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Dr. Hayat Sindi. Photo credit: By PopTech from Camden, Maine and Brooklyn, NY, USA

Eureka!

The great mathematician Archimedes reportedly exclaimed "EUREKA! EUREKA!" (which roughly translates to "I HAVE FOUND IT, I HAVE FOUND IT") when the solution to a complex problem was revealed in his mind. As all scientists know, the *moment of discovery* is a cherished event; the prospect of discovery is the reason to get up in the morning, and it is what carries scientists through long nights of struggle and frustration. It is this Eureka! moment we wish to share with our students. "Eureka!" is therefore the perfect distillation of the spirit of science and an appropriate title for a journal whose goal is to provide a forum in which students can share their Eureka! moments.

To those who reviewed manuscripts for this publication

Thank you for your time and effort on the behalf of our students. We know the density of your schedule and understand the sacrifice you have made to review our work. This sacrifice is greatly appreciated. The students participating in this research program are enthusiastic, and their work strives to reveal interesting and pertinent things about the world around us. Each manuscript published herein is the result of input from at least three faculty reviewers and the interpretation of this input by the student researchers. We have done our best to address the concerns expressed in each review, and your comments and suggestions have greatly improved the quality of our manuscripts. Please understand that final manuscripts are the result of the efforts of reviewers, students and mentors, and that not all suggestions may be incorporated, but were certainly considered. We will continue to recruit new students, and therefore hope that this is not the last time we call upon you to review such work.

We sincerely thank you for your help,

EUREKA!

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Dr. Hayat Sindi, a Role Model

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Abstract

This research paper highlights the inspiring journey of Dr. Hayat Sindi as an example of how great an impact a role model can have in the scope of education and personal growth. This research examines the structural components of Dr. Hayat Sindi's life, through a narrative analysis approach. Different online sources, including websites, interviews, databases, and talks are studied to emphasize the legacy of Dr. Hayat Sindi's journey in shaping educational narratives and aspirations for the advancement of society, showcasing the broader importance of such role models in inspiring future generations.

Introduction

"A role model should be like the light at the end of a tunnel, guiding you when you are lost." (Cristina Imre)

Did you know that having a role model can substantially impact an individual's personal and professional growth? Role models instill the self-esteem to aspirate beyond one's dreams, fostering motivation and inspiration to achieve goals. However, finding a true role model can be challenging for some, as it requires identifying someone whose qualities and accomplishments resonate with one's own goals. It is important to note that role models can be mentors. Mentors offer guidance and support on a personal level. In contrast, role models are individuals who inspire and empower from a distance, often through their remarkable achievements and dedication to their fields. They also encourage people to accomplish

great things by following in their footsteps. Dr. Hayat Sindi is one such individual who found inspiration in various STEM scientists, propelling her to surpass boundaries and emerge as a role model for countless others.

In my research on Dr. Hayat Sindi, I extensively explored various websites, and databases, and watched her interviews and talks. Among many notable role models, I found Dr. Hayat Sindi to be a perfect guiding light for me. Her dedication and passion for studying science and desire to contribute to society inspired me. Dr. Hayat Sindi is a renowned scientist and innovator who has made significant contributions to society. Her notable innovations include ” Diagnostics for All” and the establishment of the ”I2 Institute of Imagination and Ingenuity”. Initially a hidden figure, Dr. Hayat Sindi has now emerged as a prominent role model for many individuals.

Methods

In this paper, all the data was collected from various online sources. The data collection period took 9 months, from August 2022 to May 2023. The sources used for this research include websites, articles, databases, interviews, and talks related to Dr. Hayat Sindi. For example, TED-TALKS, PopTech, National Geographic Society, etc. To gather the information, a thorough search was made by using keywords such as ”Dr. Hayat Sindi,“ ”achievements,“ ”innovations,“ ”journey,“ and others relevant to the topic. This approach enabled the exploration of different themes in Dr. Sindi's journey, highlighting her determination and passion for her work.

The data collection method employed a thematic analysis approach. Multiple articles, documentaries, and talks on Dr. Hayat Sindi's life were reviewed, and emergent themes were identified and analyzed. A narrative approach was chosen to delve into and comprehend Dr. Hayat Sindi's personal experiences and stories. This helped to understand her motivations, challenges, and accomplishments throughout her career.

There are some limitations of this research. Firstly, all the data collected is derived from online sources and not from direct interviews or personal interactions with Dr. Hayat Sindi. This introduces the possibility of misinformation or incomplete information in these sources. To mitigate this risk, I made efforts to minimize this risk by cross-referencing information from multiple sources. I also ensured that all the data was from reliable sources, including authoritative publications, reputable presentations, like PopTech, TEDTALK, etc. However, the reliance on online sources may limit the validity of the research findings. To address this, future research could encompass a more diverse range of data collection methods, such as interviews or direct interactions with Dr. Hayat Sindi providing a more comprehensive understanding of her journey.

Findings

While reading about Dr. Hayat Sindi's journey, it becomes apparent that she encountered numerous obstacles along the way. The most prominent theme that emerged from the data was her determination and passion, which played an essential role in her journey to success. This theme stood out as it exemplifies the qualities necessary for personal and professional achievement. Dr. Sindi's journey serves as an inspiration for individuals facing hardships, making her a role model for dedication. However, her relentless perseverance and resilience enabled her to overcome each setback and remain steadfast in her efforts.

Early Influence

From a young age, Dr. Sindi was fascinated by discoveries and had a strong desire to become a scientist. Influential figures like Al-Farabi, Marie Curie, Albert Einstein, and Isaac Newton inspired her since childhood. She used to dress up as a scientist and imagine herself as a scholar in front of the mirror. This early inspiration laid the foundation for her future accomplishments.

Family Support and Encouragement

Dr. Sindi's parents played a vital role in cultivating her dreams and aspirations, supporting her journey toward becoming a scientist. In an interview, she fondly recalled her father's encouraging words: "Hayat, with education and learning, you can do anything. You can be one of them (scientists) (TEDxCERN, 2014)." This awakened her interest in science and technology from a young age, and she often dismantled and repaired household appliances. The persistent support and encouragement from her family further uplifted her to pursue a career in science and become a role model for others.

Passion for Science

Driven by her passion for science, Dr. Sindi began her education in Saudi Arabia, where she shined academically. Her dedication to science was evident when, at the age of 5, she returned a handmade doll given by her neighbor, stating that it should be given to a younger child as she saw herself as a scientist, not someone playing with dolls. Seeing her father dealing with severe asthma, she wanted to study pharmacology. Her goal was to develop drugs that could alleviate the symptoms of asthma. Recognizing that King's College London was one of the best pharmacology schools globally, she aimed to secure admission there.

Setbacks on the Road to Success

Dr. Sindi faced significant hurdles on her path to success, mainly as a woman in a male-dominated field. Studying abroad for higher education was a substantial highlight for her, as women were not allowed to pursue it during that time. She broke social and cultural barriers as a woman of science, striving to convince her parents and family to allow her to study at recognized universities worldwide. Overcoming language barriers was just one facet of her challenges which she overcame by learning English in England. Understanding scientific concepts in a language that she wasn't fluent in presented a huge

obstacle. Imagine the complexity of grasping intricate theories and ideas when the language barrier itself could be a significant hurdle. Thus, she made an astonishing achievement by learning English and immersing herself in a different culture and civilization.

Moreover, financial challenges also arose, and she worked part-time jobs alongside her studies. The most difficult challenge was being the only woman or one of the few women in her science classes. One of the biggest challenges she faced was an article that criticized her character due to gender bias. Despite the isolation and discrimination, she strived to prove her capabilities and paved the way for herself and other women in science. These setbacks were not just barriers but a point that forged her determination and commitment in her field.

Legal Triumph Against Defamation

In 2016, Dr. Hayat Sindi took on that article attacking her character due to gender bias by filing a lawsuit against a Saudi newspaper. The article in the newspaper, published in 2014, stated that Dr. Sindi misused funds from her charity, the i2 Institute for personal use and had falsified her research data. Dr. Sindi took legal action against the newspaper and in 2018, the Saudi Arabian court ruled in favor of Dr. Sindi where the court ordered the newspaper to issue a public apology, pay Dr. Sindi a huge amount of SAR 3 million (approximately USD 800,000) in damages, and remove the articles and refrain from publishing any further defamatory material about Dr. Sindi. If we delve deeper, it is evident that these accusations may have had some gender bias, as such attacks on her character were not uncommon for a woman breaking through barriers in her field. Regardless of facing defamation, Dr. Hayat Sindi emerged triumphant. This legal victory showcases her resilience and determination in the face of false accusations and highlights her commitment to upholding her reputation and the truth.

Cross-Cultural Experiences

To pursue her dreams, Dr. Sindi embarked on a journey to England to study pharmacology, driven by her father's asthma and her aspiration to develop drugs to improve its symptoms. She learned English and immersed herself in the new culture, thus adapting to the challenges of studying abroad. Reflecting on her experience, she discovered that excelling in her studies earned her respect and friendships. She noted, "People will tend to accept you more when you are willing to offer them something, like help with studying." Motivated by this insight, she assisted her professor in founding the thoracic medicine department at King's College and received the 'Princess Anne Award.' These experiences reinforced her commitment to academic excellence.

Transformative experiences

In 1991, Dr. Sindi graduated with honors in pharmacology from King's College, London. After completing her bachelor's degree, she immediately applied to a Ph.D. program in biotechnology at the University of Cambridge for higher studies. She worked with Schlumberger Cambridge Research, focusing on combating pollution with organic materials. In 1995, she earned a Ph.D. in Biotechnology, becoming the first woman from the Persian Gulf region to achieve this feat. To further expand her knowledge and expertise, she embarked on postdoctoral fellowships at prestigious institutions like Harvard University and the Massachusetts Institute of Technology (MIT). These experiences not only deepened her scientific understanding but also broadened her cultural horizons and strengthened her resilience.

Uplifting the standards of Biotechnology

Dr. Hayat Sindi is an innovator, and this title is given to her because of her great contributions and advances that proved to be helpful for society. Dr. Sindi's illustrious career involves co-founding and co-inventing the program known as "Diagnostics For All." This initiative, in collaboration with a team from

Harvard University, that aims to develop affordable diagnostic devices for millions of individuals residing in poverty. The groundbreaking point-of-care diagnostic devices created through this program are not only pioneering but also cost-effective, as they require no power, water, or specialized medical personnel. In a few minutes, these devices can provide crucial medical results that have the potential to save lives. They are particularly valuable in areas lacking proper medical infrastructure, benefiting approximately 60% of the population who would otherwise have limited access to medical treatment. It provides care for liver function, farmer support, nucleic acid detection, child nutrition, and immunity.

Dr. Sindi's passion and determination in her career led her to play a pivotal role in leading the team of "Diagnostics For All" to achieve remarkable success. The team secured the first spot in the social enterprise track of Harvard Business School's "Business Plan Contest." Additionally, they achieved another significant victory by winning first place in MIT's $100K Entrepreneurship Competition in the same year. This extraordinary accomplishment marked the first time in history that a single team emerged as the champion in both prestigious competitions within the same year.

A Pioneer in Science, Education, and Global Development

Dr. Hayat Sindi contributed her life to promoting science, technology, education, and innovations. Some of her leadership positions are as follows.

Dr. Hayat Sindi was appointed as a UNESCO Goodwill Ambassador in 2012 by UNESCO Director-General Irina Bokova for her efforts in promoting science education in the Middle East, especially for girls. Dr. Sindi was appointed in 2013 by the United Nations (UN) Secretary-General Ban Ki-moon to the newly constituted UN-Scientific Advisory Board, which comprised 25 global experts and provided advice to the UN's leadership on science, technology, and innovation for sustainable development. Moreover, in 2020, she was appointed as a Global Ambassador for the G20 Health and Development Partnership by

Alan Donnelly, Convener of the G20 Health and Development Partnership and Executive Chairman of Sovereign Sustainability and Development.

These achievements and leadership positions showcase Dr. Hayat Sindi's profound impact on science, education, and global development, solidifying her as an influential figure and a catalyst for positive change.

Discussion

Dr. Sindi's tireless dedication to her ambitions is truly praiseworthy. Despite facing numerous challenges, she endured and achieved her dream of becoming a scientist. As a once-hidden figure, she gradually gained recognition through her relentless dedication. Her journey serves as an inspiration for individuals facing various obstacles in their pursuit of education. She inspires those who struggle to break free from social constraints and pursue education, guiding individuals, navigating cultural, and societal barriers, as well as those grappling with financial limitations in their pursuit of higher education.

One aspect that stands out is Dr. Hayat Sindi's self-determination and her ability to inspire others through her speeches and talks to do the same. Her inspiration and support particularly resonate with women and Muslim women who often encounter discrimination and fewer opportunities. Driven by her beliefs, she states, "I want all women to believe in themselves and know they can transform society." Her mission is to empower young innovators and provide them with opportunities in the field of research and innovation, enabling them to bring new ideas to STEM.

While it is true that the positive influence of role models can be difficult to determine, it is important to recognize that their impact extends beyond measurable metrics. Nonetheless, some may argue that the influence of certain people as role models is limited. However, Dr. Sindi's story represents the great effect of inspiration. By sharing her journey and advocating for education, innovation, and equal

opportunities, she creates a network of support and motivation for those facing obstacles. Dr. Sindi's impact echoes around the world, inspiring individuals from diverse backgrounds to pursue their dreams and make a positive difference in their communities.

Conclusion

In conclusion, Dr. Hayat Sindi's accomplishments and inspiring journey make her a true role model for individuals facing challenges in their educational pursuits. Her dedication, resilience, and passion for science have made her a guiding light for many people, including myself. Through her notable innovations such as "Diagnostics for All" and "I2 Institute of Imagination and Ingenuity," Dr. Sindi has not only contributed significantly to society but has also emerged as a pronounced role model for ambitious scientists and innovators. Her relentless determination and commitment to empowering women and fostering innovation have a profound and far-reaching impact.

Dr. Sindi's journey sets an example of the importance of having a role model and how it could bring positive changes to society. Through embracing her message of self-belief and equal opportunities, we can collectively work towards breaking down barriers, encouraging educational growth, and cultivating a more comprehensive and innovative society.

Finally, the remarkable journey of Dr. Hayat Sindi stands as a lasting testament to the profound significance of role models in our lives with her firm dedication and relentless pursuit of excellence, she becomes a guiding light that inspires and empowers individuals to overcome obstacles and realize their goals. Dr. Sindi's great story will continue to ignite motivation and instill empowerment in future generations, leaving an indelible imprint on the scientific landscape. She serves as a beacon of hope for all those who aspire to make a difference. In an interview with the National Geographic, Dr Sindi said, "My message is: Find a mission in life and contribute something to humanity. For me, science is a

universal language that transcends nationality, religion, and gender. It can help solve any problem our world faces."

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Species Biodiversity Utilizing eDNA Analysis in Mill Branch Creek

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Abstract

Environmental DNA (eDNA) is defined as a distinctive mashup of genomic DNA found in a variety of environmental materials from different organisms. We use graph analysis to examine trends in species diversity based on environmental DNA analysis, using four sets of organism primers (fish, organism, amphibians, and plants). Wildlife residing close to Wake Tech's campus is impacted by the construction of Highway 540. The current eDNA study assessed the species diversity existing in Mill Branch Creek following this construction. The family and species number of Homo Sapiens, often known as humans, were among the most frequently discovered in Mill Branch Creek. Identified through BLAST, a fundamental local alignment search method that identifies areas of local sequence similarity. The most common species of bacteria found was *Pseudomonas gessardi*, a highly harmful bacterial species, usually present in meat. Additional eDNA samples collected were stored in a deep freezer for downstream laboratory analyses allowing further investigation of Mill Branch Creek in the future.

Introduction

Wake Tech Community College's Mills Branch Creek campus is surrounded by construction on North Carolina highway 540 (Figure 1). The development of highway 540 influences the variety of species that live in the vicinity of Wake Tech's campus.

Figure 1: Topo Map of Streams in Wake County, North Carolina. Zoom over the area of Wake Technical Community College South Campus and the stream of interest (highlighted in dark blue) as well as of the highway 540 construction site (highlighted in orange).

The usage of environmental DNA (eDNA) focuses on its presence in environmental samples as opposed to DNA that is obtained from targeted bacteria (Nukazawa et al., 2020). Their study on eDNA intake was used to illustrate the relevance of bacteria living in river water and bed sediment to identify carp DNA. Through eDNA analysis, this project evaluates the species diversity present in Mill Branch Creek following the construction of highway 540. eDNA is characterized as a unique blend of genomic DNA from various organisms that is found in a collection of environmental samples (Taberlet et al., 2018). To help assess how species diversity changes over time we use graph analysis from environmental DNA analyses findings. This protocol led to the identification of the various species present in the creek. We wanted to answer the following question: have neighboring species been affected by the highway construction site? In this project, species diversity in a natural habitat is impacted by upstream pollution from a heavy highway construction site, including bacteria. Through eDNA analysis findings, we show

that Mill Branch Creek prevalent species diversity has decreased following the construction of highway 540.

Figure 2: Jonah Ventures Aquatic Environmental Kit (A) Jonah Ventures Aquatic Environmental Kit, syringe filter (B) Sample collection at Site E using Jonah Ventures Aquatic (C).

Materials and Methods

The water samples were collected using the Jonah Ventures Aquatic Environmental DNA Kit, which is an aquatic eDNA kit (Figure 2A & 2B). Read-Data, sometimes referred to as summary data, was used to list the species under study. Using one of the primers from each of the four organisms, ESV-Data, also known as Exact Sequence Variant, examined a particular species of interest and discovered any hits on that species. They were created by looking at the sequences that were most prevalent. READ and ESV (exact sequence variants) data were categorized by class and number of species in the Microsoft Excel spreadsheet. Biological sequences from these species, such as the DNA and RNA nucleotide sequences, were compared using the Basic Local Alignment Search Tool, or BLAST. Measurements of conductivity,

dissolved oxygen, turbidity, pH, temperature, and salinity were carried out using LabQuest Probes (Figure 2C).

Results

Spring 2022 READ and ESV data from eDNA lab findings were examined. The Jonah Ventures Aquatic Kit's READ and ESV data were separated by class and the number of species in Microsoft Excel (Figures 3,4, & 5). Each set of data was then transformed into a pie graph. Using one of the four organisms' primers, ESV data examined a specific species of interest and discovered any hits on that species.

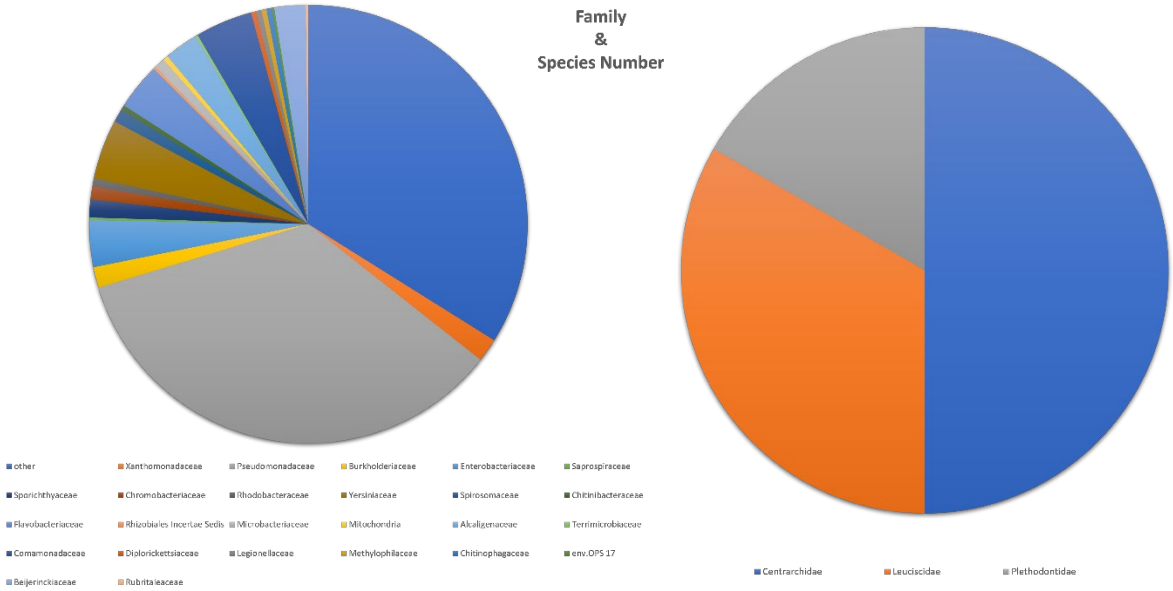

Figure 1: On the left it's 16S (Mitochondrial Organism) and on the right it's MiFishU (Fish –Lepomis).

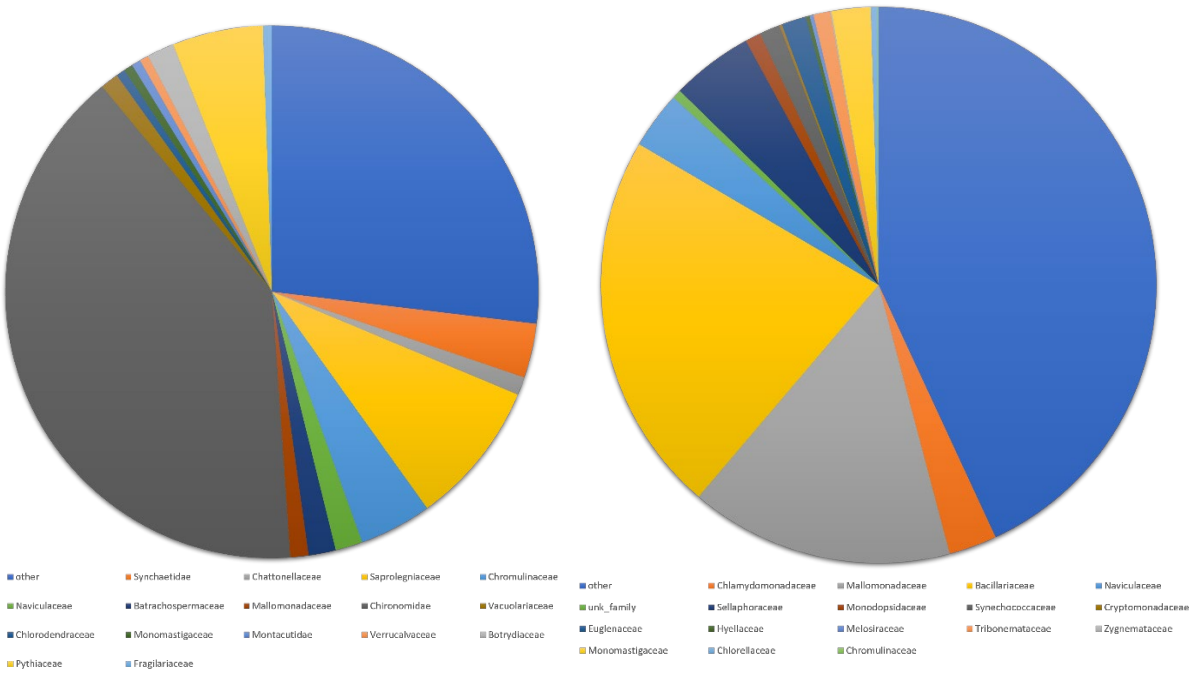

Figure 2: On the left it's COI (Mitochondrial cytochrome c oxidase subunit I) and on the right it's 23S (Eukaryote Organism – Chloroplast).

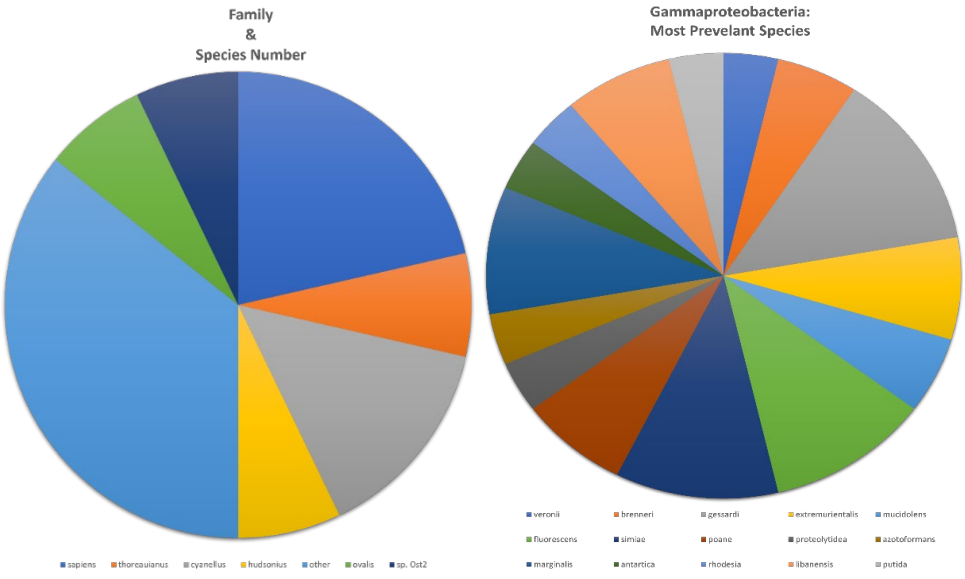

Figure 3: On the left it's BATR01 primer with the frog called Pelophylax nigromaculatus and on the right it's 16S mitochondrial DNA with Gammaproteobacteria of various sorts.

Using the BLAST computer software, we discovered that the species is a sort of frog called *Pelophylax nigromaculatus.* This primer is called BatR01 (Figure 5). *Gammaproteobacteria* of various sorts were the most frequent species discovered in the ESV data files, along with primer 16s (Figure 3).

On December 8th, 2022, a 60 mL sample of water was taken in a retention pond next to Mill Branch Creek using a Jonah Ventures Aquatic Environmental Kit. Water was drawn from a catch net and put in a sample collection cup. The 60mL of collected water was then withdrawn using a syringe, and water was then pushed into a beaker through a filter linked to the syringe. However, as we wanted to develop our own in-house pipeline for eDNA analysis, we did not send this sample for eDNA processing. Instead, we used a DNA extraction kit (DNeasy Powerwater Kit, QIAGEN) in our lab. The plasma membrane was removed from the filter and added to the PowerBead solution for lysing and homogenization. To add to plasma, the lysing agents are heated in a water bath to a temperature of 55 degrees. The DNA was extracted from the plasma's supernatant and put in a deep freezer after it was vortexed.

To conclude, the lab was able to identify the highly dangerous bacterial species *Pseudomonas gessardi*, which is frequently present in meat, by selecting the most prevalent species and focusing on bacteria. These results from earlier eDNA samples have been examined, but with more information gathered from Mill Branch Creek in the fall of 2023, the preservation of extracted DNA is retained for future students in a deep freezer. This extracted DNA sample will be utilized for PCR (Polymerase Chain Reaction) amplification using the chosen primers and nanotechnology reading.

Discussion

In this study, we utilized environmental DNA (eDNA) analysis, employing four sets of organism primers, to examine the impact of Highway 540 construction on species diversity in Mill Branch Creek, near Wake Tech's campus. Notably, Homo sapiens, representing humans, emerged as one of the most frequently detected species in this environment. Additionally, our analysis revealed the prevalence of

Pseudomonas gessardi, a potentially harmful bacterial species commonly associated with meat. These findings, obtained through BLAST analysis and facilitated by deep freezer storage of eDNA samples, provide valuable insights into the ecological dynamics of Mill Branch Creek, laying the foundation for further investigations in the future.

Graph analysis was used to make use of environmental DNA (eDNA), a special mashup of genomic DNA from many organisms that is found in a range of environmental components. Using the gathered information, four sets of organism primers were utilized to produce results that looked at trends in species diversity based on environmental DNA analysis. The four sets of primer organisms included plants, bacteria, amphibians, and fish. The development of Highway 540 affects the wildlife that lives next to the Wake Tech campus. After highway 540 was built, an eDNA study evaluated the species diversity in Mill Branch Creek and *Pseudomonas gessardi*, a potentially hazardous bacterial species that tends to thrive in meat, was the most prevalent kind of bacteria located. In this experiment, upstream contamination from a busy highway construction site—including the discovery of bacteria—has clearly had an influence on species diversity in a natural setting. Nonetheless, even with harmful bacteria present, the stream remains healthy, showcasing the ecosystem's agility.

It is plausible to assume that the construction of Highway 540, human disturbances, and other manipulated areas influence nearby habitats. Although human activity and environmental change pose a threat to ponds, they are more prone to environmental challenges than bigger water bodies with greater streams and rivers. (Biggs et al., 2016). The extent of the impacts these factors may have on species diversity remains very unclear and, at times, inconclusive. Under stagnant conditions, eDNA can settle out of suspension, but become incorporated into the water column again following sediment disturbance (Turner et al., 2015; Buxton et al., 2018). It is also unclear whether there are more identifiable species in areas farthest from development, or in streams in the most manipulated areas

because of these factors. As a result, under optimal conditions, eDNA may be detectable in ponds for several weeks (Buxton et al., 2017a), but it can also degrade quickly, with target eDNA completely disappearing within a week (Brys & Halfmaerten, unpublished results). Furthermore, there could also be a certain number of factors that affect the results of eDNA (Ariella M. Danziger et al., 2022). Maybe when the eDNA is collected there is a high degradation rate within certain species then impacting the recoverable eDNA concentration (Danziger et al., 2022). In some cases of the eDNA methods, increasing the amount of filtering water or employing several filters or using filters with larger pore sizes may correct the uncertainty in the results (Rodpai et al., 2023). Although in-house labs' eDNA results are still uncertain, based on the data, the pending eDNA test results can still be analyzed and assessed to better understand the eDNA process through larger intake of retention pond samples. Initiating the data gathering and sorting process, followed by comparative analysis and the formulation of hypotheses to explain the findings. This is aimed at maintaining consistency and accuracy in testing while keeping methods and relevant variables in check. Expanding the sample collection from retention ponds is advisable, and seeking guidance from experts and utilizing statistical methods will enhance the study's robustness. Implementing preventive measures to avoid errors and maintaining comprehensive records of the findings is essential. Recognizing that persistent analysis, even in uncertain circumstances, will lead to a deeper understanding of the eDNA process.

From this experience, my knowledge has significantly broadened. I have gained valuable insights into the process of conducting fieldwork to collect firsthand data samples, which has deepened my understanding of the complexities involved in merging field and laboratory work. Additionally, I have honed my skills in utilizing Microsoft Excel for data analysis, which has been instrumental in my research endeavors. One of the key aspects of my learning has been the extraction of DNA from retention ponds using research methods that were initially outside of my expertise. This hands-on experience has provided me with a solid foundation upon which future students in the lab can build their own

understanding of eDNA analysis procedures. Furthermore, this experience has highlighted the importance of interdisciplinary approaches in scientific research, as it involves both fieldwork and laboratory analysis. As the Aquatic Flora & Fauna Lab looks forward to incorporating eDNA analysis into its repertoire, I have gained valuable insights into the potential applications and benefits of this technique for advancing our understanding of aquatic ecosystems. My knowledge has expanded through this experience, allowing me to contribute to the broader goal of enhancing students' abilities to comprehend and execute eDNA procedures effectively in the future.

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Aedes albopictus Larval DNA Extraction for F1534S KDR Mutation in the Voltage-Gated Na+ Channels

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Abstract

In medical entomology, some species of mosquitoes are closely examined because they are significant biological vectors of several devastating diseases. Among the 900 species that encompass the Aedes genus, the *A. aegypti* and *A. albopictus* are of a particular concern in the United States because these invasive species can spread dangerous diseases (such yellow fever and dengue fever) to vulnerable, naïve populations. In addition, mosquitoes can develop resistance to chemical control through genetic mutations that modify the target site of the insecticides, decrease their absorption, or improve their capacity for chemical detoxification. This research focuses to investigate if there is the presence of the F1534S, a type of knock-down resistance (KDR) mutation in the voltage-gated sodium channels (VGSC) which is a protein on the mosquitoes' neurons. This mutation in the mosquito's nervous system confers resistance to pyrethroid insecticides, commonly used for mosquito control in the U.S.. To detect KDR mutations, this study used DNA extracted from wild Aedes albopictus as the template for the VGSC polymerase chain reaction. Therefore, the aim of this research is to assess the prevalence of this mutation in Wake County, which is important for developing efficient vector control methods. Moreover, the findings from this research hold significant implications for

mosquito control programs in North Carolina and in the rest of the country, as well as other aspects related to population dynamics and species distribution.

Introduction

Among many species of *Aedes spp.,* two species, *Aedes aegypti* and *Aedes albopictus*, are concerning vectors for diseases in the United States, with *Aedes aegypti* considered the primary vector and *Aedes albopictus* the secondary (Reed et al., 2018). Among the diseases these mosquitoes transmit are Dengue virus (break-bone fever), Yellow fever, Chikungunya virus, Zika virus, West Nile virus, Eastern equine encephalitis virus, and La Crosse virus (the primary cause of pediatric encephalitis in the U.S.) (Abernathy et al., 2022). Although there has been no record of *Aedes aegypti* in North Carolina, the species has been found in Washington D.C. and coastal South Carolina (Lima et al., 2016). In contrast, *Aedes albopictus* has a high prevalence in North Carolina (Reed et al., 2018). In addition, *Aedes albopictus* is considered one of the world's most dangerous invasive species (Lee et al., 2022). The female sucks blood from mammals, especially humans, in order to have nutrients to lay her eggs (Lee et al., 2022). Besides the major epidemiological factor, these mosquitoes are known for being truculent biters which is alarming to the population inhabiting the areas where they are present (Abernathy et al., 2022). North Carolina has several different approaches in controlling mosquito surveillance, because the state manages it at either regional, county, or municipal levels and is located under public works, health agencies, or other environmental service agencies (Del Rosario et al., 2014). The average yearly budget of North Carolina's mosquito

control programs, including salaries, chemicals, and equipment, is typically small, being $66,303 in 2009 (Del Rosario et al., 2014) and $36,373–$741,377 in 2023 (Stroops et al., 2023).

Chemical insecticides are commonly used for mosquito control. Pyrethroids are a class of chemical insecticides such as permethrin, deltamethrin, and bifenthrin that have an effect on mosquitoes known as knock-down, causing paralysis and death. With that in mind, this research aims to look for a mutation of the gene encoding the voltage-gated sodium channel (VGSC), a protein responsible for synapses to occur within the nervous systems. Pyrethroids target the VGSC, and mutations in that protein can result in knock-down resistance (KDR) therefore interfering with electrical signals in the nervous system (Chen et al., 2016). This study aims to find the F1534S in wild *Aedes albopictus* in Wake County since the mutations have been found there (Abernathy et al., 2022). Among the many mutations conferring KDR, three have been well studied: at codon 1534 in domain 3 that has phenylalanine instead of serine, cysteine, or leucine; at codon 1532 threonine is substituted with isoleucine and at 1016 glycine is substituted with valine (Abernathy et al., 2022). The current study will focus on detecting the F1534S KDR mutation in eggs from wild caught mosquitoes using polymerase chain reaction on larval specimens after hatching these mosquitoes' eggs. This is important because KDR is correlated with the overuse of pyrethroids since it is most common and cost-effective insecticide in the U.S. for the past twenty years. This observational study was conducted throughout three Wake Technical Community College campuses to examine the prevalence of the F1534S mutation in Wake County.

Material and Methods

Mosquito Collections

Mosquito's eggs were collected on two different Wake Technical Community college campuses. Although only mosquito eggs of *Aedes albopictus* were collected, four CDC Miniature Light Trap Model 512 were set up on three campuses (Figure 1) in the attempt to collect adults. Hence, no adult *Aedes albopictus* were collected. Mosquito egg traps were also put out and constitute of black plastic cups, germination paper stuck to the border of the cup, and ~350 ml of water in each cup. Germination paper is used for the *Aedes* mosquitoes to stick their eggs on. Therefore, germination paper is used in ovitraps to collect the eggs because it mimics natural breeding environments, due to its exceptional water absorption and retention qualities, which make egg collecting and counting simple. It also offers a reliable and stable surface for egg laying. Approximately ten to fifteen cups were placed at each time on the area wished to collect eggs for three days. In addition, these cups were placed approximately five to ten meters apart since *Aedes aegypti* and *Aedes albopictus* display a behavior called skip-oviposition behavior, which is a preference to lay eggs in when there are multiple cups available. They lay 40% more eggs in one cup than in others (Reinbold-Wasson et al., 2021). Thus, this would allow for different females to lay their eggs in different cups, creating more chances of finding the mutation on a larger number of mosquitoes. After collecting the eggs, they were hatched in white trays filled with water, one tray for each collection site. The germination paper along with TetraMin Tropical Flakes Fish Food were put inside the trays for seven days, approximately, until larvae were identified. Since fish food contains vital nutrients that encourage the growth of microorganisms and algae, which serve as food for mosquito

larvae, simulating natural conditions and supporting their development, hatching the mosquito eggs. The choice of TetraMin as the source of nutrition is due to reduced conductivity, pH, ammonium and phosphate concentrations. Additionally, TetraMin diet results in the biggest body sizes and calorie intakes of adult mosquitoes (Müller et al., 2013).

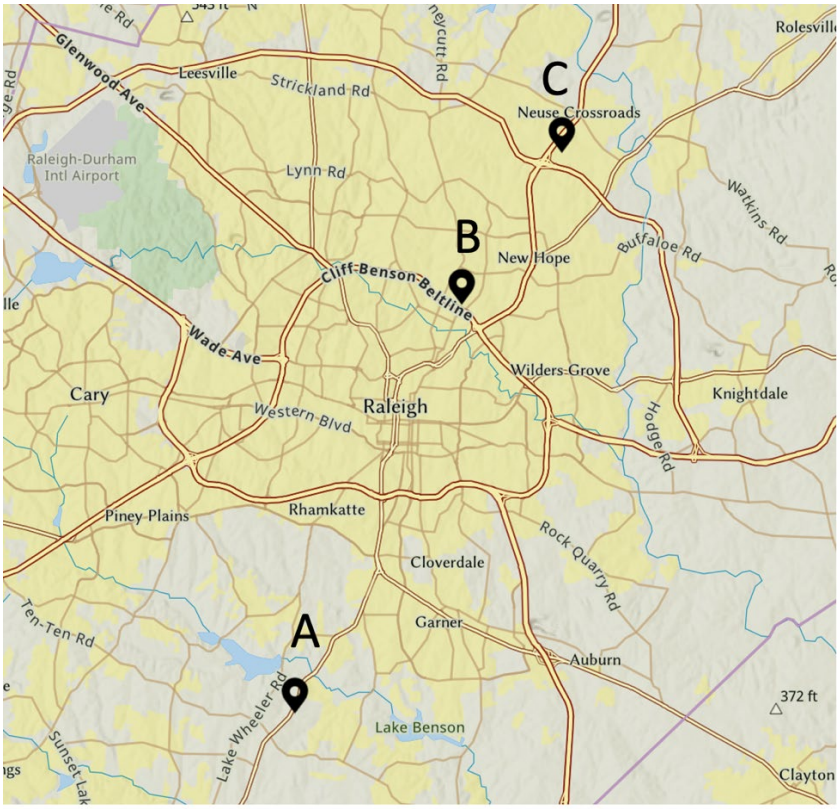

Figure 1. Map with the locations of all three campuses where the ovitraps and light traps were displayed. (Map generated by https://mapmaker.nationalgeographic.org/map/05ee0056dfa242a59da98ecab197f777/edit)

Location A: Southern Campus, 9101 Fayetteville Rd, Raleigh, NC 27603

Location B: Beltline Education Center, 3200 Bush St, Raleigh, NC 27609

Location C: Scott Northern Wake Campus, 6600 Louisburg Rd, Raleigh, NC 27616

DNA Extraction

After separating the larvae into specimens one through seven (Table 2), a sterile blue mini pestle was used to thoroughly grind each sample, containing the number of larvae indicated in the table 1. Without removing the pestle, 500 µL of extraction buffer (200 mM Tris, pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added to the sample, and the pestle was used to continue to grind the sample. After removing the pestle, the tube was vortexed for five seconds, then centrifuged for three minutes at maximum speed. A 200 µl pipettor was used to transfer 150 µL of the supernatant twice to a new microcentrifuge tube, and 300 µL of isopropanol was then added to the tube with the DNA. With the lid closed, the tube was inverted 3-4 times to mix, and then the tube sat at room temperature for two minutes. The tube was centrifuged at full speed for five minutes. Most of the isopropanol was dumped into a waste container without disturbing the pellet and 300 µL of 70% ethanol was added. The tube lid was closed, and the tube inverted two to three times. The tube was centrifuged for one minute and most of the ethanol was removed. The tube was centrifuged at full speed for five to 10 seconds to bring all the remaining ethanol to the bottom. A 20 µL micropipette was used to remove the last few microliters of solution, avoiding the pellet. The tube was left open and after any residual ethanol evaporated 25 µL of TE buffer (10 mM Tris, pH 8, 1 mM EDTA) was added to the tube to dissolve the DNA pellet. The tube was left at room temperature for two minutes to allow the DNA to rehydrate.

Table 1. Summary of *Aedes albopictus* eggs collected, hatched, and larval DNA extraction from different locations and dates.

Locations		Date (Collection)	Number of ovitraps	Eggs	Hatching Date	Total Larvae	Larvae	Extraction Date
Location A	Creek	9/6/23	15	25	10/6/23	9	Specimen 1: 5 larvae Specimen 2: 4 larvae	10/11/23
	Creek	10/4/23	5	69	10/11/23	8	Specimen 4: 4 larvae Specimen 5: 4 larvae	10/18/23
Location C	Pond	9/9/23	4	23	10/6/23	2	Specimen 3: 2 larvae	10/11/23
	Forest	9/9/23	2	40	10/11/23	11	Specimen 6: 5 larvae Specimen 7: 6 larvae	10/18/23

PCR and Sequencing

PCR was conducted to amplify the desired region. In previous research, the primers V3F and V3R were used to amplify the domain 3 of the VGSC (Abernathy et al., 2022) (Bowman et al. 2022). The Primer concentration (23 µL) was added to a PCR bead and allowed the bead to dissolve for 1 minute and then 2 µL of DNA was added directly to this mix. Afterwards, the samples were placed in a thermal cycler for 35 cycles of the following profile: Denaturing step: 95ºC for 15 seconds; annealing step: 58ºC for 15 seconds; extending step: 72ºC for 30 seconds; and final extension step: 72 ºC for 5 minutes.

Table 2. Primer sequences that were utilized to amplify *Aedes albopictus's* voltage-gated sodium channel genes in domain 3.

Primer	Sequence
V3F(domain 3)	GAG AAC TCG CCG ATG AAC TT
V3R(domain 3)	TAG CTT TCA GCG GCT TCT TC

The PCR products were visualized using gel electrophoresis as previously described by the Biology Instructors of Wake Technical Community College, 2023. Briefly, aliquots (5 µL) of each PCR reaction were mixed with loading dye and loaded into the wells of a 2% agarose gel and electrophoreses at 120 V for 30 minutes. A DNA ladder was simultaneously electrophoresed as a size reference (Biology Instructors of Wake Technical Community College, 2023).

Results

Mosquito Control:

Due to limited adult collection, this research was only able to extract DNA from the mosquitoes' larvae. Two hundred and fifty-nine eggs were collected from ovitraps on locations A and C. In these locations, the light traps did not collect any adult *Aedes albopictus.* Neither adults nor eggs of *Aedes albopictus* were collected in the location B from the four ovitraps and four light traps set on campus. Only adult *Culex pipiens* were collected in all 4 light traps. Since *C. pipiens* deposit their eggs on the surface of the water rather than on germination paper, no eggs from this species were retrieved (Kauffman et. al. 2017).

PCR and Sequencing Results

Gel electrophoresis indicated the presence of bands of approximately the size 300 to 400 base pairs, indicating successful amplification of VGSC in specimens one, two, three, five, six, and seven (Figure 2). These PCR samples were then sent to Genewiz for sequencing, but the results were inconclusive, due to lack of priming with the primer used.

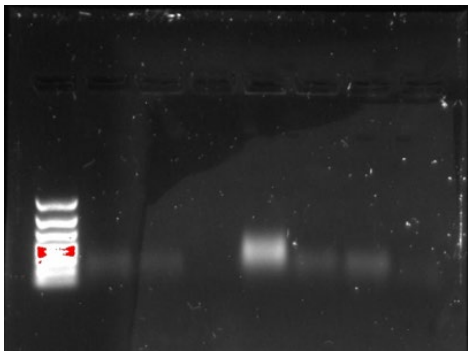

Figure 2. PCR gel electrophoresis shows the glowing bands of the samples one, two, three, five, six, and seven that have DNA and that were copied from the primer used.

Discussion

In the past, the F1534S KDR mutation has been found in Wake County in adult *Aedes albopictus* (Abernathy et al., 2022). Controlling these mosquitoes is crucial for public health, as they are biological vectors for many diseases. Knowing the prevalence of the F1534S mutation helps North Carolina combat this invasive species. With climate change, the number of mosquitoes, especially *Aedes albopictus*, is increasing drastically (Ryan et al., 2019). Nevertheless, mosquitoes can be controlled through biological and chemical methods.

Some natural insecticidal *Aedes spp*. control methods can be a large number of aquatic organisms including fish, like the *Gambusia affinis,* amphibians, copepods, odonate young instars, water bugs, and even larvae of other mosquito species (Benelli et al., 2016). On top of that, bats also eat mosquitoes and reside in North Carolina. *Myotis lucifugus*, most known as Little Brown Bat is an example. While mosquitoes are a part of *Myotis lucifugus*'s diet, they are not its primary prey (Gonsalves et al. 2013). In addition, the Germacrene D-4-ol, an essential oil derived from fresh leaves of *Zanthoxylum monophylum*, not only demonstrated efficacy in combating *Aedes spp.* larvae but also showed no harm to *Gambusia affinis* (Pavela &

Govindarajan, 2017). In contrast, some chemical control methods are currently used in mosquito control. For example, permethrin which is a synthetic chemical widely used as an insecticide, acaricide, and insect repellent. Permethrin-impregnated clothes, such as the uniforms used by the U.S. military personnel, act as insecticide to protect soldiers. This use of Permethrin is one of the possible explanations of the reason no KDR mutation was found in a study done in Fort Liberty in comparison with Wake County, due to these uniforms reducing landings and preventing bites, therefore repelling and killing the mosquitoes (Abernathy et al., 2022). Therefore, there is a need to find other environmentally safe methods to combat the population of *Aedes* without leading to the selection of mutants, addressing alternative methods for the use of pyrethroids that effectively controls mosquitoes with minimal environmental impact.

In the current research, there were some limitations in obtaining adult *A. albopictus.* The weather at the time the traps were set out is one of the potential limitations. Both location A and C had rain on the period the traps were active. Location A had 0.1 inch of rain expected in the twenty-four-hour period and 0.9 inch on location C. Additionally, there were winds at locations A and C of eight and four miles per hour, respectively. In addition, the batteries on the traps only lasted for approximately 48 hours while conducting the experiment, leaving a short window to collect the adults. Equally important, the absence of eggs on location B might be due to both light traps and ovitraps being displayed next to the interstate 440, a busy highway, since *Aedes. spp* has a preference on crossing calm and small roads over busy and large highways (Regilme et al., 2021). Nevertheless, there is a strong correlation between *Aedes* preferring to lay their eggs in household containers (Kraemer et al., 2015). Also,

limitations have occurred when sequencing the samples to search for the mutation. In molecular biology, the primers M13 forward (M13F): TGTAAAACGACGGCCAGT and reverse (M13R): CAGGAAACAGCTATGAC are often used for polymerase chain reaction (PCR) amplification and DNA sequencing. In other words, these primers position themselves on each side of the desired DNA segment, enabling amplification or sequencing of that specific region. The M13 sequence was attached to our primers by Genewiz for sequencing. Moreover, the lack of proper sequencing could be a problem with our primers or it could have been a problem with their primers. They used the M13 to try to sequence so it might not have sequenced properly because of that. Besides, since the PCR gel showed the glowing bands of 300 to 400 base pairs, it means that the samples with DNA replicated from our primer as indicated by the light bands. Therefore, even though the DNA samples bound to the primers, a full sequencing would be needed in order to look at the sequence to confirm the mutation.

Research must more thoroughly assess the mutation rates among *Ae. albopictus* throughout North Carolina and identify the underlying mechanisms of resistance selection. Also, further studies should gauge the aspects on how the weather impacts adult's distribution, and how long traps should be placed in each location in order to collect the mosquitoes. Understanding these phenomena is crucial as the identification of wild mutants becomes essential for public health. In addition, the increase in resistant mosquito populations will make it harder to manage these vectors, which will eventually cause a rise in illnesses carried by mosquitoes. Thus, it is imperative that public organizations take a proactive and comprehensive strategy to reduce this growing threat in order to protect the public health.

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