of mussel was one food unit) in trays at either end of the sea-tables, approximately 2.25m apart. We covered the food with a porous plastic container so the crabs could sense the food, but not eat it, and waited for a 10-minute habituation period before removing the covering. We placed three *C. maenas* and six *H. nudus* (equivalent to one green crab’s feeding capacity), in the center of each sea-table. We then recorded the distribution of the crabs in the good and poor patches every twenty minutes for one hour, for each of the three tanks. This generated an average distribution of individuals for each of the three replicated tanks. We compared these experimental distributions to the theoretical predictions generated by the IFD interference model.
4.6. Testing how the distribution of *H. nudus* changes with variable *C. maenas* density

We repeated the above experiment five times with varying numbers of *C. maenas*. We ran the experiment with zero, three, four, six, or nine *C. maenas*, each replicated three times, to gain an understanding of how the animals would distribute under a gradient of *C. maenas* densities. Zero *C. maenas* was our control: it represented an environmental system without the introduction of *C. maenas*. We chose to run the experiment with six *C. maenas* to mimic a scenario with equal numbers of both species and nine to mimic a system with the high green crab densities observed in some areas of the PNW. The other numbers of *C. maenas* were chosen to fill out the gradient from zero to nine. With four, six, or nine *C. maenas* there was less total food in a sea-table than competitive units of crabs. At zero or three *C. maenas* the food was in excess. We kept the number of *H. nudus* constant at six individuals and did not vary the amount of food present. In the final trial, in which there were nine *C. maenas*, we also recorded how many of each species were feeding in each patch. This allowed us to confirm the relative competitiveness determined from our prior experiments.

4.7. Statistical Analysis

All our statistical analysis was done in R, version 3.1.2.

To determine the relative competitiveness of *C. maenas* and *H. nudus* and their percent time feeding, we tallied the number of times each individual ate during the 15 scans and divided this number by the total number of minutes in each scan sample. A two-sample Welch’s *t*-test was then used to compare the percent of time spent feeding of *H. nudus* and *C. maenas*.

To test whether *H. nudus* were distributing freely we used simple linear regression to show how the number of *H. nudus* in a patch changed with number of *C. maenas* in a patch. If individuals were distributing freely we predicted no trend.

To determine the proportion of individuals of each species present in a good patch, we divided the number of individuals of that species found in the good patch by the number of individuals of that species in the poor patch. We used ANOVA with log transformation to test whether the ratios of each species in a good patch varied across the trials of differing numbers of *C. maenas*. We also aggregated our data based on treatments with excess and limiting food. We used a two-sample Welch’s *t*-test to compare the ratios of *C. maenas* between the trials with limited and excess resources.

5. Conclusion

Although the IFD failed to predict the distributions of *H. nudus*, ecologically, our results still have implications for how *C. maenas* could affect the survival of *H. nudus*. Based on our results in stressful lab conditions, *C. maenas* distributed based on food availability whereas *H. nudus* did not. Even if this result is an artefact of *H. nudus* taking longer to acclimate to lab conditions or preferring to forage under different light or temperatures, our result still indicates that *H. nudus* are more sensitive to change than *C. maenas*. This implies that in natural environments with added stressors, such as habitat disturbance
or climate change, *H. nudus* potentially prioritize shelter, not food, while *C. maenas* prioritize food. As well, *C. maenas* consume available food faster than *H. nudus*. Thus, based on our data we expect that in resource-limited conditions with high densities of *C. maenas*, they will outcompete *H. nudus* for food with which both species have dietary overlap. Our results indicate that that *C. maenas* is less sensitive to change than *H. nudus* and so have more access to food and will eat it quicker, which has potential ramifications for survival of *H. nudus* populations as coastal systems continue to change.

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**References**


Potential impact of secondary wastewater treatment plant effluent on the concentration and antibiotic resistance of bacteria in river water

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Abstract

Effluent from wastewater treatment facilities can have a major impact on the bacterial populations in water downstream of the outfall point. We sought to assess the impact of wastewater effluent from the Northwest Langley Wastewater Treatment Plant on the concentration of bacteria and the occurrence of antibiotic-resistant bacteria in the Fraser River. We hypothesize that effluent from the plant will increase the amount of antibiotic-resistant bacteria downstream of the plant. In order to assess this, we took five samples of water downstream and five samples of water upstream from the treatment plant’s outfall point and cultured the bacteria in these samples on Mueller Hinton agar, with half of the agar plates containing Ampicillin. We then counted the number of bacterial colonies that grew on each plate. Our results displayed that there were the same amount of bacteria downstream compared to upstream from the wastewater outfall point. This may be because secondary wastewater treatment is effective enough to remove antibiotics and other toxins from the wastewater effluent. We also observed that antibiotic resistance can be detected in the bacteria before they reach the wastewater effluent. This may be due to exposure to other compounds in the water or from changes in the river flow patterns that allow for the upstream bacteria to come in contact with the effluent. Our results suggest that substances present in wastewater effluent in the Fraser river do not reduce the quantity of bacteria in river water.

Keywords — Ampicillin, Sewage treatment, Aquatic bacteria, River contamination

1. Introduction

Treatment of sewage water is important in urban societies for preserving the quality of civic water. Current sewage treatment methods, especially in secondary and tertiary wastewater treatment plants (WWTPs), were found by Environment Canada [1] to be effective at treating sewage; secondary treatment removes approximately 85% of suspended solids and biochemical oxygen demand (BOD), while tertiary treatment removes as much as 99% of wastewater impurities. Suspended solids refers to large visible solid particles in the effluent. BOD accounts for the amount of oxygen dissolved in the river water used by aerobic organisms at 20 °C in five days. These

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conventional metrics do not account for other problematic pollutants. Many studies raise concerns about the occurrence of biologically active substances, specifically antibiotic substances, in river water downstream from WWTPs [2, 3, 4]. While it is unlikely that the treatment plants themselves are the sources of resistance, as they do not use antibiotics in their filtration process, it is possible that they do not remove all of the antibiotic substances that were in the water beforehand [1]. Antibiotics from wastewater effluent have been measured in rivers at distances of up to 500m downstream from WWTPs [3]. This may have impacts on the sensitivity of the local micro-organisms to these antibiotics, thus rendering them less effective. A study conducted by Drury et al. [5] has shown a significant decrease in the overall number of bacteria downstream of two secondary WWTPs in two rivers in Chicago, IL. They hypothesize that unfiltered antibiotic substances from WWTP effluent may play a role in this decline in bacterial concentration.

Conventional medical treatment practices that promote the overuse of antibiotic agents has contributed to an increase in antibiotic resistance, which may have deleterious consequences for the future of biomedicine [5, 6]. The increased exposure of aquatic bacteria to antibiotics due to WWTP effluent may increase the prevalence of antibiotic-resistant bacteria (ARB) in receiving bodies of water. Indeed, numerous studies have demonstrated an increase in the quantity of aquatic ARB downstream from a WWTP when compared to bacteria upstream from a WWTP [2, 3, 7, 8, 9, 10]. However, there is a lack of research investigating the effect of WWTP effluent on the bacteria at the North Langley WWTP. Indeed, this is the first study to investigate the impacts of a WWTP on bacteria or the implications for antibiotic resistance in British Columbia.

Figure 1: Comparison of the number of bacterial colonies on non-antibiotic plates from the Downstream site and the Upstream site with error bars for 95% confidence intervals.

This study compares (I) the concentration and (II) the antibiotic resistance of bacteria
2.8 km upstream and 1.7 km downstream of the Northwest Langley WWTP—an urban secondary WWTP on the Fraser River in British Columbia, Canada. We chose to test at these locations because they were the most accessible. To determine bacterial concentration and resistance to antibiotics, water samples were taken from these locations and plated on Mueller-Hinton agar, half of which contained ampicillin. We hypothesize that there will be (I) a decrease in overall concentration and (II) an increase in antibiotic resistance in the bacteria downstream from the WWTP.

2. Results

2.1. Comparison (I): Non-antibiotic plates

Figure 1 shows that there was no significant difference in the number of colony forming units (CFUs)/mL on non-antibiotic plates from the Upstream site than the Downstream site; \( t(8) = 1.1339, p = 0.2897 \).

2.2. Comparison (II): Antibiotic plates

We found that there was no significant difference in the number of ARB between the Upstream site and Downstream site (Figure 2); \( t(8) = 0.5108, p = 0.6233 \).

2.3. Comparison (III): Upstream plates

We found that there was no significant difference in the average number of culturable bacteria versus ARB at the Upstream site; \( t(8) = 0.8660, p = 0.4117 \).
2.4. Comparison (IV): Downstream plates

We found that there was no significant difference between average culturable bacteria versus ARB at the Downstream site; $t(8) = 0.5523, p = 0.5958$.

3. Discussion

3.1. Comparison (I)

The results suggest that secondary treatment does not reduce the number of bacteria. This may mean that the WWTP does not release any substances that could be toxic to the bacteria in the Fraser River (Figure 1). This result may also suggest that secondary treatment is effective at removing antibiotics substances from wastewater. Another possible explanation could be that our testing site was far enough downstream for the WWTP that the effluent no longer had an effect on the CB. However, it is unknown whether this decrease in bacterial concentration is due to the WWTP or to other factors such as nutrient levels, interactions with other organisms, or sunlight exposure. Additionally, it is possible that the bacteria at both sites were already resistant to ampicillin prior to their exposure to the effluent.

Our results from comparison (I) (Figure 1) are contradicted by that of Drury et al. [5] who found a significantly lower quantity of CB downstream compared to upstream from two WWTPs. Their study collected river water and sediment at sites upstream and downstream from a major urban secondary WWTP, as well as a suburban secondary WWTP. They streaked these samples onto soy extract agar and assessed the quantity of
bacterial colonies that grew. Drury et al. [5] suggested that the decrease in bacterial populations in river water that contains wastewater effluent may be attributed to the increase in the inorganic substances in the river water. They found that the wastewater significantly increased the amount of nitrate, ammonium, and phosphate in the river water and sediment downstream from both WWTPs. It was suggested that some of these compounds may be toxic to the indigenous bacteria in the water. Although our study did not examine these compounds, this offers an alternative explanation to our antibiotic-based hypothesis. However, since our results suggest that there is no change in the quantity of CB, it may be the case that the Downstream site is too far from the WWTP for these inorganic compounds to be at a high enough concentration to have any deleterious effects on the bacterial populations. Alternatively, these contrary results may be attributed to the discrepancies in our methodology. For instance, we used Mueller-Hinton agar as opposed to soy agar because it accommodates a wide range of bacterial species. Future studies should be undertaken at the Northwest Langley WWTP to determine if the bacterial compositions between the upstream and downstream locations differ. Furthermore, since we tested different WWTPs than Drury et al., there may be variable levels of effluent output, which may have influenced the results.
Our findings are also contradicted by that of Wakeline et al. [7], who observed a higher quantity of CB downstream compared to upstream from a WWTP. Their study used the chloroform extraction technique [7] to quantify the amount of bacteria at each test site, compared to our agar-streaking technique, which may explain why their study observed a statistically significant increase in CB downstream compared to upstream from the WWTP. Additionally, their study tested the receiving waters at a maximum of 1.04km from the effluent outfall point, whereas our study collected water 1.7km downstream of the outfall point. Interestingly, they observed greater levels of bacteria at closer distances than our study did. This may indicate that the wastewater effluent does not affect bacterial concentration at greater distances from the outfall point. It is possible that this is because the effluent has become too diluted to have a significant impact. The discrepancies in our results may also be due to our different sampling methods. While we took samples of the river water mixed with some sediment, Wakelin et al. took samples of sediment that were dried before analysis exclusively. This could suggest that bacterial composition in the sediment is impacted more so than that of the surrounding waters. The results of this study also contradict that of Drury et al. [5], which may indicate that there are other factors influence bacterial concentration that have not been accounted for. Firstly, there is a substantial amount of variety between bacterial communities in general, which may explain why it is difficult to directly compare these results. Furthermore, the size of the WWTPs and other geographical factors may be influencing the results.

The quantity of bacteria in an aquatic environment may have important implications for the broader ecosystem, such as the exchange of organic material and nutrients among organisms [11] and the ability of the ecosystem to exchange CO$_2$ with the atmosphere [12]. Since some studies have found that primary WWTP effluent can significantly decrease the quantity of CB downstream from a WWTP [5], and our study suggests that secondary WWTP does not have this effect, it may be beneficial for all municipalities to upgrade their WWTPs to secondary treatment status. However, since one study on a secondary WWTP did show an increase in CB downstream [7], it is evident that further research needs be done to distinguish if this correlation is actually due to the presence of the WWTP effluent.

3.2. Comparisons (II, III, IV)

The results from Figure 3 indicate that there is no significant difference in the number of culturable antibiotic-resistant bacteria (CARB) compared to CB in the upstream site. This suggests that the bacteria in the river upstream from the wastewater treatment plant are already resistant to Ampicillin before they come in contact with the effluent. If the bacteria are indeed resistant to Ampicillin before they reach the wastewater effluent, then it is difficult to determine if the effluent is having any impact on the resistance of the bacteria to antibiotics. The presence of antibiotic resistant bacteria in river water has been well-documented in various rivers in the United States [13] and Tokyo [14], however, this is the first study to identify Ampicillin resistance in a west coast Canadian river. A study by Ash, Mauch, and Morgan [13] found that 98% of the bacteria from 22 sample sites were resistant to Ampicillin. Furthermore, they found that the resistance was plasmid-borne, which may suggest why it is observed with such ubiquity. While
we did not conduct any genome analyses on our sample bacteria, it is possible that they have developed and transferred antibiotic resistance through a similar mechanism.

Similarly, we did not observe a significant difference in the number of CARB compared to CB at the Downstream site (Figure 4). While a direct comparison between the two sites cannot be made due to potential differences in the environments at the two sites, Ash et al. [13] suggest that variations in temperature and pH does not correlate to Ampicillin resistance. This corroborates the hypothesis that the bacteria in the water are already antibiotic resistant. However, this does not definitively rule out any potential impacts of the wastewater effluent on the bacteria. If the bacteria are indeed resistant to Ampicillin prior to contact with the effluent, they may still be affected by other antibiotics that were not considered in this study. A study by Costanzo et al. [3] found that bacteria downstream from a WWTP developed resistance to six different antibiotics that were detected in the wastewater effluent, including ciprofloxacin, tetracycline, ampicillin, trimethoprim, erythromycin and trimethoprim/sulphamethoxazole. Further research should be conducted on the Fraser River to see if the bacteria near the Northwest Langley WWTP are resistant to other antibiotics in addition to Ampicillin and test to see if these antibiotics are present in the effluent.

Furthermore, it is possible that the observed resistance to Ampicillin is indeed due to exposure to antibiotics in the effluent of the river. The water may not flow in a direct downwards direction at all times, depending on the season, water volume, and wind [15]. If this is the case, then it is likely that the effluent can have impacts on the bacteria upstream from the WWTP as well as downstream. In addition to coming in contact with antibiotics in the WWTP, it is possible that the bacteria may come into contact with antibiotic-resistant strains in the downstream waters, giving them the opportunity to pass on the resistant genes via transmissible agents like plasmids. However, it is currently unknown whether the river water flow patterns vary drastically enough for any mixing to take place between sites that are large distances apart. We recommend that future studies conduct genome analyses on the bacteria at the upstream and downstream sites to identify if they are indeed resistant to Ampicillin and if this resistance is carried on the same plasmid.

However, there are numerous other factors that may be contributing to the apparent resistance of the bacteria to Ampicillin. Firstly, it is possible that the bacteria developed resistance through a naturally-occurring intrinsic pathway [16]. Secondly, we observed that both the Downstream and Upstream sites were regular sites of human activity—both recreation and industrial. These activities offer a multitude of sources of contaminants that may be affecting the bacteria. To further investigate this hypothesis, further studies should be conducting at more remote areas of the Fraser River to determine if Ampicillin-resistance is still present.

4. Materials and methods

We collected water samples upstream and downstream of the Northwest Langley WWTP and then plated these samples onto Mueller Hinton agar containing standard nutrients required for bacterial growth. The samples were monitored for nine days and the number of colonies on the plates were recorded.
4.1. Collection of water samples from the Fraser River

On June 29th, 2015, we collected water samples from two sites that are near the Northwest Langley WWTP outfall into the Fraser River, in Langley, British Columbia, Canada. The temperature outside was 28 °C. The Downstream Site was located at the end of 104th Avenue near a ferry parking lot in Surrey, British Columbia, Canada – approximately 1.7km downstream of the wastewater outfall. We chose this distance because these sites were readily accessible for testing. The Upstream Site was located at Derby Reach Regional Park, Langley, along a recreational beach and campground site – approximately 2.8km upstream of the wastewater outfall. The shoreline at the Upstream Site was rockier than the Downstream Site.

At each location, we collected five water samples at a distance of 2.5m apart parallel to the shoreline and a depth of approximately 10cm, in sterile 50mL Greiner Bio-one Cellstar® Tubes (Cat #210-261, USA). We chose to sample water at this depth as it is more likely to contain bacteria from both the surface water, sand, and sediment [17]. We also measured the temperature of the water at each site and found both to be 21 °C using a thermometer at a depth of 10cm. To ensure that we were gathering bacteria from both the surface and bottom of the river, we mixed the water and underlying sediment using a meter stick by jabbing the sediment with the stick for approximately five seconds before collection. Then the samples were collected approximately 5cm below the surface of the water. All samples were stored in a beaker that was on ice in a cooler, at an air temperature of approximately 4 °C, to ensure that the bacteria did not overheat during transportation [3].

4.2. Preparation of Mueller Hinton agar plates

To provide a medium that could accommodate a wide range of bacteria, we prepared 60 petri dishes (Fisher 08-757-1, Fisherbrand™, Canada) with 15mL each of Mueller Hinton II Agar (Ref #211438, Fisherbrand™, USA) with a concentration of 50.16g/L. To ensure that only bacterial colonies grew on the agar, we added 0.0132g/L of crystal violet dye (Aldrich Chemical Company Inc., USA) to act as an anti-fungal agent. We chose this concentration based on a recommendation by Atlas & Snyder [18]. To determine the concentration of culturable antibiotic-resistant bacteria (CARB), we also prepared 60 agar plates following the same procedure as above, with the addition of 0.051g/L of Ampicillin A9393-5G (Lot #103M4844V, Sigma-Aldrich, Canada) based on the recommendation from the article "Addgene" [19]. To ensure that the Ampicillin was not degraded by heat or light, we kept it in a refrigerator at 4 °C and covered it in aluminum foil (Alcan, USA) until we added it to the agar solution. We added the Ampicillin to the agar solution once the agar had cooled to 55 °C, to prevent heat-degradation [11]. Additionally, the plates containing Ampicillin were covered with aluminum foil for four hours after their preparation.

4.3. Agar plating methods to determine concentration of culturable bacteria and antibiotic-resistant culturable bacteria between sites

For comparison (I), we plated 25µL of each sample onto separate Mueller Hinton agar plates containing Ampicillin. For comparison (II), we repeated the above procedure for
the Mueller Hinton plates that did not contain Ampicillin. For each sample, there were 6 replicates which included 1 original concentrated sample + 5 dilutions with a 20X dilution factor. We prepared a total of 120 agar plates. Afterwards, we stored all of the plates under aluminium foil in a dark cupboard at room temperature (20-26 °C). We chose this temperature to mimic the conditions of the Fraser River (see subsection 4.1). We counted the number of colonies on the plates nine days after inoculation.

4.4. Statistical Analysis

To determine if there is a statistically significant difference in the quantity of culturable bacteria (CB) in the Downstream site versus the Upstream site, a t-test was performed. We graphed the average number of colony-forming units (CFUs)/mL on the non-antibiotic plates from each site. We also plotted and compared the average number of CFUs/mL on the ampicillin-treated plates from the Downstream site and the Upstream site to determine if there was a statistically significant difference in the quantity of antibiotic-resistant culturable bacteria between the sites. In each graph, we included 95% confidence intervals.

5. Conclusion

Our data suggest that the concentration of naturally-occurring bacteria in the Fraser River was not significantly affected by the presence of wastewater effluent in river water downstream from a secondary WWTP. Additionally, we found no significant difference in the quantity of CARB from the Downstream site compared to the Upstream site. Since the bacteria upstream of the WWTP seem to already show antibiotic resistance, it is not surprising that the downstream bacteria also display resistance. Our results may also be due to our testing at large distances from the WWTP or because we tested water rather than sediment communities. Further research should be conducted on the mechanism of antibiotic resistance in these bacteria as well as on the effect of tides on the spread of WWTP effluent in the river to see if it can explain the ubiquity of antibiotic resistance in the river.

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Relationship of tree growth to climate in the Nechako region of central interior British Columbia

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Abstract

Relationships between tree growth and climate can be found using dendroclimatology, and are important as a basis for understanding regional limiting factors of growth and projecting how forests might be altered by climate change. This study aims to determine factors limiting growth of coniferous trees in the Nechako region of sub-boreal Central-Interior British Columbia by studying tree growth-climate relationships at the Carrot Lake Experimental Fire Study area. Trees cores were collected from the study area in 2012 then processed and analyzed in 2014. Ring-counting of cores from Lodgepole Pine and White Spruce trees indicated samples had ages of 127-136 years. Tree ring chronologies were standardized, verified by cross-dating, and pre-whitened for dendroclimatology analysis. A simple linear regression comparison of ring widths against summer temperature and precipitation data from nearby weather stations showed there was a statistically significant, positive correlation between annual ring growth and precipitation in the month of May (standardized $R^2 = 0.06128$, pre-whitened $R^2 = 0.05635$; $n = 9$). This indicates a growth-precipitation relationship during the beginning of the growing season, where more rain results in greater growth. Due to the small, localized sample size used in this study these findings may only represent the mesoclimate of the Carrot Lake Experimental Fire study site. Nevertheless, this study may be the basis for future research that can provide better insight into the climate history for the region, as well as projections of climate change impacts on the forests of British Columbia.

Keywords — Dendrochronology, Dendroclimatology, Tree ring-growth, Sub-boreal forest

1. INTRODUCTION

Knowledge of the factors that limit the growth of trees is fundamental to our understanding of forest productivity and how forests may be altered by climate change. Dendrochronology is the study and dating of tree growth rings, which are formed through differing rates of tree cell division, creating the annual alternating light-coloured "earlywood" (late spring–early summer) and dense, dark "latewood" (late summer–early fall) [1]. Dendroclimatology combines dendrochronology and climate data, and is used to examine relationships between tree growth and the environment.

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The cell number and size in earlywood and latewood vary from year to year depending on environmental factors, such as temperature and precipitation, which affect photosynthesis and cell division, thus creating annual variation in growth patterns [2]. This annual variation of ring growth can be used to infer past climate conditions (prior to the instrumental record), such as the precipitation variability across the Great Plains [3] and the southern Canadian Rockies [4] over the past 600 years, or the temperature variability in the Northern Hemisphere over the past 1000 years [5]. Dendroclimatology may also be useful for projecting tree responses to future climate change, such as a potential reduction in Douglas-fir (Pseudotsugamenziesii) productivity at high elevations based on a projected increase in the climate-based heat moisture index [6].

Tree ring width is controlled by sensitivity to the growth component (i.e., water, temperature, light, nutrients) that is least available to the plant – a limiting factor [2]. Ring widths in warmer and wetter regions tend to be less sensitive to climate factors due to more stable environmental conditions, whereas trees in arid environments are often more sensitive to variability in precipitation and trees in cold regions are sensitive to temperature [1]. For example, in Alberta, increased radial growth of Lodgepole Pine (Pinus contorta) in mountainous areas is positively associated with early spring temperature and late-summer precipitation [7]. Limber Pine (Pinus flexilis) in the Great Plains shows a positive relationship between growth and precipitation in May, June, and August [8].

![Temperature and Precipitation Chart for 1981 to 2010 Canadian Climate Normals](image)

**Figure 1:** Average temperature and precipitation data chart based on data from the Vanderhoof weather station, near the study site, from 1981 to 2010 [9]. The months of May to August are considered the growing season in our study. This plot was obtained from the Government of Canada’s Climate Normals Data for the Vanderhoof Weather Station [9].

Although there have been studies done to determine the limiting growth factors and seasonal timing for tree growth across Canada, there are differing results depending on the location of the study [6, 7, 8]. A broad environmental study of the Sub-boreal Spruce Zone of British Columbia (BC) was completed, [10] which detailed general vegetation, soil types, and air temperature patterns in order to characterize environmental factors that affect vegetation growth. However, to our knowledge, no studies have been completed specifically assessing climatic controls on growth for coniferous trees that
dominate the forests of the sub-boreal region. The forests in this region experience distinct seasonality in temperature and precipitation (Figure 1), with an average growing season (May to August) temperature of 14.3°C and an average precipitation of 46.8mm [9]. Since the growing season is relatively short, there is reason to believe that this region may have strong limiting factors that affect the growth of trees.

Figure 2: The Carrot Lake Experimental Fire Study area is located in Central Interior British Columbia, approximately 75km southwest of Vanderhoof, BC. Tree core samples analyzed in this study were taken from plot2, labelled in the figure [11].

Our study aims to determine the tree growth-climate relationship in the Carrot Lake Experimental Fire Study area located in the Nechako region of sub-boreal forest in Central Interior BC (Figure 2). To determine this, we tested for a correlation between tree growth, via annual ring widths, and annual climatic factors of precipitation and temperature of the growing season months.

2. Results

Annual growth of trees in the study area was positively correlated with May precipitation, based on both standardized and pre-whitened radial tree growth (Table 1). These results can be viewed visually through scatterplots, as illustrated in Figures 3 and 4. Three relationships had test statistics close to the critical p-value of 0.05 (Table 1): standardized growth vs. Klusklus May precipitation, pre-whitened growth vs.Vanderhoof May mean temperature, and pre-whitened growth vs. Vanderhoof June mean temperature.
Table 1: Calculated $R^2$, slope estimate, standard error, and $p$-values from a simple linear regression between monthly mean temperature and precipitation measurements against standardized and pre-whitened radial growth values. Results are shown for data from the Kluskus and Vanderhoof weather station. Statistically significant relationships ($p < 0.05$) are shown in italics.

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3. **Discussion**

We found a positive correlation between annual tree ring growth and May precipitation. This growth-precipitation relationship coincides with the seasonal timing for the beginning of the growing season in Central Interior British Columbia. A similar sensitivity of tree growth to the early summer environment was found for Lodgepole Pine [7] and
Figure 3: Scatterplot with slope from a linear regression for annual standardized tree growth of the master chronology plotted against precipitation in May ($R^2 = 0.0613$, estimate = 0.0019, standard error = 0.0009, p-value = 0.0323). The relationship is based on precipitation data from the Vanderhoof weather station (1916-2006).

Limber Pine [8] in mountainous and prairie areas of Alberta. Together, these results suggest early summer conditions play a key role in conifer tree growth across broad regions of western North America.

Dendroclimatology has its associated limitations [12], and certain assumptions were employed in our study in order to address them. First, the two different species sampled, Lodgepole Pine, and White Spruce, were assumed to both grow the same way in response to climate factors, i.e., have the same climate sensitivity. However, pines are generally more efficient at water conservation and also grow better under warmer and drier conditions compared to spruce [13]. Further analysis where each species is analyzed independently would allow us to verify the consistency of response we observed. Secondly, the obtained climate data is assumed to be representative of the study site. Although the nearer Klusklus weather station would likely better reflect the study area’s local climate, it had a shorter time series (1992 to 2013) compared to the more distant Vanderhoof weather station (1916 to 2006). Third, no confounding factors are assumed to have influenced the radial growth of the trees aside from temperature and precipitation. However, other climatic factors, such as light availability or above-freezing-degree days, which would provide more energy for increased photosynthesis and growth, were not tested. Finally, the study site is located within a Mountain Pine Beetle (Dendroctonus ponderosae) infestation zone and therefore could have other environmental conditions, such as insect damage, that may potentially have an effect on tree growth.

In order for the results of this study to be applied in any future research, the
relationship must be confirmed to be an accurate reflection of the region. This study is a preliminary investigation in the tree growth and climate relationship of sub-boreal forests in British Columbia, and contains a small sample size in a single area. Future research to confirm the growth-climate relationship would benefit from having a larger sample size taken across a larger region of the sub-boreal forest, as well as comparison against other climatic factors. Within the samples, separating conifer species may reflect more accurately how different species respond to climatic conditions, and may show differing limiting factors. Given that the average temperature for the region in April and September is above 0°C [9], the growing season months examined could be extended to include late spring and early fall, when earlywood and latewood may be forming. Finally, work by both Chhin et al. [7] and Case and MacDonald [8] also examined the effect of temperature and precipitation from the previous year on the current year’s growth and found a negative correlation with late summer temperatures. Future work could include performing this correlation test to include any residual effects from previous years on the tree growth-climate relationship.

4. Methods

4.1. Study Area

The study area was located at the Carrot Lake Experimental Fire Study in Central Interior BC in the sub-boreal spruce biogeoclimatic zone [14]. The sub-boreal spruce zone is characterized by dense coniferous forests dominated by Lodgepole Pine (Pinus contorta),
and also includes White Spruce (Picea glauca) and Subalpine Fir (Abies lasiocarpa) [14]. The region has distinct seasons (Figure 1), allowing for tree ring formation.

Climate data were obtained from two weather stations near the Carrot Lake Experimental Fire Study area and included daily temperature and precipitation data. The Vanderhoof weather station data were available for 1916 to 2006 (90 years), and the Klusklus weather station data were available for 1992 to 2013 (21 years).

4.2. Tree and Core Sample Selection

The tree core samples were taken during the summer of 2012 from sample plots established for the Carrot Lake Experimental Fire Study. Tree core samples were taken from conifer trees in plot 2 (Figure 2), which was approximately 100m by 100m, on relatively flat terrain. The tree cores were taken at the base of each sample tree, from plots located 20m distant from one another. Cores were taken using an increment borer and stored for transport. At the lab, cores were mounted and sanded with progressively finer sandpaper to improve the visibility of narrow or faint growth rings. Of the tree cores taken, nine were selected for analysis based on whether the wood was intact with no disintegration, if the core contained the pith, if the tree was alive, and if the growth pattern did not have sudden growth spurts that would likely not be attributed to climatic conditions, but rather to localized stand dynamics. The cores were selected from 108 samples taken from live Lodgepole Pine and White Spruce trees, aged between 127-136 years.

4.3. Sample Processing

The tree rings were counted and rings widths were measured using a binocular microscope and Velmex™ measuring stage (precise to 0.001mm) along with MeasureJ2X™ software [15]. Each individual ring width was measured perpendicularly to the earlywood-latewood boundary, starting from the bark boundary to the pith. This method built a record of intra-boundary measurements that reflected the annual growth rate and patterns of each tree.

4.4. Chronology Development

We developed a master chronology of the tree ring data for comparison with the climate data. The master chronology was assembled using an iterative cross-dating process with standardized ring width data, then by averaging all the cross-dated width measurements from each tree core sample across each corresponding year to produce a collective of average width measurements showing the inter-annual variability in growth characteristic of trees in the study area. The initial cross-dating process was important to ensure that the tree ring count was accurate and that tree ring widths from identical years were being compared with climatic variation [16]. All analyses, standardization, and the verification process outlined below were done using the dplR library package in the statistical analysis program R [17] and following the steps given in Bunn [18] and Bunn [19].

We used a detrending process that standardized all tree ring width measurements by applying a cubic smoothing spline curve to eliminate the sigmoidal curved signal of
increased growth that occurs at the beginning of each tree’s lifecycle (Figure 5). Using the standardized measurements, a graphic correlation chart (Figure 6) was created that “flagged” segments of core measurements where standardized ring width did not have a high correlation when compared with the current master chronology.

**Figure 5:** The master chronology of standardized tree ring width, with the standardized and averaged tree ring width measurements corresponding to the year of growth.

**Figure 6:** Graphic correlation chart showing different cores (y-axis) and core segment lengths (x-axis) compared with the master chronology generated by pooling information from all cores together. Blue segments show positive correlations in standardized ring widths, red segments are negative correlations, and green segments lack enough information for correlation. The segment length of 30 years was chosen as it would be long enough for comparison, but short enough to narrow down any negative correlations indicative of offset growth patterns among cores.
For each "flagged" segment, a lag plot (Figure 7) was used to better visualize the correlation of growth for each year. Shifts in the lag plot may be due to miscounts or a missing ring. Using an iterative process, adjustments were made to the assigned years of the core, e.g., if there were any miscounts or missing rings, by reviewing the tree core and adjusting/correcting dates in the data.

Figure 7: Lag plot for example core "C-2-03-SE" showing an overall positive correlation in annual growth between the core and the master chronology. Red markers indicate a positive correlation in standardized ring width, and blue markers indicate a negative correlation. Overall, one would expect to see high values of tree ring growth for a particular year occur in years when the pooled, master chronology shows high values; and correspondingly, low values shared between a core and the master. In this example, although there is the lower correlation between 1975-2004, the correlation is still positive, and is thus attributed to the natural variability in growth of the tree, and not due to miscounting or incorrect dating.

After any low correlations were verified to be variation in growth of the individual trees and not due to miscounts, the master chronology verification was complete. Each core's standardized measurement was then pre-whitened as a second standardization step where autocorrelation, or any influence on growth due to previous year growths, is removed from each series before averaging the width measurements.

The final, verified master chronology measurements of tree ring growth were correlated against mean temperature and mean precipitation for the months of the growing season of all operating years of the weather stations (Vanderhoof: 1916-2006, Klusklus:}
A simple linear regression for tree ring growth vs. temperature and tree ring growth vs. precipitation in May, June, July, and August for both Vanderhoof and Klusklus weather stations was performed. We created visual graphs and used a t-test to determine whether we should reject the null hypothesis, and these suggested the estimated slope of the relationship was statistically different from zero, at alpha = 0.05. No Bonferroni correction of significance was used in this analysis.

5. Conclusions

The results of this study indicate that conifer tree growth responds to May precipitation in the Nechako region of the sub-boreal forest of British Columbia. The growth and May precipitation relationship allows for a historical estimate of precipitation going back beyond weather station operating years, based on tree ring data. This long-term record can provide insight into the regional climate history. Thinking to the future, annual mean temperatures are projected to increase by 2-4°C, and annual precipitation is expected to increase in North America by the end of the 21st century [20]. The growth-precipitation relationship allows us to make an educated guess at how coniferous trees in the British Columbian sub-boreal forest may respond to climate change; with increased precipitation we may see increased growth.

6. Acknowledgements

The study was completed during a work-study semester as a Directed Studies course (GEOG 405) at Simon Fraser University. The research was done under the direction of Dr. Meg Krawchuk, with support given by members of the Landscape and Conservation Science Research Group (Kim House, Michael Ton, Phil Camp, and Marc Edwards); all of whom, as well as Lucas Green, I thank for the guidance, patience, energy and laughter that they have given me.

References


Management of *Dosidicus gigas*, a large, pelagic predator in the eastern North Pacific Ocean

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**Abstract**

The Humboldt squid (*Dosidicus gigas*) has been expanding its geographical range in the eastern North Pacific Ocean over the past 20 years. This species of squid has advanced from the most southern part of their native range, off the Chilean coast, northward to southern Alaska. This expansion of a fast-growing pelagic predator is concerning and should be evaluated. *Dosidicus gigas* has been known to negatively affect native species of fish populations, such as Pacific hake (*Merluccius productus*), when expanding its range. The effects of an establishment of a *D. gigas* population in Pacific Canadian waters on both ecological systems as well as commercial fisheries should be assessed to develop management plans to protect native species and commercial fisheries. The reduction of potential effects of *D. gigas* establishment on native species of the eastern North Pacific is essential to conserving current native populations. A complete list of trophic interactions between *D. gigas* and species native to the eastern North Pacific is still underdeveloped. To qualitatively assess what is driving the migratory behaviour of *D. gigas*, the physiological, reproductive, and ecological traits of the species are reviewed here. The results indicate that warming water temperatures, reproductive plasticity, and prey/predator interactions are the leading causes thought to be driving *D. gigas* northward.

1. **Introduction**

Scientists have found that Climate Change has shown to affect a variety of natural ecosystems around the world including marine ecosystems [1]. One of the effects of Climate Change is the alteration of geographical distributions of organisms throughout the globe [2]. This has been documented in the North Sea as marine fishes are moving northward with the warming of water temperatures resulting from Climate Change [2]. The shift in distribution Northward was present in commercially fished species such as Atlantic cod (*Gadus morhua*) and common sole (*Solea solea*) as well as fish species that are not commercially fished such as scaldfish (*Arnoglossus laterna*) and snakeblenny (*Lumpenus lampreataeformis*) [2].

The Humboldt squid (*Dosidicus gigas*) is an abundant oceanic predator [3] that has been steadily expanding its range since the late 1990s [4]. *Dosidicus gigas* was once endemic to the Humboldt Current System (HCS) of south and central America but is now observed as far north as Central California (Figure 1) [3]. Few occasions of *D.
gigas stranding have been observed as far north as British Columbia, Canada (BC) and Alaska [4]. This unprecedented and sudden change in distribution has deemed D. gigas a highly migratory predator that must be managed to avoid negative effects on native ecosystems should it become established in Canadian waters.

Figure 1: The current geographical distribution of Humboldt Squid (Dosidicus gigas) in the Eastern Pacific. FAO 2015 [5].

The first large migration of D. gigas was seen in Monterey Bay in 1997/98 after a strong El Niño occurrence [6]. After 1998, D. gigas sightings were rare off the coast of California until 2002 when the squid were present in large numbers after a weak El Niño event [6]. Dosidicus gigas have been present near Monterey Bay ever since 2002 [6]. Thus it can be inferred that an indirect effect of El Niño events contributed to the establishment of D. gigas along the Californian coast [6]. In 2009, large numbers of D. gigas washed up on the shores of BC [7]. In the years to follow, D. gigas was a rare sight off the coast of BC [8], similar to the events that took place in Monterey Bay ten years prior.

2. Ecology and Biology of D. gigas

To better understand the reasons behind the first large migration and what to expect if D. gigas begins to become established in Canadian waters, a review of their basic biology and ecology is required. The diet of D. gigas varies on prey availability [9]. In the California Current System (CCS) the diet of the squid varies based on habitat use and geographical location [9]. The main dietary components of D. gigas in the CCS were analyzed from stomach content samples and were found to be two species of lanternfish (Tarletonbeania crenularis, Stenobrachius leucopsarus) [9]. Many other species were also present in the stomachs of these squid including crustaceans, Pacific herring (Clupea pallasii), Pacific hake (Merluccius spp.), Pacific sardine (Sardinops sagax),
salmon (*Salmonidae* spp.), rockfish (*Sebastes* spp.), flatfish (*Pleuronectidae* spp.), and other cephalopods including its own species [9]. The wide diversity of diet can be observed among individuals within the same school of *D. gigas* [9].

The diet of *D. gigas* off the coast of BC has been found to be different than the diet of *D. gigas* in the CCS [9, 7]. Multiple stranding events occurred in late 2009 off the west coast of Vancouver Island, BC which provided the opportunity for their stomach contents to be analyzed [7]. After stomach analysis of the stranded *D. gigas* it was evident that the main prey species were *C. pallasii* and *S. sagax* [7]. Other prey species in the eastern North Pacific Ocean included Dungeness crab (*Metacarcinus magister*), Coho salmon (*Oncorhynchus kisutch*), and kelp greenling (*Hexagrammos decagrammus*) [7]. Even though *D. gigas* is a generalist predator, it is also prey for many species [3]. Large sharks, North Pacific Spiny Dogfish, tunas, billfish, and marine mammals such as sperm whales, pilot whales, porpoises, sea lions, and seals are known to predate on most life stages of *D. gigas* [3, 10].

*Dosidicus gigas* is a fast growing squid that can reach 0.75 metres within the first year of its life [3]. They live for one to two years and reproduce only once in their life time with the potential to produce up to 32 million eggs [3]. Reproduction occurs year round and peaks during the summer of the southern hemisphere (October through January) [3, 10, 11]. Recent work has shown that *D. gigas* is capable of switching reproductive patterns. After the El Niño that took place in 2009, the squid population around the Gulf of California seemed to collapse [12]. Typically *D. gigas* reproduce near shore at around 1.5 years old [3]. Shortly after 2009 the squid were found reproducing further north and offshore at a younger age of six months and a smaller size of 30 centimetres [12]. This faster reproductive pattern saw positive returns as the *D. gigas* population near the Gulf of California doubled by 2011 [10, 12].

*Dosidicus gigas* are known to spend most of their time in the Oxygen Minimum Zone (OMZ) at depths of around 200–700 metres [13]. The squid undergo diurnal vertical migrations between dusk and dawn to hunt [13]. The OMZ that the Humboldt squid occupy has been steadily approaching the surface over the past 50 years [13]. The average depth of the OMZ changed from 638 metres in the 1950s to 500 metres in 1997 [13]. Physiological changes in temperature, oxygen availability, and water pressure that the squid experience daily suggest that abiotic factors may not be the direct driving force in the *D. gigas* vagrant behaviour [13].

### 3. Reasons behind range expansion in *D. gigas*

*Dosidicus gigas* have moved their geographical distribution northward from the Humboldt Current System (HCS) in South and Central America to as far north as Alaska [3]. *Dosidicus gigas*’ variation in physiological adaptations and reproductive patterns make the abiotic factors of their range expansion harder to determine. The OMZ where *D. gigas* spends most of its time has been approaching the surface in recent years [13]. This change in OMZ depth is thought to be largely due to the effects of Climate Change and could benefit *D. gigas* as a predator by changing prey distribution [13]. El Niño events are thought to increase deoxygenation of ocean water and cause the OMZ to occur at shallower depths [13]. This effect will become more prevalent in northern latitudes.
with increasing water temperatures [13]. As prey move north to stay at a deeper OMZ in warm temperatures, *D. gigas* will likely follow resulting in a migratory pattern.

The reproductive patterns of *D. gigas* appear to change with warmer water temperatures [12]. The faster reproductive pattern would make a more adaptive population of *D. gigas*. Species with faster life histories are known to shift their distribution more than species with longer generation times [2]. Due to increasing ocean temperatures, the reproductive peak of *D. gigas* could extend into September and February in the South Pacific. Work with stable isotopes show that all *D. gigas* captured at northern latitudes are in fact from one origin close to the Gulf of California [14]. This recent work shows that large and varying migration patterns of a single population can be responsible for the distribution changes of *D. gigas*. The abiotic fluctuations that accompany El Niño events seem to affect *D. gigas* on a population level rather than individually.

4. **Implications of *D. gigas* range expansion**

Many problems could arise with the establishment of *D. gigas* into Pacific Canada. One trophic interaction that is known to occur is a decline of Pacific hake (*Merluccius productus*) with an increase of *D. gigas* [6, 15]. Dietary evaluation of *D. gigas* shows that there would be pressure on economically important fish species such as Pacific herring (*Clupea pallasi*), Pacific sardine (*Sardinops sagax*), and salmon to a small extent [9]. The predatory activity of *D. gigas* would compete with local predators such as harbour porpoise (*Phocoena phocoena*), harbour seal (*Phoca vitulina*), salmon (*Oncorhynchus spp.*), and shark species. Alternatively, *D. gigas* would be a prey item for larger predators in the eastern North Pacific Ocean [3, 10].

The landings of Pacific sardine (*S. sagax*) in Pacific Canada have been declining since 2006 [16]. Over the last 6 years the landings of *S. sagax* in British Columbia declined from 22,000 tonnes in 2010 to 0 tonnes in 2013 [16]. The Pacific herring (*Clupea pallasi*) is an economically and ecologically important species off the Pacific coast of Canada [17]. *Clupea pallasi* is preyed upon by multiple species in the local area and is very abundant close to shore [17]. The regions of Pacific Canada that land the most herring are the Strait of Georgia (SOG) and the Prince Rupert District (PRD) [17]. The SOG produces much higher amounts of herring due to a large local population [17]. The Pacific hake (*M. productus*) total allowable catch (TAC) for 2013/2014 was set at 87,000 tonnes [18]. As *M. productus* is a ground fish, it is caught by bottom trawling [18]. Management would be needed to protect these commercially important fish species if *D. gigas* becomes established in the eastern North Pacific.

5. **Management actions and future work**

There are management steps that can be taken to reduce the likelihood that an establishment of *D. gigas* will drastically affect the abundance of ecologically and economically valuable marine species. Anthropogenic mediated Climate Change is not something that can be fixed immediately but must always be considered a top priority. Reducing the burning of fossil fuels and methane production from activities like cattle farming can help slow the production of greenhouse gases that contribute to Climate Change.
After reviewing the research that shows a strong correlation between warmer El Niño events and large migrations in *D. gigas*, there is evidence that suggests potential negative consequences of Climate Change can lead beyond simple range expansion if not remedied promptly.

Should *D. gigas* successfully establish itself in Pacific Canada some economically important fish must be managed to avoid overexploitation and local extinction. The *S. sagax* fishery is small, sensitive, and declining and steps should be taken if a new voracious predator is present in local waters. Having a healthy *S. sagax* population helps the ecosystem stay stable by supporting higher trophic levels that prey on *S. sagax* [16]. Larger *S. sagax* populations would also reduce top-down cascading effects on lower trophic levels and potentially prevent the establishment of a *D. gigas* population [19]. The *C. pallasii* fishery should remain open but should consider cutting the TAC by a significant amount in the event of a *D. gigas* establishment. Extra attention should be paid to the SOG region as it is the southernmost region that would most likely see the largest amount of migrating *D. gigas*.

The expansion of *D. gigas* overlaps with the range of North Pacific hake (*Merluccius productus*). Since the increase in *D. gigas* in Californian waters there has been a coincidental decline in *M. productus* [6]. The coincidence may provide evidence for the presence of a top-down trophic pressure from *D. gigas* on *M. productus* [19, 15]. The predatory pressure of *D. gigas* could change the distribution and abundance of *M. productus* [15]. Similar effects of *D. gigas* are present on rockfish species in the same habitat [15]. It is recommended that the amount of bottom trawling in all fisheries is drastically reduced. The reduction of bottom trawling will help Pacific hake and long-lived, slow growing rockfish populations remain stable as they are often caught as by-catch.

Since 2003 the global *D. gigas* fishery has grown from 400,000 tonnes per year to over 900,000 tonnes [20]. The fishing pressure on *D. gigas* has been most prevalent in Chile and Peru and has been recently increasing in Central America and Mexico [5, 21]. The increasing pressure on *D. gigas* from fishing mortality in combination with climatic change and El Niño events may help explain the drastic range expansion. It could be that southern fishing pressure is lowering the genetic variability in *D. gigas* and possibly creating two distinct populations. Other types of fishing involving large pelagic species could also help explain the change in distribution of *D. gigas*. The abundance of large tuna species, billfish, sharks, and mackerel have declined due to overfishing in the temperate Pacific [22]. The reduction in large pelagic predators, including toothed whales, provided a mesopredator release for *D. gigas* [3, 6, 19]. The juvenile *D. gigas* are directly released from predation by the removal of large pelagic predators while the adult *D. gigas* are released from predation as well as competition for food resources [3, 6, 19].

A recent analysis of *D. gigas* off the coast of BC has shown high levels of paralytic shellfish toxins in *D. gigas* tissue [7]. High levels of toxins would make *D. gigas* inedible for humans and other animals. If toxin concentrations are not consistent for all *D. gigas* in the area, then there is a possibility to open a fishery for *D. gigas* in Pacific Canada to help manage its effect on the local ecosystem. The current *D. gigas* fishery has been growing in recent years and is exported to places such as Asia and eastern...
North America [5, 21].

6. CONCLUSION

With large migrations of D. gigas occurring in congruence with El Niño events, there is evidence that warmer ocean temperatures have some positive effect on D. gigas movement to the North. Other marine species have also changed their own geographical distributions in response to increasing ocean temperatures [2]. The prevention of anthropogenic mediated increases in ocean temperature is imperative to conserving local and native ecosystem structures.

The interactions between D. gigas and local species in the eastern North Pacific Ocean must be better understood to properly evaluate and manage the effects that a possible establishment would have on the ecosystem. Dosidicus gigas should be studied as a predator as well as a prey item in trophic food webs. The removal of large pelagic predators must be avoided to prevent the establishment of foreign predators such as D. gigas.

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