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The CROW (Campus Research and Obervational Writings) is an annual journal of student research. We accept submissions throughout the year, with a priority deadline of January 1 and a final deadline of March 1 for each upcoming issue. You must be a student at the University of Washington Bothell to submit. Submissions are accepted by email to publish@uw.edu.

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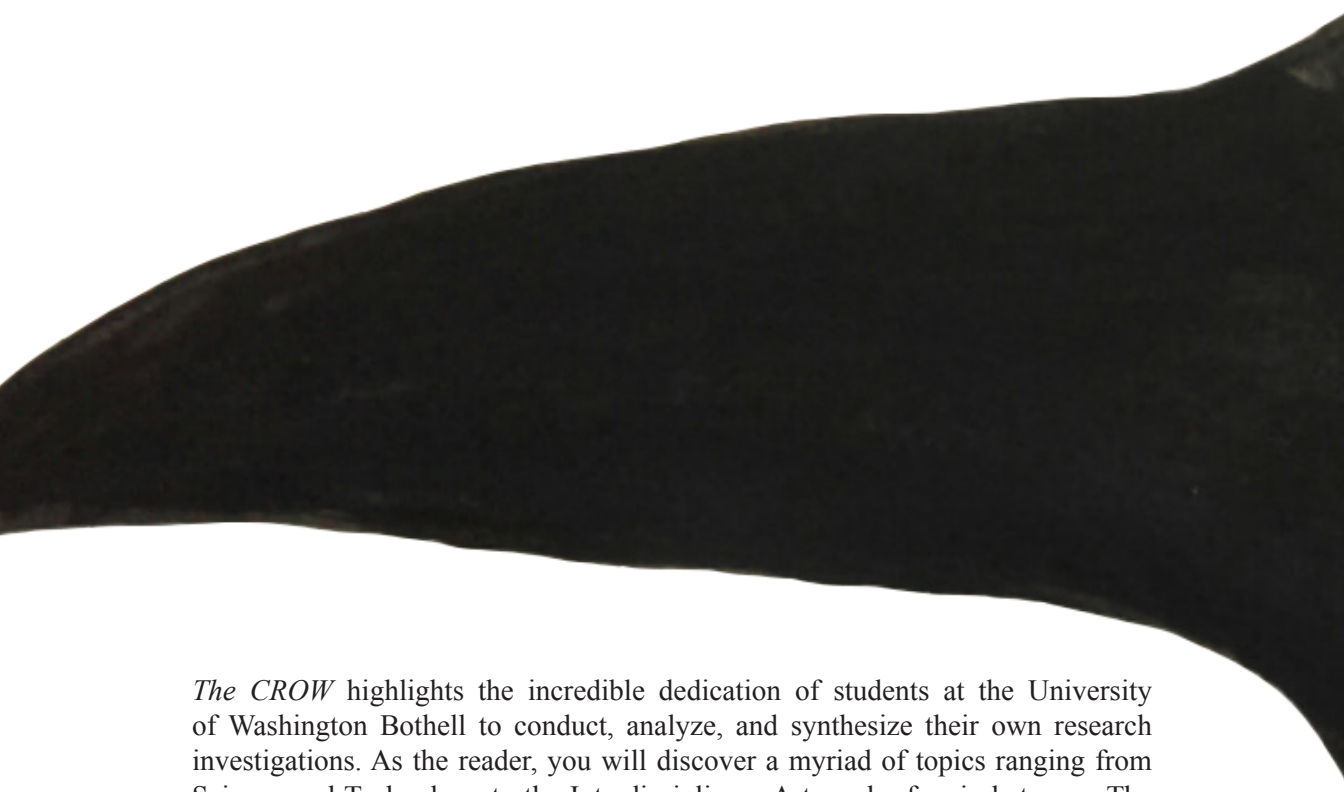
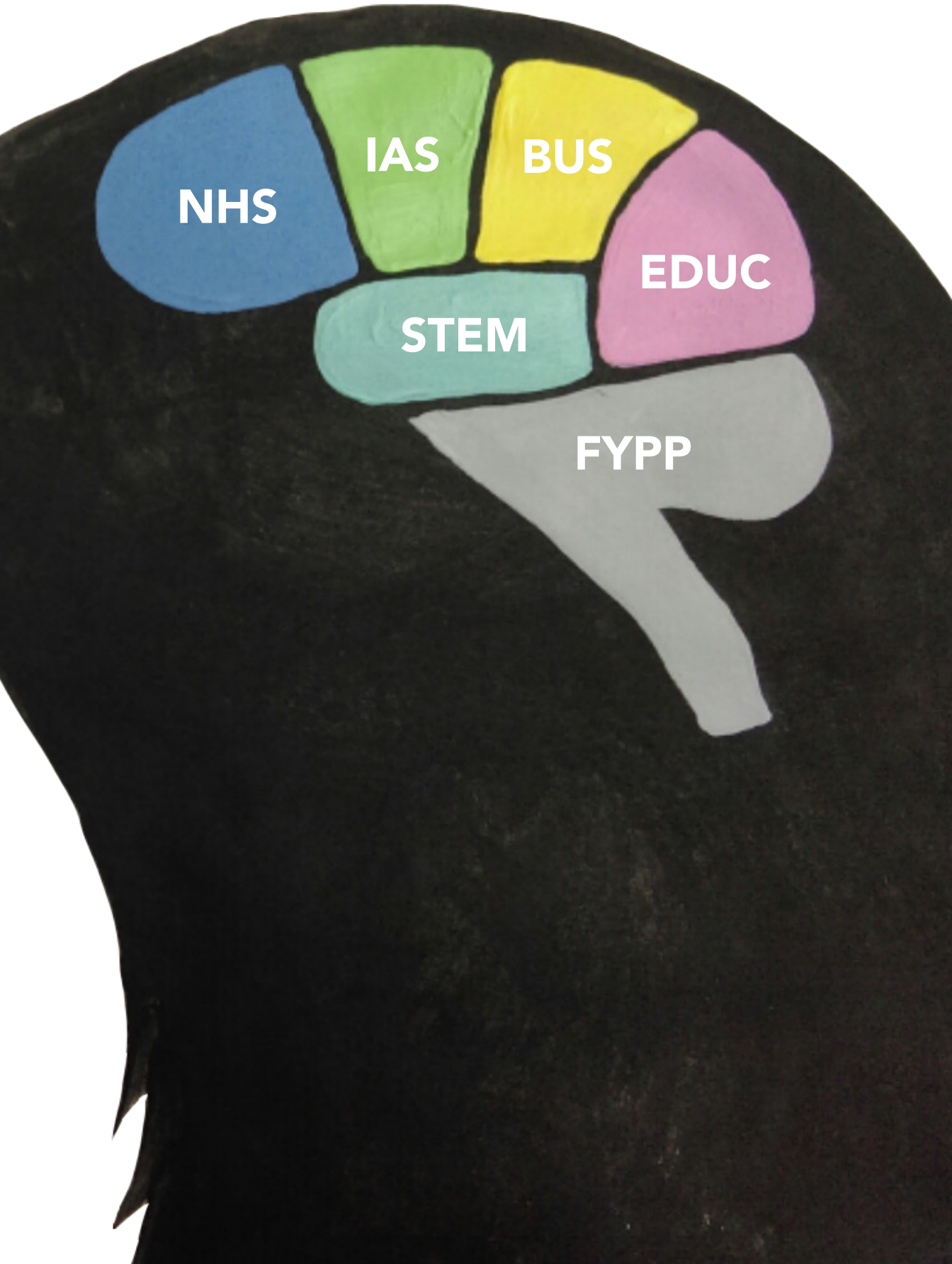


The CROW highlights the incredible dedication of students at the University of Washington Bothell to conduct, analyze, and synthesize their own research investigations. As the reader, you will discover a myriad of topics ranging from Science and Technology to the Interdisciplinary Arts and a few in between. The multiple submission types and various writing styles feature the campus research and observational writings that should spark any reader's interests. The act of conducting research is proven to be a highly impactful learning practice that engages students outside of the classroom setting and allows them to think more critically about the topics they wish to discover. Having their work published will forever encapsulate and preserve their work while also transforming it into a powerful tool which can be used by the next academic who seeks new ideas. By taking this monumental and often daunting step of subjecting their work to be reviewed and critiqued by their peers, the authors featured in this journal have progressed towards becoming contributors to the academic discourse of their particular field of study. The Editorial Board was overjoyed to have had the opportunity to review all the incredible submissions this year. The selection process was undertaken with extreme thoughtfulness and sensitivity for the overwhelmingly positive and well written submissions we received. We want to praise all the students for the hard work they put into their research and to everyone who submitted their work for review. We also want to thank the faculty and staff mentors who foster students' passions and talents, while working with them to develop into becoming published researchers.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SPRINGTIME ON MOUNT EVEREST

Denae Weigelt

The world is propelled by the commercialization of human endeavors ranging from the use of cell phones to American football. Since Mount Everest was discovered to be the highest mountain in the world, it has emerged as the missing marketable commodity of Nepal. Nepal's unsurpassed natural wonders include a stunningly sublime tableau of peaks, valleys, rivers, and biodiversity. Because Nepal is situated in Southern Asia between China and India, the Himalayas and vast jungles serve as hindering borderlines. The commercial enterprise of Mount Everest mountaineering, however, has normalized the treatment of humans as disposable. This has also created a definitive line between those who seek personal gain and egotistical bragging rights, and those who simply wish to feed their families with more than rationed bread.

The latter is representative of the common draw for Sherpas and porters. These individuals make up an ancient ethnic community that lives in the shadow of Mount Everest. Prior to the popularity of the mountain, Sherpas worked as farmers and hunter-gatherers within the foothills of the Himalayas (Weathers 9). "Today, a Sherpa can earn a couple of thousand dollars or more lugging gear up and down the mountain for a typical two-month climbing expedition" (Weathers 10). In addition to carrying more than their personal body weight up and down the trail, Sherpas leave their families, establish camps, tend to the hundreds of pack animals, cook and serve their fellow climbers, and risk their lives on a daily basis. Springtime on Mount Everest is no walk in the park for Sherpas.

The exploitation of Sherpas depicts a fundamental, yet somewhat overlooked, role in the process of commercialization. This is because they are the mechanism that keeps the commercial enterprise of Mount Everest mountaineering afloat. Apa Sherpa is a man who just recently summited Mount Everest for the 21st time, the highest number of successful attempts in the world. His name, though, goes unknown as the dozens of climbers he assists every year receive praise for their intrepid feat of conquering the highest mountain in the world. These are the same individuals who expend $50,000-$100,000 or more for a single expedition (McCurdy 139). This monetary amount is often insignificant to climbers seeking egotistical reward because for them, standing atop of the world for a brief second or two is seemingly priceless. The true cost of conquering Mount Everest, though, lies with those who truly know the mountain — the Sherpas.

If the number of annual deaths of various professions per 100,000 people with full-time equivalents were to be calculated, only 25 miners have died between the years of 2000 and 2010. This can be compared to the death rate of 124 commercial fishermen, and 1,332 Mount Everest Sherpas. If this time frame is shifted to 2004-2014, the death rate for Sherpas would be over 4,000 (Ogles).

At what point does leading an egotistical life begin to diminish your overall effect on the world? Some may argue that maintaining an ego initiates a necessary sense of self-worth or pride that can be used as a source of motivation. This means that one's ego allows one to develop an array of standards that direct our decisions in life. As one's confidence has the power to propel our potential and personal success, the world is continually propelled by the commercialization of these human endeavors. But at what point is it acceptable to ask others to risk their lives for the sake of our ego? I'm going to give the answer away: never.

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HAPPINESS IN RELATION TO SPIRITUALITY

Malak Shalabi

Many Americans appear to be preoccupied with pursuing happiness, their God-given right according to the *Declaration of Independence*. What is often neglected in this pursuit is the long-lasting and authentic sense of happiness (Dambrun et al. 1) that spirituality has been proven to grant individuals through neurological and physiological means. Spirituality is the deliberate mental engagement and solitude exercised by secular or religious means and has been proven to have the potential to prevent depression and reduce stress. Educational institutions should approach this data with programs promoting exploration of and engagement in spirituality.

Numerous studies have found spiritual activity to have the ability to prevent the development of depressive symptoms. A correlational, longitudinal study conducted over a time span of ten years kept records of adults with a genetic predisposition to depression recorded a "90% decreased risk in major depression in adult offspring who reported that religion or spirituality was highly important to them" (Miller et. al. 89). MRI testing also found "self-associated attributed importance of religiousness or spirituality to be associated with an increased grey matter in the left and right parietal regions of the cortex, as well as thickening in the occipital region, right mescal frontal, and left cuneus and precuneus regions" (Miller et. al. 89). This neurophysiological change creates thicker cortical regions that demonstrate as a "buffer for the development of depressive symptoms" (Miller et al. 89).

Spiritual engagement has also been found to stimulate regions of the brain directly associated with happiness, as the basal ganglia is activated during spiritual acts (Mcnamara and Butler 215) as well as when positive emotions are elicited (Wager et al. 521). The anterior cingulate cortex is also stimulated during meditative states (McNamara and Butler 215; Cahn, Rael and Polich 199) as well as during feelings of pleasure (Rolls, Grabenhorst and Parris 1508) and happiness (Engstrom and Soderfeldt 599). Further evidence that affirms the biological reflexivity of spirituality and happiness is the higher than average resting alpha wave rate found in those practicing spirituality for ten years (Tenke et al. 426). Alpha waves have been found to be strongly associated with positive emotions, including positive well-being (Petlock par. 3) and decreased anxiety and stress levels ("Five Types of Brain Wave Frequency" par. 8). Studies have also found heightened activity of the parasympathetic nervous system during spiritual activity (Seeman, Dubin and Seeman 59), a system triggered during soothing situations which "foster(s) the calming and easing that underlie many positive states of being" (Hanson par. 2).

Misconceptions of happiness derived from spirituality is that the happiness is gained from the social context associated therein and that they are limited to certain organized religions. However, research finds that internal spiritual engagement is the source of increased well-being, not the frequency of visits to a place of worship (Kelley and Miller 225). Results find the positive effects of spirituality to be applicable to a wide demographic, positively impacting one's well-being regardless of their gender (Maselko and Kubzansky 2856), faith (Cohen and Hill 293), or personality (Aghababaei 141). Much evidence supports the inclusiveness of spirituality in benefit to happiness, and "religious/spiritual practice, secular meditation, and spiritually-oriented meditation all hold potential to reduce symptoms of anxiety and

depression, increase resilience and empathy, and improve well-being" (Barnby et al. 229). These indiscriminatory results may encourage people to explore and ponder their beliefs, and then embrace them through spiritual exercise.

We as a society should approach this data with the intent of preventing depression and high stress among adolescents, who are vulnerable to such mental hardships. It would be extremely beneficial to expose students, starting from middle school, to explore world religions and secular practices. Universities should also provide courses pertaining to world religions, and an authentic description of the various practices of spirituality around the world may inspire students to practice so on their own, drastically decreasing their risk for depression and stress while increasing their overall well-being.

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UNDERSTANDING THE ORIGINS OF ANIMAL MULTICELLULARITY THROUGH THE STUDIES OF CHOANOFLAGELLATES

Isaac Kim

ABSTRACT: Choanoflagellates are the closest living relative of animals (Alegado, 2014; King, 2015; King 2016; Rokas, 2008). This has been confirmed through multiple lines of phylogenetic analyses, comparative genomics, and similarities in cell biology (Alegado, 2014; King, 2001). Previous studies of the choanoflagellate Salpingoeca rosetta suggest that bacteria may have played an important role in the early origins of animals (Alegado, 2014; King, 2016; Levin, 2011). One such study showed that the Bacteriodetes Algoriphagus machinoponensis can release lipid signaling molecules that induces choanoflagellates to grow into multicellular colonies known as rosettes (Alegado, 2016; Beemelmanns, 2014). Another study showed that when grown in the presence of Vibrio fischerii, choanoflagellates will exhibit swarming behavior and sexually reproduce (Levin, 2013). Choanoflagellates have been repeatedly proven to be an experimentally tractable, phylogenetically relevant model system for investigating the unicellular ancestry of animals. Through the use of molecular and comparative genomic approaches, we can possibly study the origins and evolution of animals through choanoflagellates.

Multicellularity may be defined as the differentiation and organization of cells into functioning tissues within an organism (Anderson, et al., 2016; Rokas, 2008). Multicellularity arose independently several times in life's history (Abedin, 2010; Alberts, 2002; King, 2016). The morphological diversity of multicellular organisms such as animals, fungi, and plants stem from the fact that each lineage obtained multicellularity in different ways (Abedin, 2010; Alberts, 2002; King, 2016). One way each lineage varies is in their method of adhesion or cell-cell connections (Abedin, 2010). Another property crucial to all multicellular organisms is their method of intercellular communications (Alberts, 2002). How each eukaryotic lineage achieves these unique multicellular challenges is partly what distinguishes them from each other (Abedin, 2010). Surprisingly, the origin and evolution of animal multicellularity is still largely unknown (Anderson, et al., 2016; King, 2016). However, by studying closely related extant species, we may be able to learn more about the current state of life today (Anderson, 2016; King & Carroll, 2001; King, 2016).

For animals, the closest living relative is considered to be the choanoflagellate, a single-celled eukaryote with a complex lifestyle (Alberts, 2002; King & Carroll, 2001; King, 2016; Rokas, 2008). There are multiple lines of evidence that support the idea of animals sharing a common ancestor with choanoflagellates, including several independent phylogenetic analyses, comparative genomics, and similarities in cell biology (Alegado, et al., 2014; King & Carroll, 2001).

Choanoflagellates are also characterized by their complex life cycle, which consists of a single celled form, a chain form, and a multicellular spherical form known as a rosette (Alegado, et al., 2014; King, 2016). Rosette formation is induced by its prey, *Algoriphagus*, which releases lipid based signaling molecules that induce incomplete cytokinesis to promote rosette formation (Beemelmanns, et al., 2014).

By identifying the molecules responsible for rosette formation, it is becoming possible to create defined media to use choanoflagellates as a model system for studying the origin of animals (King, 2016). Furthermore, choanoflagellates have demonstrated interesting behaviors in the presence of different species of bacteria, namely *A. machipongonensis* and *Vibrio fischeri* (King, 2016; Levin & King, 2013). These bacterial interactions may hold some clues to the origin of animals.

Choanoflagellate's phylogenetic relevance and similarities to modern animal cell structures

Many animal genes not found anywhere else in the tree of life have been found in choanoflagellates. For example, the first tyrosine kinase receptor found outside the animal kingdom was found in the choanoflagellate *Monosiga brevicollis* (King & Carroll, 2001). Another example of an animal gene shared with choanoflagellates is the GK protein interaction domain (GKPID), which enables animals to orient their mitotic spindles to maintain organized tissues (Anderson, et al., 2016). Studies like these suggest that the last common ancestor of animals and choanoflagellates probably possessed many of the genes necessary for multicellularity (King & Carroll, 2001; King, 2016; Rokas, 2008). Thus, by studying the genes of choanoflagellates and comparing them to the genes of animals today, we may learn something about the evolution and origins of animal multicellularity (Anderson, et al., 2016; King, 2016).

Not only do choanoflagellates share genes with animals that are not found anywhere else, they also share similarities to modern animal structures. One similarity is between the single-celled form of choanoflagellates and the eukaryotic sperm cell. Just like a sperm cell, the main parts of a choanoflagellate are the ovoid head and a singular flagellum (King, 2016; King, 2015; Alegado & King, 2014). The second similarity is when a single cell undergoes a series of incomplete cytokinesis to form a ball shaped rosette which bears resemblance to morula stage embryos (Alegado & King, 2014; Beemelmanns, et al. 2014). Finally, when linearly arranged as chain colonies, they resemble and function similarly to the epithelial cells of the gastrointestinal tract (Beemelmanns, et al., 2014). Through the undulations of the flagella, choanoflagellates can trap prey within its microvilli before engulfing it (Alegado & King, 2014). In fact, sponges—choanoflagellate's closest related Metazoan relative—use a form of epithelia that has several hallmarks of eumetazoan epithelia, including a basement membrane and proteinaceous intercellular junctions (Alegado & King, 2014; Dayel & King, 2014). The conserved roles and phylogenetic distributions of epithelia suggest that Metazoans shared a common ancestor with choanoflagellates.

Bacterial influence on choanoflagellate behavior

One of the most fascinating aspects of choanoflagellates is their interaction with bacteria. It has been shown that rosette formation is actually induced by the presence of its prey, *Algoriphagus* (Beemelmanns, et al., 2014). *Algoriphagus* is a Bacteriodetes similar to ones found in the human intestinal microbiome, and produces a novel class of signaling molecules related to sphingolipids that promotes rosette formation in choanoflagellates (Alegado, et al., 2012). Furthermore, electron microscopy has imaged *Algoriphagus* producing outer membrane vesicles composed of these important signaling molecules (King, 2016). This has fascinating implications on possible methods of interactions between animals and bacteria.

The main signaling molecule produced by *Algoriphagus* is Rosette Inducing Factor 1 (RIF-1) (Beemelmanns, et al., 2014). By itself, however, it is only able to induce 1% of the cells into rosettes (King, 2016). For complete rosette formation, it also needs a second lipid based signaling molecule lysophosphatidylethanolamine (LPE) (King,

2016). Together, they can recapitulate full rosette development. This is because LPE promotes proper development and maturation (King, 2016). The presence of these two lipid based signaling molecules enables the choanoflagellate to form robust rosettes, which may increase its efficiency at capturing and consuming prey (Alegado & King, 2014).

To prevent rosette formation, *Algoriphagus* also produces IOR-1 (Inhibitor of Rosettes-1) to decrease choanoflagellate's predatory capabilities (Cantley, 2016). IOR-1 looks similar to the chemical structure RIF-1 if it were to be cleaved down its symmetrical axis (Cantley, 2016; King, 2016). This suggests that there may be coevolution between the two species that may resemble the Red Queen Hypothesis, with the prey constantly trying to change its lipid composition to avoid its predator's relentless adaptations.

Bacteria can also induce choanoflagellates to exhibit strong, unmistakable swarming behavior (King, 2016). This behavior is promoted when choanoflagellate cultures are grown in the presence of *Vibrio fischeri*. In addition to swarming, *V. fischeri* encourages sexual reproduction between the single-celled choanoflagellates (King, 2016; Levin & King, 2013). Although the molecular signal that induces this phenomenon is still unknown, there is strong evidence that choanoflagellates are reproducing sexually. Under a light microscope, it is possible to see videos of a larger "female" choanoflagellate engulfing a smaller "male" choanoflagellate (King, 2016). Furthermore, it is possible to obtain Punnett squares that exhibit Mendelian inheritance (King, 2016). Additionally, just like sexual reproduction in animals, choanoflagellates experience a change in ploidy from diploid to haploid during this process (Levin & King, 2013).

As mentioned before, in the presence of *Algoriphagus*, choanoflagellates will grow into multicellular rosettes. Grown in the presence of *V. fischeri*, they will exhibit swarming behavior and reproduce sexually. Thus, it may come as no surprise that when grown in a culture with both *V. fischeri* and *Algoriphagus*, choanoflagellates will grow as colonies of rosettes exhibiting strong swarming behavior and sexual reproduction (King, 2016). In other words, simple multicellular structures are reproducing sexually due to the presence and influence of bacteria. This has strong implications on the importance of bacteria during the early evolution of animals.

Discussion

Studies of choanoflagellates have shown that these voracious bacteriovores are a promising model system for understanding the early evolution of animals. These studies have shown much evidence to demonstrate the close relationship between choanoflagellates and animals. By studying this closely related extant species, we may learn how animals first evolved (King & Carroll, 2001). Furthermore, these studies suggest that bacteria may have played an important role in animal origins (Alegado, et al., 2012). Understanding the regulation and mechanisms behind these processes may also serve to elucidate current biological mysteries. For example, it has been shown that the gut microbiome has a significant impact on the development of children, the appetite in mice, and the shaping of the immune system (Nikoopour, 2014; Vijay-Kumar, 2010). However, the exact mechanisms of interaction between host and microbiome is still unknown. Coincidentally, *Algoriphagus* is a Bacteriodetes similar to those found in the human gut microbiome (Alegado, et al., 2012). By studying the lipid based signaling molecules between choanoflagellates and *Algoriphagus*, we may learn something about the communication between humans and their gut microbiota.

Still more work has to be done to definitively conclude that these lipid based signaling molecules are causing behavioral changes in choanoflagellates. Although a recent study has identified the gene necessary for rosette formation,

we have yet to uncover a receptor for the signaling molecules (Levin, Greaney, Wetzel, & King, 2014). Identifying this receptor and outlining its molecular effects may help us understand the gene regulation of early animal evolution (Alegado & King, 2014; Levin & King, 2014).

Other experiments may look to see if the multicellular rosettes gain a fitness advantage over its single-celled form. Previous studies in other model organisms have shown that multicellular forms can increase resistance to predation, UV tolerance, and ability to grow in new ecological niches in relation to their unicellular counterparts (Boraas, Seale, & Boxhorn, 1998; Goldman & Travisano, 2011; Koschwanez, Foster, & Murray 2011). It would be interesting to see what kind of fitness advantage (if any) multicellular colonies of choanoflagellates gain.

Another interesting experiment would be to study the coevolution between choanoflagellates and bacteria. The current state of knowledge of these interactions is still nebulous and it is unclear why bacteria would produce the RIF-1 and LPE. At first glance it would appear that bacteria are expending energy for the benefit of choanoflagellates. However, it is extremely rare in biology, if not impossible, for one species to be completely altruistic for the sake of another. One hypothesis may be that RIF-1 and LPE are waste products that choanoflagellates use to sense the presence of *Algoriphagus*. By identifying and defining the symbiotic relationship between bacteria and choanoflagellates, we may learn more about our own relationship with bacteria and how bacteria can influence us.

Because of the identification of the signaling molecules for rosette formation, it is becoming possible to create defined media to grow robust colonies of rosettes. This will make studying questions of mutualism, fitness advantage, and gene regulation in choanoflaellates easier and more accessible. In conclusion, there are several lines of evidence to support the fact that choanoflagellates are the closest living ancestor to animals. These similarities include but are not limited to similarities in cell biology, phylogenetic analyses, and genomic analyses. By studying this organism, we may learn something about the early evolution of animals and the role of bacterial influence.

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FOOD ENVIRONMENT IN HEALTHCARE

Samantha Frati, Jessica Jacobsen, Rachel Li, and Shelby Lubchuk

ABSTRACT: The public relies on health care professionals to provide solutions to chronic disease. With chronic disease prevalence rates continually increasing, a public health response in prevention is needed. Maintaining one's health is dependent upon diet and nutrition, in addition to physical activity. Dietary and nutritional behavior that prevents disease and promotes health is directly linked to the quality of food consumed. The lack of knowledge and access surrounding quality nutrition is an increasing concern both within healthcare and the community at large. Currently, hospital administrations are signing contracts with fast food corporations and processed food suppliers, skewing an assumed alliance with healthy living requirements for chronic disease patients. By hosting a counter productive food environment, medical facilities fail to align with patient's nutritional behavior requirements.

Medical students receive very little, if any, nutrition education in medical training. Medical administrations harness the ability to promote healthy diets but are failing to educate and motivate patients. Moving forward changes in policy development, healthcare food environments, and medical school nutrition education can realign with public health disease prevention/health promotion initiatives. By addressing the point of chronic disease intervention, medical facilities can also act as a point of public health intervention, we build trust with patients and the community by demonstrating beneficence.

Public health has led the development of vaccines and proper sanitation, both of which have greatly reduced infectious diseases in the United States, saving millions of lives. Now, public health faces a new era: combating the rising incidence of chronic diseases such as cancer, diabetes, obesity, and cardiovascular disease. Poor nutrition is a leading risk factor for the growing number of chronic illnesses in the United States and is the reason that the Affordable Care Act called for an increase in nutritional awareness, assessments, and counseling (Kris-Etherton et al., 2015). Still, the importance of nutrition is often overlooked in areas of medical practice, education, and the food environment within healthcare settings. To fully address the growing incidence of chronic disease in the United States, the food environment in healthcare must be assessed and reformed to include proper education for medical professionals and nutritionally dense foods in hospital cafeterias.

Dietary interventions have been known to reduce the incidence, severity, and associated morbidity of hypertension, diabetes, and some cancers. Nutrition not only shows potential in the treatment and prevention of chronic disease, but is also safer and more affordable than pharmacologic interventions (Kris-Etherton et al., 2014). Research increasingly shows that proper nutrition is critical for the prevention and treatment of chronic disease, yet medical professionals, and more specifically medical doctors, do not receive the adequate training to counsel in nutrition. The Accreditation Council for Graduate Medical Education, which oversees specialty programs, requires very little nutrition education for most specialties. Without proper education, health care professionals are not getting all the tools they need to succeed in furthering the health of patients and the public.

Healthcare settings are an intervention location primed for demonstrating to the public what healthy eating looks like. Fast food restaurants

provide foods high in salt, fat, and sugar, which are extremely unhealthy and counterproductive in the treatment of chronic diseases. Therefore, the presence of such franchises within the walls of medical facilities is, at the very least, troubling.

Although proper nutrition is greatly lacking in the United States, some medical schools and hospitals are taking the call to action. Nutrition education equips graduates to counsel patients in food intake and preparation. These steps are critical to changing the current food system within health care and are allowing for more changes to be made all over the United States.

Literature Review

Nutrition & Chronic Disease

Understanding the risk factors for chronic disease is a pathway toward a prevention framework. Lifestyle behaviors that increase a patient's risk of cardiovascular disease include unhealthy diet, physical inactivity, obesity, excess alcohol and tobacco use (Centers for Disease Control and Prevention, 2015). When a cancer patient seeks advice on diet guidelines, they are encouraged to eat smaller portions, choose vegetables and legumes, while avoiding calorically dense foods such a fried potatoes, ice cream, and sweetened treats (American Cancer Society, 2015). A newly diagnosed diabetic will likely look to their pantry as a point of management, they will be encouraged to eat less unhealthy fats, keep portions in perspective, and reduce their sodium intake (American Diabetes Association, 2014). This advice is valid in the treatment and prevention of disease, as it targets the reduction of obesity and maintaining a healthy body weight.

The consensus is in, a range of interventions for treatment and prevention of diabetes, among other chronic diseases, requires innovative interventions (Kyle, 2015). The American Medical Association, National Institutes of Health, Obesity Society, American Association of Clinical Endocrinologists and the Endocrine Society have joined in one communal voice to give precedence to combating obesity related disease with evidence-based care opposed to continually throwing prescriptions at a lifestyle disease (Kyle, 2015). Intensive behavioral therapy is a primary tool for evidence based obesity care (Kyle, 2015). Aligning nutrition behavior guidelines from the prestigious diabetes, cancer and cardiovascular disease organizations previously mentioned, with intensive behavioral therapy is supported within the constraints of the doctors office. The next step is to reinforce those same behavioral values within healthcare food environments.

Current Food Environments

It is apparent that the current food system within the healthcare setting is flawed, creating a missed opportunity for public health improvement. Cafeterias within hospitals not only feed the patients but also the visitors and medical staff. The Physicians Committee for Responsible Medicine (PCRM) reports that hospitals are signing lease contracts with fast food companies. In recent years the number of hospitals working with fast food restaurants and large corporations has been on the rise (Physicians Committee for Responsible Medicine, 2015). The PCRM is exposing hospitals, calling for responsibility and compares the collaboration of fast food and hospitals to allowing physicians to smoke (Physicians Committee for Responsible Medicine, 2015). As previously mentioned, fast food is high in fat, sugar, and salt, all of which are harmful to the health of patients, visitors, and hospital workers. These sources are especially dangerous for patients with chronic disease. The PCRM recommends a shift to food such as fresh produce and whole grains, which will help in the prevention and recovery from chronic disease. Healthcare facilities are places where patients go to regain health. Increasing patient exposure to chronic disease risk factors does not align with public health prevention initiatives.

With the high volume of public interaction within hospitals, food service providers must

respond to customer demand to stay in business (Stanton, 2015). As nutritional knowledge becomes increasingly widespread, more patients will look for healthier options, which encourage and reinforce healthy food choices. Preference for healthy food options is a current and increasing trend, which indicates food behaviors are moving in a healthier direction (Stanton, 2015). This momentum provides an advantage in encouraging physicians, nurses, and dietitians to maximize the point of intervention with the public, to educate and provides sources for long term benefits of healthy food options.

Lack of Medical Nutrition Training

Currently, the majority of healthcare professionals receive insufficient nutrition education, which has created a gap in patient treatment plans aimed at healthy lifestyle behaviors (Kris-Etherton et al., 2015). The Affordable Care Act has shifted focus onto prevention and treatment of chronic diseases (Kris-Etherton et al., 2015). "Policies and programs that support a public health focus on prevention and treatment of chronic diseases through better nutritional awareness, assessments, and counseling," (Kris-Etherton et al., 2015, p.85). The gap in professional staff nutrition education is attributed to a competition for time, indicating other subject areas are more dominant in medical school preparation (Kris-Etherton et al., 2015). However, with nutritional knowledge, healthcare professionals become intervention points for patients to learn about disease-targeted nutrition and gain the proper knowledge to prevent chronic disease, all of which support the ideal healthy lifestyle.

Numerous health benefits associated with proper diet and nutrition further support nutrition intervention at the health care level (Kris-Etherton et al., 2014). Current medical degree requirements provide physicians with less education in nutrition than nurses (Kris-Etherton et al., 2014). The importance of advocating for nutritional patient care and the health of the patients is a top priority (Kris-Etherton et al., 2014). Registered dietitians are a great resource and are often easily accessible to other healthcare professionals, researchers have suggested utilizing dieticians as onsite educators for physicians and other healthcare professionals (Kris-Etherton et al., 2014).

Progress and importance is being supported amongst current medical students. According to a medical student in Chicago, patients are more and more concerned about preventing disease before it occurs (Eng, 2015). While the demand for medical professionals to counsel patients in nutrition is increasing, many medical doctors continue to lack nutritional education and do not feel comfortable discussing it with their patients (Kris-Etherton et al., 2014). Health care professionals are often seen as role models for the public and have an opportunity to promote proper nutrition by aligning with public health disease prevention interventions.

Public Health Interventions

It is imperative that as knowledge increases regarding the role of nutrition in the prevention of chronic disease and promoting health, interventions in medical education and the food environment within health care follow suit. Current movements within both health care and education are playing an important role in increasing awareness and bringing about change. The article *Menu of Change: Healthy Food in Healthcare* states, "Changing the culture around health and food habits is one of the most difficult things to do. It is important to understand that it is a long journey...Changing culture is about chipping away at it one step at a time, always finding a new way to involve a new group of people," (Kulick, Nathanson, & Sirois, 2011). Fortunately, small changes are being made to improve the current food environment.

After recognizing that current, practicing physicians are not equipped to advise their patients on proper nutrition, medical students at the University of Chicago set out to find their own nutrition education (Eng, 2015). In their own time, without credit, students began

taking culinary classes with Dr. Sonia Oyola and Dr. Greeta Maker-Clark (Eng, 2015). The University of Chicago does not offer culinary classes in their medical program, but students valued the importance of this type of education for their future as clinicians and their ability to effectively treat, diagnose, and counsel patients (Eng, 2015). This class, modeled after Tulane University, required a culinary nutrition course, and is therefore unique as it teaches components of proper diet in conjunction with practical preparation of healthy foods (Eng, 2015). It is students like these that are willing to take initiative over their own education and who recognize their responsibility to improve health who will ultimately bring about change.

Approaching change in the food culture within hospitals is no small feat, but is necessary for improving the health of patients and the medical professionals who oversee them. Health Care Without Harm, an organization dedicated to responsibly advancing medicine worldwide, stresses the importance of providing hospital patients, visitors, and staff, with healthier food options (Health Care Without Harm, n.d.). They are advocating to incorporate local and sustainable foods, at an affordable price, making it easier for people to make healthy food choices (Health Care Without Harm, n.d.). Two hundred and fifty hospitals have signed a pledge with Health Care Without Harm and are now vowing to provide antibiotic and hormone free foods as well as fresh produce (Conis, 2009).

Plan of Action

Policy

Looking locally, the greater Seattle area is home to more than twenty-three major medical centers, identifying the region as suitable and optimal for maximizing the benefits of a program that brings healthy food behaviors into healthcare. Moving toward a healthier, less toxic food environment will require changes in current policies such as the hospital food system, sourcing and education intervention. By combining these areas of focus, the Pacific Northwest can lead the medical world by improving the nutrition and health of our patients, community and providing a successful example for the rest of the country.

This Healthy Food Program will require an observational study, both quantitative and qualitative, as well as implementation of continuing education courses in nutrition and culinary medicine. We propose an observational study at each participating area medical center to assess the current food environment, health ratings of food offered, and measurement of toxic exposures. Once areas of concern have been identified within the current food system, the Healthy Food Program will make recommendations tailored to fit the needs of each medical center.

As the food environment concerns are addressed and remedied, it will also be essential to increase physician, nurse, and dietician knowledge in nutrition and culinary medicine. The program plans to create continuing education courses in culinary medicine available to all medical staff. We plan to approach the University of Washington School of Medicine to incorporate culinary medicine courses into required electives for first and second year medical students. As the program improves, knowledge of the relationships between disease, medicine, and nutrition will become intrinsic. Professional staff will be prepared to approach dietary solutions to health conditions with a multidisciplinary perspective. As staff offer solutions to patients, they will in turn be equipped with essential tools and knowledge which can be leveraged in their own life.

Food Environment

With results from the observational food environment study, the Healthy Foods Program can target areas of needed improvement. Generally, medical centers will then tend to follow the example of hospitals which have made the change to organic produce, hormone free, and antibiotic free protein sources.

Implementing organic produce in the cafeteria as well as direct patient service, supports public health messages of healthy lifestyle. Heavily processed foods containing high amounts of fat, sugar, and salt would no longer be available in the cafeteria.

With fast and processed food removed and local organic produce supplemented, the program will also see a reduction of packaging, plastics, and chemicals used to preserve produce. Reducing exposure to harmful chemicals will support the health of patients and the health of the environment as well. The most vulnerable populations in society often use emergency rooms and hospital visits as their primary source of health care. Incorporating organic foods into the food service system allows medical centers the opportunity to intervene with the larger general public as well. Within this framework medical centers will be inline with public health and empowered to lead the community by example.

Education

A robust Healthy Food Program has many demands, making cost effective budgeting a priority in maximizing the effectiveness of a new food environment. Staff must be educated in order to address patient needs for nutrition information specific to their health. Dieticians must be given additional opportunities for nutrition and culinary medicine education. Future hiring will emphasize certified nutritionists who can function both as a nutritional expert for patients, and also as educational interventionists supporting public health initiatives.

Professional staff taking on nutrition roles will become a link between tertiary and preventative care. This sends a message to patients and the public that their conditions can be improved by developing healthy lifestyle habits, supported and encouraged by their local medical centers in an effort to reduce the need for recurring visits. Another important element is that professional staff will be better prepared to make referrals to nutrition or naturopathic specialists to customize a preventative or restorative health plan for patients. Dieticians can recommend support groups, community services, and rehabilitative health specialists.

Conclusion

Collectively, the above action items support public health goals of disease prevention and health promotion. Both components offer vital public and environmental health services that vulnerable populations (i.e. “un-doctored”) may not have access to. Patients with primary care providers are unlikely to receive Health Food Program resources. Implementing this program into medical centers as a routine treatment, and applied to patients in crisis, can maximize the opportunity for intervention and positive impact for those who need it most.

Healthcare professionals and institutions have always had a large influence on the health and well-being of others. Providing these experts with the most current information and a well-rounded education is key in the spread of awareness and promotion of healthy lifestyles. In order to see our plan through, we first must educate those who are trusted most. Empowering medical schools to integrate nutrition education into programs is imperative. Replacing fast food restaurants with healthy food environments that utilize locally grown produce provides quality options and helps the health food suppliers prosper. The alignment of nutrition, chronic disease treatment, retail food environments, and medical school education with public health disease prevention programs builds invaluable trust with our patients and broader communities.

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LIVELIHOOD IDENTITY: HOW FOOD IS USED AS RESISTANCE IN THE MANGROVE ECOSYSTEMS OF ECUADOR

Hillary Sanders

ABSTRACT: Since the late 1960s, the development of the shrimp-farm industry in Ecuador has contributed to extensive ecological damage to mangrove areas. Consequently, the livelihood of those reliant on these ecosystems has been severely threatened. In response, the population mobilized a national grassroots movement. They used food to articulate resistance against the shrimp-farm industry as well as the Ecuadorian government, both of which have contributed to the destruction of coastal mangroves. The "livelihood identity," or collective sense of belonging, among ancestral mangrove people, those who are not necessarily native to the mangroves but who engage in their protection and maintenance over time, has motivated communities to protect not only their food source, but also their way of life. This paper is focused on analyzing how the expansion of the shrimp-farm industry has altered resource availability and access in the mangrove communities of Ecuador and given rise to social movements that utilize food culture as a tool of resistance.

"Mangroves are the supermarkets of the coastal poor."
– Pisti Charnsnoh, Thai campaigner for coastal ecosystems and community rights (Warne 2011, 3).

Mangrove refers to trees and shrubs that make up the overall plant community which are unique to tropical and subtropical regions (Ocampo-Thomason, 2006b). They are found along coastal tidelands and estuaries where fresh and salt water converge (McCally, 1999). They are most often characterized by their salt-tolerant evergreen forests and their aerial rooting systems, which are exposed during the day and covered by water during high tide (Ocampo-Thomason, 2006b). Mangroves provide water and soil filtration, storm barrier and surge protection, carbon storage, and habitat for flora and fauna (Latorre, 2014). Though all of this is important to understanding the value of these coastal environments, mangroves are so much more than the lush, bio-diverse ecology that they embody or the ecosystem services that they provide. To the ancestral people of coastal Ecuador, mangroves are a livelihood. To those looking to extort natural resources for profit, mangroves are money ponds just waiting to be tapped.

Around the late 1960s, when mangroves began to be exploited for shrimp production in Ecuador, "society and the scientific community only valued mangroves for what they could be converted into" (Hamilton, 2013). Ecuador was the first Latin American country to invest in the shrimp aquaculture industry. The Ecuadorian government began leasing out the land to the shrimp-farm industry in a process of concessions (Latorre, 2014). Due to the major boost that shrimp exports gave to the country's economy, the "consequences on the way of life of those populations linked to the mangrove ecosystem were overlooked" (Latorre, 2014). Between 1969 and 1984, the average loss of mangroves per year was 1,439 hectares (ha). The destruction continued into the 1990s and early 2000s as shrimp became Ecuador's most profitable export. The immediate costs of mangrove deforestation have been "mainly borne by the poor, who depend directly on these natural resources for their livelihoods" (Latorre, 2014). As a result, it has been in impoverished, isolated

communities where local resistance movements have gained their momentum.

As more ancestral mangrove people began to recognize the harm being done by industrial shrimp-farm operations, a notion of "livelihood identity" formed among mangrove communities (Latorre 2014). This concept can be described as a means of basing collective and territorial rights on a sense of belonging ancestrally within a natural ecosystem (Latorre 2014). Additionally, "livelihood identity" has to do with distancing from racial connotations normally associated with the dominant conception of "indigeneity" (Latorre 2014). The politics of identity are aimed at "unifying and mobilizing a racially heterogeneous" group that is connected by their shared reliance on mangrove resources for food and income (Latorre 2014). Therefore, the actions taken by these groups are conceptualized as "livelihood movements"or as part of the "environmentalism of the poor" (Latorre 2014; Martínez-Alier, 2002). Their struggle is a response to the depleting resources in the environment that serve as their source of livelihood.

It is through this collective struggle that food culture becomes a method of resistance. By continuing traditional practices of gathering and fishing while also openly standing in opposition to the shrimp industry that threatens to destroy them, ancestral mangrove people are articulating their resistance. Mangroves are to livelihood as deforestation is to poverty. Without access to the mangroves, the ancestral people of these areas cannot maintain their way of life. Under these circumstances, the loss of mangroves to deforestation is a very real threat to the majority of the population. Grassroots resistance movements emerging from the mangrove communities of Ecuador are engaged in a collective formation of identity. Their shared livelihood identity strengthens opposition to the shrimp-farms' invasion of mangrove environments.

Food (In)Security and the Ecuadorian Government's Role in the Shrimp Industry

Shrimp-farms were perceived to have many benefits in the early years of development, one being a boost to the national economy, another being increased food security for the Ecuadorian people. The former worked out smoothly. Ecuador reached the "highest export level of its history in 1998, when shrimp contributed to 26% of total private exports of the country" (Rivera-Ferre, 2009). The same success cannot be said for the latter, however.

Despite government initiatives to make food security a priority over the last few decades, actions have not been consistent with policy. In 2008, newly elected president Rafael Correa rewrote Ecuador's constitution in which it was specified as the State's responsibility to prevent ecosystem destruction. This new constitution ultimately determined that "communities have the right to benefit from environmental resources" (Warne, 2011). However, before the international community had even finished applauding the historic decision to institutionalize environmental rights, Correa issued Decree 1391, which legalized the country's illicit shrimp farms (Warne, 2011). It was a significant step backwards in that it reflected decades-old Ecuadorian policy, which prioritized monoculture and the export of non-sustainable products (Giunta, 2014). In exchange for cooperation from the shrimp companies in funding minimal mangrove reforestation, the decree "absolved them from punishment for violating mangrove-protection laws," not to mention the human rights of local populations (Warne, 2011). The decree regularized illegal farms, essentially gifting land to shrimp farmers on the principle that mangroves are "national assets of public use" (Yépez, 2008). According to mangrove activists and inhabitants, this undermined the progress made in the new constitution and violated environmental, water, and human rights (Yépez, 2008). What it didn't do was promote food security, a declared

"strategic goal" of the Ecuadorian government since the 1980s (Giunta, 2014).

Food security is an admittedly difficult goal to achieve. There are three major pillars of food security: availability, access, and use (knowledge of nutrition and care). Unfortunately the introduction of shrimp aquaculture in Ecuador has not been shown to uphold any of these critical pillars. Shrimp is one of the most profitable branches of the seafood industry today. Demand has increased, with the U.S. consuming 40 percent of the world's shrimp, Europe and Japan as close seconds (Ocampo-Thomason, 2006b). The way the current system works, shrimp is produced in the global South to be exported to "grace the tables of consumers in the North" (Yépez, 2008). Meanwhile, the 0.6 percent of the Ecuadorian population who are employed by the shrimp companies receives little to no benefits (Ocampo-Thomason, 2006). In reality, the development of shrimp aquaculture has never been about food security (Warne 2011, pp. 34). Elaine Corets is the Latin American coordinator of the Mangrove Action Project[3] (MAP), a nonprofit organization based in Port Angeles, Washington. According to Corets, shrimp is "an exotic species farmed in ponds created by destroying local ecosystems and exported to wealthy countries for the consumption of over-weight people who don't need any more protein or cholesterol in their diet" (Warne, 2011). There's really nothing left to add except for the fact that the globalization of the shrimp-farm industry had led to food *in*security for the countries actually producing the food.

This has not gone unnoticed by the ancestral mangrove people of Ecuador. They recognize that shrimp-farming is a "capital-intensive enterprise" rather than a labor-intensive one (Ocampo-Thomason, 2006b). Case-in-point, employment is often limited to low-wage jobs in the labor and security sectors (Ocampo-Thomason, 2006b). Activists have argued that one hectare of shrimp farm "provides 0.1 employment while one hectare of mangrove produces enough resources to feed at least 10 families" (Ocampo-Thomason, 2006b). According to those same activists however, the concern is not so much how many jobs the shrimp industry *creates*, but rather how many livelihoods it *destroys* (Ocampo-Thomason, 2006b).

The Gendered Impact of Shrimp Farming in Mangrove Environments

The areas that have been transformed into industrial shrimp operations have gone from "a multi-use public resource to a single-use private asset to a derelict waste" (Warne, 2011).

In some areas of present-day Ecuador, over 90 percent of mangroves have been wiped out since the early 1970s (Warne, 2011). Clear cutting has contributed to shoreline erosion and loss of fragile habitat. In addition, existing shrimp-farm operations use chemicals and antibiotics to prevent disease in the shrimp ponds. The excess fluid is dumped into the estuaries flowing through the mangroves, resulting in extensive fish and crab kills (Ocampo-Thomason, 2006). Despite the uncertainty and danger, women do most of their labor in these contaminated estuaries.

Veuthey and Hamilton point out the importance of recognizing the disproportional effect pollution and deforestation events have on particular groups, especially women. Those most at risk include: 1) Women who live in the interior of the mangrove where most of the clear-cutting has oc-curred, 2) those without boats who cannot access open-ocean fishing, and 3) the poor (Hamilton, 2013). Anyone existing at the intersection of these three groups is in a very vulnerable position and faces a higher risk of poverty than others living in the mangroves. Across Ecuador, it was women who became most active in grassroots movements to defend the mangroves. According to Veuthey and Gerber, poor women disproportionately bear the consequences of industrial shrimp farming activities. This is why women became so critical in organizing resistance movements against the

seafood corporations; they had everything to lose if the destruction was allowed to continue (Veuthey, et al. 2012).

In considering women's involvement in the protection of mangroves, it is important to note that initially it was a considerable challenge for women to meet at all (Veuthey, et al., 2012). Husbands would forbid their wives from attending grassroots meetings or events (Veuthey, et al., 2012). Due to the gendered division of labor and power, women were expected to either stay in the home or be working in the estuaries. In becoming community leaders and activists, women participants challenged power relations "on one hand, between local poor communities and regional or national elites, and, on the other hand, between men and women in their own villages" (Veuthey, et al., 2012). Their assertion of their right to a livelihood resisted the assumption by both the shrimp industry and men in their communities that women would remain complacent.

The Livelihood Impact and Community Response

In 1996, the Ecuadorian government allocated 52,000 hectares in the northern Esmeraldas province for the creation of the Cayapas-Mataje Ecological Mangrove Reserve (Reserva Ecológica de Manglares Cayapas Mataje, REMACAM in Spanish). Although the reserve is one of the most pristine mangrove ecosystems in Ecuador, the shrimp industry maintains 45 farms within the reserve's borders, occupying a total of 3,114 hectares (Ocampo-Thomason, 2006; Warne, 2011). The vast majority of these farms, 90 percent, are illegal, but little is done to enforce the law. The farms are located in the central and southern areas of REMACAM, impacting fishing and cockle gathering-reliant communities such as Tambillo. In response, the National Coordinating Committee for the Defense of the Mangrove Ecosystem (C-CONDEM² in Spanish), grassroots resistance movements such as the Foundation for the Ecological Defense of Muisne (FUNDECOL in Spanish) and local communities devised a stewardship practice called "*custodias*" (Ocampo-Thomason 2006, pp. 150). Through this practice, sections of mangrove forest are designated to each community "for their traditional use and management" (Ocampo-Thomason, 2006). This strategy is based on evidence that mangrove communities know how to manage natural resources in a sustainable way, the reason being that their futures depend on the health of mangrove ecosystems (Ocampo-Thomason, 2006). Losing the mangroves would also mean losing their culture, their homes, and their source of food and income.

Tambillo, a community of 130 households and approximately 600 people, is the largest *custodia* in REMACAM (Warne, 2011). The ancestral users of this community rely mostly on fishing and cockle collecting as their source of income (Table 11.1, Ocampo-Thomason, 2006). The gendered division of these two activities determines that men are the fishers and about ¾ of the women are *concheras* (Warne, 2011). *Concheras*, cockle collectors, often need a large harvest in order to provide for large families. According to one *conchera* in Tambillo, women often have several male partners throughout their lives, but the "children from those relationships always stay with the woman" (Warne, 2011). The larger the family, the longer the days of cockle gathering in between caring for children, performing housework and other forms of domestic labor (Ocampo-Thomason, 2006). According to Warne, the pressure to provide for one's family is so great that pregnant women will often work right up to when the baby is due, some have given birth on the wooden boats that transport *concheras* into the mangroves (Warne, 2011).

Due to the high dependence of cockle gathering in REMACAM communities (an average of 90 percent across the reserve), any further strains on *concheras* put entire families at a much greater risk of poverty (Table 11.1, Ocampo-Thomason, 2006). Potentially due to of shrimp-farm expansion, an increased percentage

of men have moved into the estuaries to collect cockles, having been forced out of areas typically reserved for fishing. This poses a problem not because they are men, but because the influx of displaced people becoming collectors stresses the already limited natural resources. Also, the itinerant collectors are often not coming from local communities that follow traditional and sustainable harvesting practices (Warne, 2011). They will cut the mangrove roots to make collecting easier, they often don't respect closed seasons, and refuse to leave behind juvenile cockles that are necessary to the replenishment of stocks (Ocampo-Thomason, 2006; Latorre, 2014).

Other than the challenges of availability of food supply in the mangroves, access has also become a threatening, if not fatal, issue. In the southern province of El Oro, and other parts of Ecuador as well, the buffer zones surrounding legal shrimp-farm operations have been deemed no trespassing zones to locals (Latorre, 2014). Guard dogs are sent on anyone who gets too close and armed security guards fire at trespassers. According to Latorre, "over the years, a number of deaths and disappearances have occurred in suspicious circumstances, the causes of which are presumed to be linked to the shrimp industry" (Latorre, 2014). In one incident, a cockle collector was killed when an attack dog was let loose on him (Warne, 2011). In 2008, a *conchero* was shot and killed by a guard on the border of a shrimp farm (Warne, 2011). The guard responsible said the man was stealing shrimp, but only cockles where found in the victim's possession. Though many have died, the goal is often to shoot to maim, thereby halting the process of gathering while also cutting off the family's food and support. The assumption is that collectors will eventually have to leave the mangroves. In small towns like Gauchal in the Esmeraldas, where there are a total of nine households, the loss of a single collector can prove disastrous to the entire community (Table 11.2, Ocampo-Thomason, 2006). The attacks are also gendered in that women are more often harassed by armed guards because of the proximity of shrimp-farms to estuaries where women work (Veuthey, et al., 2012).

Despite the risks, collectors breach shrimp-farm boundaries to collect food as their access to resources continues to decrease. Mangrove communities across Ecuador are joining together to continue to collect food in the ways that they have for generations. According to Latorre, there is a strengthening "sense of belonging among mangrove gatherers in opposition to the others (shrimp-farm owners)" (Latorre, 2014). They are determined to resist rather than flee their home.

Similarly, the *cangrejeros*, crab collectors, operating out of the mangroves of Huaquillas in the El Oro province are also an example of a local resistance movement. They not only oppose the deforestation of mangroves, but the loss of local access to mangrove areas as well. A motto for the group of *cangrejeros* is *El manglar es nuestra casa. Protégelo y nos alimentará* [The mangrove is our home. Protect it and it will feed us] (Warne, 2011). Pedro Ordinola, the founder of the environmental group of *cangrejeros*, has received death threats and bribes, and he is not the only one (Warne, 2011). Many outspoken opponents of the shrimp farms have been threatened, sometimes forcing them and their families into hiding in the mountains (Warne, 2011). Despite the monopoly that the shrimp industry holds on the liberation of mangroves and its resources, activists have refused to back down. According to Warne, "Money talks, but it doesn't drown out the voice of those who have been killed or maimed for asserting their right to make a living" (Warne, 2011).

Food as Resistance and Grassroots Movements to Reverse Mangrove Destruction

On the morning of July 26, 1998, several hundred people from the west Esmeraldas province gathered at the site of an illegal shrimp farm (Warne, 2011). Using whatever tools they had, including their bare hands, the crowd dismantled the pond wall, letting the shrimp pour into the sea. They then planted mangrove

seedlings in the empty pond basin (Warne, 2011). This was the first time such a concerted, visible act of resistance was organized against the shrimp farms in Ecuador. The participants were made up of local activists, mangrove defense organization members, and the fishers and gatherers themselves. This collective action made a number of key statements, 1) it was a denouncement of the government's unwillingness to act on illegal shrimp-farming operations, 2) by dumping the illegal food product into the open sea, they were depriving the shrimp-farm of its profitability – a retaliation against the shrimp-farms attacking the environment and the ancestral users of the mangroves – and 3) planting the mangrove seedlings stood as a symbol of the collectors' livelihood. July 26, 1998 was a historic day for mangrove activists around the world and is now known as the International Day of Mangroves.

Though this act of resistance took more drastic measures to convey opposition to the shrimp farms, every day forms of resistance have continued in the years since the protest. The work of grassroots organizations like FUNDECOL have "aimed at increasing power over the mangrove common-pool resources" as a more collective defense against mangrove destruction (Latorre, et al., 2014). Despite the challenges, the efforts of ancestral people to engage in traditional gathering and fishing practices have led to a strengthened sense of belonging within mangrove communities. This contributes to the argument that food is used as a means of disrupting the control that the shrimp-farm industry has over the mangroves and the people who rely on it for their livelihood.

Conclusion

In Ecuador's coastal communities, there is a collective "livelihood identity" that supports grassroots social movements in their mission to prevent mangrove deforestation. Ancestral mangrove people have united to overcome shrimp industry giants that have subordinated and nearly destroyed "a cultural way of living with nature" (Latorre, 2014). Through the use of food as both a means and a symbol of livelihood, mangrove gatherers and fishers have articulated their resistance against the shrimp-farm industry and the Ecuadorian governmental policies that enable it. Furthermore, they have pushed for collective land rights as a population heavily dependent on mangrove resources. Even as the shrimp-farm industry has expanded into the far reaches of Ecuador's mangroves, the people have asserted their 'livelihood identity,' and in the process, motivated social movements that prioritize mangrove culture over monoculture.

Notes

[1]According to testimonies of mangrove activists, 'Ancestral Mangrove People' is a more comprehensive term than 'indigenous' because it is not "necessarily or exclusively related to a native status. Elements such as the defense of the mangroves (against the shrimp farmers) and the adoption of environmentally sustainable practices and uses play a more determinant role in choosing who is included or excluded from this political category" (Latorre, 2014). Therefore, mangrove inhabitants are referred to throughout this paper as 'ancestral mangrove people'.

[2]C-CONDEM is a collection of associations and grassroots communities of traditional gatherers and artisanal fishers, as well as environmental and social NGOs that have formed an interconnected network to resist mangrove destruction at the hands of the shrimp–farm industry (Yépez, 2008).

[3]The Mangrove Action Project is a nonprofit organization with an approach focused on education, networking, advocacy, and research. They have partners around the world and release regular reports on the status of mangroves from Asia to Latin America. For more won MAP, visit http://mangroveactionproject.org/

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ANALYSIS OF CO_2, CO, $PM_{2.5}$, AND PM_{10} FROM FLAMING AND SMOLDERING COMBUSTION IN A HOME WOOD STOVE

Sara Wells

ABSTRACT: It is advantageous to study carbon monoxide, carbon dioxide, and particulate matter smaller than 2.5µm because these pollutants have known negative health and climate implications. We will use three instruments: a trace level gas CO analyzer from Thermo Environmental Instruments, a non-dispersive infrared gas analyzer from Licor Biosciences for CO_2, and an aerosol monitor from TSI for particulate matter, to continuously measure the output of these pollutants from a wood stove with two types of combustion, flaming and smoldering. The CO instrument model 48C has a lower detectable limit of 0.04ppm, with linearity ± 1% of readings ≤ 1000ppm. The CO_2 instrument model LI-820 has measurement range is 0-20000ppm with an accuracy of <3% of the reading. The DustTrak, using gravimetric and photometric analysis to filter and analyze particulate matter, has a flow rate accuracy of ±5% of factory set point and can measure concentrations from 0.001-150 mg/m^3.

In heating homes, using wood for fuel in wood stoves is becoming an increasingly common practice in the Pacific Northwest (Tonn & White, 1990). The combustion of wood in stoves releases pollutants which may have a connection with respiratory illness in households that use them and a definite connection with atmospheric pollution (Larson & Koenig, 1994). Major pollutants of atmospheric importance are carbon monoxide (CO), carbon dioxide (CO_2), sulfur and nitrogen compounds, and large and small particulate matter (PM_{10}, $PM_{2.5}$). The carbon-containing pollutants contribute to ozone increases in the lower atmosphere (which worsens smog in densely populated areas) and decreases in the upper atmosphere (Haagen-Smit & Fox, 1954). The connection between carbon monoxide and ozone formation can be shown by the following overall equation (there are more steps and intermediate species, but the net is shown) (Reeves et al., 2002):

$$CO + 2O_2 \longrightarrow hv + CO_2 + O_3$$

From this equation, we can see how carbon monoxide released from the combustion of wood in wood stoves may contribute to atmospheric issues. Particulate matter with diameter smaller than 2.5µm is of particular importance has been shown to be linked to respiratory health because it is small enough to bypass the nose into the lungs (Lea-Langton et al., 2015). There are many other respiratory illnesses associated with wood smoke from stoves: emphysema, anthracosis (build-up of carbon dust in the lungs), lung fibrosis (scarring of lung tissue), and asthma are some issues (Bruce, Perez-Padilla & Albalak, n.d.).

Under ideal conditions, wood burns according to the combustion reaction:

$$C_XH2_{X+2} + O_2 \longrightarrow H_2O + CO_2$$

Under real conditions, though, this reaction is never complete and depending on the burning conditions and type of wood, side reactions occur to release different pollutants. Smoldering combustion can be characterized by "slow, low temperature, and flameless … combustion" which takes place on the surface of a material, whereas flames arise as material goes into the gas phase, instead of solid (Chao & Wang, 2001; Moghtaderi & Fletcher, 1988; Rein, 2009).

Gas chromatography, dilution tunnel, and mass spectrometry have all been used to test emissions of

varying fuel types and combustion environments for particulate matter and carbonaceous matter (Hedberg, 2002; Lea-Langton et al., 2015; McDonald et al., 2000). A dilution tunnel is a useful method to measure flow of particulate matter from combustion. This method was used to monitor particulate size from a combustion reaction running for 90 minutes. The results of this experiment showed an average value of 4.22E14 particles of particulate matter with diameter less than 0.9μm per kilogram of wood taken over 10 minute periods (Hedberg, 2002). In a recent study, researchers used gas chromatography to identify the amount of carbon monoxide produced from wood combustion. This method, testing for semi-volatile and volatile organic compounds showed an increase in carbon monoxide release as compared to other similar studies (McDonald et al., 2000). Mass Spectrometry has proven especially helpful in identifying the different types of particulate matter that result from wood stove combustion. One study used aerosol time of flight mass spectrometry (ATOFMS) to measure the particulate matter (large and small) products of flame and smoldering combustion of wood (Lea-Langton et al., 2015). This method gave results that there is a higher concentration of particulate matter from smoldering than flaming combustion for different wood types.

In comparing fuel types, soft has been tested against hardwood for emission types and amounts (McDonald et al., 2000). This study showed that when examining carbon monoxide and particulate matter, softwood generally expelled more carbon monoxide, and hardwood generally expelled more particulates of varying sizes. Another study compared combustion of wood in different environments: an open fireplace, a closed fireplace, and a traditional stove (Ozgen et al., 2014). This research showed that, when comparing emissions factors for carbon monoxide versus particulate matter, there was not a significant difference in emissions factors for the two pollutants. That data comes from combustion cycles not shorter than 45 minutes. In a different part of the combustion cycle, the air valve was closed and the fuel load increased by 50% at the latter part of the cycle. This change resulted in a higher emission factor for CO for a closed fireplace than in a stove.

We are presenting research and data to contribute to the study of particulate matter and carbon monoxide resulting from the combustion of wood in wood stoves. Two parts in the combustion cycle were studied: flaming and smoldering. We wish to examine the question: What is the effect on the concentrations of pollutants during flaming vs. smoldering combustion? We hypothesize that for flaming combustion, concentrations of particulate matter will be relatively low compared to concentrations of carbon monoxide and carbon dioxide, due to a more complete reaction. For smoldering combustion, we hypothesize that concentrations of particulate matter will be relatively high compared to low concentrations of carbon dioxide and carbon monoxide due to the incomplete burning of fuel.

Materials and Methods

1. Overview/setup

We will monitor three pollutants with a respective instrument to examine the hypothesis that smoldering combustion in a wood stove produces high concentrations of particulate matter and flaming combustion produces high concentrations of carbon dioxide and carbon monoxide because smoldering combustion results in an incomplete breakdown of organic material and flaming combustion gives a more complete reaction. We will analyze pollutant data by comparing concentrations from smoldering combustion with concentrations from flaming combustion. Data will be collected using a wood stove in Covington, WA. The stove is located in a back room of a 6-member family home, and is only used for heating. The Rainier 90 model stove from Avalon Firestyles will be used. A diagram available on the company's website shows an inner area of 58.89 square centimeters, and other specifications of the

stove (Avalon Firestyles, 2016).

The stove we will be using has an exhaust flue connected, which leads up through the ceiling to the roof. For consistency, wood burned will be from a single source and stored indoors away from moisture.

The exhaust will be monitored for CO, $PM_{2.5}$, PM_{10}, and CO_2 using the setup described below. We will use a trace level gas CO analyzer from Thermo Environmental Instruments (model 48C). For CO_2, we will use a non-dispersive infrared gas analyzer from Licor Biosciences (model LI-820). Finally, to measure particulate matter, we will use an aerosol monitor from TSI (model DustTrak DRX 8533/8534). The instruments will be connected to the gas source via a rubber tube which is connected to the exhaust flue with a gasket. We will place a particulate matter filter downstream from the DustTrak and upstream from the Licor and 48C, to ensure that the latter two do not take in any particulates. A pump will be placed upstream of the Licor because it is not included in the instrument itself.

2. Theory of Operation

2.1. Theory of Operation of the DustTrak

The DustTrak works to measure the size and amount of particulate matter using photometry and gravimetric analysis. Air is drawn into the instrument using an included pump, and some of it is diverted to a HEPA filter to be used as sheath air. The unfiltered air is passed through a laser emitted from a laser diode. The amount and intensity of light that is not absorbed is measured by a sensor and then recorded as a voltage. This signal is separated into the photometric signal and the single particle pulses. The air that passes through the laser then is passed through a gravimetric filter, which separates particles by size (TSI Incorporated, 2012).

2.2. Theory of Operation of the 48C

Sample gas is passed through an infrared light source set to 4.6 microns, because CO absorbs at that wavelength. The absorption is measured with an IR detector, and from this, the concentration of CO in the sample gas can be calculated (TSI Incorporated, 2012).

2.3. Theory of Operation of the LI-820

The LI-820 instrument is an NDIR (non-dispersive infrared) gas analyzer. It works to analyze air for carbon dioxide by directing infrared light through an air sample to a detector. There are two chambers, one with sample and one with reference sample which consists of ambient air. Depending on the amount of light absorbed, and our knowledge that carbon dioxide absorbs at 4.24 microns, we can measure the amount of carbon dioxide in a given sample. There are two absorption bands, one at 4.24 microns, and one non absorption band (Licor Biosciences, n.d.).

3. Calibration of Instruments

3.1. Calibration of the DustTrak

The DustTrak comes calibrated to Arizona Test Dust (ISO 12103-1, A1). This dust is used because it covers the size range of particles that the DustTrak can measure. To blank the instrument, we will use a zero air filter provided by the manufacturer. For our collection, we will calibrate according to the manual, by using a reference instrument and calibration gas of a known concentration of PM2.5 and PM10 (TSI Incorporated, 2012). The LI-820 and reference instrument will be simultaneously sampled and their average concentrations of aerosol recorded.

3.2. Calibration of the 48C

The 48C will be calibrated using NIST/EPA certified CO and zero calibration gas and relating flow rate to CO concentration using equations provided in the manual by the manufacturer (Thermo Environmental Instruments, 2007). “Blank” or zero air must have less than 0.01ppm CO, so we must remove any CO from the zero gas by scrubbing and drying it according to procedures detailed by the manufacturer. We will connect a flow rate meter to the instrument and by changing the flow rate, we can change

the concentration of CO in the standard gas to create a calibration curve by recording and plotting the standard CO concentration and the instrument's response.

3.3 Calibration of the LI-820

To blank the instrument, a CO2 free gas will be passed through it. Similar to work done by Gibert et al. (2009), we will choose two standard gases (one at high and one at low CO2 concentration) chosen to contain the high and low readings expected from measurements. In the study, the low standard was 365.922 ± 0.045 ppm, the high standard was 401.292 ± 0.045 ppm, and were chosen to reflect the range of concentrations expected from what they measured. Reference gas was dried ambient air. We can expect our highest concentration to be higher than 400 ppm because we will be measuring direct products of combustion and not atmospheric concentrations as in Gibert et al. (2009), so we will choose a higher concentration standard for the upper limit of our calibration range. Calibrations for the LI-820 can either be done manually or at set time intervals to create a linear calibration curve using the voltages from the detector.

4. Data Collection and Error Reduction

4.1. Overview

Data will be collected in January 2015, over the span of three continuous weeks. Data will be collected continuously and recorded electronically for each instrument, at a minimum of two repetitions per instrument (one for each burn type). Similar to an experiment done by Grieshop et al. (2008), we will cut wood into small pieces (in the previously mentioned study dimensions of 4 x 4 x 20 cm were used). For each repetition, the combustion chamber will be cleared of all ashes, charred wood, and other reside. As in Grieshop et al. (2008), wood will be allowed to burn for approximately 30 minutes before recording data, to keep as many variables (temperature, flow rate, etc.) as constant as possible. All instruments will be calibrated in the lab before transport to field site, and brought back to the lab when not in use to prevent damage. No instrument will be left unattended.

4.2. Data Collection and Error Reduction for the DustTrak

For the DustTrak, the voltage across the detector is proportional to the $PM_{2.5}$ fraction of the total sampled aerosol. A calibration constant is determined using the equation in section 3.1 of the manual, and the recorded voltage from $PM_{2.5}$ is multiplied by this factor. The aerosol stream is surrounded by clean, filtered air (sheath air), to prevent particles from circulating and changing results. A study by Ramachandran et al. (2003) shows possible error due to the method of analysis of the DustTrak. This study mentions that because the instrument samples at ambient humidity, concentration measurements change with increased humidity due to increased average size of particles. To correct for this error, a correction factor using relative humidity has been developed by Laulainen et al. (1993), cited in Ramachandran et al. (2003).

We expect to see data similar to that collected in an experiment by Wang et al. (2012), in which they studied pollutants from wood fires, and developed a test for instruments measuring those pollutants. For smoldering combustion, they showed high relative concentrations of $PM_{2.5}$ compared to smoldering combustion using the DustTrak. This is the opposite result in regards to our hypothesis, but it is useful to see what data we could possibly obtain using the same instrument.

4.3 Data Collection and Error Reduction for the 48C

We expect to see carbon monoxide data similar to that collected by Grieshop et al. (2008). For a certain wood type, their data shows high relative CO concentration released by flaming combustion vs. smoldering combustion. Even though they used a different instrument than we will and recorded data under laboratory conditions while we will be measuring at a field site, they are researching wood combustion

	Parameter	Flaming	Transition	Smoldering	Overall
Burning Phase Average	VOC (ppm)	121	147	119	128
	CO (ppm)	202	346	264	265
	CO_2 (ppm)	3353	2895	1385	2546
	NO (ppm)	5.59	9.15	8.85	7.73
	PM Number (cm^{-3})	8.52E+06	9.19E+06	4.41E+06	7.30E+06
	BC (mg/m^3)	3.42	3.10	0.83	2.44
	$PM_{2.5}$ by DRX (mg/m^3)	109	107	47	87
	MCE (-)[a]	0.95	0.90	0.86	0.90
	$BC/PM_{2.5}$ (-)	3.95%	3.15%	1.13%	2.75%
Emission Ratio	$\Delta VOC/\Delta CO_2$ (%)	4.07%	6.06%	14.26%	8.14%
	$\Delta CO/\Delta CO_2$ (%)	7.30%	15.04%	29.10%	17.01%
	$\Delta NO/\Delta CO_2$ (%)	0.20%	0.40%	1.09%	0.56%
	$\Delta CPC/\Delta CO_2$ (#/cm^3/ppm)	3.00E+03	3.92E+03	4.74E+03	3.86E+03
	$\Delta BC/\Delta CO_2$ (mg/m^3/ppm)	1.24E-03	1.28E-03	6.46E-04	1.05E-03
	$\Delta PM_{2.5}/\Delta CO_2$($mg/m^3$/ppm)	4.01E-02	4.64E-02	4.07E-02	4.21E-02

[a] MCE: modified combustion efficiency defined as MCE = $[CO_2]/([CO_2] + [CO])$

Figure 1: Table 4 from Wang, et al., 2012. The Burning Phase Averages of PM2.5 measured by the DustTrak is relatively high for flaming combustion as compared to smoldering combustion.

and found results that are consistent with our hypothesis.

The detector in the 48C is responsive to the intensity of light hitting it, and the response is proportional to the light's intensity. Span error can be calculated for this instrument as detailed by the manufacturer. This is done by testing with a gas with CO level around 80% of the upper range limit. It ensures that span error can be recognized and minimized.

4.4 Data Collection and Error Reduction for the LI-820

CO_2 absorbance in the LI-820 is measured by comparing the output of the two detectors. From these values we can calculate the mole fraction of CO_2 in the gas we pass through the instrument, using equations specified in the instrument manual (Licor Biosciences, n.d.). The measurement range for the instrument is 0-20000ppm with an accuracy of <3% of the reading.

Broader Impacts

This is a useful experiment in that there are many possibilities for expansion. For instance, future researchers could change variables such as fuel type, combustion environment, length of combustion, etc. There are many pollutants aside from CO, CO_2, and particulate matter that, depending on the conditions, can be detected from combustion. So, it's possible to study and analyze other compounds and their effects on the environment and respiratory health.

We will use this experiment to teach non-scientists about the effects of the pollutants we'll be studying. Doing the data collection in a family home will provide an opportunity to demonstrate what we'll be doing and the methods used to collect and analyze data. As we're actually collecting data, we can talk a small audience of five college-age people through the setup and operation of the instrument(s). Because of the home setting of the experiment, the audience will be residents of the home who have previously expressed an interest in science. We will describe and display the instruments we will be using to help gain attention, and then describe how we will analyze the data and use it to evaluate a hypothesis. Allowing the students to learn not only about instruments, but also about data analysis, will help them to decide to pursue a STEM major or career.

Another way to share findings would be to display a poster at the University of Washington Bothell, and speak about the findings. This could be used to convey information to a

	Experiment	MCE	POA	OC:EC	NO_x^a	ΔCO^b	Injection $\Delta CO/\Delta CO_2^b$	ΔBenzene/ΔCO^b	ΔToluene/ΔCO^b	ΔAcetonitrile/$\Delta CO^{b,c}$
			$\mu g\ m^{-3}$	ratio	ppb	ppm	molar %	ppb ppm^{-1}	ppb ppm^{-1}	ppb ppm^{-1}
	Laurel Oak									
1	smoldering and flaming	0.90	40	1.6	113	19	17	0.8	0.2	0.1
2	flaming w/ embers	0.95	90	1.9	150	13	7	1.3	0.2	0.3
3	smoldering and flaming	0.93	40	1.1	60	6	11	1.1	0.1	0.5
	Yellow Pine									
4	flaming w/ embers	0.92	770	2.2	103 (39)	10	14	4.3	1.5	0.6
5	smoldering/dying flame	0.79	50	13	63	37	41	0.1	0.0	0.1
	High NO_x (Pine)									
6	flaming w/ embers	0.69	70	13	244 (18)	2	71	3.7	1.1	0.8

Figure 2: Table 1 from Grieshop, et al., 2008 We expect to obtain results similar to those from experiments 1, 2, and 3, column 5 for change in CO in ppm.

more technical audience, which would allow us to include most of the details and technical language of the research. Specifically, the audience would be STEM students and teachers who have a background in scientific language.

Timeline

Week	Completed Work
1	- Make sure instruments are calibrated correctly according to the needs of the experiment detailed in the methods section. - Collect test data in the lab to make sure that the instruments are calibrated and running. - At the field site, make sure that the plumbing/setup fits the stove, and no parts are missing (trip 1, no instruments). - Record experiences in lab book.
2	- Check that instruments work at field site by collecting test data (trip 2, with instruments). - If necessary, resolve any errors in function. - Collect smoldering combustion data (trip 3, with instruments). - Record experiences in lab book. - Start data analysis: convert raw data into concentrations, organize raw data into tables, and find basic statistical values of data sets: averages, standard deviations, etc.
3	- Collect flaming combustion data (trip 4, with instruments). - Record experiences in lab book. - Start data analysis: convert raw data into concentrations, make sure data is organized, and find all basic statistical values of data sets: averages, standard deviations, etc.
4	- Collect remaining necessary data. - Analyze CO data: 1. CO and flaming combustion 2. CO and smoldering combustion 3. Make visuals: graphs, tables, etc. 4. Format visuals 5. Answer questions regarding CO and combustion. - Use data to write discussion on the effects of changing combustion type on CO output.
5	- Analyze CO_2 data: 1. CO_2 and flaming combustion 2. CO_2 and smoldering combustion 3. Make visuals: graphs, tables, etc. 4. Format visuals 5. Answer questions regarding CO_2 and combustion. - Use data to write discussion on the effects of changing combustion type on CO_2 output. - Lab book should have all necessary notes to write up paper/make poster.
6	- Analyze PM data: 1. PM and flaming combustion 2. PM and smoldering combustion 3. Make visuals: graphs, tables, etc. 4. Format visuals 5. Answer questions regarding PM and combustion. - Use data to write discussion on the effects of changing combustion type on PM output.
7	- Finalize discussion of the effects of smoldering vs. flaming combustion and pollutant output. - Use the data and analysis to come to conclusions about predictions. - Write abstract. - Revise introduction and methods written in 495 to match the method that was actually used.
8-10	- Put together paper/poster.

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OVEREXPRESSION, PURIFICATION, AND INHIBITION OF HELICOBACTER PYLORI ALDO-KETO REDUCTASE (HPAKR) USING DESIGNER INHIBITORS

Taryn Meachem

ABSTRACT: Helicobacter pylori infects the gastric mucosa of over half of the world's population, and is implicated in the genesis of many gastric pathologies. Current treatments for H. pylori infections are becoming increasingly ineffective as antibiotic-resistant strains of H. pylori become more prevalent. The purpose of this project is to discover a competitive inhibitor for an aldo-keto reductase enzyme (HpAKR) that is required for H. pylori to survive in the human stomach. The plasmid containing the HpAKR gene will be transformed into Rosetta™ E. coli cells (Novagen) for overexpression, and the purified HpAKR molecules will be assayed for activity and inhibition.

Helicobacter pylori, a bacterium which primarily colonizes the gastric mucosa, infects over half of the world's population, and is one of the most common agents of chronic bacterial infection in humans (Garcia, Salas-Jara, Herrera, & Gonzalez, 2014; Krah et al., 2004). Although most infected individuals are asymptomatic, *H. pylori* infections are associated with the majority of gastric pathologies, particularly chronic gastritis and gastric and duodenal ulceration (Garcia et al., 2014). It is estimated that 90-95% of duodenal ulcers in Europe are due to *H. pylori* infections (Krah et al., 2004). Additionally, *H. pylori* has been classified by the World Health Organization (WHO) as a Class I carcinogen (Ernst & Gold, 2000), and *H. pylori* infections have been associated with a 2.7– to 12–fold increase in the risk of developing gastric cancer (Krah et al., 2004).

Socioeconomic status (SES) plays a pivotal role in determining the risk of contracting an *H. pylori* infection (Malaty & Graham, 1994). This is particularly evident in developing countries where infection rates can be as high as 90%. (Garcia et al., 2014). A study conducted by Malaty and Graham (1994) on the prevalence of *H. pylori* infections among individuals living in the Houston metropolitan area found that 85% of individuals in the lowest social classes were infected with *H. pylori* versus only 11% of individuals in the top two social classes. The correlation between low SES and higher rates of *H. pylori* infection is due to a variety of factors often associated with low SES including poor hygiene and overcrowded living conditions (Laszewicz, Iwańczak, & Iwańczak, 2014).

H. pylori infections are currently treated using antibiotics and a proton pump inhibitor, however, such treatments are becoming increasingly ineffective due to the evolution of antibiotic-resistant *H. pylori* strains (Megraud, 2004). Antibiotic resistance rates vary depending on geographical location, but resistance rates for clarithromycin and metronidazole, two of the antibiotics most commonly used to treat *H. pylori* infections, have been reported to be as high as 25% and 76%, respectively (Megraud, 2004). Although other antibiotics such as tetracycline or amoxicillin may also effectively treat *H. pylori* infections, the current trend of increasing resistance rates for the common antibiotics may eventually render these antibiotic options equally ineffective (Megraud, 2004). Thus, the development of alternative treatment methods for *H. pylori* infections is paramount.

The key to discovering an alternative treatment lies in understanding how *H. pylori* is able to survive the harsh acidic conditions of the human stomach. Initial studies indicate that *H. pylori* colonization is facilitated by the use of urease, an enzyme capable of converting urea into ammonia and bicarbonate (Marshall, Barrett, Prakash, McCallum, & Guerrant, 1990). As shown in the reaction below, urease removes H^+ ions from the bacterium's immediate environment in order to create ammonia and bicarbonate. This reaction neutralizes the H^+ ions present in the gastric juice, thereby increasing the local pH (Marshall et al., 1990). However, urease-negative *H. pylori* strains are still able to colonize the gastric mucosa, indicating that other factors are required for *H. pylori* to survive in acidic environments (Mine, Muraoka, Saika, & Kobayashi, 2005).

$$(NH2)2CO + 2H2O + H^+ \xrightarrow{\text{Urease}} 2NH4^+ + HCO3^-$$

A recent study by Bijlmsa et al. (2000) identified ten different genes whose products may enable *H. pylor*i to survive in acidic conditions. One of these genes codes for an aldo-keto reductase (HpAKR). HpAKR functions over a broad pH range (pH 4-9), but displays optimum activity levels at a pH of 5.5, which is similar to the pH of the gastric mucosa of the human stomach (Cornally et al., 2008). Although the exact function of HpAKR is unknown, it appears that the enzyme is required for growth at a low pH. Isogenic mutants—mutants with nearly the same genotype as wild type—of *H. pylori* without the HpAKR gene were unable to grow at a pH of 5.5 (Cornally et al., 2008). Given that HpAKR is necessary for the pathogenesis of *H. pylori* within the human stomach, it serves as a promising target for pharmaceutical development. This particular study describes the expression, purification, kinetics, and inhibition of HpAKR with the hope of finding a competitive inhibitor for the enzyme.

Methods And Materials

Plasmid Preparation of HpAKR/pET28b

In order to create the necessary amount of HpAKR enzyme, the pET28b plasmid (which harbors the HpAKR gene) will be transformed into DH5α, a storage strain of *E. coli*. The DH5α cells will be grown on Lennox Broth (LB) agar plates containing 50 mg/mL Kanamycin (KAN) and 34 mg/mL Chloramphenicol (CAM) (antibiotics used to prevent other types of bacteria from growing on the plates). One of the DH5α colonies will be used to start an *E. coli* culture containing the pET28b/HpAKR plasmid and will be grown in 5 mL of LB containing 5 µL of KAN (50 mg/mL) and 5 µL of CAM (34 mg/mL). The culture will shake in the Excella™ E24 Incubator (New Brunswick Scientific) at 180 rpm and 37°C for approximately 15 hours. Following incubation, the pET28b/HpAKR plasmid will be purified using a Qiagen plasmid purification kit and then transformed into Rosetta™ *E. coli* cells (Novagen) for overexpression.

HpAKR Overexpression

To overexpress the HpAKR plasmid, transformed Rosetta™ cells will be grown at 37°C in 1 Liter of LB broth with 5 µL of KAN (50 mg/mL) and 5 µL of CAM (34 mg/mL). A UV-Vis Spectrometer (BioMate) will be used to measure the absorbance of the cells at 600 nm. When the absorbance value is between 0.80 and 1.00, the cells will be induced with 1M IPTG (final concentration 1mM) and grown at 37°C for approximately 15 hours. The cells will be isolated via centrifugation at 5000 x g for 10 minutes (Beckman Coulter, JA17 rotor). Following centrifugation, the cell pellet will be re-suspended in a 25mL Lysis buffer containing 20 mM Tris-HCl (pH 7.9), 0.5 M NaCl, and 5 mM imidazole. The cells will be sonicated for five 30-second intervals with a 1 minute rest period in between each sonication period. The sonication step will lyse the cells to release all of the HpAKR molecules produced during

the overexpression phase. Cell debris will be removed via centrifugation at 15,000 x g for 20 minutes.

The supernatant will be loaded onto a Nickel-Nitriloacetic Acid resin column (Novagen) to separate HpAKR from any other proteins present in the supernatant. The column will be washed five times with Wash Buffer containing 20 mM Tris-HCl (pH 7.9), 0.5 M NaCl, and 30 mM imidazole to remove the undesired proteins. HpAKR will be eluted from the column using Elution Buffer containing 20 mM Tris-HCl (pH 7.9), 0.5 M NaCl, and 150 mM imidazole. The eluted fractions will spin in the centrifuge twice, first for 20 minutes at 3500 RPM, then at 4000 RPM for 30 min with 7 mL of the Lysis buffer. Following the final centrifugation, the sample will contain pure HpAKR, which can be used to run activity and inhibition assays. The purity of HpAKR will be evaluated using 12%-SDS-PAGE.

Activity Assays for HpAKR

Following the literature procedures (Cornally et al., 2008), the UV-Vis spectrophotometer will be used to monitor the reduction of benzaldehyde to benzyl alcohol by NADPH at 340nm. The assay (total volume 1 mL) will consist of 50 mM potassium phosphate buffer, 500 mM NADPH, 10 µM HpAKR, and benzaldehyde concentrations ranging from 0.5 mM to 20 mM. The molar extinction coefficient (ε) that will be used for NADPH is ε_{340}= 6.2 x 10^3 $M^{-1}cm^{-1}$. K_M and k_{cat} for the purified HpAKR will be determined by fitting initial rates to the Michaelis-Menten equation and by using the linear regression of a Lineweaver-Burke plot in Microsoft Excel™. If the calculated K_M and k_{cat} are similar to literature values (Mine et al., 2005), then the HpAKR molecules produced during the overexpression and purification phase are sufficiently active and can be used in the inhibition assays.

Inhibition of HpAKR

Potential inhibitors for HpAKR will be determined by Dr. Peter Anderson (University of Washington Bothell) using Autodock Vina (Trott, & Olson, 2010), a program designed determine the binding affinity (i.e., docking ability) of potential inhibitors to a particular enzyme. Dr. Anderson will screen thousands of potential inhibitors and recommend at least 10 potential inhibitor molecules with K_i values (binding affinity values) between 15 and 200nM. Two of these inhibitors will be selected and used for inhibition assays.

Similar to the activity assays, the UV-Vis spectrophotometer will be used to monitor the reduction of benzaldehyde to benzyl alcohol by NADPH at 340nm. The assay for each inhibitor (total volume 1 mL) will contain 50 mM potassium phosphate buffer, 500 mM NADPH, 10 µM HpAKR, benzaldehyde concentrations ranging from 0.5 mM to 20 mM, and inhibitor concentrations ranging from 0 to 5 µM. Kinetic parameters (K_M and k_{cat}) for HpAKR will be computed by fitting initial rates to the Michaelis-Menten equation and using the linear regression of a Lineweaver-Burke plot in Microsoft Excel™.

The shape of the Lineweaver-Burke plots will indicate whether the inhibitors are competitive, non-competitive, or mixed inhibitors (Figure 1). Ultimately, we hope to find a competitive inhibitor for HpAKR since a competitive inhibitor will only interact with HpAKR while a non-competitive or uncompetitive inhibitor may interact with other proteins present in the sample.

The methodologies described above are commonly used in protein purification and the kinetic analysis of proteins and can easily be completed by undergraduate students under the advisement of a faculty mentor. A list of required materials can be found in the Appendix.

Objectives and Deliverables

Objective 1. Express and purify HpAKR. The deliverables for this objective will include a

photograph of the SDS-PAGE gel showing the transformed DNA plasmid with the HpAKR gene, a photograph of the SDS-PAGE gel showing the purification of the HpAKR, and a fully detailed protocol for the expression and purification process.

Objective 2. Run activity assays using the purified HpAKR. The deliverables for this objective include a data table showing the raw absorbance vs. time data and kinetic scan plots of the absorbance vs. time data, as well as detailed calculations for determining reaction velocity values and substrate concentrations used in the assay. Additionally, we shall create Michaelis-Menten and Lineweaver-Burke plots using the reaction velocity data and use the equation generated by the Lineweaver-Burke plot to calculate K_M and k_{cat}. We will also write a fully detailed protocol for the activity assays.

Objective 3. Run inhibition assays using Inhibitor 1 and Inhibitor 2. The deliverables for this objective will be the same as the deliverables for Objective 2, including a fully detailed protocol for the inhibition assays.

Broader Impacts

The primary focus of our education and outreach efforts will be teaching our fellow undergraduate students from all disciplines about the importance of finding alternative methods to treat *H. pylori* infections. First, the members of the HpAKR project will create and present a brief summary of our findings along with any relevant background information to the other members in Dr. Robins' research lab. This presentation will inform our colleagues on the status of our project as well as increase their knowledge on the topic. Additionally, the HpAKR project members will create a poster which will be presented at the University of Washington Bothell's Undergraduate Research Fair in the spring of 2016. This will allow us to share the knowledge gained from the project to other undergraduate students who conduct research in a wide range of disciplines.

In order to foster an interest in biochemical research in first and second year students at UW Bothell, the HpAKR team members will partner with the on-campus entities such as the Office of Undergraduate Research and the Pre-Medical Club at the University of Washington Bothell. These entities will help to recruit new students to conduct further research in Dr. Robins' lab. Furthermore, the HpAKR team members will hold several information sessions throughout Winter Quarter. These sessions will provide interested students with background information about the current state of the project and answer the students' questions regarding our experiences working within the lab. The current HpAKR project members will also assist in teaching the new research students the skills and techniques necessary to conduct further research on HpAKR.

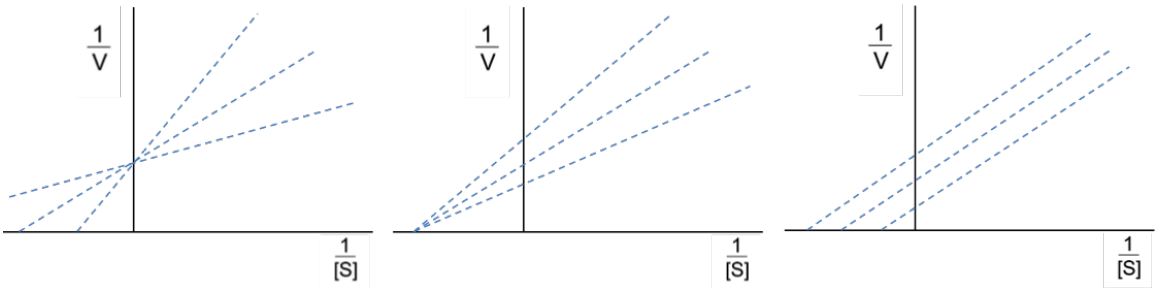

Figure 1: Lineweaver-Burke Plots for Competitive inhibition (left), Non-competitive inhibition (center) and Uncompetitive inhibition (right).

With the assistance of Dr. Robins, the HpAKR project members will write and publish a paper detailing our methodologies and findings in order to disseminate what we have learned to other scientists who are working with *H. pylori*. If we are successful in finding an effective competitive inhibitor for HpAKR, researchers in the drug development field can explore the feasibility of incorporating the inhibitor into a treatment for individuals who are infected with *H. pylori*. If we find that the inhibitors are not competitive in nature or do not effectively inhibit HpAKR, then future researchers will know to either test other potential inhibitors or to improve upon the method we used to test our inhibitors. Ultimately, our research will provide current and future *H. pylori* researchers with new insight into how to resolve the *H. pylori* predicament.

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Appendix

Required Materials

Instruments	Glassware, Containers, and Pipettes	Reagents and Cells	Miscellaneous
New Brunswick Scientific Excella E24 Incubator Shaker Series	125 mL, 200 mL, and 500 mL Pyrex Erlenmeyer Flasks	DH5α cells	Fisher Scientific 3mm solid glass beads
BioMate 3s UV-Vis Spectrometer	0.2-20 µL, 0.5-10 µL, 20-200 µL, and 100-1000 µL micropipettes with tips	Lennox Broth (Fisher Bioreagents®)	Qiagen Plasmid Purification Kit
BioRad Gel Doc EZ Imager	Microcentrifuge tubes	Agar pellets (Fisher Bioreagents®)	Nickel-Nitrolotriaectic Acid resin column (Novagen)
Avanti® J-E Centrifuge by Beckman Coulter	Large centrifuge tubes	Kanamycin and Chloramphenicol (Fisher Bioreagents®)	Quartz cuvettes
BioRad Electrophoresis Kit	Glass test tubes with lids and test tube rack	Novagen Rosetta® Singles Kit	
Microfuge® 20R Centrifuge by Beckman Coulter	100 mL Pyrex Beaker	Tris-Borate-EDTA (TBE) (Fisher Bioreagents®)	
Fischer Scientific Vortex Mixer	Filter tube	1M IPTG (Fisher Bioreagents®)	
Fisher Scientific Isotemp® Refrigerator		Nanopure water from NANOpure Infinity	
Autoclave		Lysis buffer [20 mM Tris-HCl (pH 7.9), 0.5 M NaCl, and 5 mM imidazole]	
		Wash Buffer [20 mM Tris-HCl (pH 7.9), 0.5 M NaCl, and 30 mM imidazole]	
		Elution Buffer [20 mM Tris-HCl (pH 7.9), 0.5 M NaCl, and 150 mM imidazole]	
		Benzaldehyde stock (200 mM)	
		NADPH stock (500mM)	
		Potassium phosphate buffer (50 mM)	
		Inhibitors (eMolecule)	

ANALYSIS OF FLOURIDE, CHLORIDE, CARBONATE, AND SULFATE IN FILTERED, TAP, AND GROUND WATER SAMPLES BY ISE AND TITRATION

Bunraj Grewal

ABSTRACT: This study aims to compare natural groundwater to tap water and filtered water in order to identify whether or not the groundwater has a significantly different concentration of FCCS from the household water. Consuming high concentrations of fluoride can lead to health issues such as fluorosis and reduction of IQ; both do not have effective treatment options, therefore, prevention of these health issues are necessary. Additionally, chloride, carbonate, and sulfate can make water less desirable to drink due to changes in the taste and texture. The concentration of fluoride, chloride, carbonate, and sulfate (FCCS) ions will be measured (with emphasis on fluoride due to its effect on human health) in tap water and filtered water for two households in the Pacific Northwest and will be compared to natural potable groundwater from the Artesian Well in Lynnwood. Fluoride and chloride will be measured using an ion-selective combination electrode, while sulfate and carbonate will be measured using the method of titration. The largest source of error will be from the interference of hydroxide when detecting concentrations of fluoride and chloride using the ion-selective combination electrode.

Fluoride ions are attracted to the positively charged calcium ions in teeth and bones. Too much fluoride can result in major dental, organ, and skeletal health issues such as skeletal fluorosis and kidney failure. The World Health Organization (WHO, 2008) set a maximum limit for the concentration of fluoride allowed in potable water at 1.5 ppm or mg/liter. Most of the fluoride intake gets stored in bone, dentin, enamel, and cementum (Gonzales et al., 2011). The cementum is a calcified bone-like substance that covers the root of a tooth. As one ages the concentration of fluoride in the cementum increases (Gonzales et al., 2011). Fluorosis, one of the most common effects of increased fluoride intake, causes mottling in teeth and is usually cosmetic, but could also result in severe enamel and cementum damage (Beltrán-Aguilar, Barker, & Dye, 2010). In the United States during 2004 the dental fluorosis was present in 40.6% of adolescents aged 12-15 and the prevalence was lower for adults at only 8.7% for ages 40-49 (Beltrán-Aguilar, Barker, & Dye, 2010). Human teeth are most vulnerable to fluorosis during the early ages of life when fluoride intake requirements are minimal and can be easily exceeded (Bottenberg, 2004). Although potable water have a concentration of fluoride below 1.5 mg/liter, consuming anywhere around 1 mg/liter can increase the chances of fluorosis, especially when consumed in the first six years of life (Bottenberg, 2004). The treatment of skeletal and dental fluorosis is difficult and usually ineffective, therefore, preventing a high fluoride intake is necessary (Huber, Tobias, & Mosler, 2013).

This study aims to compare the fluoride, chloride, carbonate, and sulfate concentration in groundwater from the Artesian Well in Lynnwood, Washington to the fluoride, chloride, carbonate, and sulfate concentration of unfiltered tap water and water filtered through a refrigerator in order to determine if the groundwater is safe and beneficial to drink. Many residents in and around Lynnwood gather to fill gallons of water from that well and take it home to the rest of their family because the taste is preferred. This research applies specifically to that small population who consume untreated groundwater on a daily basis. Most potable water sources in the United States have enough fluoride, carbonate, chloride, and sulfate added into them to help prevent tooth decay and improve dental health, but the water in the community well is

untreated and could vary in concentrations of fluoride, carbonate, chloride, and sulfate. One study gathered over 60,000 measurements of fluoride concentrations in groundwater from 25 countries and created a global probability map using statistical modeling and concluded that there is a 20% probability that the groundwater in the Seattle area contains fluoride levels over the 1.5 ppm guideline from WHO (Amini et al., 2008, WHO, 2008). This research has implications of increased fluoride uptake for residents who drink untreated groundwater in the Artesian Well.

The concentrations of fluoride, chloride, sulfate, and carbonate in filtered water will be compared to the concentrations in tap water to determine if the water filters are actually reducing the concentration of the undesired chemicals. The Artesian Well, unlike household water supplies, contains continuous running groundwater that does not undergo a treatment process. Water samples from several different tap water sources (some from private in home sources others from community faucets and water fountains) will be examined for the concentration of fluoride, chloride, carbonate, and sulfate. The results will be compared to the levels in the Artesian Well to determine if there is a significant difference in concentration and whether it is beneficial to health and taste or whether it leaves citizens with too many undesired chemicals in their water.

Background

Exposure to fluoride in water has been associated with reduced performance, verbal, and full-scale IQ scores (Rocha-Amador et al., 2007). The effect of fluoride on IQ was tested by a study in China with children of age 10-12. After analyzing the data, results showed that children with high fluoride concentrations--over the 1.5 ppm--scored a lower average IQ (92.27) than the IQ of children with a low fluoride concentration (103.05). The difference between the groups was statistically significant, therefore, the scientists concluded that fluoride can cause damage in brain function if too much is consumed from an early age (Lu et al., 2000). A more recent study concluded that there was an average decrease of 6 points in IQ in children who consume fluoridated water (Cheng & Lynn, 2013). Fluoride is also considered a neurotoxin linked to neurobehavioral disorders in children, and studies have shown that excessive intake cause central nervous system dysfunction (Shuhua et al., 2012; Yan et al., 2013).

Conventionally, fluoride was removed from contaminated water by liming and accompanying precipitation of fluoride. Various other methods used for the defluoridation of water are ion exchange, precipitation with iron(III), activated alumina, alum sludge, reverse osmosis, and electro coagulation. Adsorption is the most effective method and removes 80% - 94% of fluoride from an aqueous media such as water (Tomar & Kumar, 2013). However, removing too much fluoride in water is also problematic as it can lead to fluoride deficiency due to the fact that fluoride concentrations from other sources such as food are minimal, ranging from 0.02 mg/kg in milk to a maximum of 5 mg/kg in certain fish (WHO, 2008).

Bodies of water that are fluoride rich have been noted all over the world. One study collected groundwater data that involved concentrations of fluoride and created a predicted probability map of fluoride concentration exceeding the WHO guideline for drinking water (Amini et al., 2008). Based on this map, it is predicted that Africa and the Middle East have fluoride concentrations in their groundwater over the 1.5 ppm limit that is considered safe (Amini et al., 2008). Most fluoride in groundwater is not in ionic form. Fluoride-rich minerals like fluorite (CaF_2) and fluorapatite ($Ca_5F(PO_4)_3$), through the process of dissolution, are commonly the cause of fluoride ions being left over on rocks and soil that interact with water (Laurent & Marie, 2010). A recent study done in Brazzaville, Congo (Africa) showed that while the average concentration was 0.49 ppm of fluoride, some

areas had exceptionally high concentrations of fluoride, and the maximum concentration of fluoride in sampled groundwater was 2.90 ppm (Laurent & Marie, 2010). Another recent study done in Pakistan showed an average fluoride concentration of two different cities, Lahore and Sialkot, at 2.603 ppm and 2.819 ppm respectively (Tariq, 2014). These studies show that fluoridation in water is an international concern affecting many bodies of water across the world.

Water samples will also be tested for chloride, carbonate, and sulfate. Consumption of water with a high concentration of chloride can result in dehydration, and the presence of chlorine can affect the taste of water making it bitter. High levels of chloride are used in water treatment and not all of the chloride is removed afterwards which leads to a change in the taste. The WHO (2003) set a limit of chloride in drinking water to 250 ppm, which prevents dehydration and keeps the taste satisfactory. It was also determined by the WHO (1979) that chloride reacts with metal ions and creates soluble salts that increase the levels of metals in drinking water, which could be problematic for citizens drinking out of the Artesian Well (1979). Sulfate in drinking water has been known to cause short-term laxative effects with a sulfate concentration of 600 mg/L or more (Chien, Robertson, & Gerrard, 1968). There is no official concentration limit of sulfate in drinking water, although the WHO (2004) indicates that sulfate concentration over 500 mg/L causes a difference in taste. Carbonate in drinking water also affects the taste and whether the water is hard or soft. Hard water is created due to the excess presence of calcium and/or magnesium carbonate. The magnesium concentration is much lower than the calcium concentration in drinking water, which indicates that hardness due to calcium predominates. The concentration of calcium carbonate in potable water varies from 10 ppm to 500 ppm. The WHO (2003) has no limit on the amount of carbonate in drinking water because there is no conclusive evidence of a link between the presence of carbonate and water hardness and negative health effects. Carbonate can also make water more corrosive resulting in the presence of heavy metals such as lead, copper, and zinc (Assembly of Life Sciences, 1977). These three chemicals are also biologically important and will be examined in this study.

Methodology

To measure the concentration of fluoride ions in the water, we will use a fluoride ion-selective electrode (ISE) from Thermo Fisher Scientific™. This procedure is based on a fluoride detection method using an ion-selective electrode published by the EPA (1974). The fluoride selective electrode produces an electric potential measured in volts, which can be related to the concentration of fluoride by using the Nernst equation. Lanthanum trifluoride (LaF_3), a solid inorganic surface, acts as the membrane of the electrode and attracts fluoride ions, but because the size of a hydroxide ion is similar to fluoride there is a possibility of interference if the solution is too basic. Fluoride ions pass through the membrane and cause a change in electrical potential inside the instrument by forcing the anions in the solution to move towards the membrane, which is then measured with respect to the reference. The fluoride ion-selective electrode contains an internal reference element, an internal reference electrolyte that will be 4 M KCl with AgCl, and the ion exchange crystal (Cheng, 2015; Bialkowski, n.d.). An external reference electrode is not necessary because there is an internal reference electrode included within the combination electrode.

We will collect tap water samples from the kitchen faucets of two different households in western Washington, and filtered water will be collected from the refrigerators of those exact same households after replacing the old refrigerator filters with similar brand new filters. Samples will also be collected from community water fountains and the Artesian Well in Lynnwood, which is a community water

source that runs using a reservoir of potable groundwater. Data from the well will be used to compare the results from the filtered and tap water sources to a source of natural untreated groundwater. Each sample will be collected by letting the water run for one minute and then placing similar one liter plastic (polyethylene) bottles into the flow to collect the running water. Each type of water sample will be collected five times and stored in one liter polyethylene bottles until analysis which could be up to four weeks. Since the tap, filtered, and ground water contains trace amounts of many different chemicals, the ion-selective electrode is a reliable method that can minimize interference easily.

Possible interferences could arise when determining the fluoride concentration if the pH of the sample is below 5 or above 10 due to fluoride ions bonding with hydrogen ions and also due to interference from OH^-. However, the water samples collected should be just under neutral pH (Annual Water Quality Report, 2008). Other chemicals that may be present in water such as Cl^-, Br^-, I^-, SO_4^{2-}, HCO_3^-, and PO_4^{3-} will not interfere with the electrode. The pH of the water being tested is predicted to be within 6-8 pH units, which indicates interference due to pH will be very minimal. The manufacturer has provided a detection limit of 0.02 ppm given that the solution in which fluoride ions are measured is neutral (User Guide Fluoride Ion-Selective Electrode, 2011). A study determining the fluoride concentration in the Antarctic krill successfully calculated the concentration of fluoride using this same ion-selective electron method at ~0.01 ppm, which is below the method detection limit given by the manufacturer (Lu et al., 2015). We expect our tap water samples to have a much higher concentration around 0.8 ppm due to a balance of limitations on the amount of fluoride allowed in drinking water and the addition of fluoride for oral health (Annual Water Quality Report, 2008). We predict that filtered refrigerator water would have a concentration of fluoride less than 0.8 ppm, assuming that the filter removes fluoride ions, and we predict the groundwater from the Artesian Well is going to have a concentration of fluoride ions that is significantly greater than 0.8 ppm because it is unregulated.

The combination electrodes from Thermo Fisher Scientific™ will be blanked using deionized distilled water (DDW). Three standards will be created from a stock solution of 50 ppm sodium fluoride and will be diluted down to a range of 0.2-2 ppm based on the WHO (2008) limit of 1.5 ppm of fluoride in drinking water. To create the 0.2 ppm standard, we will pipette 1 mL of the stock solution into a 250 mL volumetric flask and dilute it using DDW. A standard of 1.1 ppm will be created by pipeting 2.2 mL of the stock solution into a 100 ml volumetric flask and diluting using DDW. We will create the 2 ppm standard by pipeting 4 mL of the stock solution into a 100 mL volumetric flask and diluting with DDW. All dilutions will be performed using a pipette with the range of 0.500 - 5.000 mL.

The concentration of chloride will also be determined in each water source using a chloride selective electrode that works similarly to the fluoride selective electrode. Chloride concentrations vary largely in drinking water sources, especially groundwater (WHO, 1979). To blank the chloride selective electrode, DDW will also be used. Four standards will be created from a stock solution of 500 ppm sodium chloride and will be diluted down to a range of 25 - 250 ppm based on the WHO (2003) suggested maximum limit of 250 ppm of chloride in drinking water. To create the 25 ppm standard, 5 mL of the stock solution will be pipetted into a 100 mL volumetric flask and diluted using DDW. A standard of 100 ppm will be created by pipeting 20 mL of the stock solution into a 100 mL volumetric flask and diluting using DDW. The 175 ppm standard will be created by pipeting 35 mL of stock solution into a 100 mLvolumetric flask and diluting with DDW. We will create the 250 ppm standard by pipeting 50 mL of the stock solution into a 100 mL volumetric flask and diluting it with DDW.

Dilutions will be performed using a pipette ranging from 0.500 - 5.000 mL along with a 15 mL and 25 mL pipette.

We will generate a calibration curve using the results from the standards of fluoride and chloride by graphing the log of the standard concentrations (x-axis) against the millivolt values (y-axis) that come from the ISE. Concentrations will be calculated based on the Nernst equation. We will use a standard t-test to determine if the concentrations of the groundwater samples are significantly different from the filtered and tap water samples. T-tests will also be used to compare tap water with filtered water in order to determine whether filters actually remove a significant amount of fluoride and chloride. Using a t-test will provide a clear statistical analysis on whether the set of data collected from one potable water source is statistically significant from a different set of data collected from another source of water, therefore, a clear conclusion can be drawn with the data resulting in either a significant difference between chemical concentrations of the water sources or no significant difference observed. As shown in figure 1 below, t-test results will be displayed in tables, while concentration results will be displayed in a graph similar to those found in previous studies to make it easier to compare the collected data to previous data (Fojo, Figueira, & Almeida, 2013; Marrero, Torre, Fernández, Armendáriz, & Gironés, 2015).

To determine the concentration of carbonate we will titrate the water samples with 1 M hydrochloric acid along with 4 drops of bromcresol green indicator (Eng, 2001). To accurately determine the concentration of HCl used in the titration, we will titrate it with a known concentration of 1.000 M NaOH in triplicate. The HCl will be poured into the burette until the volume reaches the 50 mL mark for every reaction and the acid will be dispensed dropwise until the end point is reached. We will pipette 25 mL of the rainwater samples into a 200 mL beaker containing a magnetic stirrer and the beaker will be placed on a hot plate with a magnetic stirring option. As the drops of HCl are added the stirrer will mix the sample with the acid readily until the solution turns into an intermediate green color after which the volume of HCl that was dispensed will be recorded. The results will be determined in molarity and will be converted to ppm afterwards. We expect the concentration of carbonate to range from 10 mg/L to 500 mg/L depending on whether the samples is ground water or filtered/ tap water; most water samples previously tested averaged around 100 mg/L of carbonate (Hardness in Drinking-Water, 2003).

Sulfate will also be analyzed using direct titration. We will titrate the water samples against a standard barium solution (Schroeder, n.d.). When using a barium solution, barium sulphate precipitates out. The method for determining sulfate concentration by titration that is going to be discussed next was developed by the Indian Standard Institution (Bhavan, Shah, & Marg, 1980). A barium perchlorate solution will be used for this experiment and is created by weighing 220 grams of barium carbonate and suspending it in water. Perchloric acid will be added to the solution drop wise until the carbon dioxide has bubbled out. The solution will then be heated gently on the hot plate while the carbon dioxide slowly boils out. After the solution cools it will be transferred into a 1 L volumetric flask, dissolved in 500 mL of water, and diluted to the mark with acetone. Acetone is used to speed up the titration time and helps the barium sulfate precipitate faster. The product will be a ~1 M barium perchlorate solution that we will standardize our 1 M barium perchlorate solution by titrating drop by drop against a known concentration of 0.100 M sulfuric acid. The 0.100 M sulfuric acid standard will be created by pipeting 10 mL of a 1 M stock sulfuric acid solution into a 100 mL volumetric flask and diluting to the mark with DDW. We will pipette 25 mL of our water samples into a 200 mL beaker containing a magnetic stirrer, place the beaker onto a hot plate with a magnetic

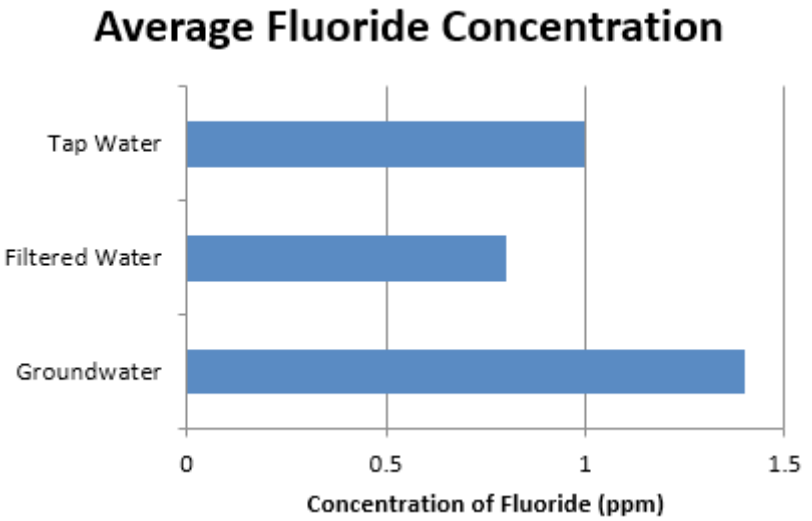

Figure 1: An example of a visual product of the data that will be collected based on graphs made by other scientists in past studies (Fojo, Figueira, & Almeida, 2013; Marrero et al., 2015).

stirring option, and titrate drop by drop against the barium perchlorate solution. The end point is detected by a blue-green color formed by sulphonazo III indicator. The results will be determined in molarity and will be converted to ppm afterwards. We expect the concentration of sulfate to be less than 250 mg/L for the tap water and filtered water, while the ground water could range anywhere between 400 mg/L -770 mg/L (WHO, 2004).

The concentration of carbonate and sulfate will be determined using the volume of the sample, the volume of the titrant was needed to reach the end point, and the concentration of the titrant used, which will be determined by the standardization step in each method. Results will be displayed in a table and as titration plots. A standard t-test will determine if the concentrations of the groundwater samples are significantly different from the filtered and tap water samples. Also to determine if filtered water removes a significant amount of sulphate and carbonate compared to tap water. A t-test is a reliable and accepted statistical examination for comparing two or more samples. The results from t-tests will clearly explain whether there is a significant difference between the chemical concentrations calculated in the filtered water and the tap water and if there is a significant difference between the chemical concentrations calculated in the groundwater and the filtered and tap water.

See Appendix 1 for a list of materials that will be necessary for this experiment.

Communicating the Results

The scientists we will mostly be addressing will be dentists, but our paper will be available on the internet to all researchers. We plan to spread our information by educating dentists in local clinics about fluoride and other chemicals in drinking water so that they can address the topic with their patients appropriately. Dental clinics will be visited and a presentation will be given along with a print-out of the poster for dentists for reference. Most citizens that have fluorosis or are at risk of high fluoride intake will most likely visit their dentists, therefore, educating dentists can help them to advise their patients on the amount of fluoride they should intake and health choices they should avoid. Every dentist will also get a copy of the paper in case they wish to explore the topic in further

detail and choose to read through the references that were used in the paper.

For the general population, we plan to give demonstrations (in the form of hands-on activities and videos) of the connection between fluoride and dental health at local western Washington high schools. For our presentation, we will also find and refer to studies that are more applicable to the general population (such as fluoride in toothpaste), along with our study about fluoride in drinking water, due to their similarities. By adding more relative information and other causes of high fluoride intake into our high school outreach plan these presentations will better relate to non-scientists in the general public, and we can better help them understand the ubiquitous nature of fluoride. The study will be simplified and there will be a heavy focus on the results and the impacts to dental health that arise from an overabundance of fluoride in the system.

We will also set up a website or social media page (on Facebook or Twitter) and display both simplified and scientific information along with dentist information that could be easily found by the general populous or government officials. It is our goal to reach the population of citizens that could be affected by large intakes of fluoride and explain to them what they can do to reduce exposure and where they can find reliable information. If the groundwater samples at the Artesian Well clearly show a high concentration of fluoride, we will send (by mail and email) our paper and our poster to the state health department so they can evaluate our work and plan actions accordingly.

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Appendix 1:
A list of supplies that will be necessary for this experiment.

Equipment / Materials	Size / Specifications	Quantity
Pipettes with tips	0.500 ml – 5.000 ml range 15 ml and 25 ml pipette	1 of each pipette, several boxes of tips
Thermo Scientific Orion pH/ISE meter	STAR A324	1
Volumetric flask	1 L and 250 ml and 100 ml	1, 2, & 2 respectively
Water	Deionized and Distilled	Copious
Beakers	50 ml, 100 ml, 200 ml, and 500 ml	3, 5, 2, and 1 respectively
Polyethylene containers w/ lids	1 liter	25
Thermo Fisher Orion Fluoride Combination Electrode	Model: 9609BNWP	1
Thermo Fisher Orion Chloride Combination Electrode	Model: 9617BNWP	1
50.00 ppm Sodium Fluoride	Pure stock solution	50 ml
500.0 ppm Sodium Chloride	Pure stock solution	250 ml
4 M KCl with AgCl	Fill solution	100 ml
1 M HCl Standard	Stock Solution	3 L
1.000 M NaOH Standard	Stock solution	500 ml
Hot plate with magnetic stirring option		1
Magnetic Stirrer	Small enough to fit into a 200 ml beaker and stir properly	1
Burette	Standard 50 ml burette	1
Bromcresol green	Indicator	100 ml
Barium Carbonate	Solid state	500 grams
2 M Perchloric acid	Stock Solution	100 ml
100 % Acetone solution		1.5 L
1 M Sulfuric acid	Stock solution	500 ml
Sulphonazo III	Indicator	100 ml

Appendix 2: Timeline

The experiment will be carried out over a course of ten weeks. Each week will have set goals to meet. The goals are:

Week	Goals	Calculations & Progress
1	Get instruments running - Calibrate and blank	- Test water samples from the lab sink to determine if the readings are reasonable
2	Run standards - Create working standards - Determine precision (R.S.D.) of I.S.E.	- Create calibration curve - Determine method detection limit
3	Collect samples - Label and store samples	- Write up sample descriptions and storage conditions (temp, cloudiness of water, particulate matter present, etc.)
4	Run samples - Create raw data tables	- Calculate averages, standard deviations, C.I. and R.S.D.
5	Run additional samples if needed & check for mistakes or outliers	- Gather additional data to improve S.D. & R.S.D. - Record outliers and apply statistical tests such as the Grubbs test
6	Analyze results - Determine relationships between water sources with statistical tests such as a t-test	- Create tables of concentrations - Run T-Tests and C.I. tests
7	Refine & interpret data - Create labeled graphs & titration plots - Provide strong statistical support for hypotheses	- Start writing the discussion section - Reject or accept hypotheses based on data
8	Get peer reviews from mentors and other scientists	- Finish writing paper
9	Create poster - Add visual appeal with a balance of text and white space	- Turn heavy scientific writing from the paper into more visual and focused scientific writing
10	Revise poster and paper for grammar, topic transitions, and organization	- Look at peer reviews & proof read paper and poster

ACCESS & AFFORDABILITY IN PUBLIC HEALTH POLICY TO INCREASE ADHERENCE OF CANCER PREVENTION GUIDELINES

Jessica Jacobson

ABSTRACT: Children residing in low income, food insecure neighborhoods are faced with increased obstacles in adhering to cancer prevention guidelines (CPGs). CPGs focus on nutrition and physical activity to maintain a healthy body weight. The geographic, economic, structural, and social conditions place low socioeconomic status (SES) youth at increased risk. Targeted public health policies and programs are needed to increase adherence within low SES communities. What are the unmet needs of children living in low SES, food insecure neighborhoods, that prevent adherence to cancer prevention guidelines? Research efforts must include qualitative methodology, to identify unique obstacles that present health risks. Exploratory methods will likely uncover social determinants of health (SDH) facing populations and therefore require ethnographical field research for greater depth in understanding and theory development. Results indicate that cancer prevalence is increasing globally, many cancers are preventable, higher SES households adhere to CPGs more easily than those in low SES households, children rarely meet daily fruit and vegetable recommendations, both cancer survivors and families without a cancer history are largely unaware of CPGs, and lastly, living, working, and recreational environments hold great capacity in a community's ability to adhere to CPGs. The overwhelming conclusion: a need for development and implementation of targeted intervention programs for specific community obstacles to increase access to and quality of nutrition, recreational space, and the resilience to meet and maintain a healthy bodyweight.

Keywords: cancer prevention guidelines, socioeconomic status, public health, cancer

Poor, limited access areas, which lack sources of healthy and affordable food, will not meet cancer prevention guidelines without intensive public health intervention at the government, state and community levels. The World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) developed a widely accepted set of guidelines for cancer prevention (Miles, 2008). While models for assessing population adherence to the guidelines exist, there are obvious obstacles, supported by the results of previous research, indicating the most impoverished and limited neighborhoods cannot achieve without intervention (Masset et al, 2009).

This paper begins with the definition and development of cancer prevention guidelines by the WCRF/AICR. Then an analysis of the current research and results in adherence to those guidelines will be discussed. Which leads to the final section of this paper, gaps in the current research will be examined to identify the obstacles in adherence to cancer prevention guidelines. This will conclude in support of the argument that further research is needed in poor, limited access areas to assess the unmet environmental needs, which prevent adherence to guidelines.

Literature Review

Development of Cancer Prevention Guidelines

In late 2007 the WCRF/AICR presented a report called: Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective, based on the most comprehensive review of all studies since 1960 on diet, exercise, and cancer (Miles, 2008). What resulted are eight guidelines to implement for cancer prevention, they can be seen in Table 1.

The WCRF/AICR, who developed the guidelines above, took a new approach in 2014. The development of the Continuous Update Project (CUP), which is the largest, continuous review on food, physical activity, and cancer globally, brings focus to the need of

Table 1. Cancer Prevention Guidelines and Adherence Recommendations (Miles 2008).

Cancer Prevention Guideline	Adherence
1. Be as lean as possible within the normal range of body weight.	Maintain body mass index within normal range from childhood through adolescence and into adulthood
2. Be physically active as part of everyday life	Walk at least 30 minutes a day, as fitness improves aim for 60 minutes of moderate or 30 minutes vigorous exercise
3. Limit consumption of energy-dense foods and avoid drinks with high sugar content	Consume energy dense food sparingly, avoid sugar drinks, consumer fast foods sparingly, if at all
4. Eat mostly foods of plant origin.	Eat at least five portions of non-starchy vegetables and fruits each day, limit refined starches, relatively unprocessed grains or legumes with every meal
5. Limit intake of red meat and avoid meat that's been processed	If eating meat, consume less that 18oz a week and little to no processed meat
6. Limit alcoholic drinks	If consumed, limit to no more than two drinks per day for men and one drink per day for women
7. Limit consumption of salt, avoid moldy grains or legumes	Avoid salt preserved foods, limit processed foods with added salt to ensure intake of less that 6g/day, do not eat moldy cereal or beans
8. Aim to meet nutritional needs through diet alone	Dietary supplements aren't recommended in the prevention of cancer

public access to the guidelines and policy plan development, furthering the emphasis on public implementation methods is of utmost importance (Simmonds, Mitrou & Wiseman, 2014). By 2030 cancer prevalence is estimated to reach 22 million globally and an estimated 30% of cancers are preventable with guideline adherence—now is the time to direct focus on public needs for adherence. Furthermore, the strongest links to cancer risk are body composition, growth development, and maturation (Simmonds, Mitrou & Wiseman, 2014). With ever increasing obesity rates, youth and young adolescents are at a great risk. In the 1970's, childhood obesity among 12 - 19 year olds was 5%, but in the 2007-2008 school year prevalence of obesity reached 18.1% (Holman & White, 2011). Conversely, if guideline implementation can begin with youth, it could reduce future cancer rates and overall health (Holman & White, 2011).

Analysis of Adherence Studies

Understanding cancer prevention guidelines, the organizations involved, and how they were developed, as well as adherence studies should be analyzed as to mine knowledge regarding function in various communities and cultures. The University of Washington utilized the WCRF/AICR guidelines to develop diet optimization models (Masset et al, 2009). Two plans were created, the first meeting guidelines 3-5 and 7 (Table 1), which consisted of higher consumption of fruits and vegetables and a reduction in refined grains, against the current consumption patterns (Masset et al, 2009). The second plan met requirement 8 (Table 1), that all nutrient needs were met through diet alone, which required a large change in both volume consumed and patterns of consumption (Masset et al, 2009). The cohort consisted of 161 (n=161) adult men and women in the Pacific Northwest, the mean age for women was 42.2, and 38.0 years for men, 60% of women and 50% of men

had annual incomes greater than or equal to $55,000.00/year, and largely Caucasian (84% men and 82% women) (Masset et al, 2009). Adherence results were largely successful with 93.5% of men and 94.0% of women meeting dietary optimization goals (Masset et al, 2009).

A contrasting adherence study was conducted among 18 African countries, which also assessed alcohol intake and smoking habits (Akinyemiju, McDonald, Tsui & Greenlee, 2014). An adoption of Western lifestyles has been increasing in African countries and with it an increase in chronic diseases, including cancer (Akinyemiju, McDonald, Tsui & Greenlee, 2014). The generalized results reveal populations were able to limit alcohol and tobacco consumption but largely failed at meeting the diet, physical activity, and BMI guidelines (Akinyemiju, McDonald, Tsui & Greenlee, 2014). For sake of comparison to the University of Washington study, it's worth noting that less than 5% of the population reported any college education (Akinyemiju, McDonald, Tsui & Greenlee, 2014). Adherence was highest in young women of high SES, who were in good overall health and residing in urban areas (Akinyemiju, McDonald, Tsui & Greenlee, 2014).

Obstacles in public knowledge

The Oncology Nursing Society conducted a national telephone survey of cancer survivors and those with no family history of cancer, to inquire on both adherence and knowledge of guidelines. Participants were selected from a sample taken from the Health Information National Trends Survey (HINTS) (Mayer et al, 2007). Only 21.6% of subjects without cancer and 29% of cancer survivors identified a better diet as a factor in risk reduction of cancer diagnosis (Mayer et al, 2007). Regarding physical activity, only 4% without cancer and 3% of cancer survivors identified regular physical activity as a method to prevent cancer (Mayer et al, 2007).

A physical activity levels (PALs) study is used to portray another obstacle in adherence, which proposes a hypothesis that energy expenditure requires energy consumption to create a balance, opening up the opportunity to consume the suggested recommendations of dietary intake (Csizmadi et al, 2014). Conversely, those of healthy weight with lowest PALs would not meet the dietary intake needs as to keep an energy balance and prevent weight gain (Csizmadi et al, 2014). Those who eat less to avoid obesity risk in CPGs, do not allow opportunity to consume enough calories to meet nutrient requirements. (Csizmadi et al, 2014). Even in healthy looking individuals, risk is still prevalent

When focusing on intended populations, it is important to consider results of a survey on youth and adolescent behaviors. The Division of Cancer Prevention and Control at the Centers for Disease Control conducted a survey assessing National Health and Nutrition Examination Surveys (NHANES) and Youth Risk Behavior Surveys to measure cancer prevention guideline adherence in U.S. youth from ages 8-18 (Holman & White, 2011).

The results indicate that 6.2% of adolescents between 12-18 met fruit consumption recommendations and 2.2% met that of vegetables, while only 0.9% met recommendations for both (Holman & White, 2011). Whole grain results were also low, with 3.4% of adolescents meeting recommendations (Holman & White, 2011). So where are calories coming from? The results above suggest that they must be coming from energy dense foods (Holman & White, 2011). As for red meat, with the WCRF/AICR recommendations of a 18oz serving per week (Miles, 2008), the NHANES report of adolescents ages 12-16 consumed 1-2 servings of red meat per day and nearly one serving of processed meat per day, which was recommended to avoid altogether (Holman & White, 2011). Although dietary guidelines recommend sodium consumption of 2300 mg/day or less, the NHANES reported males consumed approximately 4266 mg/day and females consumed 2950 mg/day (Holman & White, 2011). This rationale is why I would like

to focus on adolescents aged 8-18 for further study.

Gaps between prevention guidelines and reality of adherence

According to the American Cancer Society's annual report, Cancer Facts & Figures 2014, the obstacles of adherence to cancer prevention guidelines surface with recommendations from large organizations for public policy change (American Cancer Society, 2014). Now more than ever, research has agreed that the environments in which people live, learn, work, and play have an increasingly large impact on their ability to eat healthy foods and practice adequate physical activity (American Cancer Society, 2014). Obesity rates in adults and young children have tripled over several decades, in 2010 children between 2- 19 years of age, 17% were obese, of that group African Americans represented 24%, Hispanics 21%, and non-Hispanic whites 14% (American Cancer Society, 2014). Adoption of the guidelines to a physically active lifestyle, the challenges reported were that 25% of adults had no leisure-time activity (American Cancer Society, 2014). Only 49% met the minimum recommendations for moderate activity, while youth represented 37% of the same measure (American Cancer Society, 2014).

According to Cancer Facts and Figures 2014, barriers that contribute to obesity are: limited access to affordable and healthy foods, large portion sizes notably in restaurant servings, marketing of food and beverages high in caloric, fat, and sugar content - especially aimed at children, schools, and worksites that do not offer healthy options or activities, poor community design that does not offer safe or accessible space for physical activity which in turn promotes sedentary activity, and lastly, economic and time constraints (American Cancer Society, 2014).

Overall, conclusions for cohort studies agree that adherence to guidelines in populations in poor, limited access areas of low SES have an increased and disproportionate disadvantage in adhering to cancer prevention guidelines; and that development and implementation of targeted intervention programs are needed at all levels (Akinyemiju, McDonald, Tsui & Greenlee, 2014; American Cancer Society, 2014; Csizmadi et al, 2014; Holman & White, 2011; Masset et al, 2009; Mayer et al, 2007; Simmonds, Mitrou & Wiseman, 2014). With that I would like to steer toward a geographical perspective, honing in on "food deserts" in the United States. Food deserts are poor, urban areas that lack sources of healthy, affordable food (Caspi et al, 2012). Within food desert neighborhoods, the obstacle can be distance to a supermarket or a perceived lack of access to healthy foods (Caspi et al, 2012). Perceived notions of access have a great impact on fruit and vegetable consumption, with intake around 0.5 servings/day (Caspi et al, 2012).

Summary

Next is a method for observation, survey, and analysis; identifying the gaps in available nutrition, access to safe parks and areas for physical recreation, advertisement of fast foods and, public health campaigns. In the analysis of adherence studies, we learned that the higher the SES the greater adherence to guidelines. Another discovery is that children are the least likely to meet weight, nutrition, and physical activity guidelines, while being a population whose diets are largely controlled by parents and schools, and whose success in meeting guidelines has the most promise for reduction of lifetime cancer prevalence. What are the unmet needs of children living in low SES, food insecure neighborhoods, that prevent adherence to cancer prevention guidelines?

Proposed Methodology

Purpose of Research

While the adherence studies above demonstrate that populations of higher SES are more likely than those of low SES to adhere

to CPGs—with children showing the lowest consumption of healthy foods, research does not take explanatory measures. I propose qualitative research into groups of lowest adherence, to identify obstacles that present health risks. Once obstacles are identified, observed knowledge of communities can be further studied to develop effective public health policies.

Due to the exploratory search for greater depth into social problems facing the population in food deserts and the inductive search for obstacle clarity for theory development, ethnographical field research is the appropriate mode of observation. The variables require direct observation, data processing, and analysis, however in a cyclical process which other modes would not allow (Babbie, 2011). The gaps in knowledge are evidence that hypothesis testing is not singularly adequate for creation of social change. An understanding of the reality the population faces will provide a plethora of data for analysis. From that analysis a new theory for focused and effective public health policy can unfold, but with greater validity for these geographical populations. The variables will allow me to perform as an observer, in line with goals for positive social change, which allows for more transparency and development of rapport.

Population and Sampling

The population will be children and families in schools with greater than or equal to 40% of children receiving free meals via the National School Lunch Program within designated food desert neighborhoods. This will allow for a controlled group of analysis, where Income Eligibility Guidelines are controlling for participants at or below the 100% Federal poverty guideline (Food and Nutrition Service, USDA, 2014). Due to the ethnographical nature of the proposed research, I would like to observe various populations matching this description in school districts with highest rates of childhood obesity. The population will include students from ages 8 to 18, to account for a variety of needs at different levels of interest. As for sample size, I would like to measure at least 50 students or families within each school district observed.

Units of Analysis and Observation

Units of analysis in this study are neighborhoods. The units of observation are individuals. The community-based nature of this inductive proposal requires gathering information from individual accounts for improved analysis and definition of needs facing specific neighborhoods. More directly, the neighborhoods are defined as those holding the food desert designation. Individuals for observation will be those students registered on the NSLP within their districts.

Sample Type and Strategies

I will be using purposive sampling in the initial stages of my research. As we are seeking detailed validation of personal experience for a specific disadvantaged group with intent for clarity of obstacles preventing equal opportunity in health advantages, it is best to study the population directly. Purposive sampling is the mode of selecting the population that will be studied. Once on location, the school district will have a list of students utilizing the NSLP, which will allow for a shift into systematic sampling.

Sampling from the National School Lunch Program as the strategy for observation will unfold as each school is approached. Being that the NSLP is a Federal program, every school district will have the information available, and have controlled for income inclusion. Based on the size of the NSLP list, a sampling interval will be implemented to select the specific units of observation. The initial selection will be done at random, with the systematic approach utilized thereafter.

Variables

The measurement of each variable will be conceptualized with the holistic intent to identify the naturally occurring social organization within each neighborhood by assessing the availability and quality of social programs, public spaces, and availability of healthy food choices.

Access to healthy foods, produce as an independent variable will measure a social-ecological approach of access to food stores based on distance from one's home. Access to food stores, being supermarkets, grocery stores, or markets, has an effect on individual well being and the ability to adhere to diet recommendations (Sharkey, Horel & Dean, 2010). Indicators will be distance in miles to nearest food store with unprocessed produce available. Another indicator will be presence of a usable vehicle, which would compensate for distances greater than one mile. The level of measurement for this variable will be a scale of 1 to 5.

Advertising within community (of fast and processed foods) as an independent variable will be measured indirectly via access to historical data and directly via in person observation. The presence of marketing materials within school grounds via vending machines, school lunch programs, and marketing in areas within the community via billboards and other visual simulations. The indirect measure will be historical data of dollars spent within these communities to advertise fast or processed foods via television and radio. The level of measurement will be decided during data analysis, as the results will have to be post coded.

Age as an independent variable will be measured indirectly through individual response via questionnaire. Age is a concept that will offer more conceptualization through interviewing and questionnaire response. Meaning, over responses to other variables, patterns of access, dietary habits, and other responses may prove to show more patterns with age correlation. Operationally, the variable will be measured as reported via questionnaire in Appendix I.

Physical activity in school as an independent variable will be measured from the availability and level of engagement in physical education

Table 2. Type and List of Variables.

Cancer Prevention Guideline	Adherence
1. Be as lean as possible within the normal range of body weight.	Maintain body mass index within normal range from childhood through adolescence and into adulthood
2. Be physically active as part of everyday life	Walk at least 30 minutes a day, as fitness improves aim for 60 minutes of moderate or 30 minutes vigorous exercise
3. Limit consumption of energy-dense foods and avoid drinks with high sugar content	Consume energy dense food sparingly, avoid sugar drinks, consumer fast foods sparingly, if at all
4. Eat mostly foods of plant origin.	Eat at least five portions of non-starchy vegetables and fruits each day, limit refined starches, relatively unprocessed grains or legumes with every meal
5. Limit intake of red meat and avoid meat that's been processed	If eating meat, consume less that 18oz a week and little to no processed meat
6. Limit alcoholic drinks	If consumed, limit to no more than two drinks per day for men and one drink per day for women
7. Limit consumption of salt, avoid moldy grains or legumes	Avoid salt preserved foods, limit processed foods with added salt to ensure intake of less that 6g/day, do not eat moldy cereal or beans
8. Aim to meet nutritional needs through diet alone	Dietary supplements aren't recommended in the prevention of cancer

programs, the requirement for student participation and the perceived importance of participation from the individual perspective. The availability of physical education programs will be operationalized via direct observation, noting the presence of the program, variety of activities offered, presence of gym recreation, and/or fields for outdoor play. A measurement of 1 will be given to schools showing all options (program, activities, gym and fields), 2 if the school is displays three of four options, 3 if they have two of four options, 4 if they have one of four options and 0 if they have no options available.

Table 3. Level of Measurement for Access to Food Stores, Produce.

Measure	Distance to Food Store
1	0 - 1 mile
2	>1-2 miles
3	>2-3 miles
4	>3-4 miles
5	>4 or more miles

Physical activity programs in the community as an independent variable will be measured by presence of recreation centers offering intramural sports to children and accessibility to parks via distance to one's home. The presence of recreation centers offering intramural sports to children will be operationalized directly, by visiting each site and gathering data of sports offered and to what age groups. By interviewing program staff, a collection of enrollment participation for each sport will be measured, again, having more definition after cite site visit. Accessibility to parks will be measured in miles of distance from one's home (Table 4).

Dietary habits as an independent variable will be measured directly within the school community and indirectly through the construct of a questionnaire given to students (See Appendix 1). The direct in school observation will require multiple site visits during meal times to each school in the study, allowing for measurement of food choices available and choices made over time. If possible, collection of historical ordering and discard will be attained for the lunch program, indicating what the school district is ordering, what's being sold, and what is discarded at the end of each meal period. A questionnaire will be developed for the sample student population with aim of their perception of their dietary habits, questions will be asked from a variety of angles to attempt increased reliability, while maintaining a maximum of 10 questions, to increase participation and completion of the questionnaire.

Family Size, Presence of a primary caregiver and Sex as independent variables will be measured indirectly. If allowed and accessible, the information will be gained through the school district NSLP database. There is a degree of assumption indicated after literature review that the presence and value of these control variables has a degree of correlation with adherence to dietary guidelines. With family size demanding increased quantities of food and therefore increased financial resources from the family, the quality of food in the home is likely affected. The presence of a primary caregiver would represent the likely preparer of the food. Their presence in the home, and the degree in which they spend time in the home is a likely indicator of quality of food available to

Table 4. Level of Measurement for Access to Parks.

Measure	Distance to Park Space
1	0 - 1 mile
2	>1-2 miles
3	>2-3 miles
4	>3-4 miles
5	>4 or more miles

children. Lastly, the sex of the individual may show correlation to food choices and, physical activity both within and outside of school.

National School Lunch Program will operate as a control variable, keeping the sample within this federal program ensures that all observed individuals come from families within the same poverty level income brackets, which is also based on family size (Food and Nutrition Service, USDA 2014). Using this control maintains consistency both within and outside the school district. Reliance on this system allows for each school observed fitting within the same social parameters.

Adherence to cancer prevention guidelines ultimately serves as the dependent variable. Adherence will be measured as a result of collection and analysis of responses of independent variables. Once all data is cleaned and analyzed meaningful results will be produced. The guidelines available and low adherence by school aged children shows promise for compelling results. As Holman and White indicated in their 2011 study, 0.9% of U.S youth and adolescence meet adherence to both fruit and vegetable consumption per CPGs. This fact alone indicates we have selected an appropriate population to study. And wWith the accessibility issues facing food deserts and the clear impact on health advantages, the place of study is ultimately important (Sharkey, Horel & Dean, 2010). The results of this variable will give clarity to the degree at which this population faces adversity in terms of health inequality.

Appendix I: Proposed Project Timeline.

Task	June-August	Sept.-Oct.	Nov.-Jan.	March-May	June-August	Sept.-Oct.	Nov.-Jan.	March-May
Complete Approved Proposal								
Operationalize Variables								
Selection of School Districts								
Physical Integration								
Sampling for Units of Observation								
Direct Observation								
Data Processing								
Data Analysis								
Development of Results								

Appendix II: Sample Student Questionnaire-
Obstacles of Adherence to CPGs

1. Where do you normally shop for your groceries? Please name the grocery store and location below

2. Who goes to the grocery store with you?

3. What transportation do you use to travel to the grocery store?
   Select ONE that you use most often:
   [ ] Walk
   [ ] Bike
   [ ] Bus
   [ ] Drive
4. Do you participate in PE activities?
   [ ] Yes
   [ ] No
   Give a reason for your answer:
5. On the weekends and after school, do you go to the park?
   [ ] Yes
   [ ] No
6. If you do, what is your favorite park to play at?

7. What kind of lunch do you eat most days?
   [ ] Hot (from school cafeteria)
   [ ] Cold (brought from home)
8. What is your favorite food to eat at lunch?

9. What is your least favorite food to eat at lunch?

10. If you could pick one food to be added to the lunch menu, what would it be?
11. Sex

12. Family Size
    How many people live at your house?
    [ ] Male
    [ ] Female
13. Age

14. Who cooks dinner at home?
    [ ] 5 -7
    [ ] 8-10
    [ ] 11-13
15. Do you like to help?
    [ ] 14-16
    [ ] Yes
    [ ] 17-19
    [ ] No

Appendix III: Script for Qualitative Interviews

1. Where do you normally grocery shop? What's the name of the store? Where is that located?

2. Do you go to the grocery store? Who usually goes along?

3. How do you get to the grocery store? Do you walk, ride your bike, take a bus ride or ride in the car with your family?

4. Do you go to a PE class? Do you go every day? What do you do in PE? What is your favorite activity in PE?

5. On the weekends, do you go to the park with your friends or family? Who do you go with?

IF a negative response: Why don't you go to the park? What would make the park a better place to play?

6. Do you have a favorite park? What would your dream park look like?

7. Do you usually eat a hot lunch or do you bring your own from home?

8. What is your favorite food that you eat from the cafeteria? **OR** What is your favorite lunch that you pack from home?

9. What is your least favorite food to eat at lunch? Why?

10. If you could add one item to the lunch menu at school, what would that be? Why?

11. Direct observation of sex - no script

12. How many people live at home with you? Do you have brothers or sisters at home? Do y o u have a Grandma or Grandpa at home?

13. How old are you?

14. Who cooks dinner for you most nights?

15. Do you like to cook? Do you ever help cook dinner?

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DETERMINING POTENTIAL INHIBITOR(S) OF THIOREDOXIN GLUTATHIONE REDUCTASE, KEY ENZYME OF SCHISTOSOMA MANSONI PARASITE

Mengkhy Lay and Peter Anderson

ABSTRACT: Schistosomiasis is a parasitic worm disease that affects more than 200 million people worldwide, and the drug praziquantel remains the only treatment. Recently there have been reports of patients showing signs of resistance to praziquantel. Consequently, there is an urgent need to develop a new drug to serve as an alternative to praziquantel. In this experiment, we will determine potential inhibitor(s) of Thioredoxin glutathione reductase (TGR), an essential enzyme that is responsible for the parasite's survival. We will use three phases of computational modeling techniques including virtual screening, lead optimization, and down-selection to determine potential drug candidates. As a result, we predict the top hit compounds to have binding affinities of about -10.0 kcal/mol after virtual screening. Furthermore, we expect to improve the binding affinity of the hit compounds to -13.7 kcal/mol after lead optimization. Eventually, we will proceed to the down-selection phase to determine potential drug candidates with highest probability of positive biological processes and negative toxicity levels. These drug candidates could potentially progress into preclinical and clinical development and eventually serve as a marketable drug.

Schistosomiasis (bilharzia or snail fever) is one of the major neglected tropical diseases that affecting populations in developing countries and is caused by worms of genus *Schistosoma*. This disease has infected more than 200 million people worldwide including Africa, South America, and Asia (Hotez, Fenwick, Savioli, & Molyneux, 2009). The majority of the infected patients are women and children living in poor rural areas. According to the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), the impact of Schistosomiasis causes an estimated 200,000 deaths annually, which is second only to malaria.

The *Schistosoma mansoni* parasite is one of the causal agents of Schistosomiasis. Infected patients with *Schistosoma mansoni* showed signs of severe infection in the intestines resulting in symptoms such as abdominal pain, diarrhea, and bloody stool. Currently, there is no vaccine that can effectively prevent the infections, and praziquantel is the only choice of drug that is effective in treating Schistosomiasis (Pierce, Dubois-Abdesselem, Lancelot, Andrade, & Oliveira, 2012). However, there is evidence that patients can tolerate higher doses of praziquantel (Doenhoff, Kusel, Coles, & Cioli, 2002), suggesting that there is some level of drug resistance to praziquantel. This is an important finding because if the worm parasites develop resistance to praziquantel, those infected with Schistosomiasis may have higher mortality rates. For example, in the 1990s an antibiotic used to treat various bacterial infections, Vancomycin, became an ineffective treatment as result of bacteria developing resistant strains (Gray, Darbyshire, Beath, Kelly, & Mann, 2000). Therefore, there is an urgent need for new drug discovery for schistosomiasis before praziquantel becomes ineffective.

Adult schistosomes reside within the host's blood vessels, feed on the blood, and can reproduce for up to 30 years (Gryseels, Polman, Clerinx, & Kestens, 2006). Within the human host, the parasite produces antioxidants to protect itself from reactive oxygen species produced by the host's immune system. Previous studies

found that thioredoxin (Trx) and glutathione (GSH) systems play important roles in detoxify reactive oxygen species, cell proliferation, redox regulation of gene expression (Kuntz et al., 2007). In the two systems, the Glutathione reductase (GR) enzyme reduces glutathione disulfide (GSSG) to glutathione (GSH) and runs the GSH-dependent systems (Townsend, Tew, & Tapiero, 2003). Likewise, the thioredoxin reductase (TrxR) enzyme converts oxidized thioredoxin (Trx-S2) to reduced thioredoxin (Trx-(SH)2) (Gromer, Urig, & Becker, 2004). A study on the disulfide redox pathways of *Schistosoma mansoni* identified thioredoxin glutathione reductase (TGR) as a multifunctional enzyme that functions as both TrxR and GR enzymes (Alger & Williams, 2002). In other words, TGR serves as an essential enzyme for the parasite's survival and could be a valuable drug target (Kuntz et al., 2007).

Several strategies have used TGR as a drug target and attempted to determine the inhibitors. One study used RNA interference, a process where RNA molecules inhibit gene expression to identified TGR in *Schistosoma mansoni* and found that auranofin is an effective inhibitor of pure TGR that was able to partially cure infected mice (Kuntz et al., 2007). Similarly, another study used gene cloning to identify the presence of TGR in *Schistosoma japonicum*, and the results agreed with the previous findings for *Schistosoma mansoni* (Song et al., 2012). These studies show the important roles of TGR inhibition in curing mice infected with Schistosomiasis. However, an inhibitor that is effective in targeting TGR and is able to function in the human body has yet to be defined. Therefore, we will use molecular modeling techniques to predict the potential inhibitors of TGR with the highest binding affinity and lowest predicted toxicity. Essentially, the inhibitors will serve as drug candidates for drug development to provide an alternative to praziquantel.

Method

We will determine a potential inhibitor(s) of TGR, an enzyme in the redox system that is essential for the survival of *Schistosoma mansoni*. We will use three phases of computational modeling techniques including structure-based virtual screening, lead optimization, and down selection to filter a large database of compounds into drug candidates. Ultimately, we will generate a list of drug candidates with the highest binding affinity for the enzyme and lowest potential side effects.

Virtual Screening

Structure-based virtual screening is a modeling technique used in the early stage of drug discovery to identify series of potential hit compounds that are likely bind to a drug target (Peter Anderson, personal communication, November 20, 2015). The benefits of this technique are the low cost compared to high-throughput screening experiments, as well as the ability to bind multiple substrates and narrow down a large dataset of compounds into several lead compounds.

As demonstrated in figure 1, we will use a virtual screening process called molecular docking. This process is based on a lock and key principle, where a specific enzyme could only bind to a specific substrate. Molecular docking has three main components, identifying a protein, using algorithms to screen, and scoring a function. First, we will obtain an x-ray crystal structure of TGR in PDB format from www.uniprot.org (Figure 3). Second, the TGR file will be submitted on an idock server at http://istar.cse.cuhk.edu.hk/idock/ in a queue that will take a few weeks to complete.

The idock server will use algorithms to screen millions of substrates for the best orientation and conformations that fit the FAD binding site of TGR (Li, Leung, & Wong, 2012). Third, the idock server will generate the binding affinity of the hit compounds in a form of docking score or rank from highest to lowest negative free energy of binding (Huang, Hua, Li, & Hua,

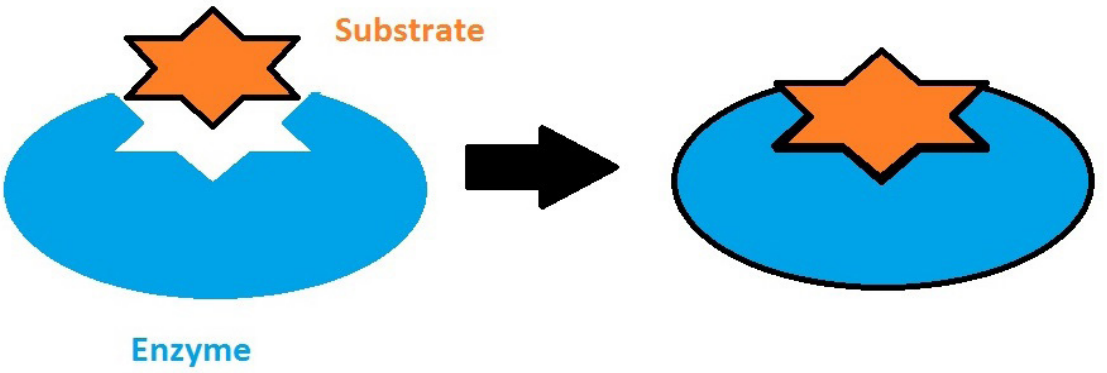

Figure 1. Illustration of molecular docking where the substrate is docked onto the active site of the enzyme.

$$dG_{bind} = k_{hb}\sum_{hb} f_{hb} + k_{ml}\sum_{ml} f_{ml} + k_{hh}\sum_{hh} f_{hh} + k_{hp}\sum_{hp} f_{hp} + k_{aa}\sum_{aa} f_{aa}$$

k: constant
f: count of atomic contact

hb: H-bond donor – H-bond acceptor
ml: ionic metal - ligand
hh: hydrophobic atom – hydrophobic atoms
hp: hydrophobic atom – polar atom
aa: any atom - any atom

Figure 2. Free energy of binding equation used to predict docking scores by adding all different intermolecular forces including hydrogen bonds, ionic bonds, and hydrophobic interactions (Peter Anderson, personal communication, November 27, 2015).

2015). The docking score is calculated based on evaluation of intermolecular forces between the enzyme and substrate (Figure 2). As a result, this phase will generate a list of the top 1000 hit compounds with the highest binding affinity (Table 1).

Lead optimization is a process used to enhance the binding affinity of the top hit compounds obtained from molecular docking into promising lead compounds. We will use a method called lead hopping to modify some of the functional groups of a hit compound by replacing with other functional groups. This technique will be performed on the AUTO_PFVS server and will take a few weeks to complete. AUTO_PFVS server required a protein-ligand complex structure in PDB format to perform CORE_GEN and CAND_GEN modules.

CORE_GEN is a tool that break down a ligand structure to fragments called pharmacophores

ZINC ID	iDock Score (kcal/mol)
9413973	-12.45
9518503	-12.283
9414156	-12.149
9518522	-12.044
44441691	-12.015
8845186	-11.965
72332034	-11.907
38632282	-11.834
14359061	-11.766
9413721	-11.758
13351455	-11.74
27664591	-11.723
.....	.....

Table 1. Example list of the top hit molecules generated from idock ranked from high to low iDock score.

and determine the binding affinities (Hao, el. al., 2012). Subsequently, a pharmacophore with the best binding affinity will serve as a core structure for generating new compounds by linking different functional groups using CAND_GEN module (Kolb and Caflisch, 2006). At the end of this phase, we will generate a list of promising lead compounds with improved binding affinities.

One of the processes of down selection is absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction, where lead compounds are filtered into drug candidates. The main purpose of this stage is to reduce costly late-stage failures in drug development by predicting potential drug side effects (Van De Waterbeemd & Gifford, 2003). ADMET prediction will be performed on admetSAR, an open source database that provide searches for ADMET properties. In admetSAR, 22 high-accuracy qualitative classification models were implemented to generate probability of ADMET properties and regression models including water solubility, permeability, and toxicity (Cheng et al., 2012). ADMET properties will predict the probability of how well the lead compounds function in biological processes such as bioavailability, intestinal absorption, permeability, and toxicity (figure 3). As a result, ADMET prediction will generate a list of drug candidates with the highest probability of positive biological processes and negative toxicity levels. For a detailed timeline, refer to appendix A.

Summary

Schistosomiasis or snail fever is a parasitic worm disease that could cause severe infection in urinary tract or intestines. Several studies had identified the TGR enzyme as an essential target for drug development (Alger & Williams, 2002; Kuntz et al., 2007; Song et al., 2012). These findings are important to our research, because

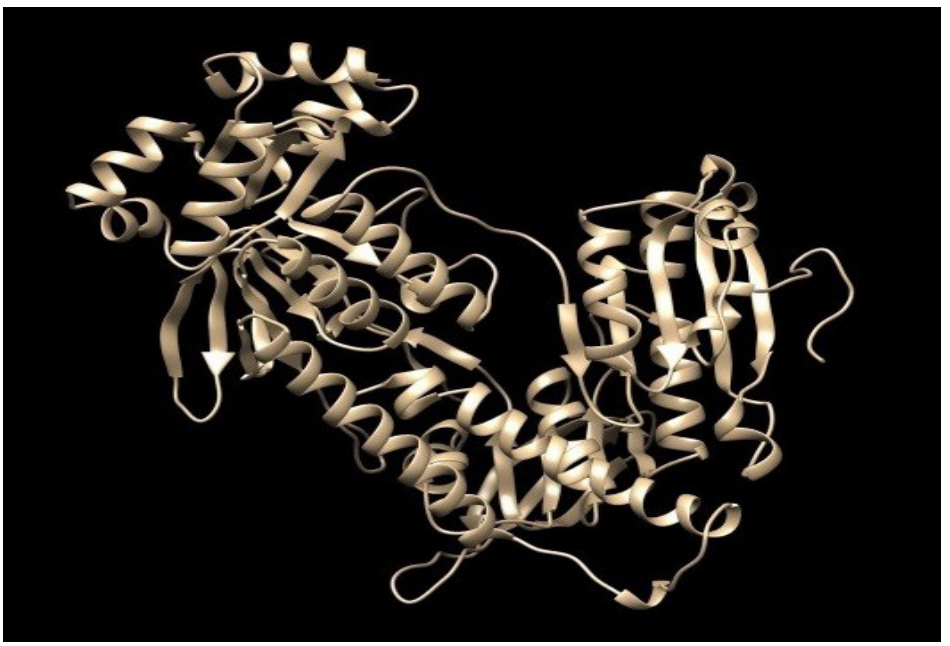

Figure 3. X-ray crystal structure of TGR obtained from Uniprot protein data bank (http://www.uniprot.org).

praziquantel remains the only drug that is effective in treating schistosomiasis. However, due to increasing drug resistance, there is an urgent need to find a new drug that could replace praziquantel. Therefore, we will use molecular modeling techniques to predict potential drug candidates. These newly identified drug candidates could be tested in clinical trials and eventually be offered as an alternative drug to praziquantel.

Our techniques will filter down a big library of biomolecules through a three phase process into a couple of biomolecules with best scores. The score tells us how well the biomolecules fit the drug target to make the parasite vulnerable and how well the biomolecules function in our body with the fewest side effects. Thus, the score will help us select only biomolecules with the highest potential to be drug candidates. Ultimately, the success of this experiment will be generating drug candidates that have the best fit and lowest toxicity levels.

Table 2. Example list of pharmacophores generated from CORE_GEN module.

Ligand	dH (kcal/mol)	-TdS (kcal/mol)	dG (kcal/mol)
Original	-43	11.514	-31
pharmacophore 1	-30	11.525	-19
pharmacophore 2	-35	12.041	-23
pharmacophore 3	-23	10.282	-13
pharmacophore 4	-40	11.884	-28
pharmacophore 5	-40	11.897	-28
pharmacophore 6	-40	11.851	-28

Table 3. Example list of newly generated compounds from CAND_GEN module.

Ligand	dH (kcal/mol)	-TdS (kcal/mol)	dG (kcal/mol)
1501	-63	11.7	-15
982	-62	13.0	-1.5
504	-65	17.0	-2.6
237	-60	11.6	-8.8
964	-60	13.1	-6.9
1761	-59	12.4	-3.2

Appendix A

Timeline

Week	Goals	Result
1-4	• Look at 3D structure of drug target and identify the active site using Chimera. • Structure-Based Virtual Screening: Perform molecular docking using iDock program • Hit to Lead	• Obtain docking scores for a set chemical structures. • Identifies the top hits with highest ΔG of binding
5-18	Lead optimization: • Perform Lead hopping to improve the binding of top hits.	• Sort and narrow down the top hits
19-20	Down selection: • Use ADMET prediction	• Sort and narrow down potential lead compounds

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EXAMINING THE EFFECTS OF DIFFERENT DIETS AND SALINITIES ON COPEPOD POPULATION GROWTH

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ABSTRACT: The coastal oceans are subject to climate impacts leading to sea level rise, increases in the frequency and intensity of storms, and increased precipitation. These events can lead to a rise in the amount of fresh water entering coastal ecosystems from runoff or rainfall, which cause decreases in ocean salinity. Understanding marine food web dynamics requires an understanding of how species interactions will respond to environmental changes of this kind. Sea urchins are key members of nearshore food webs and may help to link food availability between shallow and deep zones along coastal areas. Sea urchins posess a very inefficient digestive system, which means that their feces may possess large amounts of available nutrients which other organisms can use as a viable food source. This research studied the population growth of T. californicus copepods in both low salinity and normal seawater environments, and with diets of either fresh Ulva or urchin fecal Ulva. The calorie content for these different diets was also examined. Results show that both diet and salinity significantly affected population growth, low salinity is the better environment, and fresh Ulva is the better diet.

Climate change is affecting coastal environments in many ways, but one of the major concerns is its effects on ecosystems, as deterioration of marine community structure is increasing (Doney et al., 2012). Coastal ecosystems are sensitive to sea level rise, changes in the frequency and intensity of storms, and increased amounts of precipitation (Harley et al., 2006). In the coming years we may see heavier rainfall, which in turn can lead to increased amounts of runoff into the ocean, and decreases in ocean salinity (Curry & Mauritzen, 2005). In this rapidly changing marine environment, population-level shifts are occurring, which decrease stability and recovery potential and can lead to altered species interactions in coastal systems (Hallegraef, 2010; Worm et al., 2006).

Within marine habitats, primary producers like benthic algae and phytoplankton are essential to the food web, as they constitute the base on which all other species rely for energy. The next trophic level contains consumer organisms like zooplankton, including copepods, which eat phytoplankton. Further up this web are a variety of fish species that depend on zooplankton for their main food source (Richmond, Wethey, & Woodin, 2007). Food web dynamics are driven by the nature and abundance of primary food sources that are available to these higher trophic levels (Wallner-Han et al., 2015). Understanding these feeding dynamics requires an understanding of how species interactions will respond to environmental changes such as salinity (Norkko et al., 2007).

Coastal ecosystems have particularly complicated food webs. There are two distinct sources of primary production: phytoplankton and benthic macroalgae. Much of the organic matter entering the benthic food web derives from this material sinking to the deep subtidal

zone. Benthic communities are biologically diverse, so small shifts in their environment can create large changes in higher trophic levels (Van Oevelen et al., 2006). The importance and magnitude of this linkage between pelagic and benthic subsystems remains poorly studied (Sullivan et al., 1991). Because coastal systems are shallow, benthic fauna have direct access to primary producers, in the form of live or dead algae including detached pieces of macrophtyes. In some systems, benthic consumers may get nutrition from the fecal matter of other species (Sauchyn & Scheibling, 2009). Although it is still unclear exactly how important this fecal detritus is as an adequate food source, my experiment investigates the possible importance of this connection.

Green sea urchins (*Strongylocentrotus droebachiensis*) are a common species whose fecal matter may provide a substantial amount of nutritious particulate matter following the consumption of macroalgae. Because of their very inefficient digestion (Mamelona & Pelletier, 2005), fresh urchin feces can contain high levels of nitrogen and phosphorus (Koike, 1987). This nutrient rich byproduct can act as a beneficial source of energy for some types of marine species (Sauchyn & Scheibling, 2009), such as copepods, and in a lab setting can lead to rapid population growth when it is the only food source (Kobelt & Dethier, 2015).

A strong linkage between copepods and nearshore fish species such as juvenile salmonids exists in many coastal ecosystems, including the Pacific Northwest (Naiman & Sibert, 1979). *Tigriopus californicus* is an abundant harpacticoid copepod that is commonly located within high tide pools in San Juan Archipelago. In this habitat, they predominantly consume algae and detritus (Morris, Abbott, & Haderlie, 1980, Dethier et al., 2014). This is an ideal species to use for experimentation because it can be easily kept in culture and has a short reproductive cycle (Dethier et al., 2014). In this experiment, *T. californicus* will be used to test how food sources and selected environmental parameters interact to control population growth. By quantifying the population growth seen over at least one generation, and varying the physical environment, we can better predict how food web dynamics may be affected as greater climate change events occur along the coastal oceans.

Methods

For this study, copepod populations were raised under 4 treatments, with each treatment having four replicates, totaling 16 populations. *T. californicus* copepods were collected from a high tide pool at Cattle Point on San Juan Island. Egg-bearing females were identified and sorted under the microscope. Mason jar 'aquaria' were set up by adding 20 egg-bearing females per jar. Half of the jars were filled with normal salinity seawater directly from the Friday Harbor Labs (FHL) sea tables in lab 3. The other half were given a low salinity treatment by filling them with 50% RO water and 50% seawater. Jars were labeled and kept on the windowsill in lab 3, out of direct sunlight to minimize temperature variation, however, a gradual seasonal cooling over the course of the experiment occurred. The temperature range in the jars was between 9.5-15°C over the experimental period, with an overall average of 12 °C.

Two large-sized green sea urchins (*Stronglyocentrotus droebachiensis*) were collected from FHL sea tables located in lab 3 and lab 1, while seven more were collected from an urchin trap located off the FHL Dock. These urchins were divided into groups of three, placed in buckets, and maintained with constant water flow in a sea table located in lab 3. They were fed a consistent diet of sea lettuce (*Ulva sp*.) collected from the FHL docks. Feces from the urchins were collected with a turkey baster on a weekly basis and fed directly to the populations of copepods. The remaining collected feces were frozen for chemical analysis in the lab 3 freezer.

Each of the jars containing copepods were fed

ad libidum with either diced fresh Ulva or urchin feces. Treatments were as follows: low salinity with fresh Ulva, low salinity with urchin feces, normal salinity with fresh Ulva, and normal salinity with urchin feces. Each population's food and water were refreshed regularly as needed every 7-10 days, by pouring water through a series of different size filter sieves, then washing the filtrate back into jars with clean seawater. Water changes were performed as infrequently as possible—only when the jars started to develop a surface film and bottom cloudiness—in order to minimize the number of individuals lost.

At the end of 6 weeks, each jar was treated with 95% ethanol to kill the *T. californicus* and allow for easy counting. Fluids were drained through a sieve and manual extraction of large algal remnants was performed before counting. Counting was completed under a dissecting microscope and included all egg-bearing females, adults, sub-adults and larval (nauplii) forms. Separation of adults and sub-adults depended on size and coloration factors, as adults were larger and possessed a darker segmented appearance throughout their body. Juveniles and larval forms were distinguished by their shape and number of legs, as larval forms contained three pairs of legs and bodies that were rounder than the juvenile forms. These counts were totaled and used for statistical analysis using Excel. Final population sizes were compared with a 2-factor ANOVA (with factors salinity and food type).

Caloric content of the food for the *T. californicus* populations was considered as an additional factor that might influence population growth. Samples of fresh Ulva and urchin feces, as well as their respective frozen versions, were used for calorimetric analysis with potassium dichromate oxidation from the methods outlined by Gosselin & Qian (1998) and modified by D. Duggins. These analyses were designed to test the caloric difference between fresh Ulva and fecal matter diets, as well as examining if there are any effects on calorie content caused by freezing. Fresh blades of Ulva were tested and compared to 2 week old frozen blades. The second set of analyses examined the difference between fresh urchin feces and frozen urchin feces, as well as quantifying the calories from Ulva frozen for 6 weeks.

All samples were placed in the drying oven in foil trays to remove excess water. Ulva required drying time of between 14-18 hours whereas urchin feces only took 5-6 hours. Then 60 mg of dried sample was weighed and placed in a test tube with 10 mL of dichromate solution. The test tube was gently vortexed to mix and heated in an oven set to 115° C for 30 minutes, mixing halfway through. After the heating and mixing process, 0.5 mL of the mixture was then transferred to a new tube with 4 mL of potassium iodide solution, mixed, and left to sit for 20 minutes. Each sample was read at 575 nm in a spectrophotometer. The absorbance data was converted into calories with a regression equation generated from a glucose standard curve. Due to the expiration time for potassium and iodide reagents, analyses of Ulva samples were tested in sets 6 weeks apart, with each set run using a fresh batch of reagents and new standard glucose curve performed. We found that fresh reagents generated slightly different glucose curves, therefore, samples run with different batches were not directly compared.

Results

After 50 days, the total population size per jar for *T. californicus* had grown from the 20 original individuals to an average range of 91 individuals (in a population raised on feces from *S. droebachiensis* fed only Ulva (henceforth "fecal Ulva") and 17 PSU), to 254 individuals (for population raised on fresh Ulva in low salinity of 17 PSU). Final average population sizes at 29 PSU were 28 fed fecal Ulva and 112 fed fresh Ulva diets (Figure 1). Thus a fresh Ulva diet resulted in more growth when compared with fecal Ulva diet, and low salinity consistently had greater growth than high

salinity. The effect of low salinity was even greater when the fresh Ulva food is present, as is seen in the statistical interaction (ANOVA diet p value <0.0001, salinity p value <0.0001, interaction p value =0.003).

The population composition in terms of abundances of the individual life stages differed among treatments in both the number of individuals (Figure 2), as well as how the proportions of each life stage (Figure 3) were represented. For egg-bearing females, low salinity showed a significant positive effect over seawater with 46% more individuals seen, while diet made only a small difference among treatments and its effect was not significant (Figure 4: ANOVA diet p value=0.064, salinity p value=0.016, interaction p value=0.100). In terms of the proportions of the populations, egg-bearing females differed among the treatments; the proportion of egg-bearing females increased 10% with the fecal Ulva diets over the fresh Ulva diets. Salinity did not make a difference but there is a significant interaction between the two factors seen (Figure 5: ANOVA diet p value=0.004, salinity p value=0.125, interaction p value=0.05).

For the non-egg-bearing adults, both population abundance and proportions were significantly affected by diets and salinity. Results for abundance indicate that low salinity is more ideal than seawater, as there were 66% more adults seen. The fresh Ulva diet also resulted in a 35% increase in adults over populations fed fecal Ulva, with no interaction (Figure 6: ANOVA diet p=0.003, salinity p <0.0001, interaction p=0.210). In terms of the proportion of adults within populations, only salinity showed an effect. The seawater treatment contained a 12% increase of adults over the low salinity treatment, though diet did not make a difference and there was no interaction (Figure 7: ANOVA diet p value=0.256, salinity p value=0.003, interaction p value=0.287).

For sub-adults' abundance there were effects of both salinity and diet, and a significant interaction of these factors. The low salinity treatment resulted in 37% more sub-adults seen than in the seawater treatments, while fresh Ulva showed 30% more sub-adults than populations with fecal Ulva diets (Figure 8: ANOVA diet p value <0.001, salinity p value <0.001). When at low salinity and with a fresh Ulva diet, the maximum number of sub-adults were found among all of the treatments (interaction p value=0.008). The same pattern was not seen in proportion of sub-adults in the populations (Figure 9: ANOVA diet p value=0.109, salinity p value=0.09, interaction =0.280).

For the larval stage population abundance, there was a significant effect with salinity and diet, as well as an interaction. In the larval stage, low salinity treatments resulted in 33% more larvae growth over the seawater treatment, while fresh Ulva resulted in 28% more larvae than in fecal Ulva diets (Figure 10: ANOVA diet p value<0.0001, salinity p value <0.0001). When there was both low salinity and fresh Ulva diet, the larval stage reached maximum abundance, with a significant interaction (p value=0.02). The proportion of larval stages within the populations also showed effects of both diet and salinity, but with no interaction. The low salinity treatments resulted in a 9% greater proportion of larvae over the seawater treatments, while fresh Ulva diet showed with a 7% increase in the larval proportion over the fecal Ulva diet (Figure 11: ANOVA diet p value=0.013, salinity p value=0.003, interaction =0.062).

Chemical analysis results comparing the caloric content of fresh Ulva and fecal Ulva diets showed that the fresh Ulva was significantly higher in calories than fecal Ulva (Figure 12: t-Test p value=0.006). On average, fresh Ulva had a calorie content of 1.55 calories/mg while the fecal Ulva diet had a 1.29 calories/mg, a decrease in value of about 16%. Caloric values of Ulva also changed significantly after the freezing process. Ulva samples which had been frozen for a one week duration and for an extended 6 week duration were tested, and both showed a significant increase in calorie

content over the fresh Ulva. The one week frozen sample contained a calorie content of approximately 1.66 calories/mg whereas the fresh version had an average of 1.46 calories/mg, indicating an approximate 20% increase over the fresh Ulva (Figure 13: t-Test p value = 0.028). The 6-week extended frozen sample also showed similar results, with the frozen Ulva having a significantly higher value at 1.89 calories/mg over the fresh Ulva at 1.54 calories /mg (Figure 14: t-Test p value < 0.001), a difference of approximately 19%. The effects of freezing fecal Ulva diets did not show similar results; there was not a significant caloric difference between the frozen Ulva fecal diet and the fresh Ulva fecal diet (Figure 15: t-Test p value =0.119).

Discussion

Both diet and salinity significantly affected population growth of *T. californicus* copepods. Feces produced by urchins that have been fed only Ulva do not appear to be an effective food for *T. californicus* population growth. The quality of fecal material produced on a particular algal diet depends on the absorption efficiency of urchins, and on the chemical composition of the food (Sauchyn & Scheibling, 2009). This study determined that fecal Ulva is a poor food source possibly due to its chemical composition. Based on caloric content analyses, fecal Ulva had 20% fewer calories then fresh Ulva. This contrasts with previous research on other algal species, where the calorie content of urchin feces fed *Nereocystis luetkeana* and *Saccharina latissima* was much higher than both fresh *N. luetkeana* and fresh *S. latissimi*, and the fecal diets resulted in increased copepod population growth (Kobelt & Dethier, 2015). Further study would be warranted to determine what the calorie content difference is between fresh Ulva and these kelp species, as well as their urchin fecal counterparts.

Surprisingly, the data showed that there were clear changes to the caloric content of Ulva seen with freezing; frozen samples had a 20% increase of calories compared with the unfrozen samples. The caloric content in frozen algae has been insufficiently studied, so it is unclear exactly why it increased. Further investigation into this subject is needed.

The experiments also showed a higher rate of total copepod population growth in low salinity treatments. Both treatments testing salinity differences (17 PSU vs 29 PSU) resulted in the low salinity treatments having greater population growth than the normal seawater treatments. This could relate to the normal habitat of *T. californicus*. This copepod is found in the high intertidal pools along the Pacific coast, where it lives to avoid predation (Dethier, 1980). Tide pools are subject to physical variation on a daily basis, stemming from sunlight, waves, precipitation, and tides. Heavy rains or evaporation can change the salinity within them, and forces species there to quickly adapt to this drastic change. Species show more effective mechanisms to cope with stress if they frequently experience a more variable environment (Lewis, Brown, Edwards, Cooper, & Findlay, 2013). Although the experiment did not change the salinity of water over the course of the 50 days, it could be that this species of copepod is already better suited for a low salinity environment just due to its natural habitat. Future research could benefit from monitoring populations from different locations to see if these same trends are observed.

The proportions of life stages seen within the treatment populations suggest that different life stages are affected by different combinations of diet and salinity. Some, like the egg-bearing females, seem to be negatively affected by diet type, whereas non-egg-bearing adults were changed more by salinities. The larval stages are affected by both diet and salinities, but the sub-adults show no changes in population growth from either. These outcomes could be due to long term exposure to poor conditions, which often cause decreases in reproductive periods and delayed development (Emlen, 1966). Larval

development can be affected by differences in the chemistry of seawater, such as the absence or presence of particular dissolved compounds (Wilson & Armstrong, 1961). Running this experiment for a longer duration that spans multiple generations would clarify these trends.

Even though feces from urchins fed Ulva do not seem to provide the proper nutritional needs for *T. californicus* populations to thrive, this does not mean that Ulva is not itself a good food source. Ulva is an abundant species found through the entire Pacific Ocean, which could provide a link between intertidal zones along coastal marine communities, and many herbivore species living in the coastal subtidal zone effectively consume it as a food source. While *T. californicus* is not directly present in deep benthic communities, many harpacticoid copepod species are present, and are an essential component of deep subtidal food webs. Discovering that they showed a positive growth rate in low salinity environments gives hope that other coastal species could possess these same characteristics. As climate events are going to increase, withstanding such environmental changes is going to be imperative for marine species survival.

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Figures

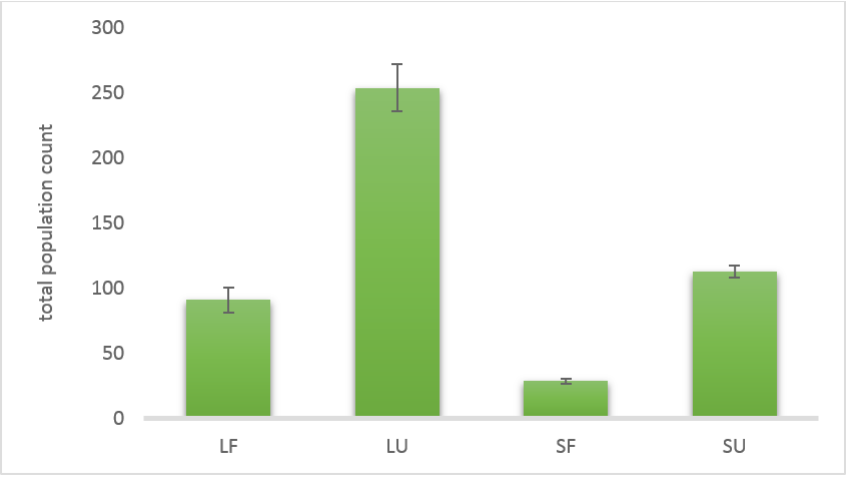

Figure 1. A comparison of total *T. californicus* population counts for each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are average among the 4 jars per treatment with standard error.

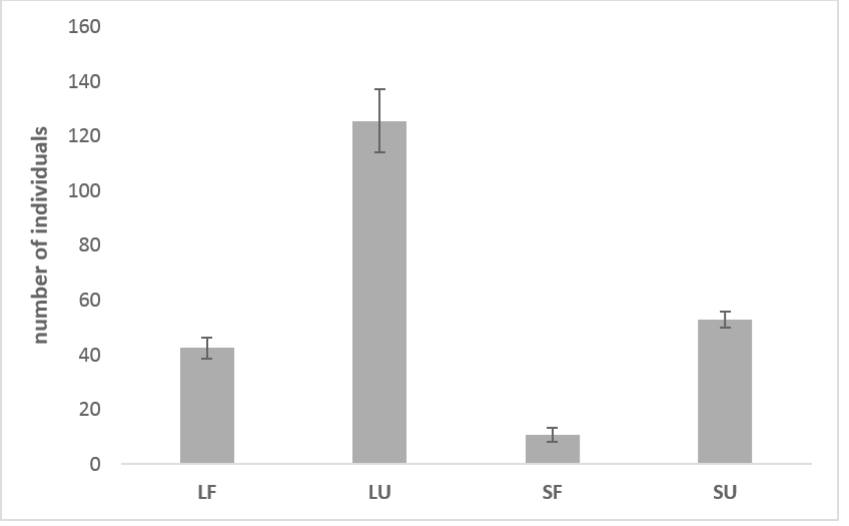

Figure 2. A comparison of total *T. californicus* population counts for each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the average among the 4 jars per treatment.

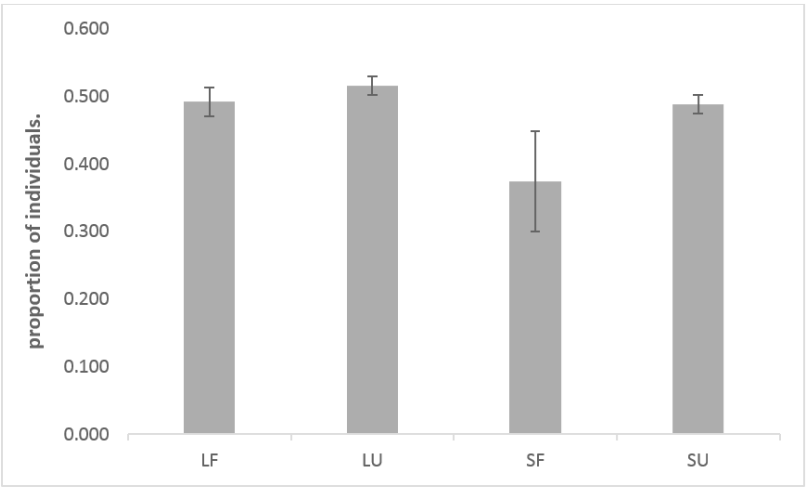

Figure 3. Comparison for the proportion of *T. californicus* populations made up each life-history state in each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Values are averages among the 4 jars per treatment.

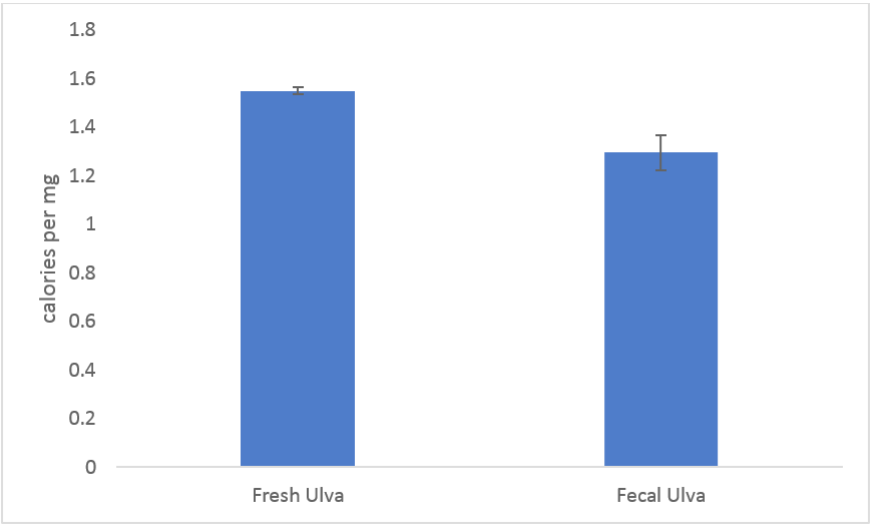

Figure 4. A comparison of total *T. californicus* population counts for egg-bearing females in each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are the SE averages among the 4 jars per treatment with standard error bars.

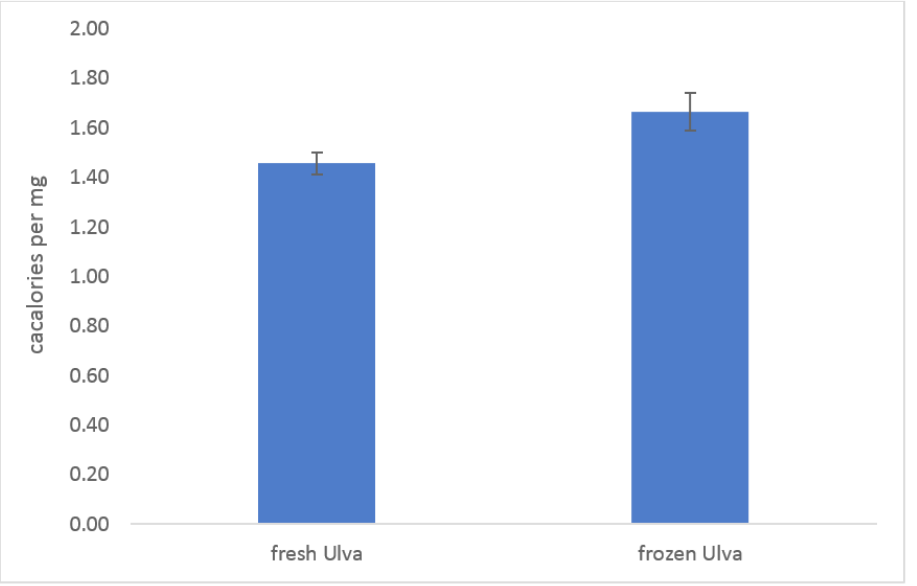

Figure 5. Comparison in proportion of *T. californicus* egg-bearing females in each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment with standard error.

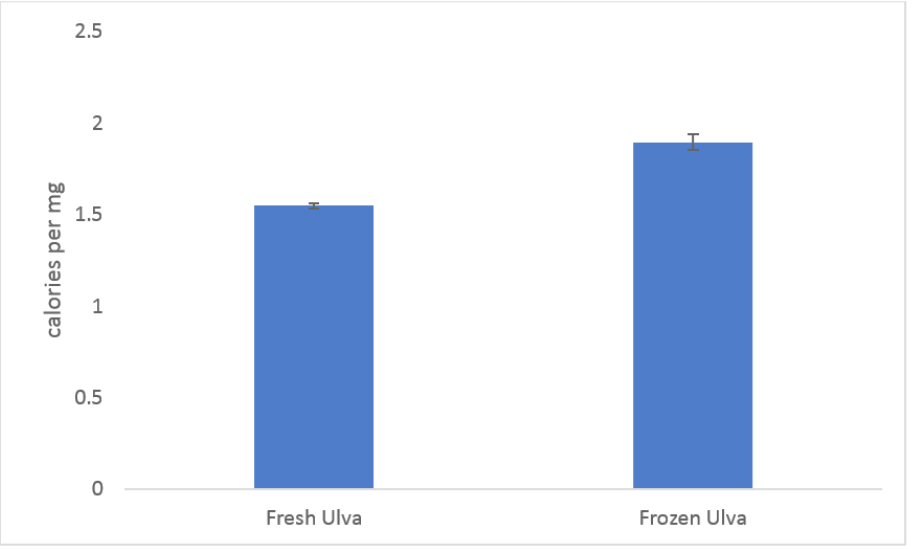

Figure 6. A comparison of total *T. californicus* population counts for non-egg-bearing adults in each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment with standard error.

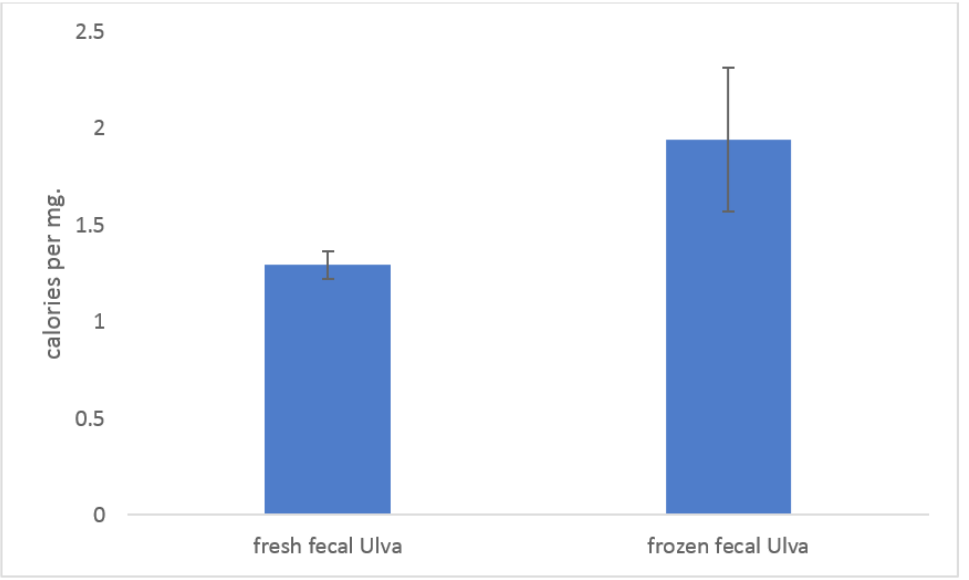

Figure 7. Comparison of the proportion of *T. californicus* non egg-bearing adults for each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU. Bars are the averages among the 4 jars per treatment with standard error.

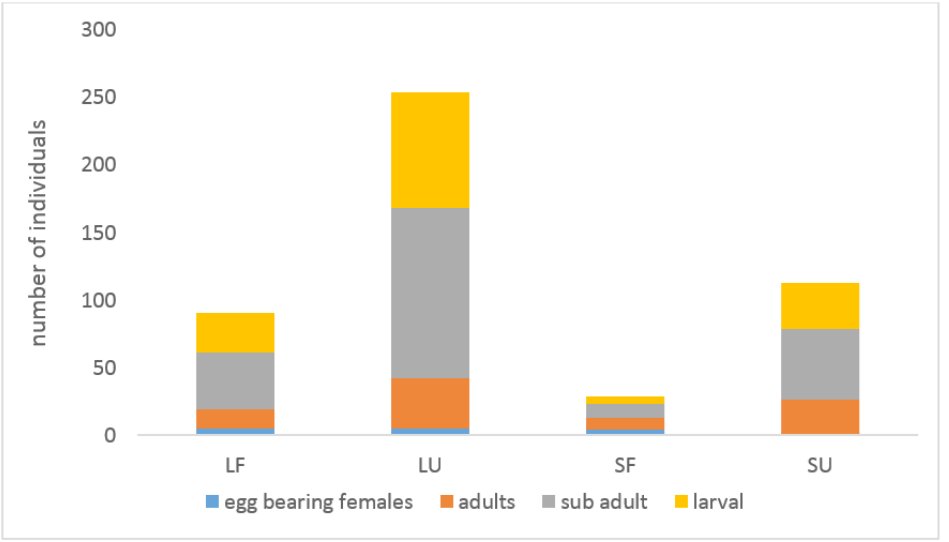

Figure 8. A comparison of total *T. californicus* population counts for sub-adult stages seen each treatment, including low salinity/urchin feces (LF), low salinity/ fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment with standard error.

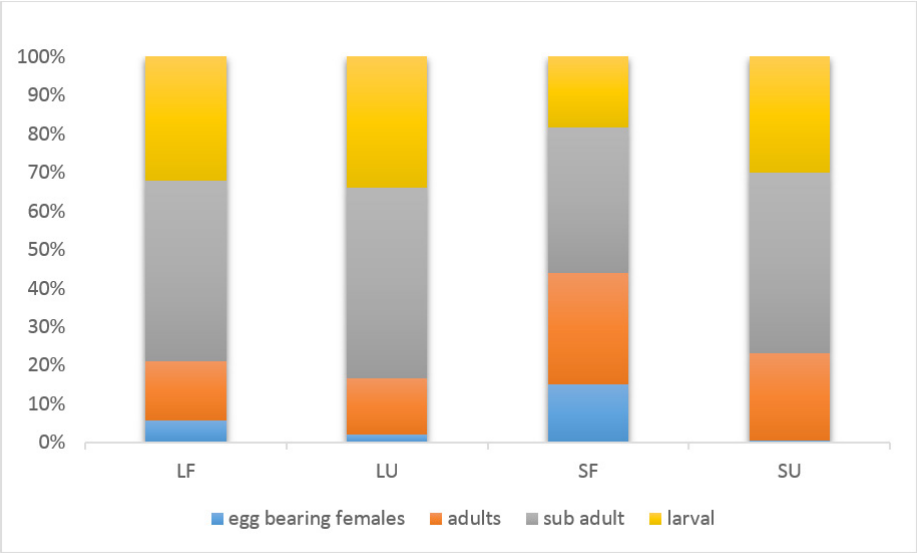

Figure 9. Comparison for the proportion of *T. californicus* sub-adults for each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment showing standard error.

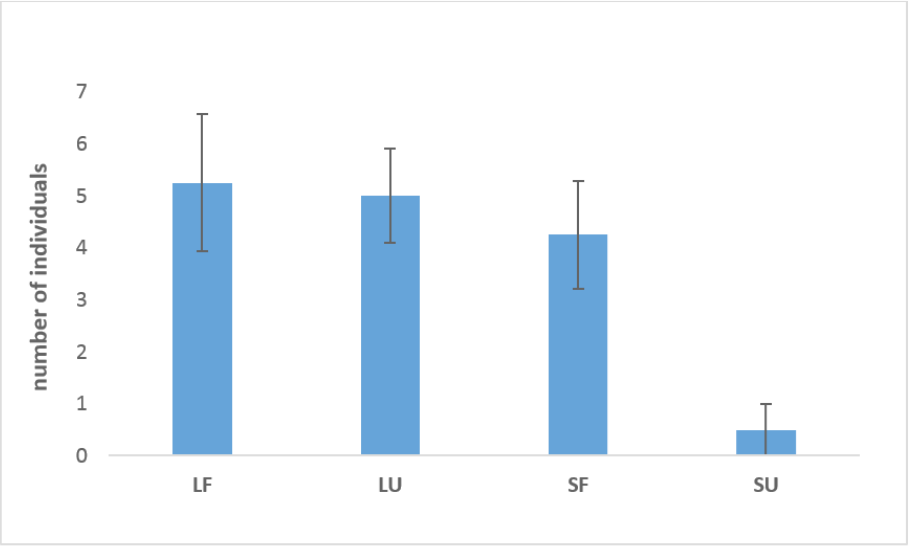

Figure 10. A comparison of total *T. californicus* population counts for larval stages seen each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF) and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment showing standard error.

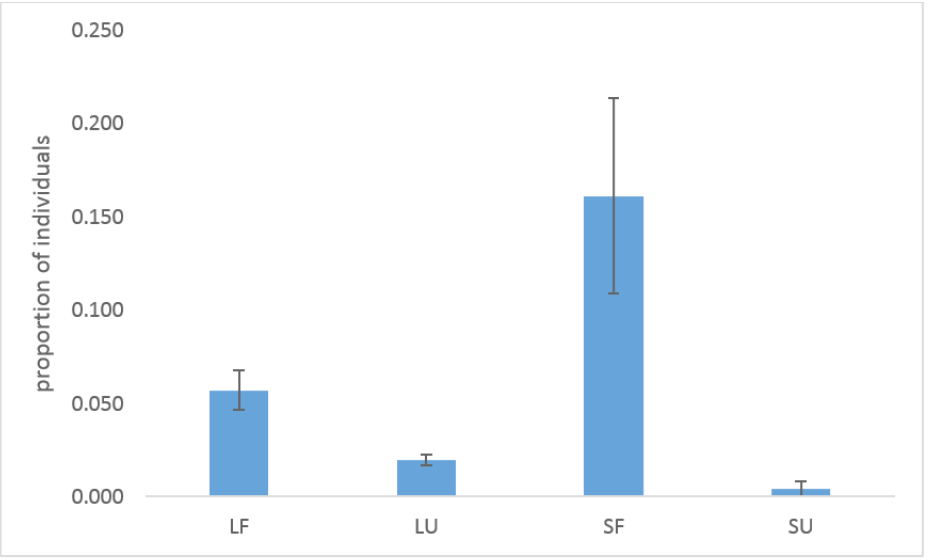

Figure 11. Comparison for the proportion of *T. californicus* larval for each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment and show atandard error.

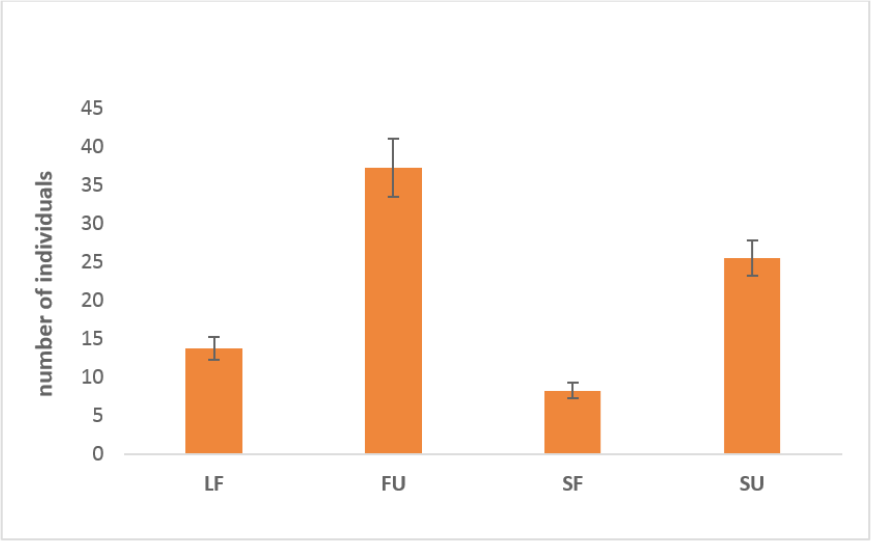

Figure 12. Comparison of the caloric content for fresh Ulva and fecal Ulva, showing fresh Ulva to be significantly higher than fecal Ulva by 16 % (t-Test p value=0.006) Bars are the averages among the 10 samples of each type and show standard error.

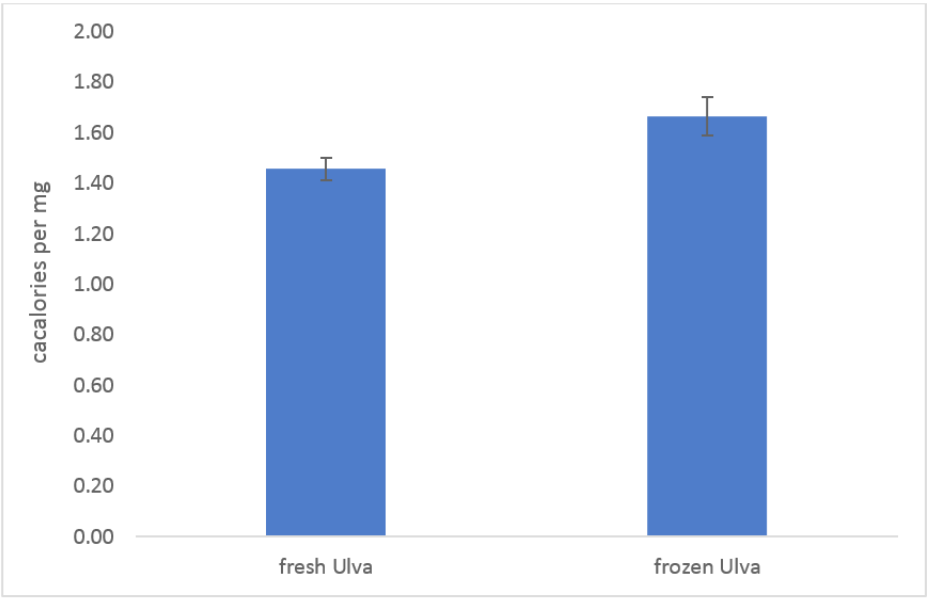

Figure 13. Comparison of the caloric content for fresh Ulva and 1 week old frozen Ulva, showing frozen Ulva to be significantly higher than fresh Ulva by 20%. Bars are the averages among the 15 samples of each type and contain standard error bars.

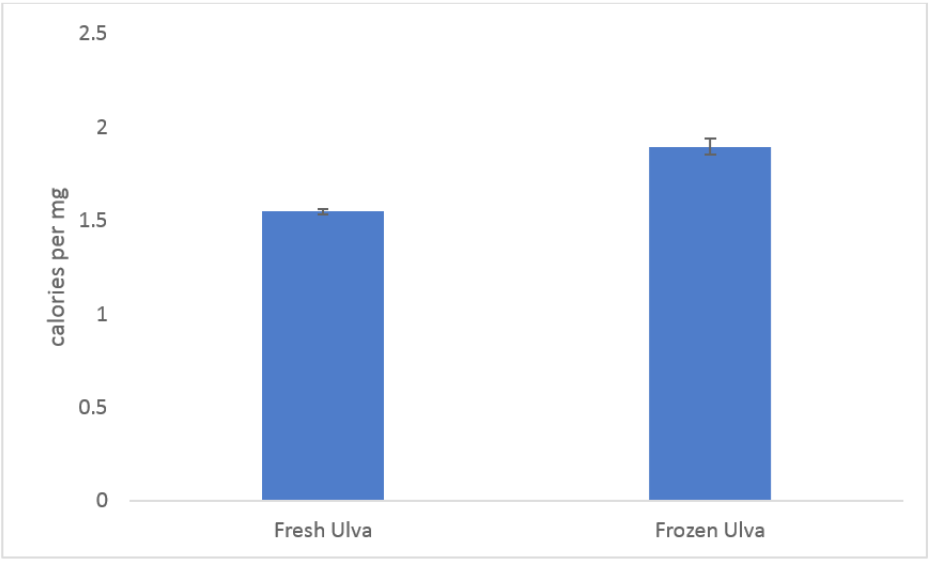

Figure 14. Comparison of the caloric content for fresh Ulva and 6 week old frozen Ulva, showing six week old frozen Ulva to be significantly higher than fresh Ulva by 19% . Bars are the averages among 10 samples per type and standard error.

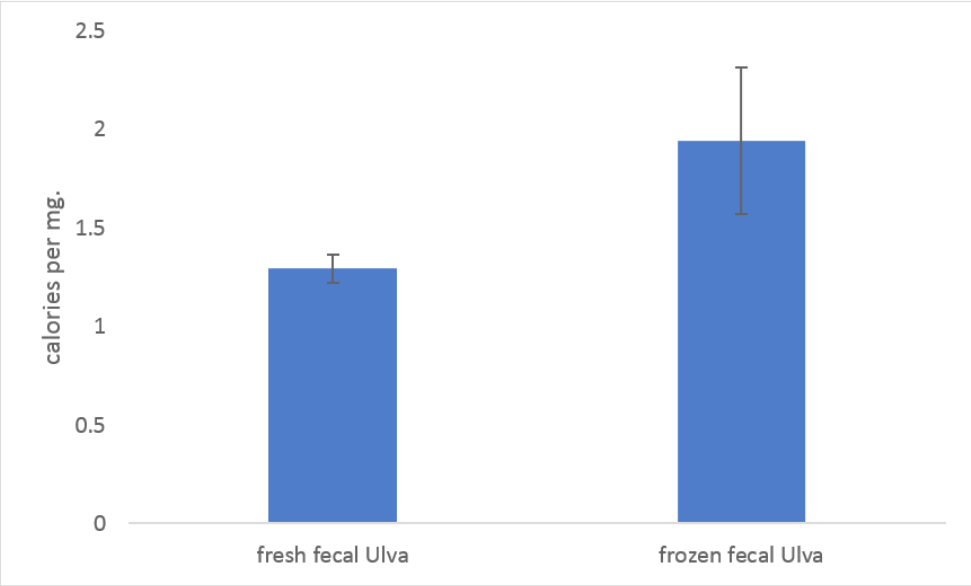

Figure 15. Comparison of the caloric content for fresh fecal Ulva and frozen fecal Ulva, showing no statistical significance in either treatments. Bars are the averages among 10 samples per type and show standard error.

DEVELOPMENT OF A THERMAL DESORPTION AND CRY-GC-MC METHOD FOR THE MEASURMENT OF VOCs IN AMBIENT AIR

Angela Angelevska, and Crystal McClure

Under the Direction of Dr. Daniel A. Jaffe

ABSTRACT: Wildfires increased across North America in recent years (Jaffe et al. 2012, 2013). Acetonitrile (CH_3CN) is chosen as a trace molecule, as it indicates biomass burning, while anthropogenic sources are limited. Previous studies of acetonitrile in ambient air required transport of massive equipment to the site of the fire, giving importance to developing a simple, portable and inexpensive method to trace the production of acetonitrile in wildfires.

We propose a new method with higher sensitivity, reproducibility and recovery for measuring volatile organic compounds VOCs, such as CH_3CN in ambient air, using thermal desorption-cryofocusing-gas chromatography-mass spectrometry (TD-Cryo-GC-MS). Focus is given on calibration and optimization of the new method, in addition to water vapor (WV) and ozone management; breakthrough tests of the sample and optimization of the conditioning of the TD tubes prior sampling.

The range for the calibration is based on previous studies, analyzing the biomass burning in rural/urban areas. We were able to detect concentrations of 0.47ng using the Cryo-GC-MS.

The TD tubes were packed with adsorbent: PorapakN, chosen for its affinity of retaining CH_3CN, high hydrophobicity and low breakthrough volume. However, PorapakN did not show the expected hydrophobicity (<1mg), making WV management necessary for sampling on the TD tubes and subsequent analysis on the GC-MS. As ozone trap, Na_2SO_4 showed high recovery (99.8%) of the samples. Breakthrough tests had recovery of 1.87 ±0.56% for sampling concentrations of up to 60ng CH_3CN, over the period of 2 hours.

The recovery of the samples was increased by conditioning the blank tubes longer and using the SIMS mode of the GC-MS instrument to look at the mass-to-charge ratio of only acetonitrile.

Finally, a validation experiment was designed, showing good first results of recovery in each step of the process. Validation experiment can be used for understanding the sensitivity of TD-Cryo-GC-MS.

1. Introduction

1.1. VOCs and biomass burning

Volatile organic compounds (VOCs) are important trace gases emitted generally from wildfires and biomass burning and are greatly studied in atmospheric chemistry. The majority of the studies done before on VOCs provide a good scope on the anthropogenic sources of these compounds (Primbs et al, 2007; Goldstain and Galbally, 2007), however the impact from the emissions from fire plumes is more difficult to determine (Friedli et al, 2001; Holzinger et al, 1999, Guenther et al, 1995). The importance in knowing this information comes from the numerous effects wild fires have on the global atmosphere, such as climate change, smog development and increased acid depositions (Guenther et al, 1995; Crutzen, Andreae, 1990), especially studied has been the impact on the second aerosol and ozone production (Jaffe et al, 2008, 2012, 2013; Wang et al, 2007; Akagi et al, 2011). Additionally, many VOCs have adverse human health effects. This includes acetonitrile's acute toxicity due to its rapid metabolism to cyanide and thiocyanate, and uniform distribution throughout the body (Jordan et al, 1995; Singh et al, 2003; Crutzen and Andreae, 1990). Therefore, exposure to acetonitrile is recommended not to exceed a time-weighted average of 40ppm in air (Singh et al, 2003).

1.2. Methods for measuring VOCs and previous uses of TD-GC-MS method

This study focuses on developing a new method for measuring VOCs, in particular

acetonitrile(CH_3CN) in ambient air. In recent studies, it has been suggested that the biomass burning emissions of CH_3CN dominate the global source of this compound, making it a unique tracer for biomass burning because of its long atmospheric lifetime (Holzinger, 1999, Loberte et al., 1990; Wang et al, 2007).

Determining the concentration of acetonitrile in air has been done using various instrumental methods, such as GC, IR, laser absorption, polarography, PTRMS etc. (Yokelson et al, 1997, 1996; Jordan et al, 1995; Holzinger et al, 1999; Christian et al, 2003, 2004; Wang et al, 2007; de Gouw, 2003; Warneke, 2004; Crespo, 2012; Apel, 2003). All of them were able to detect the compound, but with different efficiency and each method experienced several interferences.

The PTR-MS method (proton transfer reaction - mass spectrometry) used in the study of deGouw et al (2003) (and similar studies by Lindinger et al, Poschl et al and Goldan et al) had limited precision because of background signal of impurities and interference of H_3O^+ and H_3O^+ (H_2O) reagent ions from the humidity. Additionally, all the samples had to be analyzed immediately after the sampling. The only advantage of using PTR-MS over GC-MS (gas chromatography-mass spectrometry), they conclude, is measuring close to emission sources where atmospheric changes are rapid. As we are not interested in measuring VOCs close to fires and want to be able to remote sample VOCs, PTR-MS was not of interest in our study.

The use of TD (thermal desorption) tubes for collection of VOCs in breathing air was studied by Crespo et al (2012), as a substitute for Tedlar bags bags to measure off-line volatiles, as previously done in the study of Jordan et al (1995). This validation experiment of the TD tubes, showed that these tubes were able to effectively collect acetonitrile in breathing air, with the range of 32 g/mol up to 136 g/mol.

The study showed that the packing of the TD tubes was important to be selected in collecting different VOCs. The diference in sorbent packing was examined in the study done by Maria Rosa Ras-Mallorqu et al (2007). VOCs were determined using TD tubes packed with two multisorbent beds Carbograph 1/Carboxen 1000 and Tenax/Carbograph 1TD and were after analyzed with GC-MS with recoveries of 98.9%. The importance was placed on the clean blanks, as they saw better results with cleaner blank tubes packed with Tenax/Carbograph.

1.3. Optimization of TD-GC-MS

The literature review (Crespo (2012), Lee et al (2012), Peng and Batterman (2000), Jia et al (2006), Grote et al (2002), Batterman et al (2002) and Maria Rosa Ras-Mallorqu et al (2007)) showed that TD tubes can be used for determining VOCs in ambient air, analyzed by Cryo-GC-MS. The use of Cryo-GC-MS method has been extensive and well known. Additionally, the sensitivity of the GC-MS method has been proven to be increased by using selective ion monitoring modes (Jia et al, 2006).

However, the use of different sorbent packing, particularly for nitrile VOCs, the interference with humidity and ozone, the interference with background concentrations of impurities in the sample are not well understood.

The main idea of this study is to deveop a new method for measuring acetonitrile in ambient air, by increasing the sensitivity and lowering the cost of the process. Based on the papers by Woolfenden (2010a, 2010b) an extensive study of using thermal desorption tubes and optimizing the methods showed that significant attention needs to be given to the choosing of :

- the sorbent in terms of the inertness to other compounds present in the air;
- the flow at which the sampling will take place in terms of the breakthrough volume at higher sampling flows;
- the water management of the sample, which also affects the sorbent material;
- the artefacts not only while sampling but also in the conditioning of the sorbent material;

- the stability of the sorbent at higher temperature, which affects the choice of the thermal desorption temperature and conditioning of the tube;
- possible interference with ozone on the sorbent material;
- Length and temperature of storage of tubes that have been used in sampling and on tubes that are conditioned.

The need for a reliable, portable, fast and inexpensive method for quantifying VOCs in air urged us to ask the following question:

Can we create a new method with higher sensitivity, reproducibility and recovery for measuring VOCs (focus on acetonitrile) in ambient air, using TD-Cryo-GC-MS?

In this paper, the optimization and development of the calibration method for determining acetonitrile using TD-GC-MS is going to be presented. Ambient air sampling and sample analysis is going to be presented in following papers. As a reference goal for this study, the atmospheric concentrations of acetonitrile determined by Wang et al (2007) and de Gouw (2003, 2004) are being used, aiming at reaching sensitivity of the method for background concentrations of acetonitrile in ambient air below 0.1ppbv.

Table 1: Literature data on acetonitrile concentrations in ambient air, obtained from Wang et al (2007), p. 8382

Site (sampling height)	Time	CH3CN (ppbv)
Xinken, sub-urban site of Guangzhou city (8 m)	6–31 October 2004	0.6670.18 (30a)
Guangzhou downtown (50 m)	6–31 October 2004	0.6670.29 (45a)
Beijing (20 m)	2–23 August 2005	0.2870.09 (867a)
	16–17 August 2005 (right after rainstorm)	0.2170.03 (75a)
Urban areas with no distinct biomass burning		0.1–0.3b,c
Plumes strongly influenced by biomass burning		Above 0.6b,c

**Comment: a Date from Wang et al (2007); b Data from de Gouw et al. (2003). c Data for US west coast from de Gouw et al. (2004). d Data from Lobert et al. (1999). e Data from Duan et al. (2004).*

2. Methodology

2.1. Permeation source calibration

The standard for acetonitrile was prepared as a permeating gas source placing it in a setting described previously in the study by McClure et al, 2014. A G-Cal Acetonitrile permeation tube (Vici, model GC23-7912,3) was used as a permeation source in order to generate the desired vapor concentration throughout the experiment.

The tube was placed in a flow chamber with a constant flow of 100sccm, using critical orifice to control the flow rate, Nitrogen gas (5.0 UHP grade) and inserted in a housing that was held at constant temperature of 300°C. To monitor the change of the flow a mass controller (FMA 1812 Omega, 0-500ml/min) was placed upstream of the heated housing and the temperature was monitored with a thermo-controller (TE Tech PS-24-6.5) placed inside the housing.

The permeation source was measured using a scale (model Mettler AE 163), calibrated using 1, 2, and 500 mg standards, Mettler-Troemner class 1. The permeation rate was calculated using Equation 1.

$$Permeation\ rate\ \left(\frac{ng}{\min}\right) = \frac{\text{mass } loss(g) * 10^9}{time\ between\ measurements(min)}$$

2.2. Cryofocusing-Gas Chromatography-Mass Spectrometry

2.2.1 Background

The cryofocusing-GC/MS system was based on previous research done by Goldan et al

(2004) and Apel et al (2003). To calibrate the CF-GC-MS system, the gaseous acetonitrile was collected on the cryofocusing unit (model 961 GC Cryo-Trap from SIS,4 Inch) at -145°C, using a guard column (Intermediate polarity deactivated 0.53mm ID). After 5 min of cooling, the unit was heated at 100°C in 21.1 seconds average and quickly instated into an (30m, 0.25mm ID,1.4µm film DB-624) analytical column, in a (Agilent 7890B) GC system and (Agilent 5977A) MSD.

The cryotrap was cooled using liquid nitrogen (LN2). The carrier gas for the cryofocusing unit and for the transferring the sample into the GC-MS was Helium (5.0 grade).

The sample was split 99:1 using the split/splitless inlet liner (Agilent 5184-4647, 4mm ID LPD) with a gas flow of 100sccm. The GC oven was heated at 35°C for 5 min, followed by increasing the temperature 8.4°C/min to 127°C. For the analysis, MassHunter softer by Agilent was used for 5977. The calibration of this setup was done by changing the cryofocusing collection time from 5 minutes to 2.5 minutes, 1 minute and 30 seconds.

2.2.1. Blanking the instrument

The instrument was blanked each day, using three steps:

1. Autotune of the MSD
2. Oven blank, by running the carrier gas only through the GC-MS, using the parameters described in Section 2.
3. Cryotrap blank, by cryotrapping the carrier gas with the same set-up described in Section 2.

2.3. Thermal desorption tubes (TD tubes)

2.3.1. Sorbent tube packing

The sorbent bed chosen for this experiment was PoraPakN, (50/80 mesh size) from Markes International, (product number C-2PPKN). This sorbent was selected based on literature review. The selection process included several characteristics needed in air sampling of acetonitrile including:

- •Acetonitrile retention on the sorbent
- •Sampling volume (2-10 L)
- •Minimizing water vapor interference
- •Breakthrough volume less than 5%

PorapakN was suggested for use when selecting volatile nitriles (EPA Compendium to TO-17, Table 1) because of its hydrophobicity, as acetonitrile is soluble in water and the absorption of water vapor on the sorbent tube can result in loss of the initial acetonitrile concentration that was sampled. Additionally, PorapakN was selected because of its capacity related to the breakthrough volume.

The sorbent tubes were also purchased from Markes International, (product number C0-BXXX-0000), as empty glass tubes with restriction on one side at 15mm. Each package came with torsion springs (one per each tube). In addition, ¼'' tubing caps were purchased (Motion Industries, SMC KQ2C07-00A) to close each sorbent tube after packing, as well as SS nuts and ferrules from Vici-Valco. The decision to purchase the second set of caps was made because of the lower cost and easier handing of the tubes. A validation experiment of the caps was performed to check for difference between the two sets of caps.

Each tube was packed with 200 mg of PorapakN, secured on both sides with quartz wool, and a torsion spring on the side that does not have restriction. The weight of each tube was measured empty, with PoraPakN and packed, using the same scale and standards described in Section 1. A picture of the packed tube is shown on Picture 1. Each tube was then wrapped in aluminum foil and stored in an airtight container at <4 degrees Celsius, until ready for use.

Picture 1: Example of a packed thermal desorption tube, prepared for packing in the aluminum foil.

2.3.2. Sorbent tube conditioning

The setup for the conditioning of the packed tubes was done according to the EPA Compendium Method TO-17 (1999). The newly packed tubes were conditioned for 2 hours at 180°C, by passing nitrogen gas at a flow rate of at least 100mL/min.

2.3.3. Sorbent tube storage

The newly packed or used tubes were capped, wrapped in aluminum foil and placed in a clean, airtight, opaque container in a refrigerator at temperature <4°C.

2.3.4. Testing of Blank tubes

The tubes were desorbed using the same parameters for the Cryo-GC-MS described in section 2. To desorb the tubes, the same furnace for conditioning was used. Nitrogen gas was flowing through the tube with 45mL/min, at 160°C, with reverse flow. For the lines for the connections between the carrier gas, tube and the cryotrap unit, ¼' PFA tubing was used, with Swagelok Stainless steel fittings.

The SIM/SCAN mode was used in the MassHunter Softer to scan particularly at 38,39,40,41 and 42 mass-to-charge ratio (m/z) of the mass spectrums generated by the TD tubes analyzed by the Cryo-GC-MS system. The 41 m/z was checked for any peaks due to artifacts near the expected acetonitrile peak. If the blank was not satisfactory, in other words, it was showing a peak in the region of the expected acetonitrile peak, the tube was re-conditioned again.

2.4. Sampling and calibration of the thermal desorption-cryofocusing-GC/MS method

For sampling, a manifold was made to ensure proper mixing of the ambient air and the spiking of acetonitrile from the source. The sampling setup is shown in Figure 1.

By regulating the flow of the air going through the tube packed with PoraPakN using the pump downstream of the tube and the pump that was regulating the dilution of the source before going into the manifold, the concentration of acetonitrile on each tube can be calculated. The calibration is explained in Section 4.1. Based on previous studies, background concentrations of 0.1 ppbv were considered the minimum, while the maximum concentration expected to be seen in the air was 10ppbv.

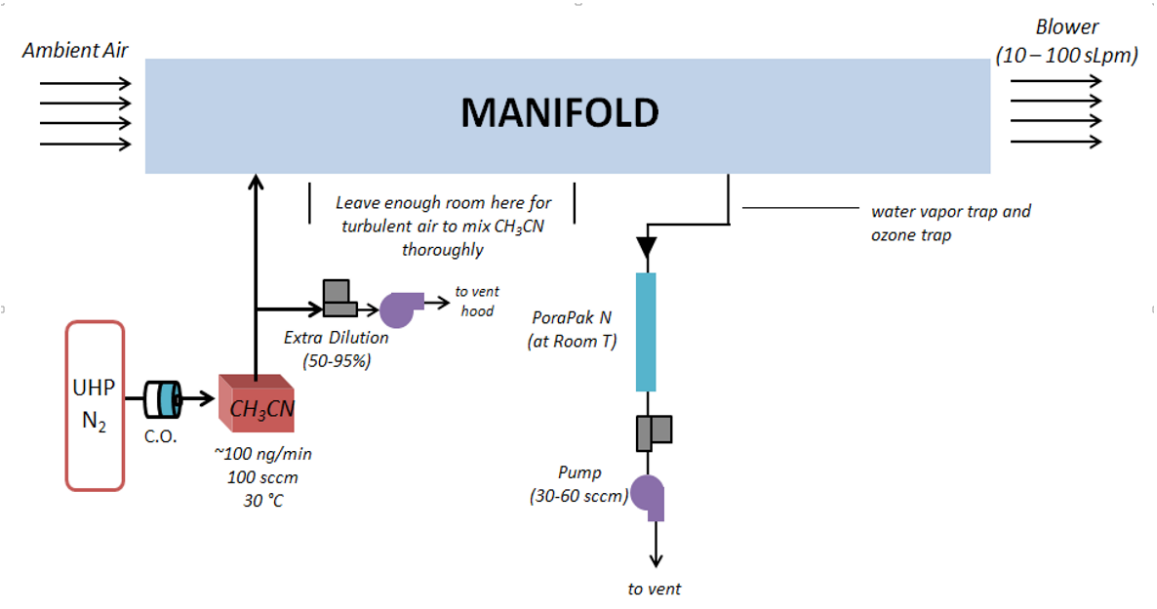

Figure 1: Schematic for the sampling procedure. The manifold and the connecting lines are Teflon tubing.

2.4.1. Collection efficiency tests

To check for consistency among tubes, acetonitrile was sampled onto 5 tubes, using the same set up shown in Figure 1. The manifold was used to make a 6-point calibration curve with acetonitrile. Each tube was sampled for 5 minutes with constant flow of 30mL/min, regulated by a pump used downstream of the tube.

The spiking concentration was decreased by reducing the flow of the acetonitrile permeation from 100 to 25 mL/min. The desired concentrations were obtained by changing the flow of the gas used in the manifold for mixing with the acetonitrile permeation gas between 0.1 L/m to 10 L/m.

The tubes were immediately desorbed after sampling. The area of the peaks was compared.

2.4.1.1. Breakthrough tests

On the first and last tube, sampled with the same concentration, a breakthrough tube was added. These tubes were chosen to check for possible difference in the flow and concentration between the first and the last tube in the experiment of the collection efficiency test. A breakthrough tube is added to measure the breakthrough volume, which is the concentration of the analyte which may be passed through the sampling tube. The breakthrough volume should not be more than 5% of the sampling volume.

2.4.2. Humidity testing

The sorbent bed was hydrophobic, however tests for water vapor uptake were performed, to test the level of humidity at which the sorbent would give incorrect results. Using breathing air (UN1002 compressed), flowing through a bubbler, several levels of relative humidity were delivered. The relative humidity was detected with a HOBO Micro Station (MAN-H21-002), also detecting temperature and pressure. Two rotometers were used to regulate the air flowing through the bubbler. The sorbent tube was placed in a mixing chamber next to the HOBO detector and a pump was added downstream of the sorbent tube, with a MFM, to control the flow going through the tube at 45mL/min. For higher humidity levels, a hot plate was placed under the bubbler. The relative humidity was then used to calculate for the absolute humidity levels using Equation 2.

$$Absolute\ Humidity = \left(\frac{6.1112 * e^{\left(\frac{17.67 * Temperature\ (degrees\ C)}{Temperature\ (degrees\ C)+243.5}\right)} * 2.164 * RH\%}{\frac{273.15+Temperature\ (degrees\ C)}{1.225}}\right)$$

The tubes were measured before exposing them to high humidity and after, using the same scale and standards used in Section 1.

2.4.3. Water vapor and ozone traps

Teflon tube filled with 26.5g Na_2SO_3, served as ozone trap. Similarly constructed Teflon tube, filled with 65 g of 3A molecular sieve trap served as water vapor trap. Both traps were tested for acetonitrile uptake. The tests were done with and without the traps in line, 3 tubes for each test, using the same concentration of acetonitrile.

In addition, for minimizing the humidity interferences, dry purging tests were done, to ensure minimum acetonitrile loss of the sample, while lowering the water vapor concentration in the tube. The dry purging was done by flowing cold carrier gas through the tube for 30 seconds, 1 minute and 2 minutes, right before desorbing the tube into the Cryo-GC-MS system.

2.5. Validation of the method

A second standard was made for validation of the method and permeation source, to understand the sensitivity of the GC-MS. Liquid stock solution of acetonitrile was purchased (UN 1648, CAS 75-05-8) with 99.9% purity. From that standard, a single–step dilution was made using methanol, to minimize the error in the diluting steps. Same concentration of the acetonitrile was inserted using a syringe into the GC column directly, into the cryotrap and collected on a tube. The syringe that was used was 0.5-5µL Hamilton Syringe (cat#24938).

The first step was done by direct injection in the GC-MS inlet, while it is held hot, immediately

after starting the GC-MS run.

The second step was done by adding a septa, wrapped in a heat tape, upstream of the cryotrap, while flowing He gas through it. The injection of the liquid acetonitrile was done using the same syringe used in the first step. The cryotrap was held cold for 2.5 min.

The third step was done by placing the tube downstream of the septa and collecting on the tube for 2.5 min. Then the tube was desorbed with reverse flow on the cryotrap for 2.5 min.

The area, height and width of the peaks from the three steps were compared to check for sensitivity of the GC-MS and for method validation of the collection efficiency of the tubes.

3. Results and Discussion

3.1. Permeation source calibration

We measured a known source of acetonitrile over a period of 7 months, to find the mass loss due to permeation of the liquid source at 300°C and flow of 100 std ccm. We found that the permeation rate is 0.0002 g/day, as seen from the slope (Fig 1), which is equivalent to 138.89ng/min. For this calculation Equation 1 was used.

The concentration this permeation rate gives in gaseous acetonitrile is calculated by dividing the rate with the known flow (100mL/min) and is equal to 1.39ng/mL, or 1.19ppm. Using this known concentration, the subsequent dilutions for the calibration of the instrument were calculated.

3.2. Cryofocusing-GS-MS

For calibrating the Cryo-GC-MS system, we collected gaseous acetonitrile on the cryotrap with immediate GC-MS analysis, using the Manifold (Figure 1) and diluting the flow with N2 instead of ambient air. The source flow was reduced to 30sccm in this step. The calibration showed positive linear correlation between the concentration of acetonitrile and the area and height of the peak from the chromatograph. This analysis also showed constant 21 second heat time of the cryo trap, for each sampling time.

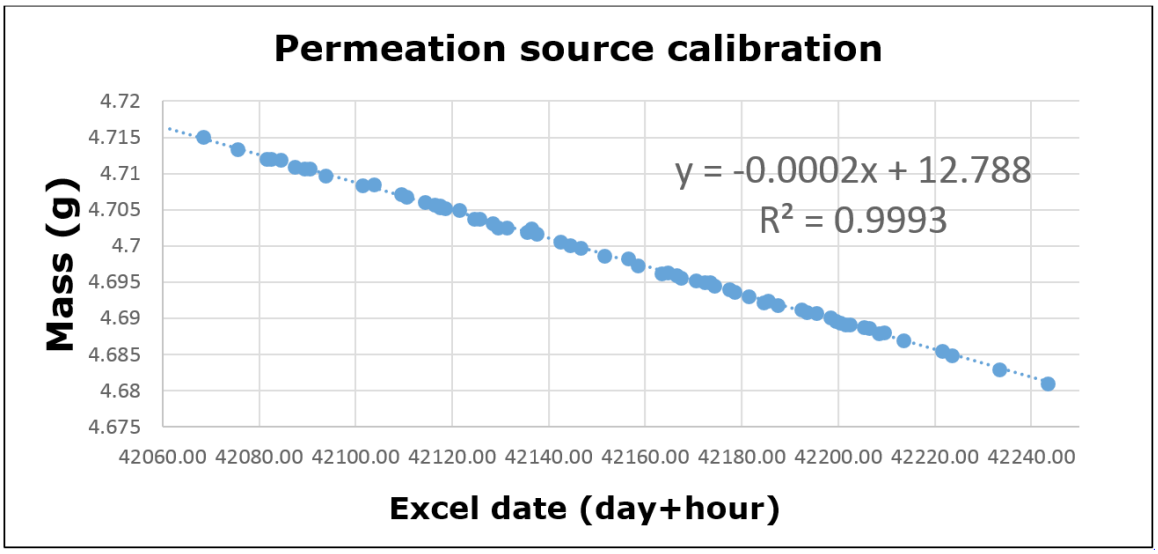

Figure 2: Permeation source mass loss over time.

Table 2: This tables contains the concentrations for the 6-point calibration for the TD-Cryo-GC-MS method, with the average area and height of 3 sample tubes. The precision for each concentration that was calculated from the results of the area.

Dilution flow (ml/min)	Concentration (ng)	Average Area	Average Height	Precision%
250	18.7	22327.96	4979.343	-8E-06
500	9.37	9473.487	2135.633	0.000331
1000	4.69	3223.795	629.47	-1.57982
2500	1.87	1471.897	297.42	-0.0134
5000	0.937	461.475	88.255	0.57858
10000	0.469	143.0667	35.46	0.971302

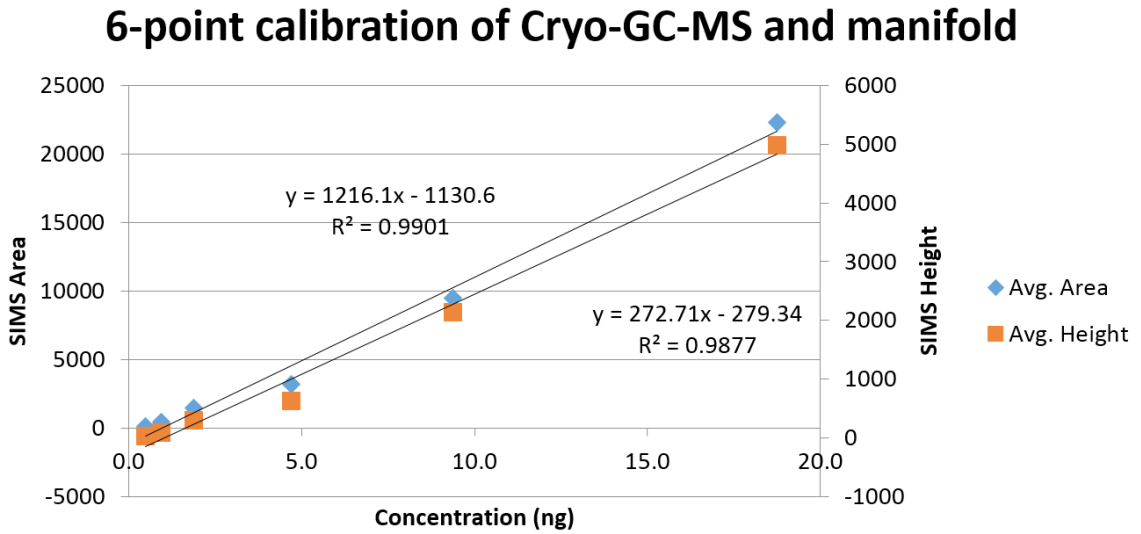

Figure 3: Graph showing the relationship between the concentration of gaseous acetonitrile in ng and the area under the chromatogram obtained from the Cryo-GC-MS

3.2.1. Blanking of the instrument

An issue we encountered using this set up was the increased level of O_2 that was especially apparent during the first Autotune of the day. By running an oven blank through the GC-MS, followed by a cryo-trap blank, which includes concentrating N_2 (5.0) for 5 min, the level of O_2 in the column decreased. In addition, the GC-MS was particularly sensitive to any impurities from the gas tanks provided by the distributors. Therefore, Ultra High Purity Helium tanks were ordered from a new lot from AirGas, instead of Praxair.

3.3. Thermal desorption tubes

3.3.1. Sorbent tube packing

The tubes were packed with PorapakN and weighted as described in section 2.3.1. Each tube had on average 200±27mg of PorapakN, as suggested by EPA (12).

3.3.2. Sorbent tube conditioning

TD tubes were conditioned according to the suggested EPA method. After conditioning, each tube was tested with the method as a blank, described in Section 3.3.4.

Table 3: Results of packing the sorbent tubes with Porapak N and measuring the weight of 62 packed tubes.

Packed tubes	Glass tube (g)	Porapak N (g)	Tube+Porapak N (g)	Tube capped (g)
Average	3.97	0.20	4.18	6.72
3σ	0.26	0.03	0.26	0.25

3.3.3. Sorbent tube storage

All the tubes were stored in a refrigerator at 0°C. Before either sampling or testing the tubes, they were left in their packaging on room temperature for 5 minutes.

3.3.4. Tests of blank tubes

The TIC/SCAN mode, the chromatogram did not show any artifacts or peaks that would suggest impurities of the TD tubes. However, in the SIMs mode, the chromatogram had artifact peaks that showed presence of hydrocarbons or other compounds that can be components of PorapakN.

Because some of these artifacts were showing peaks around the RT of acetonitrile, the TD tubes were re-conditioned. The reconditioning that showed best results was 2 more hours of flowing 100-200ccm N_2 gas through heated (160-1750C) TD tube. Figure 4 shows an acceptable example of a GC response for a blank tube, where the Abundance of the peaks in the SIMs mode is lower than 5000, and in the SCAN mode lower than 10000.\

3.4. Sampling and calibration of TD-Cryo-GC-MS

3.4.1. Collection efficiency tests

The collection efficiency test was performed by collecting specific concentration of gaseous acetonitrile, using the manifold described in Figure 2. The concentrations used in this calibration setting are presented in Table 4. The sampling flow for each tube was 45mL/min. The concentration from the source was not reduced, instead the dilution with N_2 was altered to obtain different concentrations, as shown in Table 4.

Table 4: Calibration concentrations and results of TD tubes.

Concentration (ng)	RT	Avg. Area	Avg. Height
15.31	3.043	20013.79	4384.877
30.63	3.053	31818.55	6813.67
61.26	3.053	48449.22	9011.03

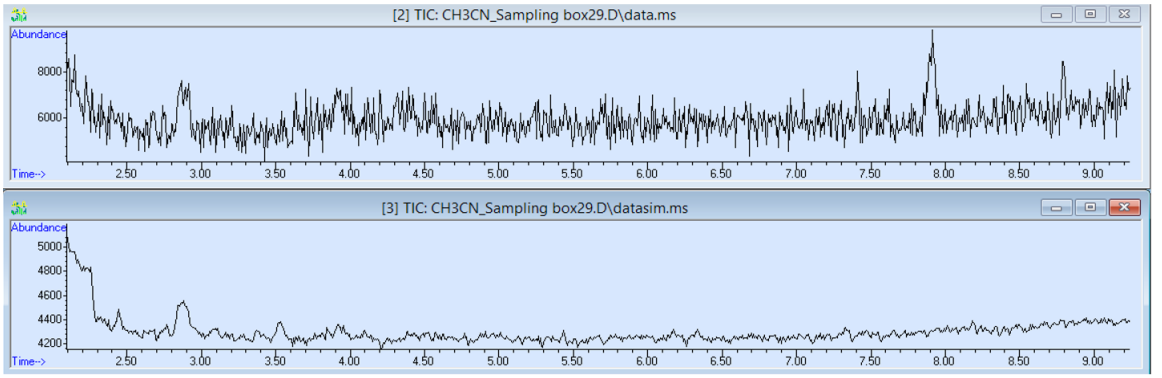

Figure 4: Acceptable example of a blank tube, showing the TIC, Scan mode on the top and the SIMs mode of the GC response on the bottom.

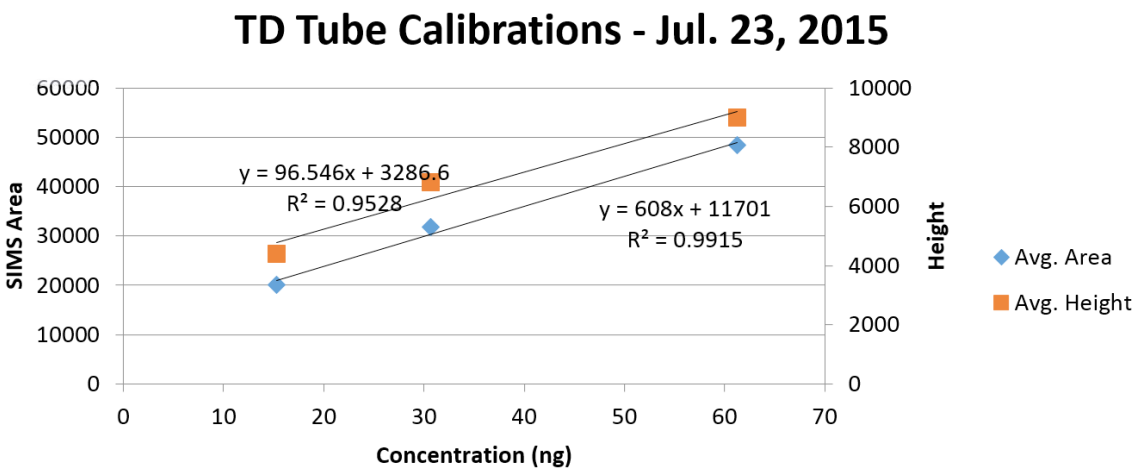

Figure 5: TD tube calibration showing the SIMS area and SIMS height response of the GC, against the concentration of each sample.

3.4.1.1. Breakthrough tests

Breaktrhough tests were performed on the first and last sampling tube from the 6-point calibration described in the previous section. The chromatograms showed no visible peaks in the SCAN mode. In the SIMS mode, the average area of the peak was 1.87% ± 0.56%. These results were satisfactory, as the BV was <5%.

Table 5.

Sample concentration (ng)	% Breakthrough
14.12547581	2.33
32.40636895	1.25
60.66692972	2.04

Table 6: Weight difference for each humidity level setting, with the average relative humidity (RH) %, read from the HOBO instrument and the absolute humidity calculated by using Equation 2, in g/kg.

	Weight difference (mg)	RH average% (HOBO)	Abs Hum (g/kg)
	2.025203	45.56147541	6.635869898
	2.178589	46.38934	6.78608428
	2.488756	50.5942623	7.366224918
	3.248376	50.65983607	7.351799764
	4.737631	61.4795082	8.986619431
	5.087456	62.06147541	9.061541074
	5.780578	68.7418	9.928719
	6.070607	68.77459	10.01317297
	11.50115	87.06148	12.93076
	10.42104	88.94672	12.95485
	10.27103	89.9959	13.10287
	11.55116	92.93033	13.83273
Max	11.55116	92.93033	13.83273
Min	2.025203	45.56147541	6.635869898
Average	6.280131	67.76639312	9.912603445
Std.Dev	3.701418	17.98957615	2.680836325

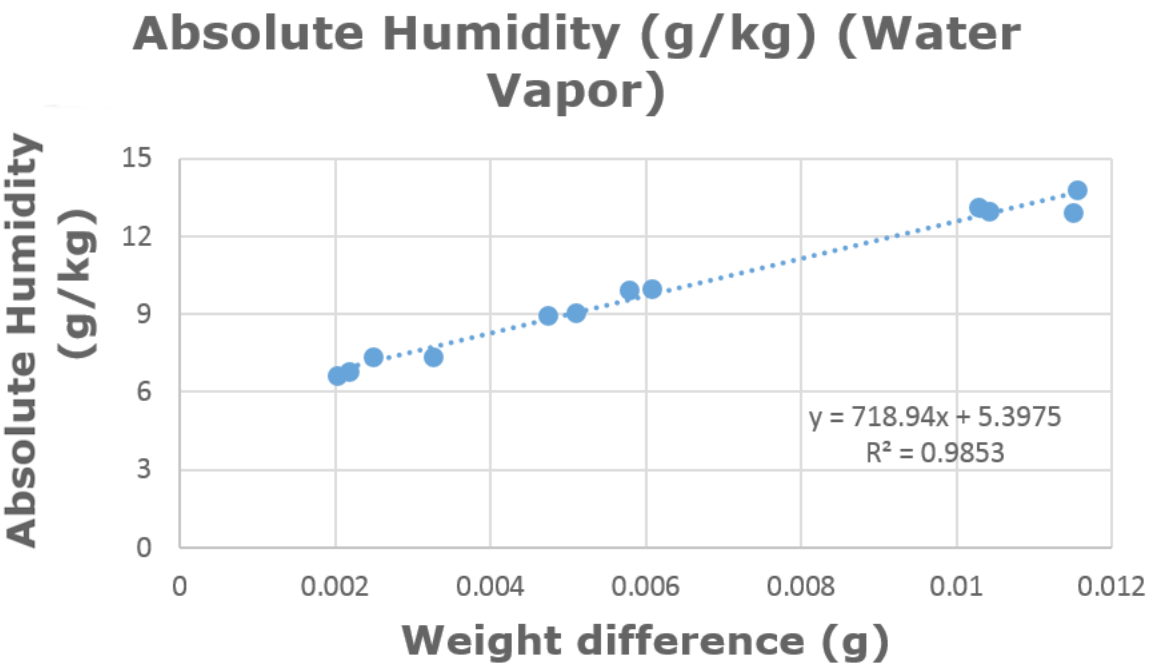

Figure 6: The weight difference of the measurement of TD tubes exposed to different levels of humidity.

3.4.2. Humidity tests

The goal for the humidity tests was determining how much water vapor PorapakN absorbs. According to the manufacturer, the maximum amount of water vapor needs to be below 1 mg. Otherwise the sorbent will not give efficient results. This tests also gives a clear picture of the requirement of further water management procedures while sampling.

Table 6 shows the results for the weight difference for each level of relative and absolute humidity. Even at average relative or absolute humidity of 45% and 6.6g/kg, the weight of the TD tube increased by 2mg. The weight increased up to 11.5mg for 92.9% relative, or 13.8g/kg absolute humidity.

This result was not expected as PorapakN is a hydrophobic sorbent, chosen as such for this study. We choose Molecular sieve 3A as a water vapor trap for the water management.

3.4.3. Water vapor and ozone traps

As an ozone trap, 26mg of sodium sulfate (Na_2SO_4) was packed in a Teflon tubing and placed upstream of the sampling tube.

Table 7: This table is showing the data obtained from the TD-Cryo-GC-MS response when using no ozone or water vapor traps in line with the thermal desorption tubes, and with using either ozone or molecular sieve trap for water vapor. The molecular sieve trap shows drastically lower area of the peak, suggesting that CH_3CN trapping in the molecular sieve.

Ozone and WV trap validation	Area	Concentration (ng)
Without ozone or molecular sieve trap	27137.03	24.76
With ozone	27085.8	24.76
With molecular sieve	722.3667	24.76

The unsatisfactory results of the molecular sieve trap validation required a new tool water management. Two different methods are proposed:

a) Dry purging the TD tubes before desorbing on cryo for 2 minutes and

b) Water vapor trap in a form of a metal tube kept on cold, positioned upstream of the TD tube when sampling.

Tests and results of these water management methods are going to be presented in other papers.

3.5. Validation method

Table 8 shows the results of the validation experiment. These preliminary results suggest that the direct injection into the GC inlet, into the cryo and onto a TD tube, give similar results. In addition, the TD tube showed full recovery during this experiment.

The results evinced acceptable method for validation of the three steps in the TD-Cryo-GC-MS method proposed in this study.

Table 8: The RT, area and height of the peaks from the validation experiment in the three steps described in section 2.5.

3ul injection of one-step dilution of 120ng liquid CH3CN	RT (min)	Area	Height	Width
1. In GC inlet	2.905	1484.7	435.61	0.261
2. In heated septa, on cold Cryo	3.028	1765.6	407.31	0.261
3. In heated septa, on room temp. TD tube	3.057	1861.0	412.92	0.290

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Angela Angelevska is the first Biochemistry graduate from the School of Technology, Engineering and Mathematics from the University of Washington Bothell. She took part in a one year research study of atmospheric chemistry with Dr. Daniel Jaffe, focusing on studying wildfires, merging her passion for science and research. Before working in a research environment she had spent her time volunteering. After graduation she is excited to combine her Biochemistry degree with the love of helping people, by working in a biopharmaceutical company focusing on cancer research.

Samantha Frati will graduate in Spring 2017 with a Bachelor of Arts in Health Studies and Law, Economics, and Public Policy. Her interests are in public health, bioethics, and policy. Samantha has been involved in undergraduate research on campus since Winter 2015. After graduation, she plans to attend graduate school and pursue a career in health law and policy specifically focusing on healthcare systems.

Bunraj S. Grewal is a senior who will graduate in the Fall of 2016 with a Bachelor of Science in Biochemistry from the University of Washington Bothell. He looks forward to continuing his education and plans to pursue a career in the medical field as a physician in the specialty of radiology or emergency medicine. After becoming an established physician in the community, Bunraj is hoping to organize a charity that will be dedicated to help pay the medical expenses for those who are in need of medical intervention and are unable to afford the procedures.

Jessica Jacobson is a senior in the school of Nursing and Health Studies at the University of Washington Bothell. She will graduate in June of 2016 with a Bachelor of Arts in Health Studies and a specialization in Health Policy, Leadership & Ethics. Jessica's interests include social determinants driving the prevalence of chronic disease, environmental exposures which burden health and policy development that serves to empower community resilience and health equality. Therefore, her mission is to build, implement and support health equality for the socially disadvantaged and the environment. In the future, Jessica hopes to bring community based research into advocacy and policy making processes, building health policies and programs that service community's unique needs.

Isaac Kim is a pre-dental student with a B.S. in Biochemistry from UW Seattle. He has always been passionate about science, and he hopes that he can utilize his scientific background to become an effective yet compassionate dentist in the future. In his free time, Isaac likes to read, hike, climb, and play with his two cats Momo and Snow.

Mengkhy Lay is a senior at UW Bothell, and will graduate in spring 2016 with Bachelor of Sciences in Biology and Chemistry with a biochemistry option. Mengkhy is currently taking part in an undergraduate research with Dr. Peter Anderson on drug discovery. After graduation, Mengkhy plans to pursue a Ph. D in Pharmaceutical Science and later work as a research scientist on drug design.

Shelby Lubchuk is eighteen years old and a junior majoring in Media and Communication Studies at the University of Washington Bothell. Shelby works as an Orientation Leader here at UWB. June of last year, Shelby graduated high school and earned her AA degree from Skagit Valley College. She was the Valedictorian of her high school class and was awarded the Carol Huber Award, the Academic Excellence Award, and the President's Medal from Skagit Valley College. Shelby is also the member of three National Honor Societies. Outside of school she enjoys training and showing dogs and horses. Shelby plans to earn her master's degree in Conservation Biology at Washington State University.

Crystal McClure is a Ph.D student at the University of Washington in the Atmospheric Science department. Her focus is on air quality which includes studies on gaseous mercury, carbon dioxide, and forest fires emissions. Crystal's current project is using acetonitrile to quantify forest fire emissions in cities to examine how that relates to high air pollution days. Her career goal is to develop and build

instrumentation that can be used to sample and classify air pollution. This research helps form policy and eventually leads to clearer air for everyone!

Taryn Meacham will graduate in Spring 2016 with her Bachelor of Science in Chemistry with a Focus in Biochemistry. After graduating, she hopes to attend medical school starting in the Fall of 2017. Outside of school and research, Taryn serves as the Program Coordinator for the Pre-Med Club at the University of Washington Bothell and manages the Pre-Med Mentorship program at UWB in addition to advising 8 first year pre-med students at UWB. She is also a student intern in the Health Scholar Program at the Swedish Medical Center in Issaquah, Washington.

Martha Raymore is a senior graduating winter of 2017. She will graduate with a Bachelor of Science degree in environmental science, with an emphasis on conservation and restoration ecology, and minor in marine biology. This research was conducted as part of a research in marine biology class at Friday Harbor Labs, and was performed under the close mentor-ship of Megan Dethier. After graduating she plans to continue on to graduate school where she can continue learning, researching, and exploring her love of everything science and nature.

Hillary Sanders will graduate in Spring 2016 with a Bachelor of Arts in Global Studies and minor in human rights. Throughout her undergraduate career at the University of Washington Bothell, Hillary has participated in a variety of student organizations. Most recently, Hillary has worked on organizing guest speaking events, public demonstrations, workshops and group dialogues, as well as an initiative to increase access to menstrual supplies on campus through Gender Equity Club. Outside of UWB, Hillary has interned with the UW Center for Human Rights and The Seattle Globalist on the main UW campus. If she had spare time, Hillary would spend it kayaking, gardening, and hiking the beautiful forests of Washington.

Malak Shalabi, a Palestinian-American, is a sophomore at UW Bothell studying Law, Economics, and Public Policy. Her interests lie in the geopolitics of the Middle East, compelling her to study international law once she attends law school. With her degree in hand and dedication to justice and human rights, Malak strives to make major contributions to the movement of Palestinian solidarity, both politically and socially.

Denae Weigelt is a junior at UW Bothell and will graduate with her Bachelor of Arts in Society, Ethics, and Human Behavior at the end of Spring 2017. Denae is looking forward to gaining her master's degree in early childhood education on her way to becoming a child life specialist. A child life specialist is a pediatric health care professional who works with families and children in a hospital setting to help them cope with the challenges of illness, disability, and hospitalization. Aside from academics, Denae proves loyal to anything relating to coffee, art, the outdoors, and athletics.

Sara Wells will graduate from UW Bothell with a Bachelor of Science in Chemistry in Spring 2016. Upon graduation, she hopes to decide on the perfect graduate program. Once there, she will apply her interest in combining aspects of chemistry and biochemistry with math and statistics to research, to expand scientific knowledge and share new ideas and findings. She is excited to be able to further her education and pursue her interest in research in chemistry.

ADDITIONAL CONTRIBUTORS
Megan Dethier and Rachel Li

ABOUT THE EDITORS

Nicholas Begley will graduate with his Bachelor of Arts from the School of Nursing and Health Studies from the University of Washington Bothell at the end of Spring 2016. His passion for research and policy will take him directly into the Masters of Arts in Policy Studies, also at UW Bothell, where he can focus on sustainable agriculture policy with an emphasis on how it impacts human and environmental health. Nick has spent a lot of time on campus conducting research in the conservatory on campus relating to hydroponics and the growing of agricultural crops. He hopes to one day create his own foundation dedicated to education, research and policy analysis that focuses on addressing issues surrounding the access to food. As an member of the editorial board, Nick hopes to encourage other students to showcase their work and drive awareness towards their research.

Bartel H. Broussard will graduate in Spring 2016 with B.A. in Society Ethics & Human Behavior. Once graduating, he hopes to go on to physical therapy school. His interests in study range from biology and physics to human rights and sociology. In the future, Bartel would like to find a way to combine all of these interests with physical therapy. Before working on the CROW, Bartel was a member of the UWB Policy Journal editorial board. He is also in his third year of working at the UWB Writing and Communication Center (WaCC), as a Peer Consultant. Bartel believes that working on the CROW is a great opportunity for him to combine his academic interests, and also provides an outlet for the skills that he has developed while working on the Policy Journal and at the WaCC.

Michelle Fessler will graduate in Summer 2016 with a Bachelor of Arts in Media and Communication Studies, and Culture, Literature, and the Arts. Throughout her time at the University of Washington Bothell she has worked on many student publications. She was an editor on Clamor, UWB's literary and arts journal, from 2013-2015, helped revive the Husky Herald in the beginning of 2015, and now she is an editor on The CROW. She also worked as a Peer Consultant at the Writing and Communication Center, and has taken up the managing editor position at The Monolith: Science Fiction Short Stories. After graduation, she hopes to pursue publishing or education.

Heather Hewitt is a senior at UW Bothell, and will graduate in the spring with a Bachelor of Arts in Health Studies. Heather is also the coordinator for two related research projects on campus. After graduation, Heather plans to work in health care while pursuing a Masters in Health Administration. As a fellow researcher and self-described grammar nerd, Heather is excited to be a part of The CROW's editorial board in its inaugural year.

Tanya Kumar is a sophomore graduating in the summer of 2018. She will be graduating with a Bachelor's in Arts from the School of Nursing and Health Studies, and a Bachelor's in Science from the School of Biological Science, Technology, Engineering and Mathematics. She is hoping to pursue pre-dental, and later become a pediatric dentist. For the past year, she had taken part in a biological research study with professor Chang, and values the unique and exciting knowledge gained from a research experience. This is her first time, being part of a journal editorial board and is thrilled to see the journal grow throughout her next few years in college.